The role of surfactants and biosurfactants in the wound healing process

The role of surfactants and biosurfactants in wound healing process: A Review

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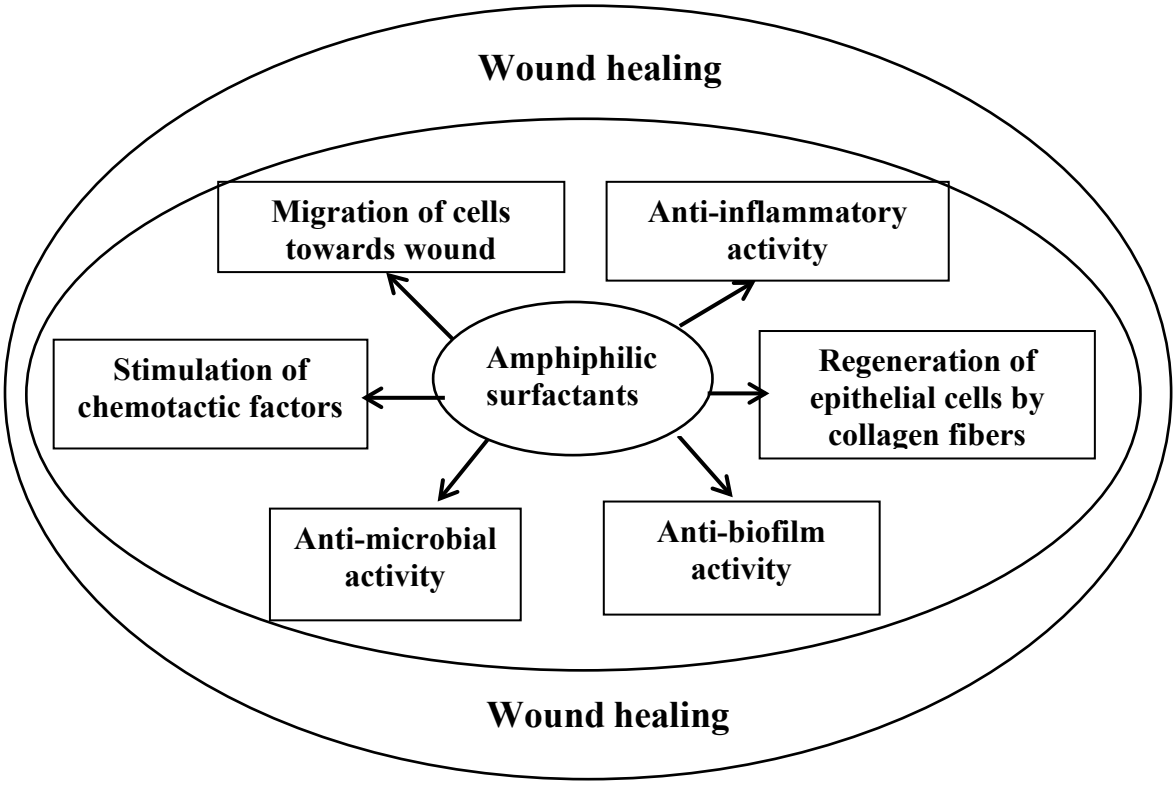
Graphical Abstract



Abstract

Wound healing refers to the complex process of restoring the forms and functions of damaged tissues. Multiple growth factors and released cytokines tightly regulate the site of the wound. The healing processes can be disrupted by any alteration that would aggravate the damage and lengthen the repair process. Some of the conditions that may impair wound healing include infections and inflammation. Surfactants are amphiphilic compounds widely used in various formulations including detergents, food, pharmaceutical, and cosmetic industries. Biosurfactants therefore are surface-active compounds produced by biological agents particularly yeast or bacteria and represent a safer and environmentally preferred alternative to chemical surfactants. Numerous studies have targeted surface-active molecules as wound healing agents for their anti-inflammatory, antioxidant, and antibacterial potential. This review focuses on surface-active molecules used in wound healing activities and analyzes their effectiveness and mechanisms of action.

