Relationship Between the Gut-Skin Microbiota and Diabetic Wound Healing

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ABSTRACT:
Diabetes Mellitus is a prevalent metabolic disorder that is characterized by hyperglycemia and can lead to health complications. One of the most common complications of Diabetes is foot ulcers which is a growing concern. A major problem in the world is diabetic foot ulcers (DFU), which can complicate regular therapeutic procedures like diagnosis and treatment. Bacterial interactions on the skin's surface play a key role in the pathophysiology of DFU and may be able to influence how quickly wounds heal. By interacting with the various cells involved in the wound healing process, our skin's microbiota directly controls cutaneous health and illness. Particularly commensal bacteria collaborate with skin cells that heal wounds to promote barrier restoration. The microorganisms found in DFU include Staphylococcus, Streptococcus, Corynebacterium, Pseudomonas, and a number of anaerobes which hinders the normal wound healing process and prolongs the period of inflammation. In this review, we tried to explain the role of varying microbes which are essential for the healing of wounds. The use of probiotics which is basically the usage of live organisms by topical or oral application is a prominent therapeutic agent for treating diabetic wounds and we have listed a few in the article which has shown improved healing mechanism in the respective models. There are more studies required for a better understanding of the gut-skin microbiota which has varying diverse mechanisms in our system. A more detailed knowledge of the microbiome-skin axis involved in diabetic wound healing may be made possible by next-generation sequencing and the development of bioinformatics tools.

Keywords: *Diabetic Wound Healing, Gut-Skin Microbiota*

1. INTRODUCTION:

Over 340 million people worldwide suffer from the complex metabolic condition known as diabetes(1). Most chronic diseases have been shown to have a pathogenesis that is related to the microbiome. Not an exception to this rule is type 2 diabetes (T2D).(2) In fact, evidence suggests that the microbiome influences glucose metabolism in both T2D preclinical animal models and in animals that are healthy(3). There are billions of bacteria in the gut, which has an intricate ecosystem made up of at least 1000 different kinds of microbes. While bacteria make up the majority of the gut microbiota, it also includes commensals including viruses, fungus, protists, and archaea. (4,5) Each of these factors must be understood in order to understand how the host and the gut microbiota interact, and each factor is pertinent and significant (6). Diabetes is expected to afflict one in three to one in 5 patients over the course of their lives, with diabetic foot ulcers (DFU) being one manifestation of a chronic, non-healing wound. The recurrence rate of DFUs is frightening (40 percent within a year and 65 percent within five years), and there are no reliable methods to forecast their progression(7).

2. COMPOSITION OF GUT MICROBIOTA

Three primary enterotypes of the gut microbiota have been identified, each of which has distinctive metabolic characteristics.(8,9) Each enterotype is identified by the proportional prevalence of among the following genera: *Ruminococcu*s, *Prevotella*, and *Bacteroides*, which are more frequent in enterotypes 1,2 and 3 respectively(10,11)

The gut microbiota in the large intestine is currently estimated to consist of two phyla, Firmicutes and Bacteroidetes,(12) according to a number of studies, most of which were conducted in developed nations. (The next most abundant phylum, *Actinobacteria*, is primarily made up of the genus *Bifidobacterium*.)(13)

The gut microbiota's makeup is well-known to be significantly influenced by diet. Some researchers sequenced mouth bacteria from the skeletal teeth of humans who lived during various eras to support this theory. (14–16) The transition from the hunter-gatherer Paleolithic to the farming Neolithic era (10,000 years ago), with a diet high in carbohydrates, and the start of the industrialized period, with a diet high in processed flour and sugar, were shown to have caused the most significant changes in human gut microbiota (about two centuries ago)(17,18)

Table 1: The list of Gut-Skin Microbiome and their functions:(19–21)

Gut-Skin Microbes	Function
1.*Bacteriodetes*	Host-mediated cell Signalling
2. *Firmicutes*	Immunomodulation
3.*Campylobacter jejuni*	Upkeep of energy homeostasis
4.*Staphyloccus aureus*	Increased insulin sensitivity and glucose tolerance
5.*Bifidobacterium Sp.*	Regulation of osmotic balance
6.*Coprococcus Clostridium*	Lipid oxidation
7.*Roseburia*	Protection against pathogens
8.*Faecalibacterium*	The control of intestinal permeability

