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Probiotics or Pro-healers the Role of Beneficial Bacteria in Tissue Repair

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Abstract

Probiotics are beneficial microorganisms, known to exert numerous positive effects on human health, primarily in the battle against pathogens. Probiotics have been associated with improved healing of intestinal ulcers, and healing of infected cutaneous wounds. This manuscript reviews the latest findings on probiotics related to their pro-healing properties on gut epithelium and skin. Proven mechanisms by which probiotic bacteria exert their beneficial effects include direct killing of pathogens, competitive displacement of pathogenic bacteria, reinforcement of epithelial barrier, induction of fibroblasts and epithelial cells' migration and function. Beneficial immunomodulatory effects of probiotics relate to modulation and activation of intraepithelial lymphocytes, natural killer cells and macrophages through induced production of cytokines. Systemic effects of beneficial bacteria and link between gut microbiota, immune system, and cutaneous health through gut-brain-skin axes are discussed as well. In light of growing antibiotic resistance of pathogens, antibiotic use is becoming less effective in treating cutaneous and systemic infections. This review points to a new perspective and therapeutic potential of beneficial probiotic species as a safe alternative approach for treatment of patients affected by wound healing disorders and cutaneous infections.

Keywords

probiotics; wound healing; chronic wounds; infection; skin; keratinocytes; fibroblasts; immune response

Introduction

At birth, diverse commensal microorganisms begin populating the human body and persist throughout ones lifetime. Commensal bacteria present in symbiotic communities are adapted

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to survive without compromising host integrity (1) in contrast to pathogenic microorganisms which can breach host barriers and contribute to disease pathogenesis. Research on the co-evolution and functional integration of microbiota and the human body has revealed that the microbiome has a major impact on multiple physiological functions including protection against infections and modulation of the immune system.

The importance of commensal microorganisms in maintaining host health was recognized early in gut microbiota research. It was demonstrated that germ-free animals are more sensitive to pathogen colonization (2), have impaired mucosal wound healing (3), and are more prone to chemical intoxication (4). Due to important local and systemic effects of gut microbiota, approaches that manipulate its composition to improve host metabolic, immunological, and physiological functions, have become of growing importance. This led to the identification of beneficial bacterial species that belong to symbiotic microbiota and improve host welfare. Lactic Acid Bacteria (LAB) and bifidobacteria are some of the most studied species that possess health benefits and are most commonly referred to as probiotics. Probiotics are defined by the World Health Organization as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (5). Probiotics have been reported to be beneficial in the treatment or prevention of cutaneous inflammatory conditions (6), prevention and management of diabetes (7), respiratory tract infections (8), various gastro-intestinal (GI) disorders (9,10,11), urogenital infections (12) and ulcerative colitis (13) to name a few. Recent studies also focused on the application of non-viable probiotic metabolites, termed postbiotics, as a safer alternative to probiotics (14,15,16). Postbiotics gained special importance in the treatment of inflammatory disorders, where the application of live bacteria bears the risks associated with excessive activation of the immune system.

Probiotics exert their positive effects through multiple mechanisms including: (a) competition with pathogenic bacteria for nutrients and binding sites on the host cell; (b) inactivation of toxins and metabolites, (c) production of antimicrobial substances that inhibit the growth of pathogenic microorganisms, and (d) stimulation/modulation of host immune response. Probiotic–host interactions involving epithelial cells (EC), dendritic cells (DC), and regulatory T lymphocytes have also been well studied particularly in the GI tract.

This manuscript reviews current knowledge on probiotics related to their pro-healing properties on both GI epithelium and skin. It also addresses their anti-microbial potential as well as known cellular and molecular mechanisms of action indicating novel approaches for the treatment of wound healing disorders.

The role of probiotics in infections and intestinal wound healing

Close association with epithelial cells makes LAB and bifidobacteria ideal probiotic candidates in antimicrobial treatment of pathogens (17). LAB and bifidobacteria utilize their association with gut ECs to directly inhibit pathogens' growth and ability to induce host mucosal defense systems and tissue repair mechanisms. These properties are of utmost importance in fighting overt and opportunistic pathogens. Below we outline the antimicrobial properties of probiotic strains and their mechanism of action.

