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Glossary

- **Apoptosis:** a controlled self-destruct mechanism within cells of the body that usually follows cellular damage; colloquially known as programmed cell death.
- **Biofilm:** blanket layer of one or more species of microorganisms, often adhered to a physical surface such as the skin.
- **Fibroblast:** creates an extracellular matrix to connect newly-formed epithelial cells in wound healing.
- **Keratinocyte:** layered within the epidermis and essential for innate immunity by providing barrier to the external environment. After skin injury, keratinocytes migrate to the wound and proliferate to repair the epithelial break. In chronic wounds, keratinocytes are dysregulated (Pastar, 2014).
- **Microbiota:** community of microorganisms of beneficial, neutral, or deleterious consequence to a multicellular host.
- **Microorganisms of Interest:**
- *Lactobacillus spp. (Lb. plantarum, Lb. rhamnosus, Lb. fermentum, etc.)*: genus of bacteria that is naturally a part of the human microbiome, assisting in digestion and maintaining microenvironments such as vaginal acidity. *Lb. plantarum, Lb. rhamnosus,* and *Lb. fermentum* are examined as possible probiotics to facilitate the wound healing process.
- Pseudomonadaceae: family of bacteria to which *Pseudomonas aeruginosa* belongs.
- *Pseudomonas aeruginosa*: an opportunistic pathogen that can cause a multitude of dangerous infections
- *Staphylococci spp. (S. epidermis, S. aureus, etc.):* gram-positive cocci bacteria. Genus species range in detriment to the human body in that *S. epidermis* and *S. aureus* can be commensal, or beneficial, organisms typically found on the skin, whereas Methicillin-resistant *Staphylococcus aureus* (MRSA) is often responsible for a variety of hard-to-treat infections.
- *Streptococcus spp. (S. pyogenes, S. themophilus, etc.)*: gram-positive cocci bacteria. Genus species are implicated in both human infections (*S. pyogenes,*) and as a possible topical treatment (*S. themophilus*).

Abstract

Chronic wound formation is an affliction that disproportionately affects those of lower socioeconomic status on a global scale due to a variety of contributing factors, like type 2 diabetes and housing environment (Fayne, 2020). Antibiotic use in response to a cutaneous wound selects for antibiotic-resistant bacteria, posing a risk for colonization by biofilm-forming species that can result in chronic wounds. Biofilms decrease future antibiotic use efficiency and inflames the surrounding tissue, potentially resulting in necrosis of the tissues. Current studies show the possibility for probiotic application or reintroduction of commensal organisms erased by antibiotic use as a therapeutic mechanism for cutaneous wounds. Here, a two-step cutaneous wound treatment protocol is proposed involving antibiotic use and subsequent bacteriotherapy as a preventative measure for chronic wound formation via antibiotic-resistant bacteria.

Introduction

Managing chronic wounds is a challenge around the world to individuals with limited access to healthcare. A 2017 analysis of the economic burden placed upon patients who were managing chronic wounds in the United States alone showed that approximately 5.7 million people accumulate an annual cost of at least $20 billion (Järbrink 2017). In addition to the indefinite physical trauma of a chronic wound, economic and emotional trauma is experienced not only by patients, but also by their families. Individuals of lower socioeconomic status are more likely to experience medical conditions, such as type 2 diabetes or depression, that could exacerbate chronic wound development. Additionally, a patient's housing environment affects the healing rate of a chronic wound; for instance, a lack of central heating can significantly stunt wound repair (Fayne 2020). As these conditions disproportionately affect people in lower socioeconomic groups, who are often underinsured, the development of cost-effective treatment of chronic wounds is vital to the prevention of chronic wound-related emergencies, including amputation or even death (Järbrink 2017). Understanding wound physiology and the skin microbiome is foundational to curating bacterial-based therapeutics that could remedy chronic wounds.

Human bodies are covered with a vast and highly variable organ that is colloquially referred to as "skin." The expansive epithelial layer is embedded with different densities of sweat glands, oil glands, hair, and other mucus producers. Skin contains a wide range of viable ecosystems that can harbor different types of bacterial species. Sites with high sebaceous gland content contained a larger bacterial load than other regions of the skin, meaning that they contained a higher quantity of bacteria because of the moist, hospitable environment (Johnson 2018). Conversely, drier sites of the body often express more microbial diversity, or a wider variety of bacterial species (Grice 2009). Due to variations of both the body and the skin's environmental exposure, each human's skin surface contains unique microbial communities.

Depending on the host's external environment and the region of the body that is tested, up to two million bacteria can be isolated per square centimeter (Wong 2013). Although the same general bacterial species can be found in its preferred skin ecosystem, every volunteer in a 2009 study expressed different proportions of each species, suggesting that each individual contains a skin microbiota composition unique to them (Grice 2009). Dissimilarities between skin microbiota makeups can be attributed to the inherent differences of an individual host's diet, immunological capabilities, and degree of sebaceous secretion (Huffnagle 2013). Individual microbiota can be identified by a method known as 16S rRNA gene-based pyrosequencing, which identifies polymorphisms in the 16S gene that is present in all bacteria. This method maximizes bacterial diversity recognition, since

it identifies microorganism species missed through their lack of culture method compatibility (Price 2009; Huffnagle 2013). Distinguishing the presence of these unnoticed bacterial species on skin surfaces can contribute to understanding bacteria-wound interactions on the cutaneous surface.

