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Beneficial and deleterious bacterial–host interactions in chronic wound pathophysiology

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Abstract: Chronic wounds represent a major health and financial burden. Although incredible advancements in wound management have been made in the last decade, the incidences of chronic wounds continue to increase due to a rise in biofilm-associated infections. The presence of biofilm causes chronic inflammation, leading to impaired healing rates and host mortality. This review describes the deleterious bacterial–host interactions, as well as the beneficial role of pH and probiotics in chronic wound infections.

Keywords: chronic wounds, biofilms, wound pH, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, probiotics

Definition of the wound biofilm; bacterial species that colonize chronic wounds

The skin is the first line of defense for the body, providing protection against toxins, microorganisms, and chemicals in the environment. Thus, the loss of skin integrity can result in substantial physiologic imbalance and significant disability or death. The prevalence of chronic wounds associated with the loss of skin is an important concern within the health care field.[1] It is estimated that approximately seven million patients have some form of chronic wounds/ulcers that are associated with diabetes or pressure.[2–4] The health care cost for chronic wounds is estimated to be as high as $20 billion annually in the United States.[5–8] The current standard of care for chronic wounds consists of debridement, irrigation, moisture retentive bandages, and antimicrobial therapy. These treatment paradigms are aimed at promoting wound healing and the restoration of homeostasis.

Despite incredible advancements in the field of wound healing over the last decade, the prevalence and incidence of chronic wounds continues to rise.[6,7] Clinically, chronic wounds/ulcers are defined as any wound that is nonhealing after 30 days, and can be classified into three types of ulcers: vascular (eg, venous or arterial); diabetic; and pressure.[9,10] These wounds are resistant to natural healing, and may require long-term medical care.[10,11] Chronic wounds display delayed healing for a variety of reasons including diminished blood supply, uncontrolled inflammatory response, reduced re-epithelialization, and the presence of biofilm-associated infections.[10,12]

Biofilms are characterized as aggregated communities of microbes attached to a surface and/or each other, embedded in an extracellular polymeric substance (EPS) matrix composed of microbial- and host-derived extracellular DNA, proteins, and polysaccharides. These communities are often polymicrobial and dynamic, consisting of diverse species that are continuously changing.[13] Biofilms can be found in a

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number of places on and in the body including the teeth, gastrointestinal mucosa, nasal epithelium, and any implanted artificial surface (eg, orthopedic implants, artificial valves, and catheters). Though the impact of biofilms in the pathogenesis of wound infections remains debatable, certain bacterial species have clearly been shown to hinder wound healing. It is thought that wounds are first contaminated and then colonized by adherent replicating microorganisms, which do not cause tissue damage. When the bacterial load in the wound exceeds 10^5 colony-forming units/gram tissue,[14] the initial colonization is thought to have progressed to an infection. While microbial colonization of a wound alone does not prolong healing,[15] the subsequent infection can lead to tissue damage. The classical symptoms of a chronic wound infection are pain, heat, edema, and purulence. However, more contemporary signs and symptoms include pain, foul odor, and wound breakdown.[16]