9.*L. Lactis*	Regulation of transcription
10.*Klebsiella*	Regulation of translation
11.*Salmonella*	Synergy with other bioactive substances or compounds.
12.*Enterobacteria(E.Coli)*	Regulation of peptidoglycan production Modification of biofilm development.
13.*P.aeruginosa*	Intercellular adhesion by polysaccharides
14.*Clostridium Perfringens*	Regulating flagella expression
15.*S.typhimurium*	Co-factor in enzymatic processes.
16.*C. difficile*	Effects that are anti-inflammatory, anti-cancer, and anti-microbial
17.*Probacteria*	Regulations of cell membrane
18.*Actinobacteria*	Neurotransmission
19.*C.Sporogenes*	Regulation of endothelial dysfunction
20.*Prausnitzii*	Precursor to the production of phospholipids

3. GUT DYSBIOSIS:

A dysbiotic gut population is a characteristic of many inflammatory disorders, but dysbiosis also sets off processes that upset intestinal homeostasis and lead to inflammation.(22) In dysbiosis, there is an increase in bacterial translocation across the intestinal epithelium. (23)Th1 and Th17 cells, which are specifically activated by polysaccharides of Bacteroides spp. and mucosa-adherent SFB, eliminate small numbers of translocated commensal bacteria, as they exist in a healthy human gut. However, large numbers of invasive bacteria repeatedly activate TLRs and cause an overproduction of proinflammatory cytokines, which harm the gut epithelium and cause persistent intestinal inflammation(24–26)

There are several factors that contribute to gut dysbiosis, but the diet, medications, immune system, and intestinal mucosa are the most crucial ones. Reduced diversity and the development of particular bacterial taxa are the results of the effects of stress factors such oxidative stress, bacteriophage induction, and bacteriocin production.(27)

3.1 Influence of different antibiotics on gut flora:

The effects of various antibiotic classes on the gut flora differ. Numerous studies have acknowledged the short-term impacts of antibiotics; Although the hosts' occupants may have enormous socioeconomic repercussions, little is understood about its long-term implications.(10)

After the administration of temporary antibiotics accompanied with clindamycin or clarithromycin, the microbiome may change for up to 4 years, especially in regards to the establishment of resistance genes. Diagnostic techniques and data analysis methods can skew an explanation of both immediate and long-term impacts

Culture-based techniques, for instance, can more obviously demonstrate changes in clinically significant microbiota architecture, but they are less likely to spot complicated alterations in the majority of the gut's non-cultivable microbiota.(28)

3.2 Diet's impact on the gut bacteria:

Long-term as well as in the short term, studies demonstrate that nutrition plays a significant role in altering the variety of the gut microbiome.(29,30) According to recent research, many diseases, particularly those brought on by chronic low-grade inflammation like type II diabetes, are probably connected to variations in bacterial composition brought on by diet.(31)
Alistipes, *Bilophila*, and *Bacteroides* species are more prevalent in diets abundant in animal protein, while *Lactobacillus* spp., *Roseburia* spp., and *E. rectale* are less prevalent and have detrimental effects on the variety of bacteria in the intestinal bacteria.(32)

4. DIABETIC WOUNDS PROGRESSION TO SEVERE STAGES AND THE EFFECT OF MICROBES:

The complicated and sequential process of wound healing includes blood clotting and maintaining hemostasis, an immune response to microorganisms as well as cell waste, re-epithelialization within the injured tissue, development of a scarred region, and in the end tissue remodelling.(33,34) The microbes are indeed crucially significant for the diabetic wound healing process. The diversity and makeup of the DFU and the good skin microbiota are very different. As per a study comparing 23 pairs of samples of DFU microbiota with intact foot skin, intact foot skin had greater bacterial diversity than the injury, both at the genus and species levels, as well as a lower incidence of opportunistic pathogens.(35)
Microbes could potentially hinder the healing process of a wound. Wound infections and their consequences have been linked to certain bacteria, like *Staphylococcus aureus*.. *Staphylococcus*, *Anaerococcus*, *Corynebacterium*, *Porphyromonas*, and *Streptococcus* are only a few of the well-known microorganisms that are prevalent in the chronic wound microbiome. Along with cutaneous microbiota, intestinal microflora influences the healing of wounds by attempting to have an impact, either directly or indirectly, on a number of factors that aid in healing, such as the immune system, inflammation, and blood pressure.(36–38) Even though chronic wounds have high oxygen levels, anaerobes such *Fingelodia*, *Prevotella*, *Peptonipihlus*, *Peptostreptococcus*, and *Anaerococcus* continue to pose a hazard.(39)
It is well known that in western countries, the ecology of DFUs differs depending on the location. Gram-positive aerobic cocci are the predominant microorganism, but warmer climates tend to have more gram-negative bacilli (especially in Africa and Asia) . This may have an impact on the bacterial diversity of the DFU microbiome. (40)