Wounds can be defined as physical breaks in epithelial integrity together with the host's subsequent response to repair this break (Huffnagle 2013). Alterations in the cutaneous structure adjusts the physical and chemical parameters maintaining the composition of the skin microbiome; as a result, wounds reduce the production of mucus, change the construction of antimicrobial peptides, and also initiate an inflammatory response that ultimately causes recovery at the wound site (Huffnagle 2013). A typical uninfected wound response includes inflammation at the cutaneous break; this involves vasodilation and the recruitment of cells to facilitate healing. Inflammation addresses the elimination of foreign and potentially pathogenic microbes and their molecules via the migration of leukocytes, after which reparation of the wound begins via proliferation of cell types like fibroblasts (Jones 200). Although this mechanism is rapid for the sake of removing and excluding pathogenic bacteria from the exposed tissue, these processes can actually be inhibited by the presence of such pathogens. Subcutaneous tissue revealed via a wound in the cutaneous layer provides an uncolonized oasis for opportunistic pathogens that can disrupt the wound-healing process and stasis at the inflammatory stage; this is known as a chronic wound (Williams 2017).

The development of chronic wounds in patients is multifactorial and, ironically, can be due to the medical efforts to prevent infection in the first place. Understanding the microbiome of the skin can allow for specialized treatment that prevents chronic wound formation in patients, especially vulnerable populations such as low-income or elderly individuals (Price 2009). The skin, much like the gut, experiences high levels of exposure to the surrounding environment. Much of the research on the human microbiome has been centered on the populations present in the digestive tract. Fewer efforts have been made toward defining the cutaneous microbiome with 16S PCR only recently being used to better identify the microbes that were missed while using culture methods. There is little understanding of the overarching role of our skin microbiome, especially concerning wound healing promotion or inhibition (Johnson 2018). Wound management often relates to the prevention of infection. While the inherently negative impacts of our skin microbiome on wound repair have been assessed, significantly less work has been done on how our microbiota and non-commensal organisms could actually contribute to wound repair. Understanding wound microbiomes could allow us to manipulate their population proportions in order to best treat subcutaneous wounds.

In a medical scenario where multiple skin abrasions are being treated, a topical or oral antibiotic is often administered as a preventative measure against bacterial infection. However, use of antibiotics can favor the infection of antibiotic-resistant bacteria, leading to a

more severe and chronic infection of the cutaneous layer that could severely cost the patient financially, emotionally, and physically. This serious side effect of antibiotic use could be remedied using a topical application of various probiotics, such as *Lactobacillus plantarum*, that can out-compete pathogenic bacteria to promote wound healing.

Pharmaceutical Disruption of the Skin Microbiome Negatively Impacts Wound Closure

Typical Skin Microbiome Promotes Wound Healing

A 2011 study showed that keratinocytes in the skin are able to function normally in the presence of commensal, or advantageous, organisms inhabiting the microbiome (Wanke 2011). In fact, it was found that the skin microbiome is capable of amplifying the immune response against pathogenic bacteria. Commensal organisms promote the low-level expression of antimicrobial peptides, in order to prevent pathogenic bacterial growth on the skin. The study also revealed that commensal and pathogenic *Staphylococci spp.* are important in the development of antimicrobial peptides. Notably, the presence of pathogenic *S. aureus* on the skin causes a high level of antimicrobial peptide expression that is toxic to pathogenic but not commensal *S. epidermidis*, which have evolved protective mechanisms to those peptides. These findings are reinforced by similar studies that have found that *S. epidermis* itself produces molecules that promote antimicrobial peptide production by keratinocytes (Lai 2010; Wong 2013). Wanke et al. builds upon that research by showing that *S. epidermis* can simultaneously promote the efficient removal of pathogens while protecting itself.

Treatment-Induced Infection

Patients with wounds in the cutaneous layer are routinely administered a preventative dose of broad-spectrum antibiotics. If a chronic wound develops, antibiotic treatment is continued. In a study to examine the effects of treatment on the microbial community, researchers found that antibiotic use modifies bacterial communities instead of eliminating them (Price 2009). This case study found an increased Pseudomonadaceae colonization in wounds of patients that were recently treated with antibiotics, regardless of whether these antibiotics were expected to be effective against *Pseudomonas aeruginosa*. *P. aeruginosa* are known to grow planktonically, which means they do not have to bind themselves to a biofilm in order to live. However, these *P. aeruginosa* were eliminated after being introduced to antibiotics. Only biofilm-forming *P. aeruginosa* proved resistant to antibiotic treatment, providing an advantage in infecting a dermal surface. After antibiotic treatment in the hospital setting, bacterial diversity shifted dramatically to a *Pseudomonas*-dominated bacterial

composition. This selection for biofilm-producing bacteria suggests that immediate antibiotic use may contribute to formation of chronic wounds (Price 2009).