The microbial flora of chronic wounds encompasses a variety of microorganisms including aerobic and anaerobic Gram-negatives and Gram-positives, as well as fungi.[17] Some of the most common causes of wound infections are the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.).[18] However, just as every tumor is different, every biofilm infection is a unique consortium of bacteria, fungi, and host components, which can vary greatly from the initial injury to a long-term nonhealing wound. Prior to injury, normal flora inhabit the skin and differ depending on the location – eg, sebaceous areas harbor *Propionibacterium* spp., while moist areas predominantly support *Staphylococcus* and *Corynebacterium* spp. and, surprisingly, dry areas foster many Gram-negative bacteria previously thought to rarely colonize the skin.[19]
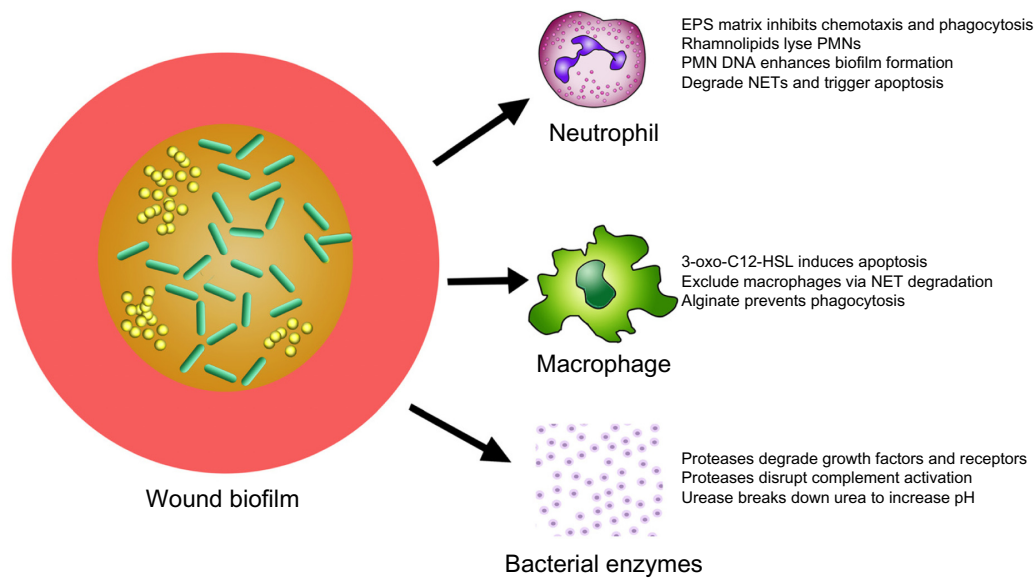
Normal flora can easily colonize wounds and lead to wound infections, as reported by a recent clinical study examining the evolution of the microflora in burn wound infections.[20] In this study, 33% of patients' wounds were already colonized upon admission. About 50% of these burn wounds were found to be colonized by *S. aureus*, while the other 50% harbored Gram-negative organisms such as *P. aeruginosa*, as well as *Acinetobacter*, *Klebsiella*, and *Enterobacter* spp., and *Escherichia coli*.[20] However, as this study was conducted using standard culturing techniques (serial dilutions, and culturing on nutrient-rich agar plates), nonculturable microorganisms were not considered. In contrast, utilizing a variety of molecular techniques (pyrosequencing, denaturing gradient gel electrophoresis, and full ribosome shotgun sequencing), the Wolcott laboratory reported the microbial populations from 30 patients with different types of chronic wound infections.[21] Regardless of the type of wound (diabetic, pressure, or vascular), the most common genera found were *Staphylococcus*, *Pseudomonas*, *Peptoniphilus*, *Enterobacter*, *Stenotrophomonas*, *Finegoldia*, and *Serratia* spp.[21] Strict and facultative anaerobic bacteria were shown to make up the majority of the microbial population (around 80%) in the wounds, but ratios of strict/facultative anaerobes differed based on the wound type: diabetic (25%/65%); pressure (58%/25%); and venous (5%/75%).

With high-throughput sequencing techniques becoming cheaper and more accessible to health care providers, a whole host of new microbes are now becoming associated with disease. Undoubtedly, large amounts of new information will be generated concerning which microbes are harmful, beneficial, and/or neutral in the chronic wound. Meanwhile, the purpose of this review is to examine recent evidence concerning the deleterious interactions between bacteria and wound tissue, discuss the possibility of using beneficial bacteria to treat wound infections, and consider the translational potential of current studies to optimize future wound management approaches.

Deleterious bacterial–host interactions in chronic wound pathophysiology

Bacterial biofilms are thought to delay wound healing for a variety of reasons, namely by shifting the wound immune response toward chronic inflammation (Figure 1). In theory, as long as the biofilm is present, the immune system will try to remove it, resulting in prolonged inflammation with collateral damage to the host tissue.[22] The host immune response is a complex multifaceted system that has been divided into the adaptive and innate immune systems. The innate immune response is made up of the skin barrier, commensal bacteria, the complement system, and both phagocytic (eg, macrophages and neutrophils) and nonphagocytic (eg, natural killer cells) leukocytes. The innate immune system recognizes a broad group of molecules specific to microbes such as lipopolysaccharide, peptidoglycan, and lipoteichoic acid via pattern recognition receptors, and is critical in fighting off bacterial infections and mounting the initial immune response to invading microbes.[23] Once the epithelial skin barrier is compromised, the microbial skin flora are typically the first to contaminate, colonize, and potentially infect the wound. Tissue macrophages and other cells respond to these invading bacteria by releasing cytokines and chemokines, which stimulate polymorphonuclear neutrophil (PMN)

