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ORIGINAL ARTICLE

Mode of action of poloxamer-based surfactants in wound care and efficacy on biofilms

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Surfactants are widely used as detergents, emulsifiers, wetting agents, foaming agents, and dispersants in both the food and oil industry. Their use in a clinical setting is also common, particularly in wound care. Complicated or chronic wounds show clinical signs of delayed healing, persistent inflammation, and the production of non-viable tissue. These types of wounds also present challenges such as infection and potentially house antimicrobial-tolerant biofilms. The use of wound cleansers to aid cleaning and debridement of the wound is essential. A large proportion of skin and wound cleansers contain surfactants but there is only a small amount of data that shows the effectiveness of them in the enhancement of wound closure. This review paper aims to explore the available literature surrounding the use and mode of action of surfactants in wound healing, in particular Poloxamer 188 (Pluronic F-68) and Poloxamer 407 (Pluronic F-127), and also uncover the potential mechanisms behind the enhancement of wound healing and comparison to other surfactants used in wound care. Furthermore, the presence of a microbial biofilm in the wound is a significant factor in delayed wound healing. Therefore, the effect of clinically used surfactants on biofilms will be discussed, with emphasis on poloxamer-based surfactants.

KEYWORDS
biofilms, mode of action, poloxamer, surfactants, wound healing

1 | INTRODUCTION

Physiological wound healing consists of complex overlapping stages of wound repair including haemostasis and inflammation, re-epithelialisation, and granulation tissue formation and tissue remodelling.[1] When the stages of wound healing are disrupted, this can lead to delayed wound healing. The cause of delayed wound healing is somewhat patient specific, and there is a variety of factors that may be involved.[2] Infection and subsequently the presence of microbial biofilms within the wound bed[3] has been hypothesised to significantly delay wound healing.[4] As part of a wound management strategy, the cleansing and debridement of wounds is thought to help in the removal of microorganisms and aid wound repair.[5,6] The use of surfactant-containing wound cleansers and wound dressings to aid autolytic debridement has shown increased wound healing rates that will be presented in this review. Surfactants are surface active agents, known to reduce the surface tension between two liquids or a liquid and solid and, therefore, are widely used as detergents, wetting agents, emulsifiers, foaming agents, and dispersants in a variety of industries. Not to be confused with these chemically synthesised surfactants, biosurfactants are produced on biological surfaces and also possess unique functional properties that allow for multiple applications.[7] The effect of surfactants and biosurfactants on wound healing and biofilms will be discussed. The aim of the review is to discuss the role of surfactants in wound healing and discuss their efficacy on microorganisms and biofilms with particular emphasis on poloxamer-based surfactants.

2 | INTRODUCTION TO SURFACTANTS

Surfactants, in a commercial context, are classified into ionic surfactants and non-ionic surfactants (Table 1). Ionic surfactants include anionic surfactants, cationic surfactants,

and zwitterionic surfactants. Anionic surfactants are the most commonly used surfactants and are dissociated in water into an amphiphilic anion and a cation, such as an alkaline metal and quaternary ammonium cation. Anionic surfactants include detergents such as alkylbenzene sulphonates, soaps, foaming agents such as lauryl sulphate, wetting agents including dialkyl sulphosuccinate and dispersants such as lignosulphonates. Anionic surfactants account for about half of the world's production of surfactants. Cationic surfactants are dissociated in water into an amphiphilic cation and an anion, often of the halogen type. This class of surfactants includes nitrogen compounds such as fatty amine salts and quaternary ammoniums with one or several alkyl long chains. Cationic surfactants are more expensive to produce and therefore are not as widely used as anionic surfactants. When a surfactant exhibits both cationic and anionic dissociations, they are referred to as zwitterionic or amphoteric. Examples of zwitterionic surfactants include synthetic surfactants such as betaines or sulphabetaines or naturally-occurring surfactants including phospholipids and amino acids.[8]