Keywords: Surfactants; Wound healing; Adjuvant; Biosurfactants

Introduction:

A wound may be considered as damage to the continuity of the skin epithelium or mucosa caused by physical or thermal injury or a disease. Tissue regeneration and development which results in wound healing takes place in four overlapping stages, including haemostasis and inflammation, migration, proliferation, and maturation [1]. The stages and their biochemical and physiological effects occurs in a specific sequence, at a distinct time, continuing for a specific duration at an optimum intensity [2]. Wound healing is affected by several factors and interfering with one or more stages in the process, leads to inappropriate or impaired tissue repair. Considering the duration and nature of the repair process, wounds can be identified as acute or chronic wounds [3]. The former is an injury to the skin occurring suddenly due to accidents, burns and chemical or mechanical injuries. They usually heal in a predictable timeframe commonly through 8 - 12 weeks according to the severity or depth of the damage. Chronic wounds are those that usually cannot be healed within the expected time frame of 12 weeks and often reoccur due to disruptions in the orderly sequence of wound healing stages. These wounds cannot be healed due to repeated tissue insults or physiological reasons, such as diabetes, malignancies, persistent infections, improper primary therapy and other parameters linked to the patient. Chronic wounds include diabetic foot, decubitus and leg ulcers [3]. It is expected to use 1–3% of the drugs indexed in western pharmacopoeias for topical use on wounds [4]. There are several medications and ointments for wound healing worldwide, of which surfactants are widely applied for removing debris, indicating their function as cleaners. Surfactants for wound healing have been long used and can be found in several wound cleansers used for wound cleaning and irrigation/hydration [5]. They act due to their micelles' formation abilities, in which polymer chains including the hydrophilic head and hydrophobic tail generate a hydrophilic outer shell and hydrophobic center. Such chemistry is

helpful for several cleaning processes [6]. Nonetheless, reports on using surfactants against other delayed factors affecting wound healing is somewhat limited. Therefore, this review will explore the literature surfactants' uses and their mods of action in managing the wound healing process.

Factors affecting wound healing

Bacterial biofilms seem to have a major role, their effects on chronic wounds have been widely considered in recent literature. They can be seen in 60 to 80% of wounds, and based on a previous meta-analysis; their presence is confirmed in 78.2% of chronic wounds [6]. They can persist in chronic wounds causing prolonged inflammation and consequently, delayed healing and enhance the risk of infection. Thus, they are crucial in the majority of chronic non-healing skin wounds [5, 6]. Pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and others such as *Proteus*, *Clostridium* and *Coliform* species are detrimental to wound healing. Improper measures for the management of infected wounds result in cellulitis (cell inflammation), bacteraemia and septicaemia which can be fatal [7]. The formation of hypertrophic scars due to increased collagen synthesis if the cells persist at the site in the last phase of the wound healing process is also challenging [8]. On the other hand, wound healing may be delayed due to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) at the wound area because of oxidative stresses, lipid oxidation, DNA damage, and inactivation of enzymes involved in free radical scavenging. Therefore, wound healing drugs with antioxidant activity are often used [9, 10].

Synthetic *versus* natural surfactants

Chemically synthesized surfactants are surface-active amphiphilic compounds capable of reducing the surface tension between liquids and the different state of matter; gas, liquid and solid. They do this by forming micellar structures encompassing the hydrophilic and hydrophobic parts of these

molecules [11]. This results in increases of the surface humidity and materials solubility or miscibility that would otherwise not be possible without. Surfactants can be categorized according to their behavior in aqueous solution, in which each category is defined according to the charge on the hydrophilic head of the surfactant molecule. Cationic surfactants have a positive charge (e.g. quaternary ammonium compounds), anionic surfactants have a negative charge (e.g. soap, detergents, sodium dodecyl sulfate), non-ionic are uncharged (e.g. poloxamer, Tween 80, Triton-X) and amphoteric surfactants (e.g. Betaine) simultaneously carrying an anionic and a cationic hydrophilic group which are able to form cation or anion depending on pH changes and ambient conditions [12].