5. MOLECULAR TECHNIQUES FOR BETTER UNDERSTANDING OF THE DFU:

The 16S rRNA gene amplified as a result of new approaches for investigating DFU can be utilised to identify particular bacterial species. The many methods at the moment include pyrosequencing, temperature gradient gel electrophoresis(TGGE), denatured gradient gel electrophoresis(DGGE), and multitarget polymerase chain reactions (PCRs).(41,42)
TGGE and DGGE are very closely related .They allow the identification of various bacterial species by separating the 16S rRNA amplicons which is followed by identifying the bacteria

by sequencing the different bands which is present on the gel.(43–45) The most recent advancement in the study of microbiota is the development of metagenomics technologies. They use massively parallel sequencing of the incomplete 16S rRNA amplicons or the whole genome from a cutaneous biopsy.(46,47) All of these methods now enable the description of the microbiota, or the overall bacterial genome capacity in a specific habitat, such as a DFU(48).

6. MICROBIOTA AND THE DIABETIC WOUNDS:

6.1 Normal Wound Healing:

Normal wound healing is basically of four stages which are haemostasis, inflammation, proliferation, and remodelling. (49) In normal wound healing all the four phases must be synced to each other and must occur in the sequence. In the hemostasis stage the platelets play a very vital role and starts the process of coagulation which is followed by the inflammation phase where the neutrophils play a major role where they phagocyte the bacteria and leads to the release of inflammatory cytokines(50–52) and then the proliferation phase where the process of angiogenesis starts and formation of extracellular matrix(ECM) takes place and last the remodelling stage where there is vascular maturation, collagen modelling and formation of the scar tissue takes place. This entire process is impaired in the wound healing.(53,54)

6.2 Diabetic Wound Healing:

In diabetic Wound healing the stage of inflammation prolongs and the injured tissue keeps releasing inflammatory cytokines and there are presence of dysfunctional macrophages,(55,56) and in the proliferation phase there is decreased angiogenesis, disequilibrium between the metalloproteinases and altered extracellular matrix(57–59) and lastly in remodelling phase there is decreased pericyte function, decreased vascular maturation , decreased wound strengthening. This all stages contribute to the delayed wound healing.(60,61)

6.3 Altered Gut Microbiota in Diabetic Wound:

The healing of wounds has been discovered to be positively impacted by the complete absence of microflora. More specifically, studies using mice raised in germ-free environments have shown that skin injuries heal faster, and without microflora, there are no scars. This is in part because wound sites exhibit improved angiogenesis, increased alternatively activated healing macrophage accumulation, and decreased neutrophil accumulation. (62–64) Microbial colonisation, however, cannot be prevented in clinical settings and starts right away after a wound. The host's immune system initially regulates microbial growth, but with time, the bacteria in the wound region form biofilms and become immune-resistant. So, a microbial infection could happen, which could result in a persistent wound and poor wound healing.(65) According to study findings, 40–70% of all non-traumatic amputations of lower extremities were caused to diabetic foot ulcers. The results of the study showed that diabetic foot ulcers were to blame for 40–70% of all non–traumatic lower extremity amputations. Diabetic patients often need to be hospitalized to the hospital when the ulcer develops to its most complex form, making treatment more challenging. Surprisingly, one of the findings examined a substantial difference in the richness of bacterial diversity in the healthy skin of the foot and forearm of 30 diabetic patients compared to 30 healthy people. The findings showed a statistically

significant shift in the microbial community and skin variety in diabetes forearms but not in non-diabetic forearms. *Firmicutes*, notably the species *Corynebacterium*, are more common in non-diabetic foot skin than *Actinobacteria*, which is more common in diabetic foot skin and has been associated with greater *Staphylococcus* aureus carriage rates. (65,66)

