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Review article

THE WAR UNDER THE SKIN - BIOFILM AND INFLAMMATION IN CHRONIC WOUNDS

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Abstract

The skin, the largest organ in the body, protects against external stimuli and microorganisms. A balanced skin microbiota, including both commensal and pathogenic microorganisms, is essential for skin health. Disruptions in this balance can lead to infections and inflammation, which are key factors in delayed wound healing. Biofilm formation further complicates the process. In chronic wounds, healing is often impaired during the inflammatory phase due to persistent activation of the immune response. This leads to increased immune cell activation, along with heightened activity of matrix metalloproteinases (MMPs), collagenase, and elastase, while tissue inhibitors of matrix metalloproteinases (TIMPs) decrease. Host factors such as wound depth, duration, local hypoxia, and immune responses contribute to healing delays. Microbial factors, including bacterial diversity, microbial load, and pathogenicity, also play a significant role. Biofilms are more resistant to antimicrobial therapy than free-floating bacteria. Its formation in chronic wounds triggers sustained inflammation, marked by elevated inflammatory mediators like IL-6, IL-10, IL-17A, and TNF-α. Biofilms not only prolong inflammation but also cause oxidative stress and protease-mediated degradation of essential receptors and cytokines, accelerating wound bed senescence. The presence and persistence of biofilm in chronic wounds affect the host's immune response. Understanding this relationship offers more opportunities for successful treatment.

Keywords: chronic wounds, biofilm, inflammation, wound healing

Introduction

The skin, the largest organ in the human body, is a multi-layered structure that serves as a protective barrier, protecting internal systems from external stimuli and preventing the entry of microorganisms [1]. A healthy skin microbiota, which consists of numerous commensal and facultative pathogenic microorganisms, is essential for maintaining skin health [2,3].

The skin hosts approximately 10^{12} bacteria, forming a well-adapted microbiome that thrives in the skin's unique environment, characterized by limited nutrients, an acidic pH, and specific temperature ranges. The microbial populations on different areas of the skin are tailored to these conditions. The native skin microbiota protects the body by outcompeting pathogenic microbes, limiting their growth, breaking down skin lipids, and regulating the immune system [4,5]. Disruptions in the skin or an imbalance between pathogenic and natural microbiota can lead to infections [5,6].

Infections, along with associated pathological inflammation, are the most common factors contributing to delayed wound healing, which results in chronic wounds [7]. A chronic wound is wound that does not heal within 30 days [8].

The human skin microbiota plays a role in wound healing and can influence the severity of infections. Chronic wounds may progress from microbial contamination and colonization to infection, involving multiple microorganisms [9]. Contamination refers to the presence of non-proliferating bacteria from the skin's natural microbiota or the external environment. All wounds are contaminated by microorganisms, but when conditions favor their survival, they can become colonized. Colonization occurs when microorganisms multiply on the wound surface [10-12]. During this early stage, the host immune defenses are not triggered, and there are no obvious clinical signs of infection. This is typically the case when the wound is colonized by Gram-positive bacteria, particularly from the *Staphylococci* genus, which do not provoke a strong immune response [9,13].

In later stages, Gram-negative bacteria, particularly rod-shaped species like *Pseudomonas sp.*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter spp.*, contaminate the wound, becoming dominant. These bacteria typically come from the urogenital tract or the surrounding environment. Antibiotic use can also influence colonization. This early colonization stage, which causes only a localized immune response, precedes deeper tissue infections and can delay healing [13,14]. In the final stage, infection occurs when microbes invade deeper tissues, overwhelming the body's control and triggering a strong immune response. Aerobic bacteria consume oxygen rapidly, promoting the growth of anaerobic microbes [13,15].