Natural surfactants also known as 'biosurfactants' have several amphipathic molecules characterized by special chemical structures naturally synthesized by different microorganisms [13]. However, as opposed to chemically produced surfactants, they are categorized according their head group which is often a sugar or protein molecule, chemical composition or overall molecular weight. Low molecular weights include glycolipids and lipopeptides while high molecular weight include polysaccharides, proteins, and lipoproteins surfactants [14]. Generally, the amphiphilic and polyphilic high molecular weight polymers have been shown to be more useful in stabilizing emulsions acting as emulsifiers, whereas the low molecular weight ones with simpler structures have better abilities to reduce surface activity. Hydrophilic moiety of natural surfactants commonly includes an acid, peptide cations or anions, mono-, di- or polysaccharides, whereas unsaturated or saturated hydrocarbons or fatty acids typically represent their hydrophobic moiety. Glycolipids and lipopeptides are the main microbial surfactants that have been investigated and explored [13]. The former includes rhamnolipids produced by *Pseudomonas aeruginosa*, sophorolipids produced by *Candida/Starmerella bombicola* and mannosylerythritol lipids

synthesized by *Ustilago* sp. or *Pseudozyma* yeasts with mono- or disaccharides associated with long-chain aliphatic acids or hydroxyaliphatic acids. The latter also includes surfactin, iturin, and fengicyn cyclic lipopeptides generated by *Bacillus* species as antibiotic or antimicrobial molecules [12, 14].

Use of synthetic surfactants in wound healing

Surfactants as wound cleaning

Surfactants are added to wounds as a hydration/irrigation agents and cleansers incorporated into surgical scrub solutions. The former includes surfactants that can properly clean and remove debris from the wound. An investigation involving 289 patients to compare the use of betaine surfactant-based saline solution impacts on bed preparation and inflammation in chronic wounds showed decreased inflammation and increased granulation tissue production and wound closure [15]. A retrospective assessment comparing the same hydration solution achieved by Ringer's solution or saline with and without surfactant on venous leg ulcers healing level, reported a 97% faster healing level in the surfactant (Betaine) containing solutions as oppose to controls without surfactants [16]. Studies have also indicated the effectiveness of surfactant-containing irrigation solutions in wound cleansing, demonstrating their importance in standard care protocols whether used with or without antimicrobial agents [6, 16]. In another investigation carried out by Burnett. et al [17] surfactants containing betaine were shown to facilitate wound cleansing and autolytic debridement, as well as supporting wound healing at the cellular level.

Surfactants as antibiofilm agent

The presence of biofilms in wounds can reduce healing rates and increase the chances of reinfection which often results in the development of chronic wounds [6]. Biofilms are able to form rapidly in wounds, as demonstrated by Kennedy et al. [18] who visualized biofilm formation

in burn wounds during 7–31 days post injury. Nakagami et al. [19] also demonstrated early signs of biofilm formation in infected wounds 3–7 days post injury. 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 typically leads to increased participation/accumulation of immune cells at the site [20]. This causes the over-production of proteases and ROS, which break down the proteins involved in the wound-healing process [21].

There are several examples of synthetic surfactants used in wound care with the most well-researched being poloxamers and betaines [5, 6]. Poloxamers are non-ionic, synthetic surfactants with a central hydrophobic chain of polyoxypropylene as well as two hydrophilic poloxyethylene chains. The chains length can be adjusted to produce different types of poloxamers [5, 6]. Poloxomer 188 has been shown to inhibit biofilm generation and development by *S. aureus* or *Acinetobacter baumannii* persisting in the wound following treatment in an *ex vivo* porcine skin model [22]. Additionally, wound dressing solution of poloxamer containing 1% silver sulfadiazine (SSD) has shown effectiveness in wounds that are more likely to be infected or those with clinical infection symptoms [23, 24]. Another case series reported such uses to be associated with favorable healing rates and reduction in pain when compared to standard care procedures [24]. Romic et al. [25] also showed that poloxamer 407 could reduce biofilm production through disrupting the attachment of *Staphylococcus epidermidis* to the wound surface. The effectiveness of surfactants on biofilm control has also been demonstrated in *in vitro* investigations by Yang et al. [21] who evaluated the effectiveness of a wound dressing containing surfactant in porcine skin explants. They reported that biofilm reduction to undetectable levels occurred one day following therapy and after cleaning the skin model using poloxamer 188-moistened gauze.