A recent study continued this work by testing the bacterial composition and wound healing of mice treated with vancomycin; ultimately, they found that there was a changed bacterial composition of the skin microbiota because of the antibiotic treatment (Zhang 2015). Decreased bacterial density and an adjustment in bacterial diversity was observed with both single-antibiotic treatment (vancomycin) and a combined antibiotic treatment (vancomycin, clindamycin, polymyxin). Measurement of the wound area five days after the wound was created showed that mice treated with antibiotics maintained a larger wound area than the control mice, suggesting that there is a correlation between antibiotic use and delayed wound healing (Zhang 2015).

Chronic Wound Development and Biofilm Formation

Antibiotic selection for biofilm-forming bacteria poses the possibility for the development of chronic wounds. *P. aeruginosa* and *Staphylococcus aureus (S. aureus)* are often responsible for the formation of such biofilms because of their resistance to antibiotics. When antibiotics wipe out a wound's microbial diversity and density, these pathogens then have more space to colonize. The existence of a biofilm creates an impenetrable defense for the infecting bacteria against immune cells, generating a state of chronic inflammation that in turn further damages the wounded tissue (Price 2009; Watters 2015). One study found that *P. aeruginosa* produces a lipid that traps bactericidal leukocytes and repurposes their lysed components like DNA and actin to further reinforce the biofilm (van Gennip 2012). The self-propagative nature of such infections contributes to the persistence of chronic wounds and their inability to heal, as the host's immune system cannot break down the biofilm of infecting bacteria (Price 2009). The presence of biofilms also decreases the ability of antibiotics to treat the infection, ultimately contributing to overuse of those antibiotics and subsequent development of antibiotic-resistant microbes, such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Venosi 2019).

The pathogenesis of MRSA and its inhibition of human dermal fibroblasts from reconstructing the epidermis after a cutaneous wound was recently examined (Kirker 2012). This research revealed that dermal fibroblasts are responsible for excreting growth factors and creating extracellular matrix molecules, and are vital for repairing a break in the skin, which ultimately contributes to the natural reconstruction of the cutaneous layer (Kirker 2012). It was found that the introduction of biofilm-forming species such as MRSA prevent dermal fibroblast migration to the wound site and promote a programmed cell death of the fibroblasts called apoptosis. The presence of MRSA within a biofilm downregulates the production of cytokines, growth factors, and extracellular matrix-adjusting molecules such as protease, preventing

wound healing at multiple stages (Kirker 2012). Using these findings as a foundation, a 2015 study increased the number of biofilm-forming species that were studied to include *P. aeruginosa* (Marano 2015). More potent, denser biofilms that were typical of later-stage chronic wounds (caused by both *P. aeruginosa* and MRSA) proved highly toxic to human keratinocytes, which are involved in similar mechanisms to wound healing as dermal fibroblasts. As a result, proliferation and migration of these tissue-regenerative cells were inhibited. Early biofilms from both species affected the proliferative nature of keratinocytes through pathogen-secreted anti-host cell compounds, but minimal effects were seen on migration (Marano 2015).

Restoration of the Skin Microbiome through Medical Applications of Bacteria

Lactobacillus spp.

A variety of bacterial applications for repairing wounds are being explored as a means of avoiding the aforementioned issues with antibiotic use and the development of chronic wounds. A prominent study in this field discovered that topical administration of *Lactobacillus plantarum in vitro* and *in vivo* fully prevented the infection of *Pseudomonas aeruginosa*, which is a primary gram-negative perpetrator of biofilms that induces chronic wounds (Valdéz 2005). *Lb. plantarum* prevents the production of *P. aeruginosa* via the production of secondary metabolites that inhibit quorum-sensing molecules, which are important for later stage biofilm formation (Valdéz 2005). While *Lb. plantarum*'s production of lactic acid via metabolism inhibited *P. aeruginosa* growth, more inhibitory activity was seen as a result of *Lb. plantarum*'s occupation of physical space with no significant detrimental effect to the host. Similarly, treatment of a *P. aeruginosa* colonization induced tissue phagocytes to phagocytose, or to engulf, *P. aeruginosa* and the *Lb. plantarum* used in the treatment. Consequentially, this decreased pathogenic bacterial counts and promoted tissue repair (Valdéz 2005). Bacterial therapies decreased premature tissue cell death (necrosis) and concentrations of inflammatory molecules in wound sites. Although this research did not see a decrease in overall healing time of the wound, the topical application of the bacterial therapy enabled the body to prevent biofilm formation at wound sites (Valdéz 2005).

