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Review article

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The wound microbiome in chronic wounds: a biomarker and therapeutic target

Bartosz Molasy[1,2], Małgorzata Wrzosek[3,4,*]

[1]Department of Surgical Medicine, Collegium Medicum, Jan Kochanowski University of Kielce, 25-369 Kielce, Poland
[2]Department of General Surgery, St Alexander Hospital, 25-316 Kielce, Poland
[3]Department of Biochemistry and Pharmacogenomics, Medical University of Warsaw, 02-097 Warsaw, Poland
[4]Department of Pharmaceutical Sciences, Collegium Medicum, Jan Kochanowski University of Kielce, 25-369 Kielce, Poland
*Corresponding author. Department of Biochemistry and Pharmacogenomics, Medical University of Warsaw, 02-097 Warsaw, Poland. E-mail: malgorzata.wrzosek@wum.edu.pl

Abstract

Chronic wounds, including diabetic foot ulcers, venous leg ulcers, and pressure ulcers, remain a major global healthcare challenge, associated with substantial morbidity, risk of limb loss, and high healthcare costs. Increasing evidence indicates that the wound microbiome modulates inflammation, tissue repair, and responses to therapy, thereby influencing clinical outcomes. This review summarizes current knowledge on the composition and function of chronic wound microbial communities and discusses their clinical relevance as prognostic biomarkers and therapeutic targets. Microbiome structure is shaped by wound etiology, chronicity, anatomical site, and host comorbidities. Dysbiosis and biofilm formation contribute to persistent inflammation, antimicrobial tolerance, and delayed healing. Advances in sequencing and multi-omics technologies have improved microbial characterization and enabled the identification of candidate microbial signatures associated with healing trajectories. Emerging microbiome-modulating strategies such as probiotics, bacteriophages, topical oxygen approaches, and nanotechnology-based interventions show potential to shift wound ecosystems toward a pro-healing state; however, robust clinical validation remains limited. Further clinical studies are needed to validate microbiome-guided diagnostics and interventions and to establish standardized protocols for their application in clinical practice.

Impact statement

This review presents the current state of knowledge on the wound microbiome and its role in chronic wound healing, emphasizing the potential of novel therapies such as probiotics and phage therapy to improve treatment outcomes.

Keywords chronic wound infections, wound microbiome, biofilm, antimicrobial resistance, bacteriophages, probiotics

Introduction

Chronic wounds, including diabetic foot ulcers (DFUs), pressure ulcers, and venous leg ulcers, represent a growing clinical and public health burden, associated with substantial morbidity, increased risk of amputation, and high healthcare expenditures (Díaz-Herrera et al. 2025). In high-income countries, chronic wounds are estimated to affect approximately 1%–2% of the general population (Sen et al. 2009). Despite advances in wound care, clinical outcomes remain suboptimal, with post-amputation mortality in patients with diabetic foot ulcers exceeding 50% within five years (Armstrong et al. 2017).

Increasing evidence indicates that the wound microbiome plays a central role in modulating inflammation, immune responses, and tissue repair (Grice and Segre 2011, Kalan and Brennan 2019). Disruption of the normal skin microbiome, known as dysbiosis, promotes opportunistic pathogens and biofilm formation, which in turn sustains chronic inflammation and delays healing (Kalan and Brennan 2019). Biofilms are of particular clinical relevance, as they confer tolerance to antimicrobial therapy and impair host immune defenses, thereby perpetuating non-healing wound states (Bjarnsholt et al. 2008). Although numerous studies have demonstrated associations between microbiome composition and wound healing outcomes, translation of these findings into clinical practice remains limited. Much of the available evidence is derived from small cohorts, heterogeneous methodologies, or cross-sectional study designs, and the majority of investigations focus predominantly on bacterial communities, often neglecting the contributions of fungal and viral components of the microbiome (Hannigan et al. 2015, Loesche et al. 2016, Verbanic et al. 2020, Cheong et al. 2021). Moreover, while emerging diagnostic and therapeutic strategies, such as next-generation sequencing (NGS)-based diagnostics and microbiome-modulating interventions, show promise, their clinical utility has yet to be established in large-scale, well-designed clinical studies. Key barriers to clinical translation include methodological heterogeneity,