EPS matrix inhibits chemotaxis and phagocytosis
Rhamnolipids lyse PMNs
PMN DNA enhances biofilm formation
Degrade NETs and trigger apoptosis

Neutrophil

3-oxo-C12-HSL induces apoptosis
Exclude macrophages via NET degradation
Alginate prevents phagocytosis

Macrophage

Proteases degrade growth factors and receptors
Proteases disrupt complement activation
Urease breaks down urea to increase pH

Bacterial enzymes

Wound biofilm

Figure 1 Deleterious actions of biofilms in chronic wounds.
Notes: Bacterial biofilms are thought to delay wound healing for a variety of reasons, including reducing local oxygen levels, mechanically inhibiting granulation, increasing tolerance to antimicrobials, and shifting the immune response toward chronic inflammation. Interactions between bacterial biofilms and dying or ineffective neutrophils and macrophages appear to significantly contribute to the chronic inflammatory state seen in chronic wound infections.
Abbreviations: EPS, extracellular polymeric substance; PMN, polymorphonuclear neutrophil; NET, neutrophil extracellular trap; 3-oxo-C12-HSL, N-3-oxo-doedecanoyl-L-homoserine lactone.

chemotaxis to the wound site. One to 2 days later, macrophages migrate to the wound site to phagocytose bacterial cells, and for efferocytosis, which refers to the engulfment of apoptotic cells. Dendritic cells and macrophages then present antigens to the correct T-cells, which begins the cascade for subsequent antibody production.[24] However, pathogenic bacteria have evolved numerous methods to evade the immune response in wound infections, which the following section will expound upon.

Polymorphonuclear neutrophils and biofilms

PMNs are critical to the host defense against bacteria, illustrated by the life-threatening bacterial sepsis problems associated with individuals who lack PMNs (neutropenia).[25] Activated PMNs rapidly migrate to the site of injury, and persist there for 2–3 days.[26] PMNs do not recognize specific antigens, but instead recognize evolutionary conserved molecules shared by numerous bacterial species such as lipopolysaccharides and bacterial DNA.[27] PMNs are able to recognize bacterial DNA and complex carbohydrates, both of which are essential components of the biofilm EPS matrix.[28,29] PMNs have been shown to kill bacteria by four specific mechanisms: phagocytosis; the release of microbicidal compounds through degranulation; reactive oxygen species (ROS) generation; and the formation of neutrophil extracellular traps (NETs).[30]

While most of the previous work examining the bactericidal abilities of neutrophils was performed with planktonic bacteria, there has been a surge in recent published studies examining the relationship of PMNs and biofilms. The majority of work on PMNs and biofilm interactions has been done with *P. aeruginosa* and *S. aureus* biofilms.[31] When *P. aeruginosa* biofilms were incubated with PMNs in vitro, the PMNs settled onto the biofilms, but they exhibited very little bactericidal activity or movement.[32] One explanation for this observation was that the alginate present in *P. aeruginosa*'s EPS matrix inhibited PMN phagocytosis and chemotaxis.[33] Rhamnolipids are another mechanism by which *P. aeruginosa* biofilms antagonize PMNs and are actively produced in the biofilm mode of growth.[34,35] Jensen et al[36] observed that rhamnolipids, produced by biofilms, rapidly lysed human PMNs in vitro. This group went on to show that *P. aeruginosa* upregulated rhamnolipid production in response to the presence of PMNs, signifying that *P. aeruginosa* actively recognizes and responds to these immune cells. *P. aeruginosa* biofilms have also been shown to produce rhamnolipids in vivo, resulting in increased PMN lysis.[37]