Direct inhibition of pathogens' growth by probiotics

Several mechanisms of direct probiotic action against pathogens were proposed, including production of antimicrobials, displacement of pathogens from epithelial cells and mucus, as well as elimination of pathogens by co-aggregation and quorum quenching (Fig 1).

Antimicrobials produced by probiotic strains include organic acids, hydrogen peroxide, diacetyl, reuterin, and bacteriocins. The production of organic acids by multiple probiotic strains, belonging both to LAB and bifidobacteria is mainly responsible for antimicrobial activity against Gram negative pathogens (Fig 1A) (18). Hydrogen peroxide producing lactobacilli, including *Lactobacillus fermentum*, *Lb. acidophilus* and *Lb. jensenii* were correlated with reduced count of fastidious anaerobic Gram positive bacteria including *Bacteroides*, *Prevotella*, *Gardnerella* and *Mycoplasma* spp. (19,20). Reuterin (3-hydroxypropionaldehyde) is a well-known antimicrobial metabolite produced by *Lb. reuteri*, and thought to exert its effect by oxidizing thiol groups in the target pathogenic microorganism (21). Importantly, reuterin can specifically inhibit the growth of harmful gut bacteria, without killing beneficial microorganisms, allowing *Lb. reuteri* to remove gut invaders while keeping normal gut microbiota intact. Reuterin also shows antimicrobial activity against the common chronic wound pathogen *Staphylococcus* (22). Diacetyl, another metabolic product of lactobacilli also exhibits broad spectrum antimicrobial potential against both Gram negative and Gram positive pathogens (23,24).

Another class of probiotic's metabolites, bacteriocins, represent small peptides produced by microorganisms that show wide range of antimicrobial activity both *in vitro* and *in vivo* (25,26,27). Bacteriocin producing microorganism, *Streptococcus salivarius*, commensal of oral epithelium, is a very potent inhibitor of *St. pyogenes*, a pathogen that causes pharyngitis and also cutaneous infections (28,29,20). Prophylactic oral administration of *St. salivarius* has shown positive effects in prevention of recurrent *St. pyogenes* infections in both adults and children (31,32).

The ability of multiple probiotic species, including *Bifidobacterium longum*, *Lb. rhamnosus* and *Lb. delbrueckii* to auto-aggregate, is thought to confer their antimicrobial ability to co-aggregate with other microorganisms (Fig 1B), including the common wound pathogens *Staphylococcus aureus* and *Candida albicans* (33,34). Although numerous *in vitro* studies have shown the potential of aggregating probiotics to displace pathogens from epithelial cells (33,35), *in vivo* studies are warranted.

In addition to production of antimicrobial substances and co-aggregation, probiotic strains can displace intestinal pathogens from gut epithelium (Fig 1C). Displacement could be attributed to specific surface molecules produced by lactobacilli, e.g. extracellular polysaccharides, which allow *Lb. paracasei* to competitively adhere to EC cells and displace pathogenic bacteria (36).

Another emerging antimicrobial mechanism of LAB is the inhibition of pathogen's quorum sensing (QS) system. Quorum sensing refers to an intercellular communication system that microorganisms utilize to alter gene expression based on cell-population density, in order to form biofilm and confer virulence (37,38). Most pathogens, including species commonly

found in chronic wounds (e.g. *Pseudomonas aeruginosa* and *S. aureus*), utilize QS for virulence, biofilm formation and resistance to host defense (38,39,40). Nevertheless, probiotics can interfere with pathogen's QS. Specifically, *Lb. plantarum* was shown to inhibit the production of QS signaling molecules (acyl-homoserine-lactone) by *P. aeruginosa*, along with reduction of biofilm formation (41). Importantly, inhibitory effects of *Lb. plantarum* on *P. aeruginosa* growth in murine burn wound model has also been documented (41).