In another study, Percival et al. [26] reported the advantages of using concentrated poloxamer-containing surfactant in breaking down, dispersing and inhibition of *P. aeruginosa*, *Enterococcus* spp., *S. epidermidis*, and methicillin resistant *S. aureus* biofilms. *In vivo* investigations by Howell et al. [27] demonstrated that pretreatment with a poloxamer-based surfactant prior to using iodine-containing surgical scrub improved povidone iodine effectiveness. However, limited information is available about poloxamer- based surfactants in clinical settings. Topical use of these surfactants is greatly tolerable and has been well accepted by patients and do not negatively affect the general wound healing process [6]. Interestingly, they also improved healing in full- thickness rat excisions wound model [6]. 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 through modulating the secretion of pro-inflammatory cytokines. It provides a moisture barrier and cleansing impact assisted by forming micelle gel matrixes [20]. The micellular component within the Poloxamer gel solutions exists in an extensively disordered state at room temperature, which forms a thin flowing gel. At increased temperatures, the micelle core is dehydrated, forming a more ordered crystalline gel state. As a result, when applying liquid poloxamer to the human body, subjecting it to slightly higher body temperatures quickly produces a more solid gel structure while at lower temperatures, disorganization occurs in the micelles and the gel flows like a liquid again [6].

Combining surfactants and antimicrobials

To further enhance surfactants impacts on biofilm removal, they are often combined with antimicrobials. For example, the surfactant Poloxamer 188 has been combined with SSD antimicrobial and was shown to eliminate all viable bacteria from the skin within 3 days of application [21]. Additionally, the use of the synthetic surfactant undecylenamidopropyl betaine and the antimicrobial polyhexanide, showed to result in biofilm elimination and dramatic improvements in wound healing in 7 out of 10 patients within 3 weeks [28]. The combination of 0.1% polyhexanide and 0.1% betaine was also used to investigate their ability to manage infected wounds. The results showed a 5.3-5.8 log reduction in the numbers and prevalence of *S. epidermidis*, *P. aeruginosa*, *Candida albicans*, *S. aureus*, *Enterococcus faecalis*, and *Escherichia coli* in addition to several other strains commonly found in wound biofilms [28].

Surfactants' mechanism of action

Surfactants are able to act as wound healing agent by various mechanisms including as facilitators at the liquid/liquid interface (water and oil) or as solid/liquid interface due to their hydrophilic and hydrophobic mobile structures. For instance, they are able to breakdown the water/oil interface promoting emulsification and holding oil in suspension. Water-insoluble molecules gather near the hydrophobic groups and the spherical micelle components are formed in a concentration and temperature-dependent manner [12]. Accordingly, debris from the wound are continuously trapped, resulting in a rinsing action. Surfactants also reduce the interfacial tension between the wound bed and cleansing liquid; therefore, a close connection is formed between the cleansing liquid and the wound bed, which can facilitate separating loose and nonviable tissues and microbial pathogens from the viable wound bed preventing biofilm production and inhibiting the existing more persistent biofilms. [26].

Wound treatment using different biosurfactant formulations

Cosmetic, wound care and pharmaceutical industries always endeavor to enhance the preservation and solubilization of the active components in their formulations; therefore, they choose surfactants and preservatives, which mostly include chemical compounds that may occasionally increase irritant or allergic reactions. Consequently, alternative natural-based products have been recently sought after and considered as replacements in cosmetic and pharmaceutical industries [29, 30]. Accordingly, some of the biocompatible preservatives with reduced side effects compared to their chemical counter parts are often considered as alternatives [31]. Thus, some biosurfactants with preservative and emulsifying properties were more commonly used. Some of these biosurfactants used as solubilizers have an additional inherent antimicrobial and anti-adhesive properties. In addition, biosurfactants are readily biodegradable due to their lipids, proteins, peptides or carbohydrates structures which make them more appealing for many applications. Several biosurfactants have potential to be used for dermatological purposes, including wound healing. They also occasionally have some synergetic activity with some antibiotics, enhancing their solubilization and antimicrobial activities in addition to have some antimicrobial and antibiofilm activities themselves [32-34]. Although, the number of studies on antimicrobial, antiadhesion, and anti-inflammatory properties of different biosurfactants have been increasing, their uses in wound healing remain quite limited.