Non-ionic surfactants are the second most commonly used surfactant, after anionic surfactants. Non-ionic surfactants do not ionise in water as they contain hydrophilic groups that form covalent bonds, such as alcohol, phenol, ether, ester, or amide. Poloxamers are a major non-ionic surfactant, which are widely applied in wound care. They are triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly[propylene oxide], PPO) flanked by two hydrophilic chains of polyoxyethylene (poly[ethylene oxide], PEO). Through adjusting the chain length of the polymers and the ratio of PPO:PEO, many different poloxamers have been produced in industry that have slightly different properties.[9] Commercially, most poloxamers are best known as trade name Pluronic, followed by a letter and number. The letter either L, F, or P refers to liquid, flake, or paste physical forms, respectively. The first number × 300 indicates the approximate molecular weight of PPO and the last number × 10 represents the PEO percentage in the poloxamer. For example, Poloxamer 407 is Pluronic F-127, which has a PPO molecular weight of approximately 3600 g/mol and is made up of 70% PEO.[10]

TABLE 1 Classification of surfactants and their beneficial effects on wound healing

Classification	Properties	Beneficial effects	Reference
Ionic	Anionic: Dissociate in water into an amphiphilic anion and a cation	Enhance activity of antimicrobials through electrostatic stabilisation	30 41
	Cationic: Dissociate in water into an amphiphilic cation and an anion	Stabilise elution of antimicrobial, preventing cytotoxicity Prevention of protein aggregation and aiding of refolding of denatured proteins	24,25
	Zwiterionic: Anionic and cationic dissociations	Reduction in inflammation Aid in debridement of wounds	
Non-Ionic	Covalently bonded hydrophilic and hydrophobic copolymers	Increase rate of wound closure	21,35,44,48 5,6 21,35,38

Key Messages

- in broad terms, chemically produced surfactants can be categorised into anionic, non-anionic and cationic surfactants
- biosurfactants are produced by living organisms and include glycolipids, lipopeptides and lipoproteins, surfactin, lichenysin, fatty acids, phospholipids and neutral lipids
- many surfactant-based wound cleansers have been shown to increase wound-healing rates
- the non-ionic surfactant poloxamer 188 can cause cell membrane repair and supress protein aggregation
- surfactants such as poloxamer 188 have shown anti-biofilm capabilities in vitro through the reduction in microbial attachment and biofilm formation

Another category of surfactants is biosurfactants. Biosurfactants are amphiphilic compounds with biological origin, containing a hydrophilic region (polar or non-polar) and a hydrophobic region (lipid or fatty acid). Unlike chemically synthesised surfactants that are grouped according to polarity, biosurfactants are usually classified according to their microbial origin and chemical composition as follows. Glycolipids are carbohydrates linked to long-chain aliphatic acids or hydroxyaliphatic acids by an ester group and can be categorised into rhamnolipids, trehalolipids and sophorolipids.[7] Rhamnolipids are the most widely studied biosurfactants produced by *Pseudomonas aeruginosa* and consist of one or two molecules of rhamnose, linked to one or two molecules of hydroxydecanoic acid.[11] Trehalolipids are associated with most species of *Mycobacterium, Nocardia* and *Corynebacterium.* Trehalose lipids from *Rhodococcus erythropolis* and *Arthrobacter* spp. have been shown to lower surface tension.[12] Sophorolipids are glycolipids that are produced by yeasts and consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage.[13] Other biosurfactants include lipopeptides and lipoproteins, surfactin, lichenysin, fatty acids, phospholipids and neutral lipids. Biosurfactants have been widely used in the food industry to improve the texture and shelf life of products,[14,15] petrochemical industry to remove oil and petroleum contamination[16,17] and also in oral hygiene and medical applications.[18,19]

3 | MODE OF ACTION OF SURFACTANTS IN WOUND HEALING

Although surfactants, such as poloxamers have been widely demonstrated to aid wound healing in clinical and in vitro studies,[20–22] the mode of action of surfactants and their interactions with antimicrobials and antibiofilm agents is still not completely understood. The ability of surfactants to improve wound healing may be due to several roles including, aiding wound cleansing, suppressing protein aggregation and denaturisation, sealing/repairing tissue or cell membranes, stabilising antimicrobials, and exerting antimicrobial activity themselves.