Probiotic effects on epithelial barrier

In addition to direct antimicrobial effects on pathogens, probiotics can enhance epithelial barrier function, therefore restricting pathogen invasion (Fig 2) (42). Probiotics have a well-described role in strengthening GI barrier by increasing expression and regulating localization of tight junction (TJ) proteins both *in vitro* (43) and *in vivo* (Fig 2A) (44). For example, oral administration of *Lb. reuteri* in newborn piglets resulted in increased occludin, claudin and zonula occludens 1 (ZO-1) expression in gut (45). Similarly, occludins and ZO-1 were recruited to the region of TJ after oral administration of *Lb. plantarum*, as demonstrated in a clinical study (44). Additionally, murine models of chronic ileitis demonstrated that a probiotic mixture of eight different bacterial strains aids suppression of chronic inflammation by fortification of epithelial barrier function (46). In addition to lactobacilli, probiotic bifidobacteria have shown similar effects. *B. infantis* increased transepithelial resistance and expression of tight-junction proteins ZO-1 and occludin in human gut epithelia. Increased transepithelial resistance was also associated with enhanced cell signaling events important for barrier formation, phosphorylation of extracellular signal regulated kinases (ERK) and p38 (47).

The effects of probiotics on wound healing in the GI tract have been extensively investigated through various experimental models, including acetic acid induced ulcers, full thickness wounds, and intestinal anastomoses. Beneficial effects of lactobacilli in these studies were mediated primarily by the activation of epithelial cells, and stimulation of fibroblast proliferation and/or migration (48,49). Aside from enhancing the repair of epithelium, presence of *Lb. plantarum* was demonstrated to correlate with increased collagen synthesis in intestine (Fig 2B) (49), and similar effects on skin were demonstrated upon oral administration of *Lb. acidophilus* in hairless mouse model of UVB induced skin photo-aging (50). Given that the functions of epithelial cells and fibroblasts are tightly regulated by chemokines, cytokines and growth factors (51), fortification of the epidermal barrier by probiotics is closely intertwined with their effects on immune components.

Probiotics can affect innate immune components of the intestinal barrier by induction of β -defensin (52), which aside from its role in fighting intestinal pathogens, is known to promote wound healing (53). *In vivo* murine studies have shown that commercially available probiotic mixture induces the expression of transforming growth factor β (TGF β) and vascular endothelial growth factor (VEGF) (54), while probiotic formulation containing *Saccharomyces boulardii* was shown to induce epidermal growth factor (EGF) and it's receptor activity (EGFR) as well as insulin-like growth factor (IGF) (55,56). Moreover, *Lb. rhamnosus* was reported to stimulate hypoxia inducible factor 2 α (HIF-2 α) *in vivo*, a master regulator of progenitor stem cell recruitment during tissue repair (57,58).

Immunomodulatory properties of probiotics

It is well documented that probiotics promote systemic and gut health through immunomodulation. Main immune modulating functions of probiotics relate to the activation of natural killer (NK) cells, dendritic cells (DC), intraepithelial $\gamma\delta$ T lymphocytes and macrophages, which are effector cells of innate immunity important for both skin and GI barrier restoration (46,59).

However, immunomodulatory effects of probiotics are species and even strain-specific, as we outline below. It was shown that *Lb. sakei* and *Lb. rhamnosus* stimulate macrophage activity (60,61), while *Lb. plantarum* increased phagocytic activity of peritoneal macrophages *in vivo* (62). These functions were mainly associated with stimulation of IL-22, TNF α , IL-6, IL-8, and IL-12 secretion (60,63). Strains of *Lb. reuteri* and *Lb. johnsonii* can also induce secretion of the cytokine IL-22, which is mainly produced by intraepithelial T lymphocytes (64,65). Metabolites of these probiotic strains have the ability to bind to and activate the aryl hydrocarbon receptor (AhR) expressed on macrophages and DC, resulting in subsequent activation of epithelial cells (37,64,66).