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inconsistent sampling strategies, and the predominance of cross-sectional analyses, all of which limit generalizability and hinder integration into routine clinical workflows (Verbanic et al. 2020, Ersanli et al. 2023, Öhnstedt et al. 2023, Morsli et al. 2024, Gabrilska et al. 2025). Furthermore, most existing reviews address either the ecological characteristics of the chronic wound microbiome or emerging therapeutic innovations. Few provide an integrated framework that links microbial composition with mechanistic insights, translational biomarkers, and targeted microbiome-modulating strategies. To address this gap, the present review integrates three complementary domains: mechanistic evidence linking microbiome composition with host immune dysfunction; diagnostic approaches capable of generating clinically actionable microbial signatures; and therapeutic strategies aimed at microbiome modulation or disruption of biofilm-mediated chronicity. By synthesizing these dimensions, this review aims to provide a structured translational perspective to support the development of microbiome-informed diagnostic workflows and personalized therapeutic pathways. Accordingly, this review bridges mechanistic insights into the chronic wound microbiome with emerging diagnostic and therapeutic applications relevant to clinical translation.

Materials and methods

This review was conducted as a narrative, integrative literature review to accommodate the substantial methodological heterogeneity across studies on chronic wound microbiomes. Although this was not a systematic review, elements of the PRISMA framework were used to guide study selection and reporting. No review protocol was registered. A fully systematic approach was not feasible due to substantial variation in sampling techniques, sequencing platforms, definitions of wound chronicity, and outcome reporting. A narrative design allowed synthesis of diverse study types while enabling a critical and clinically oriented interpretation of the evidence.

A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science for articles published between January 2008 and May 2025 using combinations of the following terms: “chronic wound,” “wound microbiome,” “biofilm,” “wound healing,” “metagenomics,” “phage therapy,” and “probiotics.” Reference lists of key publications were also screened to identify additional relevant articles. Inclusion criteria comprised peer-reviewed original research, clinical trials, and review articles focusing on wound microbiome composition, biofilm biology, diagnostic methods, host–microbe interactions, or microbiome-directed therapies. Case reports, non–peer-reviewed materials, conference abstracts, and studies lacking microbiome-related outcomes were excluded.

Given the narrative design, no formal risk-of-bias assessment was undertaken. However, study selection favored publications with clear methodological descriptions, validated sequencing or culture techniques, and clinically relevant endpoints. Conflicting findings were intentionally retained to reflect the inherent variability of the field. Such discrepancies were addressed by comparing sampling methods, sequencing depth, definitions of infection, and wound characteristics. This approach enabled a transparent and clinically meaningful synthesis while acknowledging limitations inherent to the existing evidence base.

Differences in microbiome composition between healthy skin and chronic wounds

Healthy human skin is colonized by a diverse consortium of bacteria, fungi, and viruses that together contribute to the integrity of the skin barrier and immune homeostasis. Dominant bacterial genera on healthy skin include Staphylococcus, Corynebacterium, and Cutibacterium (Grice and Segre 2011). The skin virome, composed predominantly of bacteriophages, and the mycobiome—dominated largely by Malassezia species—are thought to play regulatory roles in maintaining microbial balance and modulating host immune responses (Hannigan et al. 2015, Verbanic et al. 2022).

Chronic wounds are characterized by microbial dysbiosis, manifested by reduced microbial diversity and dominance of opportunistic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus* spp. (Kalan and Brennan 2019, Jotta et al. 2024). Next-generation sequencing (NGS) studies have revealed polymicrobial communities that vary according to wound etiology, anatomical location, and chronicity. Anaerobic genera, including Anaerococcus and Finegoldia, are frequently detected in chronic wounds and have been implicated in sustaining persistent inflammation (Dowd et al. 2008, Kalan et al. 2016, Loesche et al. 2016). Kalan et al. demonstrated that chronic wounds harbor diverse fungal communities, including Candida spp., which were associated with delayed healing (Kalan et al. 2016). Dominance of Ascomycota has been associated with a 2.1-fold increase in healing time, whereas the presence of Malassezia species has been linked to epidermal regeneration (Kalan et al. 2016). With respect to the virome, Verbanic et al. reported that bacteriophage communities differ between healing and non-healing wounds, suggesting that phages may shape bacterial populations and influence healing trajectories (Verbanic et al. 2022). Bacteriophages can modulate biofilm composition through targeted lysis of pathogenic bacteria; for example, *Pseudomonas aeruginosa*–specific phages have been shown to reduce bacterial colonization by approximately 40% in *in vivo* models, although therapeutic application still requires standardization of phage formulations (Liu et al. 2024).