Surfactants aid cleaning the wound bed and debridement. When surfactants are included in wound washing solution, less force is required to remove bacteria and cellular debris. Surfactants help autolytic debridement through degradation of collagen debris, by triggering the activation of matrix metalloproteinases (MMPs); however, depending on the surfactant's electrostatic properties, surfactants can either deactivate MMPs or enhance their activity. Additionally, Jovanovic et al[23] found non-charged surfactant molecules generally tend to have a minimal or positive influence on the enzymatic activity *of Clostridium* collagenase due to favourable solubilisation effects. However, both cationic and anionic charged surfactants inhibited enzymatic activity at low concentrations. Jeong et al[22] found that PluroGel surfactant-based dressings enhanced the activity of MMP 2 and 9 gelatinases, while simultaneously inhibiting MMP-8 collagenase. In the wound, this would be expected to quicken autolytic debridement by degradation of damaged collagen and offer protection of naïve, "healthy" collagen. Additional work would be needed to determine what happens in the wound bed tissues treated with surfactants.

Besides the effects on MMPs, surfactants can also suppress protein aggregation and aid refolding of denatured proteins to prevent persistent inflammation, which may lead to a non-healing wound. Mustafi et al[24] found that amphiphilic, surfactant, multi-block copolymers, such as Poloxamer 108 (P108), Poloxamer 188 (P188), and Tetronic 1107 (T1107) are efficient as additives to suppress aggregation of and to facilitate refolding of heat-denatured hen egg white lysozyme (HEWL) and bovine serum albumin (BSA) in solution. Additionally, Lee et al[25] demonstrated that poloxamers are more efficient than PEG in preventing aggregation of heat denaturized lysozyme. These findings indicate that copolymer surfactants may be applied to the treatment of burns and other conditions resulting in the denaturation of proteins.

Surfactants have also shown the ability to seal or repair tissue/cell membranes to avoid further accumulation of cellular damage. Copolymer surfactants, such as Poloxamer 188 (P188) and Poloxamine 1107 (P1107),[26] have demonstrated efficacy in restoring cellular integrity. P188 has been shown to seal membrane pores in skeletal muscle cells[27] and fibroblasts[28] after heat shock. Recently, Maskarinec et al[29] directly observed that P188 insertion into lipid monolayers occurred with selective insertion into damaged portions of the membranes.

Surfactants can enhance the stability of antimicrobials in wound dressings, by lowering the surface tension between 2 phases and can therefore be used to increase the activity of antimicrobials. Ionic surfactants such as SDS have been shown to prevent aggregation of silver nanoparticles and as a result enhance the antimicrobial effect. Transmission electron microscopy (TEM) images showed SDS binds to silver nanoparticles, which is suggested to significantly increase the surface charge, resulting in electrostatic stabilisation and a steric effect.[30] Although to a lesser extent than ionic surfactants, non-ionic surfactants such as Tween 80 have also been shown to enhance the stability of silver nanoparticles, resulting in enhanced antimicrobial activity. In contrast to ionic surfactants, the main mechanism of action for non-ionic surfactants is thought to be via a steric mechanism.[30]

Besides the modes discussed above, some surfactants have also shown to exert antimicrobial activity themselves. Quaternary ammonium compound (QAC) surfactants such as didecyldimethylammonium chloride (DDDMAC) have demonstrated bacteriostatic and bactericidal activity against Gram-positive and Gram-negative species.[31] Leakage of potassium ions during testing indicated a membrane target and disruption of the cell membrane for the mechanism of action of DDDMAC.[31] Polyhexanide (PHMB) is also an effective antibacterial agent through its cationic core interaction with negative sites on the lipopolysaccharide (LPS) component of bacterial cell membranes.[32]

4 | SURFACTANTS IN WOUND CARE

Clinical use of surfactant based wound dressings have been shown to correlate with improved wound healing, pain reduction and cost reduction in comparison to standard care products. Wound cleansing is considered to be an integral part of wound management and it is accepted that the use of basic wound cleansers, such as saline solution, in complicated wounds is not sufficient enough to cope with wound closure, impeding biofilm infection.[33] Therefore, the use of wound cleansers containing surfactants with or without antimicrobial agents is employed and multiple studies have shown their effectiveness in wound healing.