There is also considerable in vitro and in vivo evidence that lysed PMNs enhance *P. aeruginosa* biofilm formation. For example, PMNs were shown to eradicate planktonic *P. aeruginosa* cells, while their presence increased the number of biofilm cells.[37] The authors concluded that *P. aeruginosa* utilized the actin and DNA from lysed PMNs

to fortify their biofilms. In addition, neutrophil-enhanced *P. aeruginosa* biofilms were more tolerant to antipseudomonal antibiotics. When these biofilms were treated with DNase, the biofilm was disrupted and there was an increase in planktonic cells.[38] Furthermore, *P. aeruginosa*'s ability to take advantage of neutrophils was recently demonstrated in a murine type-1 diabetic wound infection model.[39] Diabetic mice on insulin therapy that were wounded and infected with *P. aeruginosa* were shown to have increased PMN migration as compared to both diabetic and nondiabetic mice. However, this increased migration did not lead to increased bacterial clearance. Rather, increased neutrophil migration in this group of mice resulted in *P. aeruginosa* biofilms that contained more DNA and exhibited increased tolerance to the antibiotic gentamicin.[39]

Further evidence of the deleterious interactions between biofilms and PMNs came from Nguyen et al,[40] who utilized a type 2 diabetic murine wound infection model to show that the presence of *S. aureus* biofilms significantly reduced neutrophil oxidative activity, leading to higher bacterial load and decreased healing rates. While *S. aureus* induces NETosis in PMNs, it was recently shown that *S. aureus* is able to degrade NETs to deoxyadenosine, triggering the caspase-3-mediated cell death of immune cells.[41] In addition, clinical staphylococcal infections seem to be correlated to reduced neutrophil apoptosis in diabetic patients, which leads to prolonged tumor necrosis factor-α production and reduced neutrophil clearance.[42] In summary, PMNs are effective at killing planktonic cells,[43] but biofilms appear to resist the bactericidal effects of PMNs, and may even benefit from the cellular debris left in their wake. It is clear from these recent studies that interactions between bacterial biofilms and dying or ineffective neutrophils contribute strongly to the chronic inflammatory state seen in chronic wound infections.

Macrophages and biofilms

Following the migration of PMNs to the site of injury, another innate immune cell population arrives: the macrophages. Macrophages arrive 2–3 days following tissue damage to challenge any microbial invaders, which the PMNs have not eradicated. Macrophages also phagocytize apoptotic/necrotic cells in a process called efferocytosis.[44] Macrophages kill bacterial cells by phagocytosis followed by the production of ROS, but the interactions of macrophages with biofilms are less well characterized than those of PMNs. In 2003, it was observed that *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), a quorum-sensing molecule produced by *P. aeruginosa*, induced apoptosis in macrophages.[45]

However, 3-oxo-C12-HSL has also been shown to activate phagocytosis, while not affecting ROS production.[46] When biofilms were incubated with macrophages in vitro, those lacking alginate were eradicated by macrophages in 4 hours. The killing of these biofilm cells was mediated through phagocytosis, and was dependent on the presence of interferon-γ.[47] *S. aureus* has been shown to reduce macrophage numbers by releasing nuclease and adenosine, which degrade NETs and exclude macrophages from abscesses.[41] Based upon the literature, *P. aeruginosa* biofilms seem less protected from macrophages, though there are various mechanisms that *P. aeruginosa* and *S. aureus* employ to diminish their effectiveness.

Bacterial proteases and wound tissue

Pathogenic bacteria produce a wide range of serine, cysteine, and metalloproteases that have intracellular and/or extracellular targets and can aid in colonization, the evasion of host defense, dissemination of bacteria, and tissue degradation.[48,49] Intracellular proteases are typically important in cellular and metabolic processes, while extracellular proteases are important in the ability of bacteria to interact with the host environment, such as absorbing and utilizing hydrolytic products.[50] In addition, bacterial proteases have the capability to degrade host growth factors and their receptors.[51] For example, Laarman et al[52] showed that *P. aeruginosa* alkaline protease was capable of disrupting complement activation by inhibiting opsonization of the bacteria by C3b and the subsequent formation of C5a. This protease has also been shown to help *P. aeruginosa* evade phagocytosis in the lung by degrading surfactant protein-A.[53]