Given the complexity of bacterial, fungal, and viral diversity within chronic wounds, reliance on small-scale and predominantly observational studies remains insufficient, underscoring the need for standardized investigations in large, well-characterized cohorts (Dowd et al. 2008, Kalan et al. 2016, Loesche et al. 2016). Accordingly, standardized sampling and analytical methodologies will be essential to enable meaningful clinical translation of chronic wound microbiome research. This ecological shift is illustrated in Fig. 1, depicting the transition from a balanced skin microbiota to a dysbiotic chronic wound environment.

The role of biofilm in the pathophysiology of chronic wounds

Biofilm formation is a hallmark of chronic wounds and contributes to delayed healing by protecting bacteria from antimicrobial agents and host immune responses (Bjarnsholt et al. 2008). Hurlow et al. reported the presence of biofilms in

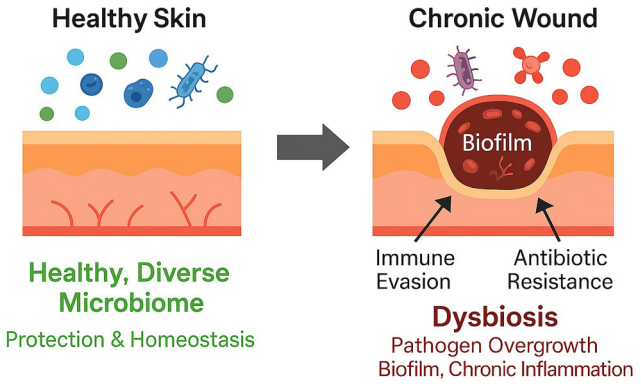

Figure 1 Transition from healthy skin to chronic wound microbiome.

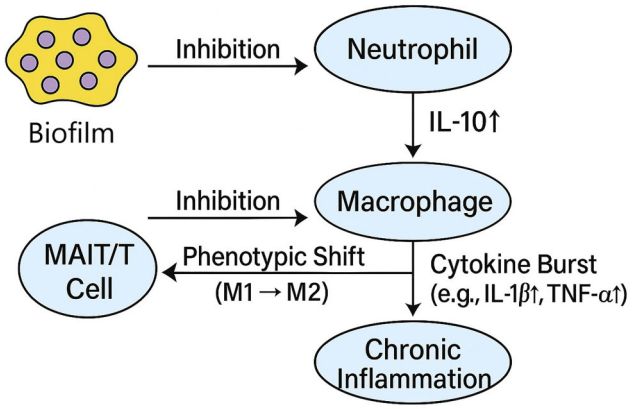

Figure 2 Immunomodulatory effects of biofilms in chronic wounds.

over 80% of non-healing wounds, with strong associations with persistent inflammation and poor clinical outcomes (Hurlow et al. 2016). Biofilms interfere with epithelial cell migration, sustain chronic inflammation, and facilitate the development of antimicrobial resistance (Bjarnsholt et al. 2008, Robertson et al. 2024). Mechanisms underlying biofilm-associated resistance include up to a 1000-fold increase in antimicrobial tolerance compared with planktonic bacteria, driven by metabolic dormancy of cells in deeper biofilm layers, the presence of persister cell subpopulations, and enzymatic inactivation of antibiotics (e.g. β-lactamase production) (Høiby et al. 2010, Almatroudi 2025).

Biofilms in chronic wounds are composed of complex communities of both aerobic and anaerobic bacteria. The presence of such biofilms is strongly associated with prolonged inflammation and impaired wound healing (Yang et al. 2024). In a comprehensive review, Versey et al. summarized evidence indicating that biofilms can modulate the host innate immune response, thereby promoting a chronic inflammatory state and delaying tissue repair (Versey et al. 2021). Collectively, these observations highlight biofilms as a critical therapeutic target in chronic wound management. Importantly, not all biofilms appear to be universally detrimental. Emerging evidence suggests that certain commensal biofilms, such as those formed by Corynebacterium striatum, may stimulate keratinocyte migration, indicating a context-dependent role of biofilms in wound healing (Iversen et al. 2024).