Another cytokine known to be induced by systemic administration of probiotics is TNF α , the most important cytokine in the innate immune response produced mainly by monocytes and macrophages, but also by epithelial cells (67). Castillo et al. showed induction of TNF α production, along with IFN γ and IL-10 in healthy mice fed with *Lb. casei* (68). *Lb. fermentum* treatment also resulted in increased expression of TNF α associated with increased neutrophil infiltration (69) which can contribute to resolution of infection.

Secretion of IL-8, a crucial chemokine for the recruitment of neutrophils, also correlated with lactobacilli treatment (70). Aside from influencing innate immune cell cytokines, lactobacilli were shown to induce secretion of IL-10 and IL-12, which are at the crossroads of innate and adaptive immune responses, leading to expansion of T regulatory lymphocytes (Treg) or Th1 cells in a strain specific manner (68,71,72). In line with this, IFN γ secretion by activated Th1 cells was observed after systemic treatment of mice with lactobacilli (68). Although IL-12 and IFN γ are cytokines that mark adaptive Th1 cell response, both IL-12 and IFN γ are important for the activation of NK cells, innate cytotoxic lymphocytes. NK cell activation was observed *in vitro* after treatment with multiple probiotic strains (71) and confirmed *in vivo* (73). Given the important role of NK cells in fighting pathogens, including the common skin pathogen *S. aureus* (74), further research regarding the protective role of probiotics in treating both intestinal and cutaneous wound infections is warranted.

To summarize, lactobacilli stimulate activation of intraepithelial T lymphocytes, NK cells and macrophages, while their effects on Treg and Th1 cell induction are highly strain specific. Hereby, some isolates of lactobacilli exhibit anti-inflammatory and immunosuppressive activity, while others have pro-inflammatory effect and can promote stimulation of the immune response aiding in pathogen clearance.

Probiotics and cutaneous wound healing - *in vitro* studies

Lactobacilli and bifidobacteria are most commonly investigated as potential probiotics for various dermatologic disorders including non-healing wounds (75). The protective abilities of probiotic strains towards skin pathogens have been highlighted through multiple *in vitro* studies with human keratinocytes (76,77,78). Similar to their effect on gut epithelium, probiotic strains, *Lb. rhamnosus* GG and *B. longum* have been shown to increase TJ function and expression of claudin 1, ZO-1, and occludin in keratinocytes infected with *S. aureus* (76). Unlike *Lb. rhamnosus*, *B. longum* increased expression of claudin 4, another major TJ protein (76), suggesting that *B. longum* can influence TJ function via an alternate mechanism by decreasing paracellular permeability and thus preventing pathogen invasion.

Additionally, activation of Toll-like Receptor 2 (TLR2) enhances tight barrier function in gut epithelial cells, as well as keratinocytes (Fig 2) (79). *B. longum*'s modulation of TJ function appears to be TLR2 dependent as transepithelial electrical resistance and TJ protein levels cease to increase when TLR2 is neutralized or blocked, respectively (76). On the other hand, the effects of the widely used *Lb. rhamnosus* GG on keratinocytes are TLR2-independent, suggesting that this strain uses an alternative method to enhance tight barrier function (76). A potential pathway involved in this process is the mitogen activated protein kinases (MAP) kinase pathway, known to enhance tight barrier function via modulation of ERK and p38 (47,80).

Other probiotic strains such as *Lb. reuteri* and *Lb. plantarum* have also shown the ability to increase tight barrier function in primary human keratinocytes (76). *Lb. rhamnosus* GG and *Lb. reuteri* also increase re-epithelialization via increased keratinocyte migration and increased cellular proliferation (78). Probiotics can also induce re-epithelialization through induction of chemokines. For example, *Lb. rhamnosus* GG increased expression of the chemokine CXCL2 and its receptor CXCR2, which stimulate keratinocyte proliferation and migration during normal wound healing (78). While most of probiotics have been beneficial for keratinocyte function, *Lb. fermentum* has been shown to reduce keratinocyte viability and re-epithelialization (76,78), highlighting again strain-specific effects.