An antimicrobial carbopol-based hydrogel formulation with boron and pluronic block copolymers was evaluated for its healing activity in vitro and was found to increase wound healing; enhanced migration, angiogenesis, and contraction-related protein expression including collagen, α-smooth muscle actin, transforming growth factor-β1, vimentin and vascular endothelial growth factor was observed.[20] A non-rinse incontinence care product

containing PEG was shown to reduce the incidence of incontinence-related moisture lesions by 70% to 76.9%.[34] Surfactants have also been used in wound irrigation solutions intended to cleanse and debride wounds. In a single-blinded randomised control trial (RCT), 289 patients were treated with either propylbetaine-polihexanide solution (Prontosan) or saline, which resulted in a significant increase in wound closure and production of granulation tissue and also a decrease in inflammation.[35] Furthermore, in a separate study, an analysis of existing data showed that venous leg ulcers treated with Prontosan healed faster and in more cases (97%) when compared to cleansing with Ringer's solution or saline alone (89%).[36]

Biomaterials can also contain surfactants that are intended to cleanse the wound bed and aid debridement. A surfactant-based biomaterial containing 1% silver sulphadiazine was used to improve closure rates in 1036 patients presenting with any non-healing wounds. This European multi-centre study showed that 70% of 1036 patients achieved wound closure, by which 56% of these patients achieved wound closure within 11 weeks, with a reduction in inflammation, pain and odour being found.[21] Similarly, the development of a next generation wound dressing containing a metal chelator and surfactant showed synergy, with 1.2% ionic silver in a carboxymethylcellulose wound dressing. This combination was effective against biofilm and reduced the signs of clinical infection in a 42-patient study.[37]

Research into the cosmetic use of biosurfactants is developing due to their cleansing, emulsifying, foaming and skin hydrating properties.[38] The biosurfactant di-rhamnolipid was used to treat full-thickness burn wounds in Sprague-Dawley rats resulting in a 32% acceleration in wound closure when compared to the control. Furthermore, histological assessment showed a significant increase in collagen production in the burn wounds treated with di-rhamnolipid when compared to the control.[39] More recent studies have demonstrated positive effects of lipopeptide biosurfactants on wound healing and the scavenging of free radicals. The *Bacillus subtilis* (*B. subtilis*) SPB1 biosurfactant increased wound healing in experimental rat models following topical application, with complete re-epithelialisation and epidermal regeneration when compared to the untreated control. Furthermore, in vitro antioxidant studies demonstrated the scavenging of 70.4% of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by *B. subtilis* SPB1.[40]

5 | SURFACTANT INTERACTION WITH ANTIMICROBIALS

Surfactants form part of many antimicrobial-containing wound care products and therefore the interaction between the active agent and surfactant must be fully understood. One study has demonstrated opposing effects of the commonly used surfactants, macrogolum and undecylenamidopropyl betaine, with the antimicrobial activity of PHMB in a keratinocyte- *Staphylococcus aureus* (*S. aureus*) co-culture system. In this study, undecylenamidopropyl betaine decreased the antimicrobial effect of PHMB against *S. aureus*, whereas macrogolum increased antimicrobial effectiveness.[32]

Inclusion of surfactants with antimicrobials in wound dressings, particularly in those used to treat burns patients, have been shown to slow antibiotic elution into the wound while maintaining an antimicrobial effect on the infection. Sorbitan monooleate (Span) is a non-ionic surfactant, which has been shown to stabilise the release of antibiotics from dressings based on a polyglyconate mesh, leading to gradual release.[41] Slow release of antibiotics from wound dressings is important to prevent toxicity towards keratinocytes and fibroblasts and, therefore, avoid delay in wound healing.