Biofilm formation is another major factor for delayed wound healing [16]. The transition from free-floating planktonic bacteria to biofilm growth involves complex signaling, including changes in gene expression. While biofilm bacteria are not always pathogenic, upregulated biofilm bacteria can become virulent, impairing wound healing and causing tissue damage. Percival *et al.* suggested that it is the genetically upregulated pathogenic biofilm bacteria, rather than the dormant commensal ones, that impair the healing process [17]. These bacteria contribute to chronic inflammation and a bacteria-driven wound environment, producing destructive enzymes and toxins. This leads to an imbalance between growth factors and lytic enzymes, affecting cell proliferation and healing. Hyper-inflammation also prevents a proper Th2 immune response, hindering adaptive immunity and the ability of the immune system to effectively fight infection [18].

Wound healing

When the human epidermis is damaged by trauma, it activates a series of cellular immunological responses and intrinsic metabolic processes [19]. During these processes, various intermediaries, including extracellular matrix (ECM) molecules, platelets, inflammatory cells, cytokines, growth factors, and chemical messengers, interact through the different phases of wound healing: hemostasis, inflammation, proliferation, and tissue remodeling [20].

Hemostasis begins with platelet aggregation and activation, which trigger the coagulation cascade and form a temporary fibrin scaffold [21-23]. During this phase, platelet degranulation releases damage-associated molecular patterns (DAMPs), cytokines, chemokines, and growth factors that accumulate within the scaffold, forming a chemotactic gradient that facilitates immune cell infiltration [21,24,25]. This immune infiltration is critical for removing dead or damaged cells, debris, and pathogens from the wound bed, preparing the wound for the next phases of healing. The innate immune system plays a crucial role in this process, particularly in the transition from inflammation to the proliferation phase, during which inflammatory macrophages (M1) shift to anti-inflammatory wound-healing macrophages (M2) [21,26]. This shift promotes the activation of keratinocytes and fibroblasts in

the wound bed, which are essential for proliferation and healing [22]. Keratinocytes are key for wound re-epithelialization [21], while fibroblasts deposit collagen to form the extracellular matrix (ECM) or granulation tissue, replacing the temporary fibrin scaffold [23,25]. Angiogenesis also occurs during the proliferation stage, restoring tissue vascularity [21]. Finally, in the remodeling phase, fibroblasts replace granulation tissue with scar tissue, leading to wound contraction and closure [10,21,24].

Biofilm and inflammation in chronic wounds

In chronic wounds, the healing process often stagnates during the inflammatory phase due to persistent activation of the innate immune response [26,27]. This ongoing inflammation results in increased infiltration and activation of immune cells, enhancing the activity of matrix metalloproteinases (MMP), collagenase, and elastase, while decreasing tissue inhibitors of matrix metalloproteinases (TIMPs) [28,29]. This imbalance leads to excessive breakdown of growth factors, anti-inflammatory cytokines, and ECM components, preventing the wound from progressing through the healing phases [30-32]. Additionally, the inflammation promotes excessive neutrophil apoptosis (NETosis) and reactive oxygen species (ROS) production, which further damages tissue and impairs healing [33-37]. A failure in the phenotypic switching of macrophages from M1 to M2 has also been linked to chronic wound formation. In venous ulcers, for instance, iron overload has been associated with prolonged pro-inflammatory M1 macrophage activation [38,39]. Studies in diabetic mice have shown that dysfunctional efferocytosis of apoptotic neutrophils by pro-inflammatory macrophages results in the accumulation of apoptotic cells, which sustains inflammation and prevents the transition of macrophages to their anti-inflammatory state [40]. Supporting these findings, the depletion of M2 macrophages in mice with surgical wounds led to increased infiltration of neutrophils and M1 macrophages, prolonging the inflammatory phase and reducing collagen deposition [41]. These observations suggest that sustained activation of the innate immune response is a key factor in chronic wound formation. However, the precise causes of chronic innate immune activation remain unclear. It is also unknown whether the mechanisms behind this immune dysfunction, including dysregulated M1-M2 switching, differ across various wound types, as emerging evidence suggests variations between antimicrobial and inflammatory responses.