Another method of protection against cutaneous wound infections is through already elaborated probiotics' antibacterial activities such as inhibition of pathogen growth and reduction of pathogen adhesion. *Lb. rhamnosus* GG demonstrated this protective effect by inhibiting *S. aureus* growth in infected keratinocytes by yet unidentified mechanism (77). Strains like *Lb. rhamnosus* GG and *Lb. casei* Shirota display antimicrobial activity that is not attributed to acid (77), bacteriocin, or hydrogen peroxide production (81), underlining the diversity of protective mechanisms by probiotics. Furthermore, *Lb. plantarum* supernatant disrupts the pathogenic properties of *P. aeruginosa*, a common chronic wound pathogen, by interfering with its quorum sensing system (82). This supernatant without live probiotics, was capable to reduce bacterial adhesion and biofilm growth through inhibition of *P. aeruginosa* virulence factors elastase, pyocyanin, and rhamnolipids (82).

Lactobacilli can also inhibit pathogen invasion into keratinocytes by competitive exclusion. *Lb. reuteri* and *Lb. rhamnosus* GG are able to inhibit the initial adhesion of *S. aureus* to

keratinocytes and displace *S. aureus* already attached to human keratinocytes (77,83). The specific molecules involved in exclusion and displacement of *S. aureus* from human keratinocytes are still unknown, but they most likely rely on moonlight proteins: a class of multifunctional bacterial adhesins that can, among many functions, bind to epithelial cells (84). An example of a moonlight protein is enolase from *Lb. crispatus* which can bind to laminin and collagen I (85), while enolase from *Lb. plantarum* has been shown to bind to fibronectin preventing *S. aureus* adhesion to epithelial cells (86). A displacement mechanism, as demonstrated by *Lb. rhamnosus GG*, would enable probiotics not only to protect but also rescue keratinocytes from infection (Fig 2) both important characteristics for potential clinical applications. In order to address safety of using live probiotic bacteria topically, lysates, supernatants and metabolites from probiotic strains have been extensively studied *in vitro* and *in vivo* demonstrating beneficial effects similar to live microorganisms (77,82).

Probiotics and cutaneous wound healing - *in vivo* studies

In vivo wound healing studies have mostly been focused on topical application of probiotics that support *in vitro* data, demonstrating improved wound healing through reduced bacterial load and increased tissue repair in rodent wound models (41,87,88). Topical application of *Lb. plantarum* impeded wound colonization by *P. aeruginosa* in a burn mouse model by clearing *Pseudomonas* from the skin, liver and spleen, through enhanced phagocytosis and decreasing apoptosis (41). Even application of kefir (containing a mixture of LAB and yeasts) resulted in improved healing with antibacterial and antifungal effects (87,88,89).

Topical application of *Lb. plantarum* interfered with pathogen colonization in human burn wounds infected with *P. aeruginosa*, *S. aureus*, and *S. epidermidis* (90). *Lb. plantarum* treatment applied topically decreased bacterial load and promoted wound healing comparable, if not better than silver sulfadiazine treatment. One potential mechanism underlying *Lb. plantarum*'s anti-pathogenic properties is that *Lb. plantarum* and *P. aeruginosa* induce opposite effects on inflammation (91). Gram-positive bacteria such as *Lb. plantarum* induce IL-12 secretion, which activates cytotoxic T cells and NK cells to secrete IFN γ , while gram-negative bacteria *P. aeruginosa* preferentially induce IL-10, which inhibits those functions (91). However, this antagonistic control of the inflammatory response may not account for *Lb. plantarum* anti-bacterial effects on Gram-positive pathogens such as *S. aureus*. Topical *Lb. plantarum* also improved wound healing in human chronic venous ulcers (92) infected primarily with *S. aureus* and *P. aeruginosa*, and promoted a continuous healing process resulting in reduced bacterial load and induced of granulation tissue formation (92). The polymorphonuclear cells (PMN) isolated from the ulcer bed demonstrated increased IL-8 production, and decreased percentage of apoptosis and necrosis upon treatment with *Lb. plantarum*. Considering its antipathogenic and immunomodulatory effects in humans, *Lb. plantarum* is thought to inhibit pathogen colonization by regulating IL-8 levels and modulating the entry and activity of PMNs migrating from peripheral blood to the ulcer (92).