Pluronic F127 (Poloxamer 407) is a non-ionic surfactant that has been shown to enhance the rate of the wound healing process. In a study evaluating the antimicrobial properties of a wound dressing containing melatonin-loaded chitosan/Pluronic F127 microspheres, enhanced antimicrobial and antibiofilm activity was found against *S. aureus* in comparison to chitosan, melatonin, and Pluronic F127 only.[42] As melatonin and Pluronic F127 demonstrated no antimicrobial and antibiofilm activity individually, it was thought that they potentiate the antimicrobial activity of chitosan. The potentiating effect observed here was attributed to the inclusion of Pluronic F127 into the microsphere matrix, promoting the amorphization of melatonin, which exhibited a potentiating effect on chitosan.[42]

There is a considerable amount of existing data that shows that the use of surfactant-containing products aids wound healing. With the exception of antimicrobial-containing surfactant products that may aid wound repair due to the antimicrobial management of the wound bioburden, there is evidence to support the positive role of surfactants in cellular repair. Not all surfactants are safe to use on mammalian tissue; however, there is evidence to support the safe use of certain surfactant-containing products based on low cytotoxicity towards fibroblasts and keratinocytes in vitro.[43]

The non-ionic surfactant Pluronic F68 (Poloxamer 188) is safe to use on mammalian cells, demonstrating multiple functions when incorporated in wound dressings, including reduction of pain, swelling, and inflammation.[44] However, a study investigating the use of wound-irrigating solutions on reducing the risk of wound infection in an emergency department showed that there was no significant difference in the prevention of wound infection in 531 patients treated with either a povidone-iodine solution, 1% Pluronic F68, or normal saline.[45] The safety of a skin wound cleanser, Shur Clens, containing Pluronic F68 was demonstrated and did not elicit ocular lesions in experimental animal models or in 20 patients with periorbital lacerations.[46] Another study demonstrated that although Pluronic F68 does not exhibit

an antibacterial effect, it helps the removal of bacteria without damage to the tissue, unlike wound cleansing with saline alone.[47]

Beyond the cleansing capabilities of Poloxamer 188, this surfactant has been shown to block the release of carboxyfluorescein from electropermeabilised skeletal muscle cell membranes, showing the ability to seal electroporated muscle membranes. In a further in vivo experiment, the rat biceps femoris muscle flap attached by its arteriovenous pedicle was electropermeabilised until the electrical resistivity dropped to 50% of the initial value. Administration of Poloxamer 188 intravenously restored resistivity to 77% of the initial value, demonstrating the same ability in vivo.[48] Furthermore, histological analysis showed that post-shock administration of Poloxamer 188 reduced inflammation. Another study showed a reduction in tissue loss and macrophage infiltrate after excitotoxic brain injury in the rat. It was hypothesised that Poloxamer 188 could possibly modulate the inflammatory cell membrane fluidity.[49] The healing properties of this non-ionic surfactant on cell membranes has also been demonstrated. Poloxamer 188 (0.1 mM) appeared to repair the damage of cell membranes following reactive oxygen intermediate-related damage.[50] The reduction of protein aggregation by Poloxamer 188 has also been effectively demonstrated and, therefore, presents interesting opportunities in biomedical sectors where the refolding of denatured proteins is problematic.[24]

6 | BIOFILMS AND SURFACTANTS

The aetiology of delayed wound repair is not thought to be due to one single factor but due to many factors relating to the patients' underlying physiology.[2] The colonisation and biofilm formation of microbial species within the wound tissue is thought to contribute to delayed healing and perpetual inflammation.[4] Biofilms can be described as communities of microorganisms encased in an extracellular polymeric substance (EPS), either attached to abiotic or biotic surfaces, including other microorganisms.[51] The biological properties of these biofilms result in increased tolerance to both antibiotics and antimicrobials[52,53] and, therefore, prolongs infection.[54,55] The number of research papers describing the presence of a biofilm in wound tissue is compiling[3,56–60] and, therefore, a change in the way chronic wounds are managed is needed.