The composition and behavior of the chronic wound microbiome are shaped by wound type, duration, anatomical site, and host-related factors such as diabetes and vascular disease (Verbanic et al. 2020, Ancira et al. 2025). Oxidative stress within the wound microenvironment promotes selection of biofilm-forming, antimicrobial-resistant organisms and contributes to reduced microbial diversity (Martins-Green et al. 2025). Commensal bacteria such as *Staphylococcus epidermidis* may transition toward pathogenic phenotypes, as demonstrated by clinical isolates exhibiting enhanced biofilm formation and antimicrobial resistance (Dinić et al. 2024).

Methodological inconsistencies—including sampling strategy (swab vs. biopsy), DNA extraction protocols, and bioinformatic processing pipelines—further hinder the clinical application of microbiome profiling in chronic wounds (Morsli et al. 2024).

Immune response in wound healing

The skin microbiome plays an active role in immune regulation. In healthy skin, commensal microorganisms interact with keratinocytes and antigen-presenting cells to activate signaling pathways, including Toll-like receptors (TLRs), which are critical for pathogen recognition and the initiation of immune responses (Chen and Tsao 2013, Belkaid and Tamoutounour 2016). These interactions trigger the release of cytokines—such as IL-1β (up to 2–5-fold increase in keratinocyte models), IL-6, and TNF-α—and chemokines, which coordinate both inflammatory defense mechanisms and tissue repair (Morizane et al. 2023, Gan et al. 2024). In chronic wounds, this immune regulation becomes disrupted. Microbial dysbiosis can lead to either excessive activation of inflammatory pathways (e.g. sustained TNF-α levels $>$ 25 pg/mL and IL-6 $>$ 100 pg/mL in infected wounds at 24–48 h) or local immunosuppression within the wound microenvironment (Loesche et al. 2016, Al-Jebouri et al. 2019, Kalan and Brennan 2019). Metagenomic analyses have shown that chronic wounds often exhibit reduced microbial diversity and dominance of opportunistic pathogens, correlating with persistent inflammation (Verbanic et al. 2020). Interestingly, some microbial species may contribute positively to healing. For example, Alcaligenes faecalis has been shown to accelerate re-epithelialization by modulating matrix metalloproteinase (MMP) activity (White et al. 2024).

The biofilm matrix significantly alters immune cell behavior within chronic wounds. Its extracellular polymeric structure impairs the activity of neutrophils and macrophages while skewing their phenotype toward a chronic inflammatory profile (Thurlow et al. 2011). Biofilms also affect T cell recruitment and promote the release of anti-inflammatory cytokines such as IL-10, which paradoxically facilitate microbial persistence (Heim et al. 2015, Cárdenas-Calderón et al. 2022). As shown in Fig. 2, biofilms impair innate immune function, suppress phagocytosis, and promote chronic inflammation through IL-10–mediated mechanisms. Notably, experimental models demonstrate that biofilm-embedded bacteria markedly reduce macrophage phagocytic capacity, often by 50%–80% depending on the bacterial species and model, and interfere with neutrophil extracellular trap (NET)-mediated killing compared with planktonic counterparts (Thurlow et al. 2011, Rathore et al. 2019, Cárdenas-Calderón et al. 2022).

Engineered probiotic strains, such as *Limosilactobacillus reuteri* R2LC, have been shown to produce chemokines such as CXCL12, thereby enhancing keratinocyte migration and promoting wound healing, with up to 40% faster wound closure reported in early-phase clinical studies (Öhnstedt et al. 2023). Similarly, commensal bacteria such as *Staphylococcus epidermidis* stimulate the production of antimicrobial peptides (AMPs), thereby reinforcing the skin barrier and enhancing protection against pathogenic microorganisms (Cogen et al. 2008, Cogen et al. 2010).