A 2014 study expanded upon this procedure, aiming to determine the effects of a wider variety of *Lactobacillus* species on *S.* aureus biofilm formation. An analysis of the topical application of live Lb. rhamnosus found that regardless of the time administered in relation to the point of wound infliction, keratinocytes that were present at the wound site were protected from *S. aureus*-induced apoptosis (Mohammedsaeed 2014). It was determined that the likely cause of this protection is the prevention of *S. aureus* growth and adhesion, as seen by the

reduction in *S. aureus* bacterial count. A similar study confirmed *Lb. plantarum*'s ability to physically prevent *P. aeruginosa* in burn wounds by displacing *P. aeruginosa* from the tissue and physically occupying the space instead (Argenta 2016). This study also showed that topical application of *Lb. plantarum* prevented pathogenic spread from the wound to distant organ systems, thus preventing sepsis (Argenta 2016).

An earlier study showed that *Lb. plantarum* topical application shows greater efficacy in the treatment of infected third-degree burns than the conventional microbicidal agent, silver sulphadiazine, which can produce adverse reactions and side effects in cutaneous burn wounds, calling for alternative treatments to be pursued (Peral 2009). These infections include *P. aeruginosa*, similar to the Valdéz study, but also encompass *S. aureus* and Streptococcus pyogenes. Overall, *Lb. plantarum* decreases the bacterial load and allows for the cutaneous wound to repair itself (Lukic 2017). The mechanism behind this pathogenic bacteria removal was proposed to include *Lb. plantarum* stimulation of immune system components previously inhibited by *P. aeruginosa* (Hessle 2000). Although not explicitly stated in Peral et al.'s 2009 examination of *Lb. plantarum* inhibition of biofilm formation, the aforementioned cytokine release and response could ultimately be responsible for the breakdown of the biofilm and thus phagocytosis of pathogenic bacteria (Mohammedsaeed 2014).

The most recent notable application of these findings expanded upon the prior studies by testing topical application and injection of probiotics on or near wound sites (Fijan 2019). The study used a variety of cutaneous wound types, including both burn and cut wounds, and found that probiotics maintain an antagonistic effect against wound pathogens, primarily *S. aureus* and *P. aeruginosa*. The probiotics used included *Lb. plantarum, Lb. fermentum,* and *Cutibacterium acnes*, all of which showed wound healing effects or inhibition of pathogenic progress (Fijan 2019).

Staphylococcus Epidermis and Friends

Infection of cutaneous wounds by *Staphylococcus aureus* has also been shown as treatable via the application of other bacterial species. *Staphylococcus epidermis*, a skin commensal organism, was shown in Sugimoto et al. to produce Esp, a protease that prevents and deconstructs biofilms created by *S. aureus* (Sugimoto 2013). This is also an effective method against *S. aureus* strains that are methicillin- and vancomycin-resistant as Esp degrades a variety of biofilm-associated surface proteins on *S. aureus* (Hessle 2000). By degrading the biofilm extracellular matrix and destruction of proteins responsible for *S. aureus* attachment and infection, host immune cells can attack pathogenic bacteria and prevent or eliminate wound infection (Peral 2009). Similarly, another study showed that *Staphylococcus spp.* can prevent prolonged, damaging inflammation in the cutaneous layer; *S. epidermis* secretes triggers for keratinocytes to produce antimicrobial peptides (Wong 2013).

A recent study assessed a clinical case of a patient with an infected chronic ischemic wound—a wound caused by stunted blood supply to the tissue—that sustained a polymicrobial infection with pathogenic bacteria *Klebsiella pneumoniae, Enterococcus faecalis* and *Proteus mirabilis*, which worsened after continued use of antibiotics (Venosi 2019). The prolonged infection began to show signs of wound recovery after a topical probiotic application of *Lactobacillus plantarum, Lactobacillus acidophilus,* and *Streptococcus thermophilus*. Those metabolites which are often associated with promotion of bacterial infection were altered, and this result could be associated with wound recovery. For example, polyamine putrescine was reduced after the application of probiotic treatment. Polyamine putrescine is involved with bacterial growth, biofilm formation, and protection from stress enacted by the immune system (Venosi 2019).

Synthesis and Conclusions

Infection of cutaneous wound trauma is a common and justified fear within the medical profession because of the possibility for sepsis or chronic wounds. However, in spite of the negative association between bacteria and physical trauma, there are aspects of our microbiome that contribute to the body's ability to repair wounds. Disruption of microbiome inhibits specific immune responses mediated by the microbiome. Similarly, broad-spectrum antibiotics that are selected for antibiotic-resistant bacteria are commonly responsible for the formation of biofilms. As shown by the aforementioned studies, commensal organisms can be involved in inducing antimicrobial peptides that eliminate the presence of pathogenic bacteria, but are also not toxic to the commensals due to evolved protection mechanisms (Lai 2010; Wanke 2011). Given that commensal organisms then modulate the microbiome and prevent regular infection of pathogenic bacteria, researchers should question the impact of immediate use of broad-spectrum antibiotics on the skin microbiome.