Another study's findings revealed that the skin of a mouse model for diabetes displayed a selective shift in the colonising bacteria present, along with a transcriptional profile indicating activated defence and immunological responses. In diabetic wounds, we noticed a long-lasting immune response together with a specific *Firmicutes* species shift after wounding (including *Staphylococcus* and *Aerococcus*). With abnormally expressed genes connected to the cutaneous defensive response, the selective Staphylococcal shift explicitly connects. It was shown that the colonising microbiota and the skin's healing process are related. In both clinical and laboratory models, Staphylococcus has been linked to poor wound healing. In all varieties of leg ulcers, Staphylococcus is easily detected by culture .After *S. aureus* or *S. epidermidis* colonisation and biofilm production, normal C57Bl6/J mice were less likely to heal a lesion. In an investigation of Infection with *S. aureus* in db/db mice showed chronic infections, an inflammatory response, and decreased phagocytic activity, demonstrating that the host with diabetes has an abnormal innate immune response. By doing a less biassed genomic scan of the microbiota and extending these findings, we have discovered altered microbial population structure inside the diabetic lesion, including a change in Staphylococcus abundance.(67,68)

The dysbiosis of commensal skin microbiome in diabetics may lead to the disturbance of immunological, a state of skin homeostasis and encourage the emergence of skin diseases. Patients with diabetes mellitus (DM) have more skin inflammation, and diabetic animal models showed a considerable increase in the number of inflammatory cells. Skin biopsies from diabetic patients have elevated expression of matrix metallopeptidase 9 (MMP-9) and protein tyrosine phosphatase-1B. Diabetes patients also have high levels of dermal infiltration of inflammatory cells (PTP1B). This may lead to growth factor resistance and contribute to diabetic individuals' struggles to cure skin lesions.(69,70)

The main motive of the comparison studies of the specimens with and without diabetes was to compare the diversity of the presence of microbiota respectively. From many studies it was concluded that the composition of the *Staphylococcus* content in the diabetic specimen was increased as compared to the non-diabetic, which is essentially the cause for the delayed wound improvement in diabetic individuals(71). The diversity and the presence of the varying microbiomes are the essential for the purpose of mending wounds. There are specific bacterias which are essential for the inflammatory responses and in support of the healing of wounds. Therefore, the composition and diversity of many different bacteria that are present in diabetic wounds are inhibited, which delays and prolongs the healing process. Therefore, the general process of wound healing depends greatly on the gut-skin microbiota.(71,72).

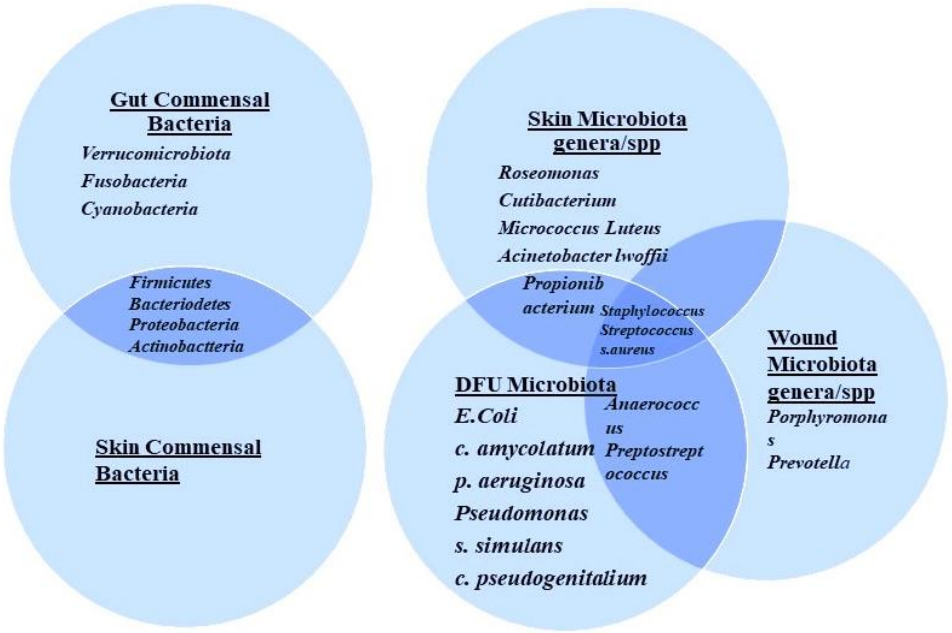

Fig 1:This Venn diagram depicts all the microbes (commensal, genera as well as spp.) present in gut, skin, wound and DFU(65)