Probiotics such as *Lactobacillus* spp. operate through dual mechanisms: competitive exclusion of pathogens and immunostimulation via gamma delta T-cell activation, which enhances phagocytosis and modulates cytokine networks (e.g. reduced IL-1β and increased TGF-β1 signaling) (Vågesjö et al. 2018). Lactic acid bacteria (LAB) have also demonstrated anti-inflammatory potential and may contribute to both immune modulation and biofilm remodeling (Li et al. 2023).

Furthermore, activation of mucosal-associated invariant T (MAIT) cells by specific microbial antigens promotes the secretion of pro-regenerative cytokines such as IL-17 and IL-22, which in turn stimulate keratinocyte migration and proliferation (du Halgouet et al. 2023). MAIT cells intrinsically express tissue-repair programs and migrate to wound sites via CXCR6-dependent signaling, where amphiregulin secretion accelerates re-epithelialization (Vågesjö et al. 2018).

An often overlooked dimension of wound biology is the microbiome's influence on wound-associated pain. For example, Campbell et al. reported that the presence of Corynebacterium and *Streptococcus* species was associated with reduced pain intensity during dressing changes, suggesting interactions between microbial and neuroimmune signaling pathways (Campbell et al. 2024).

The emerging concept of microbiome immunomodulation, using probiotics, prebiotics, microbiota transplantation, and synthetic biology approaches, shows promise for supporting tissue regeneration and modulating chronic inflammation (Tang et al. 2023a, Liu et al. 2024). However, several critical barriers to clinical translation remain. These include strain-specific variation in efficacy, challenges associated with delivery in biofilm-rich environments, and host genetic polymorphisms that may influence MAIT cell responsiveness.

Differences in the wound microbiome

The chronic wound microbiome constitutes a dynamic and complex ecosystem, the composition of which is influenced by multiple factors, including wound etiology, anatomical location, and the patient's overall health status. Understanding these variations is essential for the development of effective treatment strategies and may contribute to improved therapeutic outcomes. Figure 3 summarizes the main factors that influence the composition of the chronic wound microbiome, including wound type, anatomical location, and host-related variables.

Another important aspect is the influence of wound etiology on microbiome composition. Chronic wounds of different etiologies often exhibit distinct microbial profiles (Mihai et al. 2024). Chen et al. indicate that while wound etiology is associated with distinct microbiome patterns, it explains only a limited proportion of overall community variance, supporting the need for integrative host–microbiome profiling rather than reliance on wound classification alone (Chen et al. 2025). Nonetheless, other reports indicate that diabetic and venous ulcers tend to harbor higher proportions of anaerobic bacteria, while pressure ulcers are more frequently colonized by aerobic species (Canchy et al. 2023). These differences may be attributed to variation in anatomical location, tissue perfusion, and host immune status. For example, lower extremity wounds, such as diabetic and venous ulcers, are commonly associated with anaerobic colonization and carry a higher risk of complications, including osteomyelitis (Sachdeva et al. 2022). Wound age also influences microbiome dynamics. In early stages, wounds typically display greater microbial diversity, including commensal species that may support tissue regeneration. As wounds transition to chronicity, microbial diversity declines, and biofilm-forming and stress-tolerant taxa become dominant, thereby impeding healing (Verbanic et al. 2020, Liu et al. 2024). Longitudinal analyses have shown that wounds persisting for more than 6 months exhibit reduced microbial diversity and increased temporal stability, features that correlate with sustained inflammation and delayed healing. Temporal instability, defined as frequent shifts in microbiome composition, is associated with accelerated healing (HR = 1.8, $P < 0.01$), whereas stable microbial communities are predictive of chronicity (Loesche et al. 2016). The progressive loss of microbial diversity and dominance of resistant taxa over time is illustrated in Fig. 4.

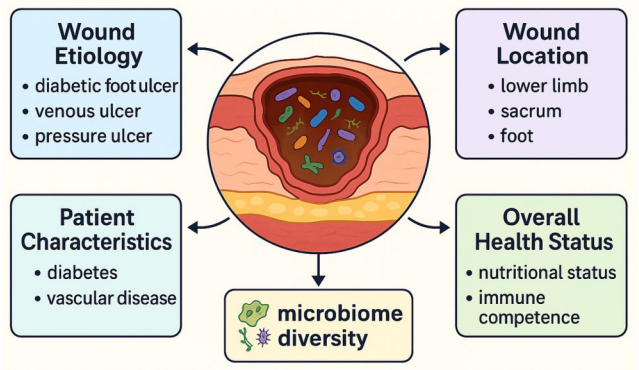

Figure 3 Key factors influencing the chronic wound microbiome.

