Skin Flora's Symbiotic Role in Wound Healing

Colonizing bacteria and probiotic therapy can help.

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Microbiomes are infinite in our world and an essential aspect of our body's homeostasis. Over the millions of years of evolution, microorganisms have adapted to our physiological ecosystem just as our bodies have adapted to them (Table 1). Research has shown that our habits, such as eating processed foods, pharmacological habits, and exercise routines, can affect the microbiome within us significantly.[1] A symbiotic relationship has developed in various area systems, such as our gastrointestinal system, coagulation cascade, neurophysiology, and even how we age.[1,2] The relationship and activity that gastrointestinal microorganisms play in the body have been well examined and studied.[3] In fact, some authors have stated that our gut microbiome is an essential factor in normal development and health maintenance.[1]

A healthy gut microbiome flora can provide a number of health benefits and ultimately lead to host protection and better health outcomes.[1] When TH17 cells in the gut flora are found to be nonfunctioning, it has been shown to lead to many autoimmune diseases such as inflammatory bowel disease (IBD), arthritis, Crohn's disease, and psoriasis.[4] Research shows that commensal organisms actively interact with the innate and adaptive immune systems to maintain a symbiotic relationship with the host. This relationship is crucial in helping regulate normal physiological functioning in many organ systems and general inflammatory responses.[1]

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The Wound Healing Phases

A microscopic lens is commonly used to examine wounds and healing. The process of wound healing can be divided from three to five phases, which overlap in time and space. The initial response to tissue disruption in the wound healing process is dedicated to hemostasis and coagulation. The inflammatory process begins with an early neutrophil phase. Within 2-5 days, a late macrophage phase occurs, and these cells phagocytose and secrete proteases. This concludes with the removal of local bacteria and degradation of necrotic tissue.

Approximately 3-10 days after the initial wound, one should be in the proliferation phase characterized by

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TABLE 1

Types of Skin	Top 3 Bacteria		
	1	2	3
Dry	β-Proteobacteria	Corynebacteria	Flavobacteriales
Moist	Corynebacteria	β-Proteobacteria	Staphylococci
Sebaceous	Propionibacteria	Staphylococci	Corynebacteria

Table 1: Top three microbacteria associated with three skin microenvironments. Skin of a healthy volunteer was used.[21]

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re-epithelization and resurfacing of the wound. Granular tissue is formed while mediators are working to restore the vascular network. Fibroblasts infiltrate the site of injury to provide structure in the form of collagen. Remodeling, which is the final phase, can last from months to years after the injury has occurred. In this phase, the formation of granular tissue stops via apoptosis. Collagen I replaces collagen III from the proliferative phase, and the angiogenic process diminishes.[5]

If any systemic factors or local factors are not functioning properly, the wound can stall and become chronic. Most chronic wounds are "stuck" in the inflammatory phase of wound healing. Three commonly referenced reasons why a wound would become chronic: systemic or physiological factors, ischemia and reperfusion injury, and a high bioburden.

Often when a wound is swabbed and cultured, a heavy bioburden is blamed for wound stalling. However, colonization does not indicate infection, and specific microbes reside in specific dermatological niches as a hallmark to our unique skin microbiome.[6] The individual microbiome of the wound is multifactorial and a complicated balance between colonization and infection.

A colonization can cross over into critical colonization or infection when the bioburden of the wound outweighs the benefit the bacteria can provide.

The Presence of Skin Flora

While the full extent of our relationship with our natural skin flora is not completely understood, both beneficial and harmful outcomes have been noted by the presence of skin flora (Table 2). A colonization can cross over into critical colonization or infection when the bioburden of the wound outweighs the benefit the bacteria can provide.[7] While it is commonly believed that any bacterial presence can stall a wound, some bacterial colonies may have advantageous effects on a patient's general well-being and wound healing abilities. Bacteria can prevent pathological species from invading through cell competition. This can lead to benign host immune stimulation and up-regulation of processes that decrease the inflammatory phase and lower the build-up of possible detrimental mediators (e.g., reactive oxygen species and overproduction of proteases).