7. TREATMENT OF DIABETIC WOUND HEALING(PROBIOTICS) WITH CONSIDERATION TO THE GUT-SKIN MICROBIOTA:

The improvement of the host's normal gastrointestinal microbiota is one of the many positive effects of probiotics, which are live microorganisms that are not pathogenic. This is especially true when they are ingested in the right amounts.(73)The local administration with probiotics, specifically the Lactobacillus type, enhanced healing of 36 DFU patients' wounds, according to research by Choundappan and colleagues. At the time of daily dressing in this trial, the probiotic solution was administered to the wound. On days 0, 5, and 10, the wound swab culture was evaluated on each occasion. The results of this study were encouraging since they showed that in both patient groups, the proportion of wounds with a positive status decreased as the disease advanced . After the fifth day, eight participants of the intervention group versus just six members of the control group reported negative wound swab cultures. On day 10, 10 members of the control group had wound cultures that were positive, 12 of the individuals in the intervention group reported negative wound cultures. As evidenced by a substantial change in the day 7 wound score, this study came to the conclusion that probiotics is used effectively with regard to the infectious diabetic wounds by accelerating the wound healing process.(74)

Skin problems, infections, and slowed wound healing are frequently brought on by factors that are internal or external to the body.(75) The development and healing of a wound exposes bacteria and pathogens to a variety of microenvironments. Microenvironments evolve more when wounds recover. As a result, microorganisms react physiologically to boost the host's innate immune system or shield the host from pathogenic infection from the main or secondary pathogens. Researchers already have published data that stress decreases AMP production and localisation, reduces barrier porosity, and increases infection susceptibility. That might slow

down wound healing, especially DFU. According to the description provided in this review, supplementing with healthy microorganisms, such as probiotics, during stressful situations or in cases of skin dysbiosis may aid in the promotion of wound healing(76,77)

The effects of probiotic on healing process, glucose homeostasis, lipid profiles, and indicators of oxidative stress and inflammation among participants with DFU were initially described in another study. Among participants with DFU, we discovered that probiotic supplementation for 12 weeks had positive effects on ulcer size, glucose metabolism, total cholesterol, hs-CRP, plasma NO, TAC, and MDA levels, but had no effect on HOMA-B, other lipid profiles, or indicators of inflammation and oxidative stress. It must be taken into account that the observed variations in ulcer size between the probiotic group and the placebo group in the current trial were clinically significant(78).

An oral probiotics supplementation trial with diabetic rats showed benefits for healing process, mature collagen expression, promoting neovascularization, reducing the inflammatory process, attenuating weight loss, and improving glycemic control. Due to higher type I collagen deposition and increased neovessel formation, the probiotic group in the current study had wound contraction that was faster than that of the control group, resulting in a reduced wound area. In a different trial, diabetic individuals with diabetic foot ulcers responded favourably to 12-week probiotic supplementation with Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, and Bifidobacterium bifidum (2109 UFC/g each). Probiotic treatment improved glucose control and decreased the size of the ulcer in that trial. According to that study, the probiotics' anti-infectious mechanisms in patients with diabetic foot ulcers included enhanced capacity to combat pathogenic microorganisms or by controlling adaptive immune system, the production of a variety antimicrobial substances, and their anti-inflammatory properties(79–81).

In this review, we discussed how probiotics, both orally and topically delivered, influence wound healing in DFU. Probiotics are known to aid in wound healing by stimulating the production of immune cells, and they also have antagonistic effects against pathogens via competitive exclusion of pathogens(82)