Wound pH and biofilm formation

The acidic pH of the skin (pH 4–6), known as the acid mantle, is one of the key mechanisms that protects the skin from microbial infections. In chronic wounds, the destruction of underlying tissue releases bodily fluids (pH 7.4) that shift the pH of the wound toward neutral/alkaline conditions.[54] The pH of the wound environment has been shown to be an accurate method of predicting nonhealing wounds (Figure 2). Clinical studies have suggested that if the wound environment does not shift toward an acidic pH early in treatment, the probability of the wound failing to heal and requiring a skin graft increases significantly. For example, a randomized clinical study found that out of 25 venous ulcers, those that were considered "non healing" had a pH of 7.42, while wounds that had a pH of 6.91 healed.[55] The relationship of wound healing and pH was further explored in a clinical study that examined the pH of 50 chronic and acute wounds. Overall, the authors reported
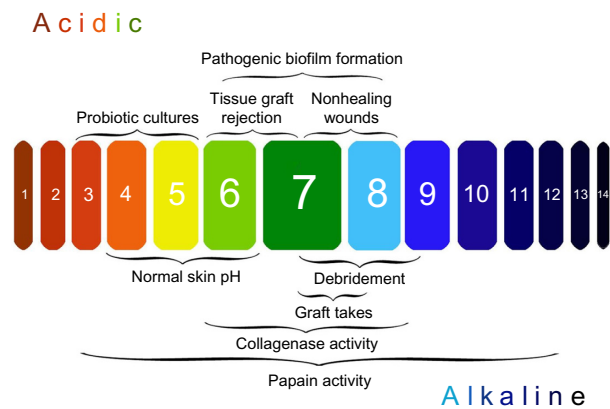
Figure 2 The importance of managing pH in chronic wound infections.
Notes: The acidic pH of the skin is one of the key mechanisms that protect it from microbial infections. In wounds, increasingly alkaline pH is a predictor that they will become nonhealing or chronic. However, wounds that are too acidic are less likely to successfully accept grafts. The activities of most biological and enzymatic therapies have pH optimums, and numerous factors can shift wound pH including debridement and bacterial colonization.

that healing wounds shifted below a pH of 8.0, while chronic wounds remained above a pH of 8.5.[56]

There are numerous factors that shift the wound toward an alkaline pH, including lowered oxygen tension, stage of healing, debridement, maggot therapy, and bacterial colonization (Figure 2).[54] For example, *Proteus mirabilis*, *Klebsiella* spp., and *P. aeruginosa* produce urease, an enzyme that catalyzes the formation of ammonia from urea. The release of alkaline ammonia promotes bacterial attachment and growth, as many wound pathogens require neutral to alkaline pH environments to form biofilms.[57] Additionally, an alkaline pH reduces the release of oxygen supporting the growth of anaerobic bacteria. For more information on the impact of pH on wound healing and biofilm formation, refer to this recent and thorough review.[57] Increased wound pH has major implications for delayed wound healing, and the next section will focus on bacterial therapies that target this problem in chronic wound infections.

Beneficial bacterial–host interactions that promote chronic wound healing

Physicians face many challenges in the management of nonhealing chronic wounds including disrupting bacterial biofilms, the global rise in antibiotic resistance, excessive inflammation, and an alkaline wound environment. Thus, the ideal therapy for wound infections is one that can reduce wound pH, dampen the immune response and target the bacterial infection. One promising and relatively unexplored treatment that meets these criteria is the use of probiotic

therapy. Utilizing commensal probiotics has shown promise in preventing and treating gut, oral, and urinary tract bacterial infections.[58] As probiotics are typically bacteria that reside in the gut, they prefer an acidic environment and grow best in pH 3–5. It has been proposed that, because probiotics can compete with pathogens for host tissue binding sites, stimulate the release of anti-inflammatory cytokines, lower pH, release antimicrobial compounds, and deactivate the virulence factors of bacterial pathogens, they are excellent candidates for promoting wound healing in diabetic foot ulcers.[59] The following section will focus on probiotic studies conducted in vitro and in vivo.