Broad-spectrum antibiotic use can select for antibiotic-resistant species responsible for biofilm production, such as *P. aeruginosa* and *S. aureus*, that ultimately colonize the wound and can induce the formation of chronic wounds (Price 2009). The removal of competing organisms allows the pathogenic bacteria to flourish and colonize the wound without restriction by resource limitation. Although the rationale for preventative antibiotic use is evident, the broad-spectrum nature of such use eliminates repair-promoting organisms that would not colonize regions of the wound successfully. To further evidence this point: it was found that antibiotic use could be associated with delayed wound healing; pure antibiotic use removes healing organisms and instead opens the gates for opportunistic pathogens to infect, colonize, and form biofilms over fresh cutaneous wounds (Zhang 2015). The infection enabled by antibiotic use and consequent biofilm formation limits the ability of the wound to repair itself, and the

presence of such biofilm-forming pathogens decrease migration of dermal fibroblasts to the wound site (Kirker 2012). This also decreases the production of molecules and compounds involved with dermal repair (van Gennip 2012; Marano 2015). This begs the question: to what degree is the removal of commensal organisms in addition to pathogenic bacteria beneficial for the repair of burn or cut wounds on the skin? Increased risk of chronic wound formation and reduced wound healing due to the use of broad-spectrum antibiotic suggests that antibiotic use needs to be supplemented with another therapeutic that inhibits biofilm growth.

The use of probiotics and reapplication of commensal bacteria lost through antibiotic exposure has been shown to promote wound repair. Probiotic use (*Lb. plantarum*) was shown to fully prevent infection of *P. aeruginosa* by inhibiting the species' biofilm production, leaving them exposed to immune cells (Valdéz 2005). In addition to acting as a preventative bacteriotherapeutic, *Lb. plantarum* can be applied in late-stage biofilm formation through the production of molecules that prevent bacterial communication in biofilms. That late-stage application also prohibits the spread of harmful bacteria to adjacent sites and distant organs (Peral 2009). Probiotics can be applied as an effective therapy against *P. aeruginosa* at any stage in chronic wound formation, resulting in pathogenic cell death and lower concentrations of inflammatory molecules (Valdéz 2005). Similar effects can be seen with *Lb. rhamnosus* in their prevention of *S. aureus*-induced keratinocyte cell death while also reducing pathogenic bacterial cell count (Mohammedsaeed 2014). More recent studies have shown that even more bacterial species can be used to promote wound healing (for example, *Lb. fermentum* and *Propionibacterium acnes*), which can open the possibility of more diversity in bacteriotherapy in the coming years (Fijan 2019).

Lb. plantarum (and potentially other species) out-competes pathogenic bacteria and creates a wound environment that is uninhabitable for organisms responsible for biofilm formation. As such, *Lb. plantarum* can be used as a means for inhabiting a wound space as a protection against harmful bacteria without inducing a chronic wound. The probiotics can eliminate pathogenic bacteria responsible for maintaining a chronic wound before the probiotics themselves are removed with treatments or the body's natural immune response. Probiotics can also be used as a shield against biofilm formation. Peral et al. also showed that infected third-degree burns treated with *Lb. plantarum* show a greater healing rate than classical treatments, posing the possibility that an additive step in conventional cutaneous trauma treatment will ultimately promote healing and further prevent chronic wound formation (Peral 2009). Potentially, a wide variety of *Lactobacillus* strains can be utilized for therapeutic purposes, as the aforementioned strain also had a positive effect on wound healing regardless of the stage of biofilm formation when it was applied.

In addition to probiotic application, there is a possibility for restoration of the patient's commensal organisms responsible for maintaining a biofilm-free wound site. *S. epidermis* was

found to produce a protease that reduces and prevents biofilms caused by *S. aureus* (Sugimoto 2013). This aligns with prior findings that *S. epidermis* is responsible for a specialized immune response, and that the elimination of the species from a wound site could delay wound healing because of this alteration in strain-strain interaction. The reduction of *S. epidermis* load at a wound site decreased the ability of the skin microbiota to prevent biofilm-forming and antibiotic-resistant *S. aureus* from colonizing the site and inducing necrosis of the surrounding tissues (Hessle 2000; Zhang, 2015).

While there are numerous benefits to utilizing probiotics in wound care and management, there are notable risks, as well. A common understanding of bacteria includes the broad generalization that all bacterial species, except those supporting our digestion, pose a risk to human health. Many studies point out the human concern that topical application of any probiotic to a cutaneous wound increases risk of septicemia, or bacterial infection of the blood (Mohammedsaeed 2014; Watters 2015). And, indeed, there is a possibility that *Lactobacillus* septicemia is more likely in immunocompromised individuals; a retroactive study conducted found that *Lb. rhamnosus* bacteremia was found in 66% of immunosuppressed patients, and 82.5% of catheterized patients, which increases the risk for consequential septicemia (Gouriet 2012). If practitioners increase probiotic use in these cases, there is also a parallel increased risk for cases of septicemia.