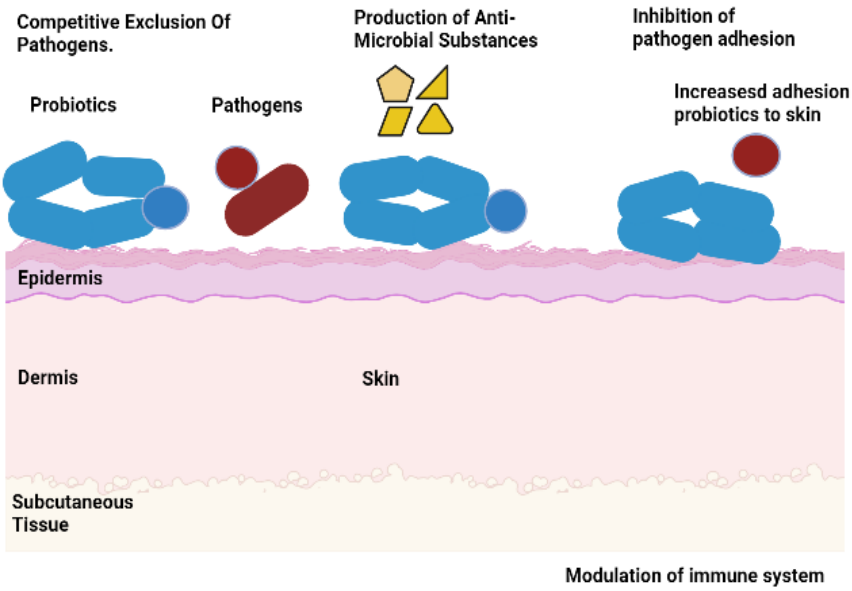

Fig2: Possible mechanism of action of Probiotics.(83)

Table2 : **The list of Probiotics used in the treatment of the diabetic wound healing:**

Samples	**Probiotics**	**Mode of administration**	**Result**	**References**
Male Wistar Rats	*L. bulgaricus* and *L. plantarum*	Probiotic Solution was applied on wound during dressing	Decrease in the wound Contraction Ratio	(84)
Male Wistar Rats	*Bifidobacteriun longum*, *Lactobacillus acidophilus,Streptococcus*	Orally	Decrease in the wound Area Measurement.	(85)
C57BL/6 mice	*Lactobacilli* with a plasmid encoding CXCL12.	Topical Application	Contraction Of The Wound	(86)
DFU patients	*L. plantarum*	Topical Application	Improvement in Wound Healing	(78)
Male Wistar Rats	*Lactobacilli*	Topical application	Increased Collagen Deposition and Improved Healing	(87)
Minipigs	*Limosilactobacillus* reuteri R2LC transformed to express CXCL12	Topical Application	Improved wound Healing	(88)

8. CONCLUSION & FUTURE IMPLICATIONS:

With the increase in the world's diabetes population, chronic non-healing wounds are becoming more common, which has a significant negative impact on society, the healthcare system, and medical professionals. Because of how intricate and synchronised the entire wound healing process is, disturbance can have disastrous consequences. The gut microbiota is regarded as a hidden organ with an active metabolism, and research on the systemic and local roles of microorganisms in tissue repair and regeneration is only beginning. Microbes regulate metabolites, inflammation, and nutritional absorption. The metabolites produced by the microbiota interact with various bodily organs, among them the immunological, hormonal, and metabolic systems of the host. Therefore, when a tissue transplant or biomaterial is designed, it must take into account the inevitable role that bacteria perform in tissue regeneration. Despite probiotics' ability to improve many parts of the healing process in diabetes patients in numerous human and animal models, there are still a lot of unanswered problems. We believe that this study will act as a springboard for the beginning of carefully planned prospective investigations to determine the potential role of probiotics in promoting efficient, secure, and repeatable

wound healing, as well as potential clinical trials. In general, bacteria like lactobacilli can fend off various infections by causing cell signalling, stepping up the immune system, and vying for binding sites in the extracellular matrix of the host. The precise workings of this defence, nevertheless, are not fully understood.

Recent research has revealed how important gut microbes are in controlling tissue health and regeneration. In this sense, the role of gut bacteria in regulating tissue health has received increased attention. The influence of microbiota has been discovered to depend on context and the microbial makeup in tissues like bone, where the presence of gut bacteria could lead to greater growth or bone loss. Having said that, it is crucial to improve microbial composition identification and characterization as well as our comprehension of how changes in the microbial community relate to changes in tissue regeneration. To gain a greater knowledge of how the gut microbiota affects various body systems, it will be crucial to more effectively utilise techniques like nutrigenomic and metabolomic approaches to better comprehend how gut microbes affect various tissue function, regeneration, and wellness. In this review, we attempted to clarify how the gut-skin microbiome contributes to poor wound healing. We have observed a difference in the microbiome makeup of healthy wounds versus diabetic wounds. Various microbiomes exist, but they are less diverse in diabetic wounds, which ultimately hinders wound healing. Probiotics are a crucial component of treatment for poor wound healing, several studies that have been conducted over the years has explained a few approaches. But more research must be done to precisely understand how the gut microbiota affects tissue regeneration and mechanism of action wound healing.

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