Both S. epidermidis and S. aureus are commonly regarded as the most common microbes found in wounds and on our skin's surface.[6] These bacteria express antimicrobial peptides (AMPs) in human keratinocytes, which can have a protective role for the skin by not allowing invasion of harmful microorganisms.[6] This maintains a skin-barrier function when these bacteria colonize the area, even if the skin is broken. One benefit unique to S. epidermidis is the capability of activating CD8 T-cells to promote keratinocyte up-regulation of toll-like receptors and downstream TNF-α.[6] This, in turn, decreases the inflammatory process and accelerates wound healing. Small amounts of S. aureus with super-antigens can also down-regulate the production of IL-17 and other chemotactic factors, resulting in decreased purulence of skin wounds and decreased skin inflammation.[6] Additionally, Pseudomonas spp. has been shown to stimulate TAK1/MKK/p38 regulation factors, leading to the repression of cell apoptosis and promoting neovascularization.[6] This ultimately improved wound healing outcomes; however, this effect was only in the absence of infection.

TABLE 2

Skin Flora	Wound Infections
S. epidermidis	Staphylococci/MRSA
S. aureus	S. pyogenes
Actinobacteria	Enterococci spp.
Corynebacteria	P. aeruginosa

Table 2: A comparison of the most common bacteria found on healthy skin flora and infected wound beds.[21,22]

Finally, even predatory organisms, such as B. bacteriovirus, have been shown to reduce biofilm formation and lower the bioburden on the wound by destroying a large number of pathogens in the wound.[6] While further research is warranted in this area, many of these studies have shown promise that our skin flora and bacterial colonization of wounds can be of beneficial presence in wound environments.

Oral probiotic supplements, or microorganisms that provide health benefits, have also shown potential in improving wound outcomes and support the idea that some or certain bacterial presence is beneficial to wound healing. Guliver was able to explore probiotics' positive influence in human subjects suffering from chronic hand dermatitis.[8] The human subjects took a daily oral probiotic of L. acidophilus, L. casei and L. rhamnosus (Bio-K+) and displayed an equivalent reduction in epidermal proliferation without negative immunological or dermal effects.[9] Bio-K+ inhibitory effects were further tested in vitro with MRSA cells by Karska, et al.

A Significant Study

As MRSA presents on the skin, it can shift the cutaneous microbiota and lead to skin diseases such as atopic dermatitis and diabetic wounds. Application of Bio-K+ inhibited 99% of MRSA cells after 24 hours of incubation.[10] In a

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mouse skin inflammation model, probiotic strains of L. salivarius, L. rhamnosus, and B. bifidum limited the development of chronic skin inflammation and showed an improvement of skin repair for diseases like psoriasis.[11]

Specifically, in this study, the L. salivarius and L. rhamnosus strains decreased the serum pro-inflammatory factors and increased anti-inflammatory factors in the host through various cytokine activity.[11] B. bifidum showed improvement as well but had lower benefits when compared to the Lactobacillus strains.[11] These two Lactobacillus strains were also shown to restore the Th/Tregs balance in Omenetti's study.[12] Overall, this research suggests that specific strains of oral probiotic therapy can break the inflammation cycle, i.e. the phase in which most chronic wounds are stalled.

Figure 1: Debridement of a wound on a diabetic patient after a superficial traumatic injury.

Topical Probiotics and Wound Care

Topical probiotics have also had beneficial effects on skin ulcerations and wounds, making them a promising potential treatment option for ulcer patients. L. plantarum has been shown to significantly affect the growth of harmful bacteria in burn wounds. Valdez et al. used burn mouse models to test the effects of L. plantarum on burn wounds infected with P. aeruginosa.[13] This study found that L. plantarum inhibited P. aeruginosa and improved tissue repair by enhancing P. aeruginosa phagocytosis.

This was further investigated in an in-vitro model study by Besser, et al. Three probiotic strains were tested against five of the most common pathogens based on the WHO research and development priorities. This study found that L. plantarum showed the most antimicrobial activity.[14] However, the antimicrobial activity of each probiotic was donor specific.[14] Sikorska and Smoragiewicz studied the effect of Lactobacillus strains against wounds infected with MRSA.[15] Their study supported that Lactbacillus strains in the wound beds can have an inhibition or reduction effect on S. aureus and MRSA colonization. These effects are attributed to cell competitive exclusion and bacterial cell secretions such as acids and bacteriocin-like inhibitors.

Application of Bio-K+ inhibited 99% of MRSA cells after 24 hours of incubation.