Battling wound pathogens with probiotics

Among the ESKAPE pathogens, probiotic therapies have predominantly been utilized against *S. aureus* and *P. aeruginosa*. The primary probiotic bacterial species that have been tested therapeutically include numerous *Lactobacillus* spp., *Bifidobacterium* spp., and *Propionibacterium* spp. For example, *Lactobacillus casei* and *Lactobacillus acidophilus* inhibited the growth of ten clinical strains of *S. aureus* by up to 99% after a 24-hour coculture.[60] In the same study, *L. acidophilus* also exhibited antimicrobial activity against 5/6 ESKAPE pathogens isolated from burn wounds.[60] In another study, *S. aureus* biofilms were reduced by 11–17 mm using the spot plate assay by several *Lactobacillus* spp. and *Bifidobacterium* spp.[61] The proposed mechanism for the inhibitory effect on *S. aureus* lies in the production of organic acids (and the subsequent lowering of pH), as this antagonistic effect was abated when culture supernatants were neutralized.[61] As recently as 2014, propionic acid, a byproduct of *Propionibacterium acnes*, was shown to kill planktonic *S. aureus* by lowering the pH of the bacterial cytoplasm.[62] Additionally, propionic acid exerted broad-spectrum activity against *E. coli* and the fungal pathogen *Candida albicans*, inhibiting their growth at concentrations >10 mM. Other studies examining the efficacy of probiotics to treat *S. aureus* are described in this recent review.[63]

In 2010, Ramos et al[64] provided some of the strongest evidence for the utilization of probiotics in the destruction of *P. aeruginosa* biofilms in vitro. The authors reported that acidic filtrates prepared from *Lactobacillus plantarum* supernatants disrupted biofilms made by a laboratory strain of *P. aeruginosa* by 43% and a *P. aeruginosa* clinical strain isolated from a wound by 35%.[64] Ramos et al[65] went on to use *L. plantarum* supernatants to both disperse and inhibit *P. aeruginosa* biofilms, and indirectly reduce the production of numerous virulence factors (elastase,

pyocyanin, rhamnolipids).[65] Overall, these studies provide the groundwork for understanding the antibiofilm properties of probiotics. However, there remain many questions as to the antimicrobial efficacy of probiotics against wound pathogens and multispecies biofilms.

Another mechanism by which probiotics have been shown to enhance wound healing is by protecting the host from pathogens like *S. aureus* and dampening the immune response. For example, the probiotic strains *Lactobacillus reuteri* and *Lactobacillus rhamnosus* were shown to protect human keratinocytes from *S. aureus*-induced cell death when applied prior to or concurrently with *S. aureus*. However, if *S. aureus* cells were incubated with keratinocytes for as little as 1 hour, the addition of *Lactobacillus* spp. did not provide any protection to the host skin cells.[66] In another recent study, when *L. rhamnosus* was simultaneously incubated with *S. aureus* and PMNs (murine and human), there was a reduction in *S. aureus*-associated PMN death and NET formation.[67] As ROS production is essential to NET formation, the authors next tested if pretreating PMNs with *L. rhamnosus* could prevent ROS production. *L. rhamnosus* incubation resulted in decreased ROS production when PMNs were activated with phorbol 12-myristate 13-acetate or H_2O_2. Some of the probiotic strains tested were able to activate NETosis alone, so this anti-inflammatory effect is clearly species specific.[67] *L. plantarum* supernatants were similarly shown to protect human PMNs from *P. aeruginosa*-associated apoptosis/necrosis via the destruction of *P. aeruginosa* quorum-sensing molecules.[68] By disrupting pathogenic biofilms and protecting key cells in the wound environment, probiotics have clearly demonstrated efficacy to provide crucial support to wound healing in vitro.

In vivo efficacy of probiotics to promote chronic wound healing

While there are numerous studies investigating the impact of probiotics on wound pathogens in vitro, far fewer have examined their efficacy in vivo. Of the studies performed, by far the most common approach has been to apply a probiotic species topically to wounds, either prophylactically or concurrently with infection, and then monitor wound healing and/or bacterial load. For example, the ability of *L. plantarum* to prevent either *S. aureus* or *P. aeruginosa* infection was investigated by treating mouse wounds with *L. plantarum* (cells or supernatant) plus *S. aureus*, *P. aeruginosa*, or the pathogens alone.[69] Based on histopathological analysis from tissue taken 2 days post-treatment, the authors concluded that