Beneficial effects of cutaneous probiotic strains

Aside from lactobacilli and bifidobacteria, other non-conventional probiotics, commonly associated with healthy skin microbiome, have shown beneficial effects on cutaneous repair. One such microorganism is a *St. thermophilus*, a LAB that increases the amount of ceramides and phosphorylcholine in keratinocytes in a time-dependent manner, through production of sphingomyelinase (93). Ceramides play a key role in developing extracellular lipid bilayers and hence improving the lipid barrier of the skin (94), and topical application of *St. thermophilus* resulted in elevated ceramide levels in the stratum corneum of healthy skin (93). Decreased levels of ceramide have been associated with barrier dysfunction in the epidermis, including lack of protection against antigens and bacteria (95). Therefore, probiotic treatments targeting ceramide have potential to improve the skin barrier function during the wound healing process.

Another well-studied cutaneous probiotic is *S. epidermis*. A skin commensal, *S. epidermis* secretes Esp, an extracellular serine protease that can inhibit colonization by *S. aureus* through multiple mechanisms. Esp can prevent *S. aureus* biofilm formation by degrading biofilm matrix and also through cleavage of host receptor proteins that are important for *S. aureus* attachment and infection, including Protein A, fibronectin, fibrinogen, and vitronectin (96). Additionally by cleaving autolysin, Esp prevents *S. aureus* from releasing its DNA as material for the extracellular matrix of the biofilm (97). Esp secreted by inhibitory *S. epidermis* can also increase susceptibility of *S. aureus* biofilm to host immune system components (98). Other staphylococci species like *S. hominis* can also have inhibitory effect on pathogenic *S. aureus*. The Gallo group recently identified coagulase-negative *Staphylococcus* strains (CoNS) from skin of healthy subjects with potent antimicrobial activity due to newly discovered antimicrobial peptides (AMPs) (99). These AMPs, mainly produced by *S. epidermis* and *S. hominis*, were highly potent in selective killing of *S. aureus*. Importantly, compared to normal controls, subjects with atopic dermatitis lacked these CoNS strains on the skin surface. Additionally, introduction of these strains to human subjects with atopic dermatitis decreased skin colonization by pathogenic *S. aureus* (99).

S. epidermis has also been shown to regulate skin homeostasis and suppress inflammation induced by *Propionibacterium acnes*, the anaerobic gram-positive pathogen linked to the pathogenesis of acne via various mechanisms (100,101). Although *S. epidermidis* has been implicated as a beneficial microorganism for management of atopic dermatitis and acne associated inflammation, its application in the context of wound healing remains to be further explored (16). In addition to commensal *Staphylococcus*, other non-pathogenic bacteria such as *Vitreoscilla filiformis* have shown ability to reduce cutaneous inflammation through stimulation of regulatory T cells and skin dendritic cells (15).

The importance of skin commensals raises the issue of widespread use anti-microbial soaps and products and their potentially harmful effects on eliminating beneficial cutaneous microbiome. While utilized to eliminate pathogenic bacteria, commonly used skin and wounds cleansers have shown toxic effect on keratinocytes and fibroblasts (102), and can indeed contribute to reduced diversity of health promoting cutaneous microbiome.

Probiotic effects through gut-skin or gut-brain-skin axes

Besides topical effects on cutaneous barrier, gut probiotics and commensals may modulate skin wound healing through effects on systemic immunity, enhanced nutrient absorption, and modulation of gut-brain-skin axis (Fig 3).