Finally, Jones, et al. examined nitric oxide-producing bacteria in vivo rabbit models.[16] They found that wounds with this topical application were 2.52 times more likely to heal when compared to the control. They concluded that this application of topical probiotics may be a safe, cost-effective treatment for chronic wounds. These studies indicate that certain strains and their byproducts can be a useful topical treatment in infected wounds.

As our understanding of skin flora, probiotics, and their systemic effects continues to advance, so does the possibility of utilizing this knowledge to improve wound health and healing potential. Numerous studies have shown the link between wound health and certain strains of beneficial bacteria.[1] Particularly, Lactobacillus bacteria and its by-products have been shown to improve wound health and inhibit harmful or infectious bacteria that can be commonly seen in infected wound beds.

Frequently used wound cleansing techniques, such as surgical debridement and cleansing, have recently undergone scrutiny (Figure 1). Constant cleansing of the wound has been shown to traumatize the tissue surrounding the wound and reduce the wound exudate that may contain bacterial properties and growth factors to help promote healing.[17] Surgical debridement has had the same hindering effects in recent literature by not allowing our natural skin flora to colonize the wound bed and limiting the growth of beneficial bacteria.[18] More conservative wound cleaning and debridement will prevent the hindering of promoter cells and allow the skin flora to remain intact to promote healthy wound healing.

Reflex antibiotic use has also been a subject of scrutiny. Zhang, et al. conducted a study on wound healing in rats with oral doses of vancomycin for seven days.[19] A decrease in the proportion of Staphylococcus spp. in the wound beds was noticed, demonstrating that the antibiotic treatment lowered IL-17, altering the bacterial composition. Ultimately, this delayed wound healing and reinforced the potentially cytotoxic effect of antimicrobial cleansers. Lukic, et al. also highlighted that recent evolutions of antibiotic-resistant bacteria have caused a decrease in antibiotic effectiveness while still having potentially harmful side-effects.[20]

Benefits of Skin Flora and Bacteria

Wounds and ulcerations are a very common problem podiatrists manage, especially in patients with other systemic ailments like diabetes mellitus. However, recent literature has brought attention to the benefits of skin flora and certain bacteria in the wound bed, as well as scrutinized common wound care practices. Common bacteria that reside on the skin can increase inflammatory responses to protect the body from invasive bacteria and produce angiogenic effects.

These and other processes have been shown to increase the rate of healing in wounds when infection is not present. Topical and oral bacterial probiotics have also been shown to improve wound healing rates and

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infection rates. Automatic antibiotic dispensing and frequent antiseptic washes are showing delayed wound healing in research due to effects on the skin flora. With the support of this literature, authors encourage you to examine your own wound care protocols. Colonization does not equate to infection, and attempting to kill every bacterium in a wound bed may be detrimental to wound healing.

In fact, colonizing bacteria and probiotic therapy may be of great benefit and aid in the healing of chronic ulcers. While more research is needed for definitive answers, the symbiotic relationship our bodies have had with the bacteria in our world deserves our respect and consideration when treating ulcerations of the lower extremity. **PM**

References

[1] Rodriguez, David Avelar, et al. "The Gut Microbiota: A Clinically Impactful Factor in Patient Health and Disease." SN Comprehensive Clinical Medicine 1.3 (2019): 188-199.

[2] Jesitus, John. (2019) "'Inflammaging' addresses internal aging processes". Dermatology Times, 40(7), 42.

[3] Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol. 2004;4:478–485.

[4] Packey CD, Sartor RB. Interplay of commensal and pathogenic bacteria, genetic mutations, and immunoregulatory defects in the pathogenesis of inflammatory bowel diseases. J Intern Med. 2008;263:597–606.

[5] Reinke, J. M., & Sorg, H. (2012). Wound repair and regeneration. European Surgical Research, 49(1), 35-43. doi:http://dx.doi.org/10.1159/000339613

[6] Johnson, T., Gómez, B., McIntyre, M., Dubick, M., Christy, R., Nicholson, S., & Burmeister, D. (2018). The cutaneous microbiome and wounds: New molecular targets to promote wound healing. International journal of molecular sciences, 19(9), 2699.

[7] Horrocks, A. (2006). Prontosan wound irrigation and gel: management of chronic wounds. British Journal of Nursing, 15(22), 1222-1228.