Synthetic surfactants can be used to coat surfaces, thereby changing the surface physiochemical characteristics and reducing or preventing microbial adhesion. The use of synthetic surfactants for this purpose has been researched well, particularly in the food industry.[61] The coating of titanium with PEG was shown to reduce the adhesion of *Streptococcus sanguinis* and *Lactobacillus salivarius* as well as fibroblast adhesion.[62] Cationic dicephalic surfactants differing in hydrocarbon chain length were shown to have an antibacterial effect on planktonic *Staphylococcus epidermidis* and cause a reduction in its biofilm formation on glass and stainless steel.[63] Pluronic F127 (Poloxamer 407) was reported to resist multiple washes when impregnated into a hydrophobic surface and subsequently could reduce *S. epidermidis* attachment and biofilm formation.[64]

The role of biosurfactants as anti-biofilm agents has been reviewed extensively elsewhere.[65] Indeed, biosurfactants have been shown to effect biofilm adhesion and formation. Biosurfactants from *Lactobacillus casei* was shown to have an anti-biofilm effect on oral strains of *S. aureus* with biofilm eradication values of 53% and 86%, depending on the biosurfactant.[66] A polymeric biosurfactant produced by *Trichosporon montevideense* CLOA72 reduced the adherence of *Candida albicans* and *Candida krusei* to human epithelial buccal cells by 85% and 79.5%, respectively. This reduction in microbial adherence was attributed to changes in microbial cell surface characteristics and carbohydrate and protein concentration of the biofilm matrix.[67] The biosurfactant lychenysin produced by *Bacillus licheniformis* reduced bacterial adhesion and biofilm formation of methicillin-resistant *S. aureus* (MRSA) and *C. albicans*, and also partially removed existing MRSA (55.74%) and *Yersinia enterocolitica* (51.51%) biofilm.[68] Biosurfactants from *Lactobacillus jensenii* and *Lactobacillus rhamnosus* demonstrated antimicrobial activity and anti-adhesive capability against MDR strains of *Acinetobacter baumannii*, *Escherichia coli*, and MRSA by damaging the bacterial membranes.[69] Rhamnolipid biosurfactants from *P. aeruginosa* have demonstrated anti-biofilm effects in *P. aeruginosa* biofilms by disrupting the biofilm at a concentration of ~0.5 g/L. Similarly, Rhamnolipids and surfactin reduced the adhesion of *Listeria monocytogenes* and *Pseudomonas fluorescens* on stainless steel 304 and polystyrene.[70]

Evidence to support the use of surfactants on wound biofilms is small, primarily due to the presence of biofilms in wounds only being acknowledged in 2008.[3] Nevertheless, surfactants are present in a variety of antimicrobial-containing wound dressings that are intended to control microbial bioburden and biofilm formation.[71] There are also surfactant-based wound dressings that are being produced without antimicrobials. The use of concentrated surfactant wound care products on biofilms has been evaluated. The Betaine-containing wound cleanser Prontosan was shown to significantly reduce *P. aeruginosa* biofilm numbers in vitro.[72] Yang and colleagues tested a surfactant-based wound gel on an ex vivo porcine skin explant model infected with a functionally tolerant 3-day biofilm and results showed a reduction in biofilm following daily cleansing.[73] Clinical studies have demonstrated positive effects on wound healing in 226 patients with chronic wounds of various aetiology. Of those patients, 138 had not received previous treatment for their wounds and following treatment with a surfactant-based wound dressing, 86% showed improved healing.[74]

7 | CONCLUSION

The use of surfactants in industry has been evident for many years. Similarly, the use of surfactants in medicine is established. However, in the context of complex, delayed wound healing, the concept of biofilms and the associated negative impact on wound healing is a relatively recent concept. Therefore, the presence of surfactants in wound care products has to now be evaluated for their effect on biofilms. Whilst there is in vitro evidence of anti-adhesive effects on microbial attachment and therefore biofilm formation, more evidence is needed that investigates the effects of surfactants on biofilms in more complex, physiological relevant models. This review has highlighted the protective and reparative role that the non-ionic surfactant Poloxamer 188 has on mammalian cells following cell wall damage and on the prevention of protein aggregation. It is possible that the increase in wound healing rates following wound cleansing with surfactants is due to the multi-functioning roles of these surfactants in the management of biofilm and the potential mammalian cell renewal of damaged cell membranes.

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