L. plantarum had prevented wound infection.[69] Similarly, *P. acnes* topical therapy was tested in mouse incisions infected with *S. aureus*.[70] Mice were given 5 mm wounds and treated with *P. acnes* that had been incubated for 3 days with either phosphate buffered saline (PBS) or glycerol. Wounds were then infected with *S. aureus* and followed for 3 days. Following the wound infection, the mice treated with *P. acnes* incubated in glycerol displayed a 60% decrease in *S. aureus* bacterial burden and increased wound healing as compared to treatment with *P. acnes* that had been incubated in PBS.[70] This study suggests that glycerol fermentation and the production of compounds, like propionic acid, provide the mechanism for targeting wound pathogens. While the studies discussed earlier used probiotic therapy to treat or prevent wound infection, topical treatment with *Lactobacillus brevis* was also shown to increase wound healing and decrease inflammation over 21 days in uninfected rat wounds.[71]

Another probiotic application was based on the idea that *Lactobacillus fermentum* plus glucose produces nitrate and protons leading to nitric oxide gas (gNO) production, which can lower pH, promote healing, and kill *S. aureus*. This concept was tested in a rabbit full thickness wound infection model.[72] One day after the wounds were infected with *S. aureus*, they were treated for 21 days with an adherent patch containing lactic acid bacteria that produced gNO. The authors observed that wounds treated with the gNO patch were 2.52 times more likely to close and displayed modest histological improvements, as compared to wounds treated with control patches. While some of the work performed in animal models is encouraging, as of yet, there have been no studies treating pre-established in vivo biofilms with topical probiotic therapeutics. Clearly there is much work to be done in this field, examining different pathogens, various probiotic strains, and optimizing treatment strategies.

Clinical studies examining the efficacy of probiotics to enhance wound healing

While mechanistic studies examining the use of topical probiotics on wounds in animal models is lacking, there have been some very thorough and informative clinical studies. For example, a study conducted in Argentina evaluated the use of topical *L. plantarum* cultures to treat infected second-degree and noninfected third-degree burns.[73] The probiotic therapy was compared to silver sulfadiazine for the treatment of 80 total burn wounds (38 *L. plantarum* treatments versus 42 silver sulfadiazine treatments), and the markers of clinical effectiveness measured were bacterial load and

wound healing rates. *L. plantarum* cultures were applied directly to the wounds for 10 days, and the patients receiving the probiotic topical therapy did not receive any other form of antimicrobial treatment. The other group of patients received a daily antiseptic bath with 0.5% chlorhexidine and treatment with silver sulfadiazine cream daily for 10 days. While there were no significant differences in bacterial load or healing rates observed between the groups, there were also no cases of sepsis in either treatment group, suggesting that *L. plantarum* can be safely used in humans and function similarly to the antimicrobial standard of care treatments for slow-healing burn wound infections.[73] Another study was performed by the same research group examining the impact of topical *L. plantarum* cultures on 14 diabetic ulcers and 20 nondiabetic ulcers.[74] Although no difference was observed between groups, treatment with topical *L. plantarum* reduced the numbers of bacteria, neutrophils, apoptotic/necrotic cells, and promoted wound healing.

The most recent clinical probiotic wound study was also conducted by Valdez et al and reported in a 2013 book chapter.[75] In this study, type 2 diabetics with foot ulcers were treated with topical *L. plantarum* cultures (number [n] =20) or with standard debridement (n=10) over the course of 30 days, and then the patients were followed for 20 days post-treatment. The investigators reported that treating the diabetic foot ulcers with *L. plantarum* doubled the rates of wound healing, granulation, and bacterial load reduction.[75] Overall, these clinical studies provide a great deal of optimism for the utilization of probiotic therapy in wound care management. While much optimization and evaluation remain to be done, the studies conducted thus far provide an excellent foundation for the clinical proof of principle for this therapy.

Applying current knowledge to optimize the management of chronic wounds

Throughout this review, we have discussed biofilms, the bacterial pathophysiology of wounds, probiotics, and wound healing, but how does this information translate to the clinic? One of the main translational concepts presented was the importance of pH in wound healing (Figure 2). The measurement of wound pH is an easy and important, but often overlooked, indicator of nonhealing wounds. Nonhealing wounds typically have a pH that is neutral to slightly alkaline,[57] and from the experimental evidence, it appears that attempts should be made to reduce pH and shift the wound toward an acidic environment. This was found to be especially true

when the pH of 30 burn wounds was measured following a second dressing change.[76] Those wounds that went on to heal had a mean pH of 7.32, while the mean pH was 7.73 in nonhealing wounds that required skin grafting.