Recent studies have demonstrated multiple links between the gut microbiota, the immune system, and skin health (6,103,104,105). Orally ingested probiotics can modulate local as well as systemic immune response (50,106,107). Here we outline known mechanisms behind systemic immune effects of orally introduced probiotics that eventually modulate skin immunity. The gut microbiome can influence skin health, as reduced or absent microbiota decreases the number of $\gamma\delta$ T cells and Th17 cells in spleen or axillary lymph nodes and the production of pro-inflammatory cytokines by T cells, which in turn may contribute to anti-inflammatory cutaneous effects. The effects of the gut microbiome on systemic and local immunity support the fact that gastrointestinal diseases such as inflammatory bowel disease and vascular disorders also have cutaneous manifestations (108). Importantly, when the gut bacterial population has an increased number of beneficial *Lactobacilli* and reduced level of pathogenic *Coriobacteriales* and *Clostridiales*, inflammation is suppressed (109). This is in line with data showing that orally administered probiotics resulted in increased frequency of Treg cells in skin lymph nodes while decreasing inflammation in the murine model of skin allergy (6). Thus, certain probiotic strains have the potential to combat skin inflammation when administered systemically by changing the composition of gut microbiome. As an additional example, *Lb. paracasei* modulates the composition of intestinal microbiota by increasing bifidobacteria and lactobacilli microbiota and also reinforces skin barrier and decreases skin sensitivity while significantly increasing T helper (Th)1 cell-dependent immune responses (110). Consumption of probiotics such as *L. reuteri* induces an upregulation of anti-inflammatory IL-10, which induces Foxp3⁺ Treg lymphocytes to minimize tissue damage at the wound edge (111) and, in turn, down-regulate IL-17A, a pro-inflammatory cytokine (112,113). In addition, *Lb. fermentum* and *Lb. plantarum* were shown to induce phagocytic activity of PMNs in peripheral circulation which be related to the induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) production (114,115) (Fig 3). It has also been suggested that the beneficial systemic effect of gut bacteria could be achieved by direct translocation of bacterial antigens to peripheral circulation via dendritic cells, instead of their transport to local lymph nodes (116). Further and ongoing studies may reveal exact processes behind the immune effects activated by gut bacteria influencing cutaneous wound healing.

Aside from influencing the immune system of the host, systemic effects of gut microbiota and orally introduced probiotics could be achieved by improved absorption of nutrients essential for skin wound healing, especially vitamins, minerals and cofactors for enzymes involved in tissue repair (117,118,119). In addition, synthesis of short chain fatty acid by probiotics improves mineral solubility by decreasing luminal pH (120). Furthermore, several lactobacilli species, including *Lb. coryniformis* and *Lb. rossiae*, have been shown to produce vitamin B12 which is beneficial for wound healing (121,122). *Lb. reuteri* and *Lb.*

acidophilus were shown to increase absorption of dietary vitamin D and E, known to be important for wound healing (123,124,125).

Recent data have shown that gut microbiota can also influence the central nervous system (126) (Fig 3). Additionally, intestinal microbes and their metabolites can interact with neuroendocrine pathways that modify stress-related responses in the skin through the gut-brain-skin axis (112,127) (Fig 3). Probiotics may offer a potential therapeutic option that could beneficially alter this axis thereby modifying systemic health for patients affected with cutaneous and wound healing disorders. *L. reuteri*-induced oxytocin is the most extensively studied example of probiotic influence on the gut-brain-skin axis (106). Encouragingly, dietary supplementation with only a lysate of this probiotic strain was sufficient to increase systemic oxytocin levels and improve wound repair (106). Oxytocin-producing cells were increased in the hypothalamus of mice after oral consumption of a sterile *Lb. reuteri* lysate. Importantly this treatment resulted in lower blood levels of the stress hormone corticosterone and faster epidermal closure of murine wounds (106). Oxytocin receptors present on both fibroblasts and keratinocytes might presumably influence this process (128). It is also known that oxytocin strengthens host immunity by increasing CD25 and IFN- γ expression in thymic and peripheral lymphocytes (129,130), thus it may enhance wound healing through improving host immune functions (111,112), *Lb. reuteri* dampened stress-induced skin inflammation and induced hair growth in humans and mice (106,126). Other gut lactobacilli were also shown to produce neuroactive molecules, including catecholamines and gamma-aminobutyric acid (GABA) (131,132). In addition, probiotic formulation consisting of *Lb. helveticus* and *B. longum* was shown to reduce stress response in humans and rats and this effect was linked to decreased urinary cortisol levels (133). Given the fact that high glucocorticoid levels were shown to impair wound healing (134), this may confirm beneficial effects of multiple gut probiotic species on cutaneous wound healing (106).