After infection, biofilm formation is the second most common cause of delayed wound healing. Bacteria can exist either as free-floating, planktonic cells or as structured, multicellular communities known as biofilms, which may or may not be attached to surfaces [42]. In comparison to planktonic bacteria, biofilms display distinct characteristics such as increased antibiotic tolerance, altered gene expression, and modified interactions with the host. Within biofilm structures, bacteria are embedded in a self-produced extracellular polymeric substance (EPS), which consists of extracellular DNA, proteins, exopolysaccharides, and water. In addition to microbial elements, the EPS may also contain host-derived substances, including proteins, DNA, immunoglobulins, and blood components [42].

Two hypotheses explain biofilm-mediated wound healing. The specific bacterial hypothesis suggests that only a few bacterial species in a diverse biofilm contribute to non-healing wounds, while the non-specific bacterial hypothesis views the entire biofilm as a unit, with its complex microflora causing delayed healing. Both theories are yet to be conclusively proven, but understanding them could guide targeted therapies to combat infection and improve healing [17,43].

Biofilms are highly complex and diverse, forming as either single-species communities or polymicrobial structures. They can be up to 1,000 times more resistant to antimicrobial agents and disinfectants compared to planktonic bacteria. Additionally, the immune system often struggles to effectively combat biofilm-associated infections. Several factors contribute to biofilm resilience, including slow bacterial growth, high cell density, the presence of

persister cells, nutrient and oxygen gradients, horizontal gene transfer, efflux pumps, and increased mutation rates. Furthermore, the EPS matrix acts as a physical barrier that hinders the penetration of antimicrobial agents and immune responses by limiting the diffusion of drugs, antibodies, and immune cells into the biofilm [42].

Quorum sensing (QS) is a bacterial cell–cell communication process that involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs). It is essential for biofilm development, with bacteria using autoinducers (AIs) to sense population density. Different species use various AIs, such as N-acyl homoserine lactones (AHLs) in Gram-negative bacteria [44], and short peptides or the autoinducing peptide (AIP) in Gram-positive bacteria [45,46]. AIs are produced at a constant rate and can cross bacterial membranes, increasing in concentration as bacterial population grows. Once a threshold is reached, AI-induced gene expression changes lead to biofilm formation.

In chronic wound healing with biofilm involvement, there are two key participants: the host and the microorganism forming the biofilm. Both sides, with their respective characteristics and properties, contribute to the delayed healing process.

The healing of chronic wounds is influenced by various host factors, including wound depth, wound duration, local tissue hypoxia, and the immune system. Studies have shown a positive correlation between ulcer depth and anaerobic environments, which support the growth of facultative anaerobic bacteria, although the relationship with *Staphylococcus* is inverse [47]. Longer wound duration correlates with greater bacterial diversity and a higher abundance of Proteobacteria, while the abundance of *Staphylococcus* decreases. Tissue ischemia, resulting from microvascular complications, delays healing. Hypoxia alters miRNA levels, impairing wound healing, as observed in murine models of ischemic wounds. This environment encourages the growth of facultative anaerobes. Chronic wounds also exhibit persistent inflammation, leading to bacterial colonization. The downregulation of TLR-2 in injured tissue impairs the immune response and delays inflammatory cell recruitment. The prolonged inflammatory state, poor angiogenic response, and reduced fibroblast function contribute to delayed healing in diabetic animal models [47].

Microbial factors that influence the healing of chronic wounds include high bacterial diversity, microbial load, and microbial pathogenicity.

Dowd *et al*. introduced the concept of functional equivalent pathogroups (FEP), emphasizing that the synergy between biofilm community members, rather than individual bacteria, drives sustained inflammation and infection in wounds [48]. Studies have shown that chronic diabetic foot wounds exhibit a greater bacterial diversity, with *Staphylococcus aureus* being predominant, and anaerobes playing a key role in multi-species biofilms, particularly in deeper, ischemic wounds [49-54]. Biofilm communities in chronic wounds also include ESKAPE pathogens (*Enterococcus faecium, S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) as well as fungi like *Candida spp.* [47].