[8] Gulliver, W. P., Hutton, A. S., & Ship, N. (2018). Investigating the therapeutic potential of a probiotic in a clinical population with chronic hand dermatitis. Clinical, cosmetic and investigational dermatology, 11, 265–271. doi:10.2147/CCID.S164748.

[9] Sikorska, Hanna & Smoragiewicz, Wanda. (2013). Role of probiotics in the prevention and treatment of meticillin-resistant Staphylococcus aureus infections. International journal of antimicrobial agents. 42. 10.1016/j.ijantimicag.2013.08.003.

[10] Karska-Wysocki, B., Bazo, M., & Smoragiewicz, W. (2010). Antibacterial activity of Lactobacillus acidophilus and Lactobacillus casei against methicillin-resistant Staphylococcus aureus (MRSA). Microbiological Research,674-686. doi:10.1016/j.micres.2009.11.008.

[11] Holowacz, Sophie & Blondeau, Claude & Guinobert, I & Guilbot, A & Hidalgo, S & Bisson, Jean-François. (2018). Lactobacillus salivarius LA307 and Lactobacillus rhamnosus LA305 attenuate skin inflammation in mice. Beneficial Microbes. 9. 1-12. 10.3920/BM2017.0084.

[12] Omenetti, S., & Pizarro, T. T. (2015). The Treg/Th17 Axis: A Dynamic Balance Regulated by the Gut Microbiome. Frontiers in immunology, 6, 639. doi:10.3389/fimmu.2015.00639.

[13] Valdez, Juan & C Peral, M & Rachid, M & Santana, M & Perdigón, G. (2005). Interference of Lactobacillus plantarum with Pseudomonas aeruginosa in vitro and in infected burns: The potential use of probiotics in wound treatment. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 11. 472-9. 10.1111/j.1469-0691.2005.01142.

[14] Besser, M., et al. "Impact of probiotics on pathogen survival in an innovative human plasma biofilm model (hp BIOM)." Journal of translational medicine 17.1 (2019): 243.

[15] Sikorska, Hanna, and Wanda Smoragiewicz. "Role of probiotics in the prevention and treatment of meticillin-resistant Staphylococcus aureus infections." International Journal of Antimicrobial Agents 42.6 (2013): 475-481.

[16] Jones, Mitchell, et al. "Novel nitric oxide producing probiotic wound healing patch: preparation and in vivo analysis in a New Zealand white rabbit model of ischaemic and infected wounds." International wound journal 9.3 (2012): 330-343.

[17] Atiyeh BS, Dibo SA, Hayek SN. Wound cleansing, topical antiseptics and wound healing. Int Wound J 2009;6:420–430

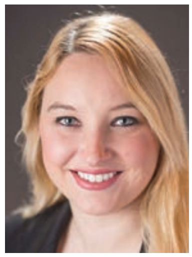
[18] Wilcox, J. R., Carter, M. J., & Covington, S. (2013). Frequency of debridements and time to heal: a retrospective cohort study of 312 744 wounds. JAMA dermatology, 149(9), 1050-1058.

[19] Zhang, M., Jiang, Z., Li, D., Jiang, D., Wu, Y., Ren, H.,... & Lai, Y. (2015). Oral antibiotic treatment induces skin microbiota dysbiosis and influences wound healing. Microbial ecology, 69(2), 415-421.

[20] Lukic, J., Chen, V., Strahinic, I., Begovic, J., Lev-Tov, H., Davis, S.C.,... Pastar, I. (2018). Probiotics or pro-healers: the role of beneficial bacteria in tissue repair. Wound repair and regeneration: official publication of the Wound Healing Society [and] the European Tissue Repair Society, 25(6), 912–922. doi:10.1111/wrr.12607.

[21] Grice, Elizabeth A., et al. "Topographical and temporal diversity of the human skin microbiome." science 324.5931 (2009): 1190-1192.

[22] Chiller, Katarina, Bryan A. Selkin, and George J. Murakawa. "Skin microflora and bacterial infections of the skin." Journal of Investigative Dermatology Symposium Proceedings. Vol. 6. No. 3. Elsevier, 2001.



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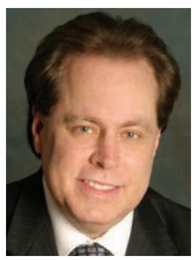

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