Lipopeptide biosurfactants as wound healing agent

Zouari et al. [29] studied *in vitro* antioxidant properties and the wound healing effect of *Bacillus subtilis* SPB1 lipopeptide biosurfactant on excision wound areas in experimental rats and reported a remarkable enhancement in wound closure rate compared to control and CICAFLORA^{TM}-administrated animals. Biopsies treatment using lipopeptide biosurfactant showed completely re-

epithelized wounds associated with excellent epidermal reproduction. The free-radical scavenging effect of the lipopeptide biosurfactant was capable of preventing inflammation and improving tissue generation, re-epithelization and differentiation of the epidermis [35]. Also, lipopeptide biosurfactant can inhibit multidrug-resistance bacteria [36] and act against phytopathogenic fungi [37]. Wound healing effects of investigated lipopeptide biosurfactants might be ascribed their ability for reducing oxidative stress through preventing ROS generation. Ohadi et al. [9] indicated that the lipopeptide biosurfactants formed by *Acinetobacter junii* B6 improved scavenging free radicals properties and enhanced histopathological remission in rats.

Glycolipids biosurfactants as wound healing agent

Gupta et al. [1] evaluated improved wound healing in rat tissue *in vivo* using glycolipids generated by *Bacillus licheniformis* SV1 containing ointment and found re- epithelization and fibroblast cell proliferation in the primary phase of wound healing leading to higher rate of deposition of collagen in the next phases. Rhamnolipids were also used for the re-epithelizing of mucous membrane tissues, especially to treat and prevent gum disorder and improve periodontal regeneration [38]. Lower levels of rhamnolipids have also been able to inhibit the phagocytic actions of macrophages allowing improved control the inflammatory phase. Stipceviv et al. [38] investigated the wound healing properties of di-rhamnolipid BAC-3 formulated as an ointment and used topically on full-thickness burn wounds in healthy rats covering 5% of the body surface. They also noted that the BAC-3 di- rhamnolipid was well-tolerated as daily subcutaneous injection, through 7 days in female mice at the maximum of 120 mg/ (kg day) [38]. In another study, Sana et al [30] assessed the wound healing activity of rhamnolipid generated by *P. aeruginosa* C2. They found that rhamnolipid could accelerate wound healing through their antimicrobial activities. This study also suggested healing support *via* increasing protein, DNA, hexosamine content, and reduced tumor

necrosis factor-alpha (TNF-α) amount. Decreasing level of TNF-α in rhamnolipid group might lead to reduction in inflammation and collagen formation. Lydon et al. [33] investigated very pure micelle-producing nonacetylated acidic sophorolipids containing 90% C18 congener and reported that acidic sophorolipids was appropriate for use in antimicrobial creams for reducing wound infection risk through healing. In another study, Sophorolipids were also reported to act as stimulators of skin fibroblast metabolism contributing to skin restructuring, repair and protection [39]. Sophorolipids act as desquamating and depigmenting agents by eliminating the affected surface of the epidermis protective layer as a part of the wound healing process. A summary of wound healing activities for different biosurfactants are listed in Table 1.

Table1. Summary of wound healing activity of biosurfactants

Type of biosurfactant	Strain	Biological activity	Ref
Lipopeptide	*B. subtilis* SPB1 LP	Anti-inflammatory Antimicrobial	[29]
Lipopeptide	*A. junii* B6	Anti-inflammatory Antioxidant Antimicrobial	[9]
Lipopeptide	*Bacillus mojavensis A21*	Anti-inflammatory Antioxidant	[10]
Glycolipid	*B. licheniformis* SV1	Anti-inflammatory Antioxidant Antimicrobial	[1]
Glycolipid	*Starmerella bombicola*	Antimicrobial	[33]
Glycolipid	*Candida bombicola*	Depigmenting	[39]
Glycolipid	*P. aeruginosa* C2	Antimicrobial	[30]