On the other hand, while a high microbial load can lead to critical colonization, which impedes healing, studies by Bendy *et al*. and Robson *et al*. suggest that the role of microbial load in wound healing remains a subject of debate [47]. Bacterial distribution is random, and microbial density alone cannot predict infection or the progression of healing.

The polymicrobial nature of biofilms in chronic wounds creates synergistic effects that transform non-virulent bacteria into virulent pathogens. This microbial community gene expression modulation allows bacteria to survive harsh conditions, resist immune responses, and evade antimicrobials [55-57]. Additionally, biofilm-forming bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* possess specific virulence traits that facilitate biofilm formation and persistence, including type IV pili, flagella, and secretion systems that contribute to inflammation and drug resistance [58,59].

In biofilm formation *P. aeruginosa* utilizes secretion systems that contribute to inflammation and invasion. Key proteins such as EPS, lipase A, and alginate are secreted, interacting within the extracellular matrix to enhance drug resistance. Quorum-sensing pathways regulate the release of virulence factors like elastase, rhamnolipids, and exotoxin A, all of which are crucial for biofilm maturation [58]. Similarly, *S. aureus* exhibits virulence traits, including microbial surface components (MSCRAMMs), fibronectin-binding proteins, and biofilm-associated proteins that promote adhesion to surfaces and host cells, ensuring the biofilm structural integrity [59]. Furthermore, the concept of 'theft biofilm' suggests that bacteria like *P. aeruginosa* exploit host lipids to enhance the production of virulence factors and biofilm formation [60].

Biofilms may trigger chronic inflammation, as an increase in inflammatory mediators such as IL-6, IL-10, IL-17A, and TNF-α has been observed in wound fluids when infections are sustained by biofilm-producing bacteria, influencing ulcer size. The ongoing immune system stimulation can exacerbate chronic inflammation, perpetuating the chronic wound cycle. Furthermore, biofilms contribute to wound bed senescence by inducing oxidative stress and protease-mediated degradation of receptors and cytokines [61].

Matrix metalloproteinases (MMPs) play a key role in wound healing by degrading the extracellular matrix (ECM), allowing immune cells to reach the site of inflammation. MMPs are initially inactive as zymogens and are later activated [62]. However, *Pseudomonas aeruginosa* produces elastase, which activates MMPs, leading to tissue damage through hemorrhage and cell death, worsening the wound and delaying healing. Overactive MMPs degrade laminin and type IV collagen, damaging capillaries and membranes [62]. Additionally, *Staphylococcus aureus* amplifies MMP activation, causing swollen lesions from neutrophil infiltration. *S. aureus* also secretes alpha-hemolysin to break down cell membrane connections and induce necrosis in the epidermis and dermis [62].

Conclusion

The presence and persistence of biofilm in chronic wounds affect the host immune response. The pathogenesis involves various microbes and their interactions with host cells in the wound environment. More research is needed to understand and counteract the effects of biofilms in wounds. Understanding this relationship offers more opportunities for successful treatment.

Conflict of interest statement. None declared.

References:

1. Pereira RF, Barrias CC, Granja PL, Bartolo PJ. Advanced biofabrication strategies for skin regeneration and repair. *Nanomedicine*.2013; 8: 603-621. doi: 10.2217/nnm.13.50
2. Lejeune P. Contamination of abiotic surfaces: What a colonizing bacterium sees and howto blur it. *Trends Microbiol*. 2003; 11(4): 179-184.doi: 10.1016/s0966-842x(03)00047-7.
3. Rendueles O, Ghigo JM. Multi-species biofilms: How to avoid unfriendly neighbors. *FEMS Microbiol. Rev*. 2012; 36(5): 972-989.doi: 10.1111/j.1574-6976.2012.00328.x.
4. Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell*. 2016;40:164:337. doi: 10.1016/j.cell.2016.01.013
5. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol*. 2011; 9:244–53. doi: 10.1038/nrmicro2537
6. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol*. 2018; 16:143–55. doi: 10.1038/nrmicro.2017.157