Although there is a reasonable concern for septicemia in individuals particularly vulnerable to infection, it can be considered that the risk of septicemia is lower than the risk posed by chronic wounds, where the degree of inflammation is so damaging that it prevents any ability to fight infection by physically retaining immune cells and also by promoting further tissue destruction (Valdéz 2005). A topical application of *Lb. plantarum* can then inhibit the exacerbated immune system to biofilm-forming bacteria in chronic wounds. Current wound management techniques are based on reducing bacterial load and preventing infection, but harsh antimicrobial protocols can negatively impact the bacterial species composition of the wound environment to ironically favor infection (Price 2009). Adjusting our approach to wound healing in order to permit a two-step method of antibiotic and subsequent probiotic application (as shown in Figure 1) can prevent the formation of chronic wounds. Three stages of wound healing are indicated. Figure 1A shows a fresh cutaneous wound has antibiotics topically applied, and Figure 1B shows a subsequent response where antibiotic-resistant bacteria begin early stages of biofilm formation. It is at this point that a probiotic bacteriotherapy is applied, the effect of which is shown in Figure 1C, where the biofilm is deconstructed by the probiotic and regenerative cells are able to begin re-epithelialization.

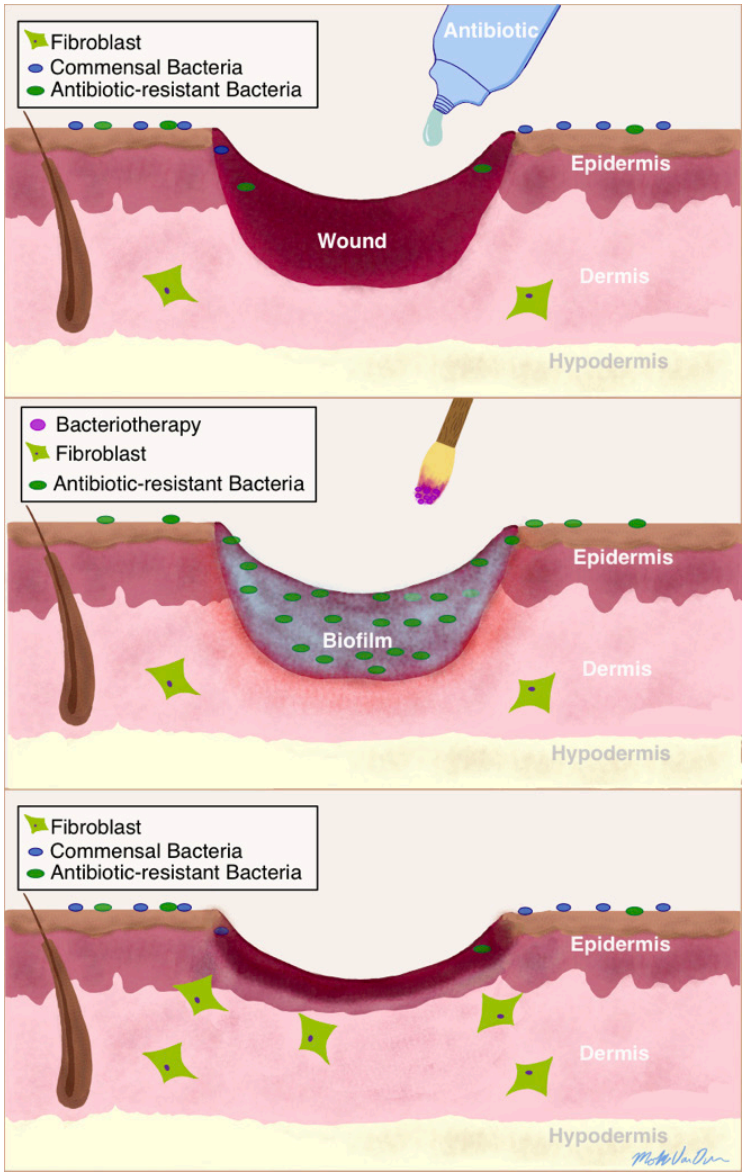

Figure 1 Proposed standard protocol for cutaneous wound management in the prevention of chronic wound formation.

Manipulation of the wound ecosystem with probiotics acts as a cost-effective alternative treatment that subverts the cost of continued antibiotic use and the healthcare that comes with chronic wound management (Johnson 2018). This would reduce the annual billions of dollars spent on chronic wounds as well as decelerate the development of antibiotic-resistance due to futile attempts at diminishing chronic wounds via chronic antibiotic administration. Easily culturable bacteria as a therapy can promote infection management for patients with income limitations, paving the way for access to more diverse healthcare methods.