Biosurfactants adjuvant/synergetic activities facilitating wound healing

Using biosurfactants for medical purposes has been increasingly considered in the past few years due to some of their unique properties [34]. Such properties are related to their amphiphilic nature increasing the surface humidity and the solubility of materials and an ability to decrease the liquids/surface tension, leading to a higher penetration of fluids, such as solvents and antimicrobials [33]. Reduction of the interfacial tension between immiscible liquids molecules makes them more 'slippery', resulting in a lower risk of adhesion to surfaces. Accordingly,

biosurfactants, allows some target molecules to be carried away upon the application of irrigating compounds such as water or saline through creating micellular structures facilitating the cleaning processes [5]. In addition to these properties, biosurfactants can act as an adjuvant or synergetic agent for enabling faster wound healing [33] in addition to their advantageous antimicrobial and antibiofilm activities [32, 34]. Investigating the effect of acidic sophorolipid as an adjuvant antibiotic effect to facilitate wound healing, Lydon et al. [32] concluded that acidic sophorolipids antibacterial, antibiofilm, and antibiotic adjuvant effects supports further consideration for applications to reduce chronic wounds infections. In another study, Tabbene et al [40] evaluated the lipopeptide bacillomycin D and amphotericin B effects separately and in combination to inhibit *C. albicans* biofilm production and accelerating keratinocyte cell migration. They showed the effectiveness of the lipopeptide bacillomycin D in combination with the common amphotericin B in inhibiting biofilm production and reducing cell viability as well as improving the closure of 'pseudo-wounds'.

Biosurfactants skin penetration enhancement techniques

Several studies indicated the effect of amino acids and peptides on reversing the cutaneous symptoms of aging and having a secondary advantage of improving the wound healing process [41]. Such peptides consist of a small sequence amino acid chains possibly stimulating angiogenesis, granulation tissue synthesis, and new collagen production. Nonetheless, proteins and peptides are hydrophilic molecules that are frequently charged at physiological pH conditions. Their molecular weights are varied from small peptides (300Da) to proteins (>1000kDa). They cause weak skin permeation and although they have high potency, are mostly therapeutically ineffective when administered transdermally due to poor permeation. To overcome the skin barrier and facilitate easier permeability of bio-drugs several skin penetration enhancement methods have

been evaluated. Formulation and chemical enhancement methods can be used for the delivery of small peptides used in dermatological and cosmetic applications [42]. The positive effects of coupling tetrapeptide attached to a short hydrocarbon chain can improve transepidermal delivery of protein inhibitors. Attached oligopeptides linked to a fatty acid for example can improve oil solubility of peptides resulting in a favorable skin penetration [43]. Cutaneous absorption of interferon alpha (INFα) attached to different palmitoyl molecules has been reported to occur at nearly five to six times that of the parent peptides [44]. Lipopeptides biosurfactants containing 8-24 carbon atoms made by a hydrophilic or hydrophilic peptide chain have been reported to stimulate the production of crucial constituents of the skin matrix (collagen and elastin) when added to the skin fibroblast culture [45].

Mechanism of wound healing by microbial surfactant

Wound repair is a dynamic series of events, in which adhered pathogens are killed, chemotactic factors stimulated, inflammatory cells are migrated and injured dermal layer is remodeled. Cellular cross-talking reactions are also initiated inside and outside the wound region that begins the repair of injured epithelial layers. The repair speed however, is affected by pathogenic infections and poor immune reactions. Therefore, the "elimination of pathogens" is of great importance for effective wound treatment [29]. Biosurfactants can enhance bacterial cell membrane permeability as well as cellular metabolites leakage preventing microbial infections. This antimicrobial effect enhances early wound healing [1, 46]. The mechanism of wound healing by biosurfactants is schematically illustrated in Fig 1. Using biosurfactants as constituent can disinfect the wound areas from potential pathogens and stimulate numerous chemotactic factors which generates chemotactic signals using different inflammatory cells, such as monocytes, endothelial cells, neutrophils, fibroblast cells at the wound area [38, 47]. These cells