7. Leaper D, Assadian O, Edmiston CE. Approach to chronic wound infections. *Br. J. Dermatol*. 2015; 173(2):351-358.doi: 10.1111/bjd.13677
8. Bertozzi N, Simonacci F, Grieco MP, Grignaffini E, Raposio E. The biological and clinical basis for the use of adipose-derived stem cells in the field of wound healing. *Ann Med Surg*. 2017;41–48. doi:10.1016/j.amsu.2017.06.058
9. Daeschlein G. Antimicrobial and antiseptic strategies in wound management. *Int Wound J*. 2013;10:9–14. doi: 10.1111/iwj.12175
10. Bowler PG, Duerden BI, Armstrong DG. Wound Microbiology and Associated Approaches to Wound Management. *Clin Microbiol Rev*. 2001;14:244–69. doi: 10.1128/CMR.14.2.244-269.2001
11. Guo S, DiPietro LA. Factors Affecting Wound Healing. *J Dental Res*. 2010;89:219–29. doi: 10.1177/0022034509359125
12. Orsted HL, Keast DH, Forest-Laland L, Kuhnke JL, O'Sullivan-Drombolis D, Jin S, et al. Best practice recommendations for the prevention and management of wounds. In: Foundations of Best Practice for Skin and Wound Management. A supplement of Wound Care Canada. 2017; 74 pp.
13. Negut I, Grumezescu V, Grumezescu A. Treatment Strategies for Infected Wounds. *Molecules*. 2018; 23:2392. doi: 10.3390/molecules23092392
14. Kingsley A. The wound infection continuum and its application to clinical practice. *Ostomy Manage*. 2003; 49:1–7.
15. Bowler PG, Duerden BI, Armstrong DG. Wound Microbiology and Associated Approaches to Wound Management. *Clin Microbiol Rev*. 2001; 14:244–69. 10.1128/CMR.14.2.244-269.2001
16. Leaper D, Assadian O, Edmiston CE. Approach to chronic wound infections. *Br. J. Dermatol*. 2015; 173(2):351-358.doi: 10.1111/bjd.13677
17. Percival SL, Hill KE, Williams DW, Hooper SJ, Thomas DW, Costerton JW. A review of the scientific evidence for biofilms in wounds. *Wound Repair Regen*. 2012;20 (5):647-57. https://doi.org/10.1111/j.1524-475X.2012.00836.x
18. Peyyala R, Ebersole JL. Multispecies biofilms and host responses: "discriminating the trees from the forest." *Cytokine*. 2013;61(1):15–25. doi: 10.1016/j.cyto.2012.10.006.
19. Wang J, Windbergs M. Functional electrospun fibers for the treatment of human skin wounds. *Eur J Pharm Biopharm.* 2017;119: 283-299.
20. Zahedi P, Rezaeian I, Ranaei-Siadat SO,Jafari SH, Supaphol P. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polym Adv Technol*. 2010; 21 :77-95.10.1002/pat.1625
21. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell Mol Life Sci*. 2016;73:3861–85. doi: 10.1007/s00018-016-2268-0
22. Nguyen AV, Soulika AM. The Dynamics of the Skin's Immune System. *Int J Mol Sci*. 2019; 20:1811. doi: 10.3390/ijms20081811
23. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound Healing: A Cellular Perspective. *Physiol Rev*. 2019; 99:665–706. doi: 10.1152/physrev.00067.2017
24. Ellis S, Lin EJ, Tartar D. Immunology of Wound Healing. *Curr Dermatol Rep*. 2018; 7:350–8. doi: 10.1007/s13671-018-0234-9
25. Cañedo-Dorantes L, Cañedo-Ayala M. Skin Acute Wound Healing: A Comprehensive Review. *Int J Inflammation*. 2019:1–15. doi: 10.1155/2019/3706315
26. MacLeod AS, Mansbridge JN. The Innate Immune System in Acute and Chronic Wounds. *Adv Wound Care.* 2016; 5:65–78. doi: 10.1089/wound.2014.0608
27. Lazarus GS. Definitions and Guidelines for Assessment of Wounds and Evaluation of Healing. *Arch Dermatol*.1994; 130:489. doi: 10.1001/archderm.1994.01690040093015