In summary, current data suggest multiple interactions within the gut-brain-skin axis and may represent a strong link between the gut microbiome and cutaneous health. However, these links are not fully explored yet and though a therapeutic potential of probiotic bacteria for wound healing disorders exists, more definitive studies are needed. Probiotics may offer a potential therapeutic that could beneficially alter the gut-skin axis and modify systemic health for patients affected with wound healing disorders in a safe manner.

Future Perspectives

This review points to a new perspective and therapeutic potential of beneficial probiotic species as an alternative and safe approach for the treatment of patients affected with cutaneous and wound healing disorders. The use of high-throughput genomic technologies to study microbiome, including beneficial microorganisms, may elucidate novel molecular mechanisms and pathways that will improve our understanding of how commensal bacteria can be utilized to approach various disorders including non-healing wounds. In addition, cross communication between the respective pathways of the host and the beneficial microbiota needs to be defined. The choice of the bacterial strains is of particular importance, as effects of probiotic bacteria can be highly strain specific. In addition to

therapeutic potential of topical probiotics, beneficial bacteria could alter the gut-skin axis and modify systemic health for patients affected with various disorders. Given the increase research on probiotics and the integral part they play in human health, their inclusion as an integrative treatment provides a new prospect for treatment of patients with wound healing disorders.

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Figure 1. Antimicrobial effects of probiotics against pathogenic bacteria at the skin and mucosal surfaces

A Probiotics release bioactive molecules which inhibit pathogen growth and/or interfere with pathogen quorum sensing system. **B.** Co-aggregation of probiotics with pathogens facilitates pathogen's removal from mucosal surfaces via peristaltic elimination of aggregated complexes. **C.** Displacement of pathogens from skin and mucosal surfaces via high affinity binding of probiotics to epithelial cell receptors.

Figure 2. Interaction of probiotics with epithelial cells and fibroblast

A Interaction of probiotics with epithelial cells leads to rearrangement of tight junction (TJ) proteins and epithelial layer restoration. Probiotic bacteria also stimulate release of TNF α , IL-6, host antimicrobial peptides (hAMPs) and growth factors (GF) from epithelial cells; this further promotes immune cell recruitment and activation, pathogen elimination and tissue regeneration. Probiotics can also restore phosphorylation of EGFR (p-EGFR) in epithelial cells upon de-phosphorylation caused by pathogens. **B.** Probiotic strains can induce fibroblast's differentiation, migration, collagen deposition and GF production.

Figure 3. Hypothetical model of intestinal microbiota influencing wound healing through gut-brain-skin axes

Gut probiotics may affect wound healing via three physiological routes: central nervous system, immunomodulation and transfer of nutrients through bloodstream. Probiotics can produce neuroactive molecules and/or modulate secretory activity of enteroendocrine cells (EEC) in gut mucosa e.g. oxytocin; this leads to release of neuromodulators with the potential to improve tissue regeneration. Intestinal probiotics can stimulate recruitment of polymorphonuclear lymphocytes (PMN) to the injured tissue as well as T-cell priming in skin lymph nodes contributing to activation of innate and adaptive immune responses. Beneficial gut bacteria enhance intestinal absorption of nutrients important for wound healing.