As has been stated, chronic wounds disproportionately affect people in lower

socioeconomic conditions. For example, individuals in lower socioeconomic groups are more likely to develop a chronic wound, and individuals who are unemployed were found to be 2.44 times more likely to develop a chronic wound than their employed counterparts (Yao 2020). Taking together the duration of chronic wounds, the increased chronic wound development rate, and the decreased recovery rate from chronic wounds experienced by lower- or no-income individuals, a statistic emerges that unemployed patients are 12.34 times more likely to be afflicted with a chronic wound than unemployed individuals (Yao 2020). Low-income individuals are also more likely to have decreased or stunted healing due to conditions that are directly related to their income and resources and thus out of their control. The increased stress of economic burden leads to an increase in stress hormones, impairing wound repair (Fayne 2020). Nutritional deficiencies, housing security and quality, marital status, and a wide range of impacts from economic hardship also extend the lifespan of a chronic wound (Fayne 2020). In summary, those in lower socioeconomic groups carry the burden of chronic wounds more than people with access to economic success.

Chronic wounds make up a large percentage of annual healthcare expenses around the world. In addition to the above 2017 US analysis stating that an annual cost of $20 billion in healthcare expenses is accrued nationally because of chronic wounds, a study of Northern China from the same year showed that annual hospital costs for a single patient treating chronic wounds amounted to around US $1,271,000 (Yao 2020). Chronic wound patients in Northern China contribute to 3.18% of all of healthcare expenses in 2017 (Yao 2020). On a global scale, lower-income individuals disproportionately carry the burden of chronic wound affliction, and as a result, they also make up the bulk of these medical expenditures. In the study that evaluated Northern China's chronic wound healthcare expenses, it was found that more than 10% of chronic wound patients, including those experiencing diabetes, infection, pressure ulcers, or even surgery, had to pay for their own medical bills (Yao 2020). The development of more cost-effective and accessible treatments will relieve the burden of these medical expenses, allowing those who are affected at a higher rate to receive the care they need without fear of further economic burden. By standardizing a method of care that will decrease further psychosocial strain, which would further impact wound repair, the cost of wound management could be reduced up to 30% (Järbrink 2017). Probiotic bacteriotherapy holds promise in providing reduced-cost treatment. Accepting the use of beneficial bacteria in the treatment of chronic wound patients may not only relieve the physical, psychological, and familial detriments caused by chronic wounds, but could also mark a broader transition to treatments that go beyond expense.

Preventative measures against chronic wound formation would ensure that the wound environment remains inhospitable to the pathogens remaining after antibiotic exposure, reducing not just inflammation but the negative psychological and physical effects associated

with a chronic wound. Adjusting our approach to wound healing will eventually benefit patients, utilizing an existing organism to decrease wound pH, reduce inflammation at the wound site, and inhibit further infection by pathogenic bacteria (Johnson 2018). While further research should investigate the susceptibility of immunocompromised individuals to septicemia due to topical application of live probiotics as opposed to their antimicrobial byproducts, the current data proposes positive effects of bacterial application as a disruptive and preventative ward against chronic wounds. Ultimately, disrupting our current paradigm regarding bacteria can pave the way for a new wave of bacteriotherapy in healthcare.

References

Argenta A, Satish L, Gallo P, Liu F, Kathju S. 2016. Local application of probiotic bacteria prophylaxes against sepsis and death resulting from burn wound infection. *PloS One*, 11(10), e0165294. doi:10.1371/journal.pone.0165294.

Fayne RA et al. 2020. The potential impact of social genomics on wound healing. Advances in wound care. 9(6): 325-331. doi:10.1089/wound.2019.1095.

Fijan S, Frauwallner A, Langerholc T, Krebs B, Ter Haar Née Younes J A, Heschl A… Rogelj I. 2019. Efficacy of using probiotics with antagonistic activity against pathogens of wound infections: an integrative review of literature. BioMed Res Intl. 7585486. doi:10.1155/2019/7585486.

Gouriet F, Million M, Henri M, Fournier P, Raoult D. 2012. *Lactobacillus rhamnosus* bacteremia: an emerging clinical entity. Eur J Clin Microbiol Infect Dis 31: 2469–2480. https://doi.org/10.1007/s10096-012-1599-5.

Grice E A, Kong HH, Conlan S, Deming CB, Davis J, Young AC…Segre JA. 2009. Topographical and temporal diversity of the human skin microbiome. Science, 324(5931): 1190–1192. doi:10.1126/science.1171700.

Hessle C, Andersson, B, Wold AE. 2000. Gram-positive bacteria are potent inducers of monocytic interleukin-12 (IL-12) while gram-negative bacteria preferentially stimulate IL-10 production. Infection and Immunity, 68(6): 3581–3586. doi:10.1128/iai.68.6.3581-3586.2000.

Huffnagle, Gary B., Scales, Brittan S, & Huffnagle, Gary B. 2013. The microbiome in wound repair and tissue fibrosis. The Journal of Pathology. 229(2): 323-331.

Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, Car J. 2017. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. Systematic Reviews, 6(1): 15. doi:10.1186/s13643-016-0400-8.