28. Wysocki AB, Staiano-Coico L, Grinnell F. Wound Fluid from Chronic Leg Ulcers Contains Elevated Levels of Metalloproteinases MMP-2 and MMP-9. *J Invest Dermatol.* 1993;101:64–8. doi: 10.1111/1523-1747.ep12359590
29. Diegelmann RF. Excessive neutrophils characterize chronic pressure ulcers. *Wound Rep Regen*. 2003;11:490–5. doi: 10.1046/j.1524-475X.2003.11617.x
30. Ellis S, Lin EJ, Tartar D. Immunology of Wound Healing. *Curr Dermatol Rep*. 2018;7:350–8. doi: 10.1007/s13671-018-0234-9
31. Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Rep Regen*. 1996;4:411–20. doi: 10.1046/j.1524-475X.1996.40404.x
32. Herrick S, Ashcroft G, Ireland G, Horan M, McCollum C, Ferguson M. Up-regulation of elastase in acute wounds of healthy aged humans and chronic venous leg ulcers are associated with matrix degradation. *Lab Invest*. 1997; 77:281–8.
33. Wlaschek M, Scharffetter-Kochanek K. Oxidative stress in chronic venous leg ulcers. *Wound Rep Regen*. 2005; 13:452–61. doi: 10.1111/j.1067-1927.2005.00065.x
34. Schafer M, Werner S. Oxidative stress in normal and impaired wound repair. *Pharmacol Res*. 2008; 58:165–71. doi: 10.1016/j.phrs.2008.06.004
35. Sen CK. Wound healing essentials: Let there be oxygen. *Wound Rep Regen*. 2009; 17:1–18. doi: 10.1111/j.1524-475X.2008.00436.x
36. Wong SL, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. *Nat Med.* 2015;21:815–9. doi: 10.1038/nm.3887
37. Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, et al. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. *Int Wound J.* 2017;14:89–96. doi: 10.1111/iwj.12557
38. Zamboni P, Izzo M, Tognazzo S, et al. The overlapping of local iron overload and HFE mutation in venous leg ulcer pathogenesis. *Free Radical Biol Med*. 2006;40:1869–73. doi: 10.1016/j.freeradbiomed.2006.01.026
39. Sindrilaru A, Peters T, Wieschalka S, Baican C, Baican A, Peter H, et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest*. 2011;121:985–97. doi: 10.1172/JCI44490
40. Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, et al. Macrophage Dysfunction Impairs Resolution of Inflammation in the Wounds of Diabetic Mice. *PloS One*. 2010;5:e9539. doi: 10.1371/journal.pone.0009539
41. Klinkert K, Whelan D, Clover AJP, Leblond A-L, Kumar AHS, Caplice NM. Selective M2 Macrophage Depletion Leads to Prolonged Inflammation in Surgical Wounds. *Eur Surg Res*. 2017;58:109–20. doi: 10.1159/000451078
42. Versey Z, da Cruz Nizer WS, Russell E, et al. Biofilm-Innate Immune Interface: Contribution to Chronic Wound Formation. *Front Immunol.* 2021;12:648554. doi: 10.3389/fimmu.2021.648554.
43. Percival SL, Malone M, Mayer D, et al. Role of anaerobes in polymicrobial communities and biofilms complicating diabetic foot ulcers. *Int Wound J.* 2018;15(5):776–82. https://doi.org/10.1111/iwj.12926.
44. Diggle SP, Winzer K,Lazdunski A, Williams P, Cámara M.Advancing the quorum in Pseudomonas aeruginosa: MvaT and the regulation of N-acylhomoserine lactone production and virulence gene expression. *J Bacteriol*. 2002;184: pp. 25762586, 10.1128/jb.184.10.2576-2586.2002