migrate to the wound area via circulating blood capillaries, collecting pathogens and dead epithelial cells through phagocytosis and acting as protective materials of cellular repair systems enhancing wound healing activities at the injured area. Fibroblast cells can be activated to begin synthesizing collagen types I and II proteins that cross-link forming a thick layer of connective tissue simultaneously (Fig. 1) [48]. The connective tissue rich in collagen is crucial for re-epithelization and neovascularization process at the wound site, which is helpful in the early wound contraction. In the Underlying connective tissues in the wound site, the epidermal part gets smaller leading to bringing the wound edges nearer to each other and assisting remodeling process of the tissue [49, 50]. Hence, biosurfactants can be regarded as a transdermal substitute causing intensive connective tissue remodeling and a greater frequency of re-epithelization with improved wound healing effects. In a recent study, Mehrabani et al. [51] investigated the wound healing mechanism of lipopeptide biosurfactants. They showed that lipopeptide biosurfactants increase the rate of wound site angiogenesis through making improvements in the levels of HIF-1α and VEGF protein expression. Lack of HIF-1α and VEGF protein expression is partly responsible for poor wound healing. However, the functional role of biosurfactants in increasing the expression of HIF-1α and VEGF protein is not well known. In normoxia, prolyl hydroxylase domain proteins (PHDs) trigger the hydroxylation of two proline residues in the oxygen degradation domain of HIF-α trigger leading to its degradation. In addition to O_2, PHDs also require other cofactors to be activated including Fe^{+2}. Therefore, through the inhibition of PHDs, iron chelating agents could be used to stabilize HIF-α and protect it from further degradation. The elevating effect on HIF-1α and its downstream genes may well be associated with the iron chelating capacity of biosurfactants. Hemlata et al. [52] demonstrated the iron chelating ability of a glycolipid biosurfactant produced by *Stenotrophomonas maltophilia* NBS-

410 11. They suggested that cells may be protected against oxidative stress through making use of the
411 iron chelating capacity of the produced biosurfactant. Also, previous studies have demonstrated
412 that iron chelators may positively affect the angiogenesis process through increasing the
413 expression of VEGF and HIF 1-α proteins [53]. ROS and RNS generation on the wounded area
414 can delay the healing process by initiating oxidative stresses, LPO, DNA damage, and
415 inactivating free radical scavenger enzymes. The anti-inflammatory activity of a range of
416 biosurfactants has been investigated in various studies [9, 10, 29]. Biosurfactants' scavenging
417 activity might be attributed to their hydrophilic and lipophilic effects that may improve the
418 radical scavenging abilities [54]. Biosurfactants have reported as antibiofilm agents although the
419 anti-biofilm effect mechanism is still unclear [32]. However, most biosurfactants are able to
420 enhance bacterial surface hydrophobicity as well as de- stabilize lipid packing because of their
421 amphipathic nature. Finally, such alterations improve the membrane cells permeability and
422 consequently, reduce microbial adhesion toward solid surfaces [46]. In addition, the mechanism
423 of anti-biofilm effect via microbial surfactant collected from lactic acid bacteria to *S. aureus*
424 CMCC 26003 has been reported by Yan et al. [55]. Biofilm-associated genes expressions, such
425 as cidA, icaA, dltB, agrA, sortaseA and sarA are affected by biosurfactants and interfere with
426 signaling molecules released by the quorum sensing systems which may prevent biofilm
427 development.
428 Diabetic wound healing is usually delayed by many factor, including high TNF-α expression and
429 low TGF-β expression which can affect the formation of new epithelial and collagen as the main
430 goal of wound healing process. The increasing TNF-α expression is associated with inhabitation
431 of cell migration, failure fibroblast proliferation, and inhabitation of angiogenesis resulting in the
432 failure of diabetic wound healing. Raihanah et al. [56] reported that biosurfactants containing

dialkyl alginate cream decreased TNF-α expression and increase TGF-β expression, and reepithelization.