Johnson TR, Gómez BI, McIntyre MK, Dubick MA, Christy RJ, Nicholson SE, Burmeister DM. 2018. The cutaneous microbiome and wounds: new molecular targets to promote wound healing. Int Jo Mol Sci, 19(9): 2699. doi:10.3390/ijms19092699.

Jones SG, Edwards R, Thomas DW. Inflammation and wound healing: the role of bacteria in the immuno-regulation of wound healing. 2004. Int Jo Lower Extrem Wounds. 3(4): 201–208. https://doi.org/10.1177/1534734604271810.

Kirker KR, James GA, Fleckman P, Olerud JE, Stewart PS. 2012, Differential effects of planktonic and biofilm MRSA on human fibroblasts. Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society. 20(2): 253–261. doi:10.1111/j.1524-475X.2012.00769.

Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichtle A…Gallo RL. 2010. Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections. Jo investigative Derm. 130(9): 2211–2221. doi:10.1038/jid.2010.123.

Lukic J, Chen V, Strahinic I, Begovic J, Lev-Tov H, Davis SC…Pastar I. 2017. 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.

Marano JR, Wallace HJ, Wijeratne D, Fear MW, San Wong H, O'Handley R. 2015. Secreted biofilm factors adversely affect cellular wound healing responses in vitro. Scientific Reports 5: 13296. doi:10.1038/srep13296.

Mohammedsaeed W, McBain AJ, Cruickshank SM, O'Neill CA. 2014. *Lactobacillus rhamnosus GG* inhibits the toxic effects of *Staphylococcus aureus* on epidermal keratinocytes. Appl Envir Microb, 80(18): 5773–5781. doi:10.1128/AEM.00861-14.

Pastar, I et al. Epithelialization in wound healing: a comprehensive review. 2014. Advances in Wound Care 3(7): 445-464. doi:10.1089/wound.2013.0473.

Peral MC, Huaman Martinez MA, Valdez JC. 2009 Bacteriotherapy with *Lactobacillus plantarum* in burns. International Wound Journal 6: 73-81. 2009. doi:10.1111/j.1742-481X.2008.00577.x.

Price LB, Liu CM, Melendez JH, Frankel YM, Engelthaler D, Aziz M…Zenilman JM. 2009. Community analysis of chronic wound bacteria using 16S rRNA gene-based pyrosequencing: impact of diabetes and antibiotics on chronic wound microbiota. PloS One. 4(7): e6462. doi:10.1371/journal.pone.0006462.

Sugimoto S, Iwamoto T, Takada K, Okuda K, Tajima A, Iwase T, Mizunoe Y. 2013. *Staphylococcus epidermidis Esp* degrades specific proteins associated with *Staphylococcus aureus* biofilm formation and host-pathogen interaction. Jo Bacter, 195(8): 1645–1655. doi:10.1128/JB.01672-12.

Valdéz JC, Peral MC, 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. Clin Microbiol Infect. 11(6): 472-9. doi: 10.1111/j.1469-0691.2005.01142.x. PMID: 15882197.

van Gennip M, Christensen LD, Alhede M, Qvortrup K, Jensen PØ, Høiby N… Bjarnsholt T. 2012. Interactions between polymorphonuclear leukocytes and *Pseudomonas aeruginosa* biofilms on silicone implants in vivo. Infection and Immunity, 80(8): 2601–2607. 2012. doi:10.1128/IAI.06215-11.

Venosi S, Ceccarelli G, de Angelis M, Laghi L, Bianchi L, Martinelli O, Maruca D, Cavallari E, Toscanella F, Vassalini P, Trinchieri V, Oliva A, D'Ettorre G. 2019. Infected chronic ischemic wound topically treated with a multi-strain probiotic formulation: a novel tailored treatment strategy. Jo Translational Med. 17: 10.1186/s12967-019-2111-0.

Wanke I, Steffen H, Christ C, Krismer B, Götz F, Peschel A, Schaller M, Schittek B. 2011. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. Jo Investig Derm. 131(2): 382-390.

Watters C, Yuan T, Rumbaugh K. 2015. Beneficial and deleterious bacterial–host interactions in chronic wound pathophysiology. Chron Wound Care Mgt Res, 2: 53–62.

Williams H, et al. 2017. Cutaneous Nod2 expression regulates the skin microbiome and wound healing in a murine model. Jo Investigative Derm. 137(11): 2427–2436. doi:10.1016/j.jid.2017.05.029.

Wong VW, Martindale RG, Longaker MT, Gurtner GC. 2013. From germ theory to germ therapy: skin microbiota, chronic wounds, and probiotics. Plastic and Reconstructive Surgery. 132(5): 854e-861e. Special Topics.

Yao Z, Niu J, Cheng B. 2020. Prevalence of chronic skin wounds and their risk factors in an inpatient hospital setting in Northern China. Advances in Skin & Wound Care. 33(9): 1-10. doi: 10.1097/01.ASW.0000694164.34068.82.

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. Retrieved January 13, 2020, from www.jstor.org/stable/24542462