This review also covered the use of probiotics to lower wound pH, which theoretically will lead to decreased biofilm formation and persistence of pathogens,[57] reduced apoptosis[67,68] and migration of PMNs,[57] and will ultimately shift wounds toward the healing spectrum. However, there are some concerns with the use of probiotics to treat wounds, specifically concerning septicemia caused by the topical application of *Lactobacillus* spp. Although there were no reports of septicemia in the clinical wound studies that used *L. plantarum* topically,[73–75] *Lactobacillus* septicemia is possible in severely immunocompromised individuals, and seems to be strain specific. Two retrospective studies comprising a total of 260 cases of *Lactobacillus* septicemia noted that the top causative strain was *L. rhamnosus*.[77,78] Thus, there is a clear need for investigators to conduct future probiotic studies to carefully consider published data before choosing their probiotic strains and study parameters.

Although the use of probiotics may take some time to become widely accepted, there are therapeutics already available, which can lower pH and promote wound healing – namely, honey and acidified nitrate creams.[57] Recently, an acidic therapeutic 3% citric acid solution was applied to diabetic and nondiabetic wounds and compared to the alkaline therapeutic, Eusol. In both the diabetic and nondiabetic patients, the 3% citric acid solution reduced the length of treatment time to nearly half that of the Eusol-treated groups.[79]

While debridement is essential in managing nonhealing wounds, it should also be noted that any form of debridement seems to immediately shift the wound toward an alkaline pH (Figure 2) due to the increased perfusion of blood components into the wound site.[54] In addition, medicinal maggots used for debridement release ammonium bicarbonate as a byproduct, which potentially shifts the wound environment toward alkalinity.[57] Though maggot debridement therapy is reported to be an effective wound therapeutic, there remains a clear gap on the impact of this therapy on wound pH. Overall, there is a therapeutic window for the application of the aforementioned acidic agents following debridement to promote wound healing.

The efficacy of enzymatic debridement and antibiotics are also closely tied to wound pH. For instance, enzymatic debridement agents like papain have a pH range of 3–12 (optimum pH: 7), while collagenase has an optimum pH range of 6–8

(Figure 2), but fibrinolysis used in conjunction with DNase only has pH activity ranging from 4.5 to 5.5.[54] Additionally, the impact of pH on new fluoroquinolones has recently been explored and it appears that acidic pH environments enhance the antimicrobial activity of delafloxacin[80] and finafloxacin[81] against *S. aureus*, but acidic environments decrease the antimicrobial activity of monofloxacin[81] and ciprofloxacin.[80] Thus, the optimal pH for different enzymatic and antibiotic agents needs to be carefully considered before applying these treatments to wounds.

Finally, wound pH also appears to be an important factor influencing grafting success. Several studies of burn and chronic wounds have noted that tissue rejection is more likely when wounds are acidic,[54,76] and the more alkaline the wound, the better the likelihood that the skin graft will take (Figure 2).[82] For example, in 18 different wounds requiring grafts, patients with a wound pH below 7.4 experienced 0% graft acceptance, while those above this cutoff experienced 99% graft acceptance.[56] Thus, acidic pH in the wound is not always beneficial and must be adjusted and evaluated properly based on the required therapy.

Conclusion

Chronic wounds persist for a variety of reasons, but the role of bacterial biofilms in preventing healing seems very clear. The continuous presence of biofilms results in a chronic state of inflammation in the wound. Collateral tissue damage occurs as the immune system tries to remove the biofilm, and this often increases bacterial pathogenesis by stimulating growth, spread, or even invasion of the pathogen. Pathogens in a wound can contribute to increased wound pH, cause neutrophil destruction, reduce macrophage infiltration, and decrease oxygen tension, all of which contribute to delayed wound healing. Manipulating the microbial ecology of wounds with probiotics appears to be a promising and cost-effective therapy with the potential to lower wound pH, increase oxygen tension, reduce inflammation, and prevent or disrupt infection by pathogens.

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Disclosure

The authors report no conflicts of interest in this work.

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