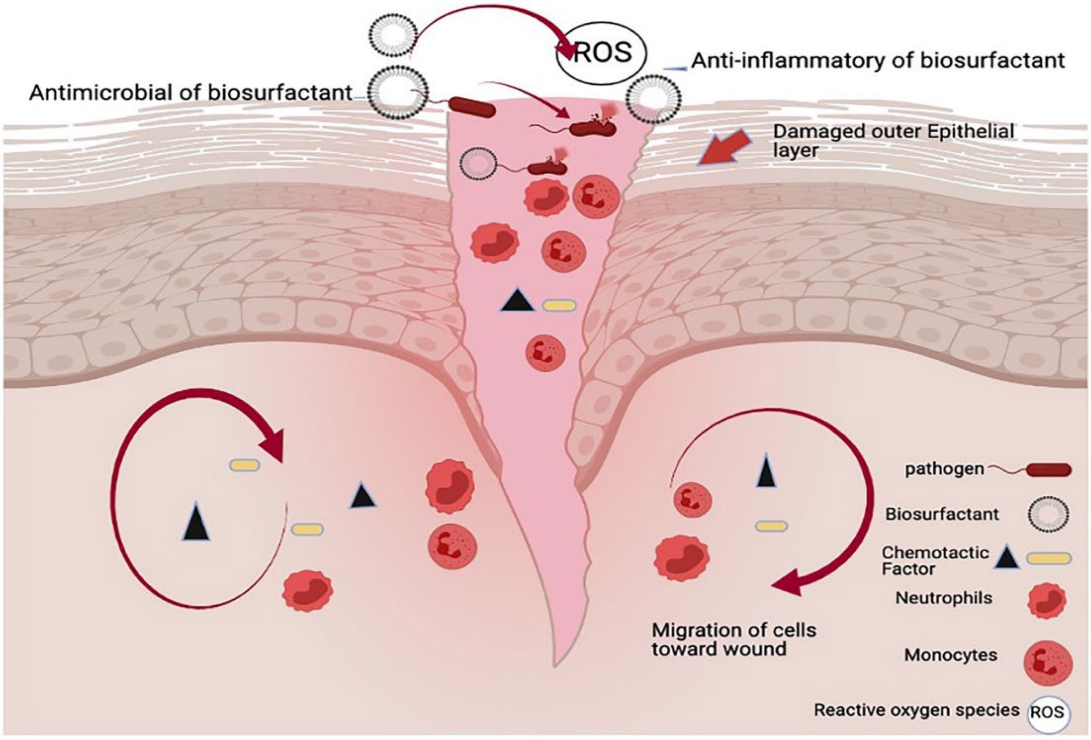


Fig1. Biosurfactants mediating *in vivo* wound repair. Figure created using BioRender (https://biorender.com/).

Challenges and recommendations for future research

A comprehensive literature search was carried out using the ISI Web of Science and PubMed search engines. Both databases were selected in order to cover all of the published peer-reviewed literature. Several studies have demonstrated the efficacy of synthetic and natural surfactants in the wound healing. Nonetheless, considering the benefits of biodegradability and the generation of renewable-resource substrates, biosurfactants may possibly replace their chemically produced compounds. Biosurfactants have not been widely used due to their high production and purification costs [57]. Biosurfactants-producing strain' physiology, genetics, downstream processing and

purification should be investigated to reduce their production costs [58]. It is also difficult to compare between studies because of variations in wound types and size, animal models, biosurfactants types, and biosurfactants mixtures (congeners variations). To increase our knowledge about the possible advantages or disadvantages of these compounds in wound healing, data regarding the constitution of biosurfactants mixtures and standardizing considered experimental wound healing model are vital [34, 59]. Additionally, the toxicity of a new biosurfactant needs to be addressed for certifying their immunity to using in cosmetics and wound care industry [9, 60]. Limited of clinical data on the use of biosurfactants in human volunteers is major challenge in preparing safe formulations in wound healing. To construct a sustainable society, several programs are now underway worldwide, of which the introduction of green technology is challenging. Regarding the today's social and technological circumstances, using biosurfactants, as environmentally friendly and highly functional components, remains an attractive proposition.

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