45. Antunes LCM, Ferreira RB.Intercellular communication in bacteria. Crit. Rev. Microbiol., 35 (2009), pp. 69-80, doi: 10.1080/10408410902733946
46. Kleerebezem M, Quadri LE, Kuipers OP, De Vos WM.Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol Microbiol*. 1997;24:, pp. 895-904, doi:10.1046/j.1365-2958.1997.4251782.x
47. Goswami AG, Basu S, Banerjee T. et al. Biofilm and wound healing: from bench to bedside. *Eur J Med Res*. 2013; 28:157. https://doi.org/10.1186/s40001-023-01121-7
48. Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). *PLoS ONE*. 2008; 3(10): e3326. https://doi.org/10.1371/journal.pone.0003326.
49. Redel H, Gao Z, Li H, et al. Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men. *J Infect Dis.* 2013;207(7):1105–14. https://doi.org/10.1093/infdis/jit005.
50. Oates A, Bowling FL, Boulton AJ, McBain AJ. Molecular and culture-based assessment of the microbial diversity of diabetic chronic foot wounds and contralateral skin sites. *J Clin Microbiol*. 2012;50(7):2263–71.
51. Banerjee T, Das A, Singh A, Bansal R, Basu S. The microflora of chronic diabetic foot ulcers based on culture and molecular examination: a descriptive study. *Wound Manag Prev.* 2019;65(5):16–23. https://doi.org/10.25270/wmp.2019.5.1623.
52. Malone M, Johani K, Jensen SO, et al. Next generation DNA sequencing of tissues from infected diabetic foot ulcers. EBioMedicine. 2017;21:142–9.
53. Johani K, Malone M, Jensen S, et al. Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. *Int Wound J*. 2017;14(6):1160–9.
54. Charles PG, Uçkay I, Kressmann B, Emonet S, Lipsky BA. The role of anaerobes in diabetic foot infections. *Anaerobe*. 2015;34:8-13.
55. Burmølle M, Ren D, Bjarnsholt T, Sørensen SJ. Interactions in multispecies biofilms: do they actually matter? *Trends Microbiol*. 2014;22(2):84–91.
56. Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S et al. Gene expression in Pseudomonas aeruginosa biofilms. *Nature*. 2001;413:860–4.
57. Boles BR, Thoendel M, Singh PK. Self-generated diversity produces ‘insurance effects’. *Proc Natl Acad Sci USA*. 2004;101:16630–5.
58. Liao C, Huang X, Wang Q, Yao D, Lu W. Virulence factors of Pseudomonas aeruginosaand antivirulence strategies to combat its drug resistance. *Front Cell Infect Microbiol*. 2022;12:926758. https://doi.org/10.3389/fcimb.2022.926758.
59. Graf AC, Leonard A, Schäuble M, Rieckmann LM, Hoyer J, Maass S et al. Virulence Factors Produced by Staphylococcus aureus biofilms have a moonlighting function contributing to biofilm integrity. *Mol Cell Proteomics*. 2019;18(6):1036–53. https://doi.org/10.1074/mcp.RA118.001120.
60. Sinha M, Ghosh N, Wijesinghe DS, Mathew-Steiner SS, Das A, Singh K et al.Pseudomonas Aeruginosa theft biofilm require host lipids of cutaneous wound. *Ann Surg*. 2023;277(3):e634–47.
61. Li P, Tong X, Wang T, et al. Biofilms in wound healing: A bibliometric and visualised study. *Int Wound J*. 2023; 20:313–327.
62. Sankar S, Muthukaliannan GK. Deciphering the crosstalk between inflammation and biofilm in chronic wound healing: Phytocompounds loaded bionanomaterials as therapeutics. *Saudi Journal of Biological Sciences.* 2024;31(4). https://doi.org/10.1016/j.sjbs.2024.103963