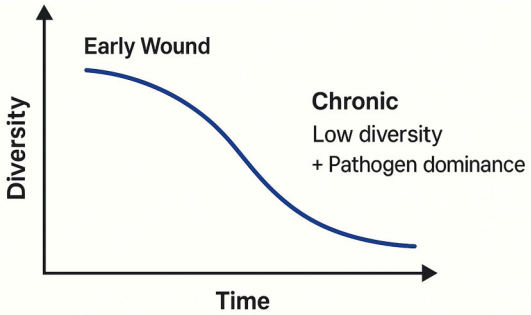

Figure 4 Temporal dynamics of microbial diversity during wound chronicity.

Unresolved microbiological aspects

Patient comorbidities, including diabetes, cardiovascular disease, and anemia, can substantially influence wound microbiome composition. Host genetics significantly shapes microbiome composition; polymorphisms in TLN2 and ZNF521 have been shown to explain up to 53% of microbial diversity variation and to predict dominance of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Tipton et al. 2020). For instance, diabetic patients often exhibit an increased prevalence of multidrug-resistant organisms, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Dinić et al. 2024). These shifts have been linked to oxidative stress and impaired immune responses (Huang et al. 2025, Martins-Green et al. 2025). Microbial dysbiosis may exacerbate inflammation and delay wound healing (Tang et al. 2023b). Excessive production of reactive oxygen species (ROS) contributes to cellular and tissue damage (Hunt et al. 2024). The interplay between oxidative stress and microbiome imbalance forms a vicious cycle that hinders skin regeneration and promotes chronic wound formation (Verbanic et al. 2020).

Despite growing interest, many microbiological factors involved in chronic wounds, including interactions among different microbial species and their collective influence on wound healing, remain poorly understood. Moreover, there is a lack of reliable microbiological biomarkers that could predict wound trajectory or complication risk. Fungal communities, particularly those dominated by Ascomycota, have been associated with an approximately 2.1-fold increased risk of non-healing, yet remain underrepresented in contemporary wound microbiome research (Short et al. 2023). Bacteriophage dynamics, which play an essential role in regulating bacterial populations, also remain insufficiently characterized, largely due to analytical challenges rather than limitations in sequencing capacity (Schönegger et al. 2023). High viral diversity, limited reference genomes, and reduced recall or precision of current alignment- and learning-based identification tools continue to hinder reliable virome reconstruction, despite emerging evidence that phage–bacteria interactions may predict wound healing outcomes (Shang et al. 2022).

Most existing studies focus on bacteria, while the roles of fungi and viruses in wound healing remain unexplored (Kalan and Brennan 2019). Methodological inconsistencies (swab vs. biopsy, 16S vs. shotgun sequencing) cause 35% variability in microbial profiles, hindering clinical translation (Kalan et al. 2016). Additionally, there is an urgent need for standardization of sampling, sequencing, and analysis protocols, as methodological inconsistencies limit inter-study comparability. Current techniques also struggle to capture the full microbial spectrum present *in vivo* (Morsli et al. 2024). Further high-resolution studies are needed to address these gaps and inform the development of targeted therapies.

Diagnostic methods for assessing the microbiome in wounds

Accurate characterization of the microbiome in chronic wounds is essential for understanding its role in wound healing and for the development of targeted therapeutic strategies. Although still widely used in clinical diagnostics, traditional culture-based techniques have significant limitations, including the inability to detect many fastidious or anaerobic microorganisms and insufficient consideration of microbe–microbe interactions under *in vivo* conditions. A comparative overview of molecular and culture-based methods is presented in Table 1, highlighting key strengths and limitations relevant to the analysis of chronic wounds.

Culture-based methods detect only approximately 20% of the microbial diversity present in chronic wounds, missing up to 52.5% of obligate anaerobes and 60% of Gram-negative pathogens that are critical to infection pathogenesis, which may result in inappropriate antibiotic selection in up to 45% of cases and delay wound healing by an average of 3.2 weeks (Rhoads et al. 2012). To address these constraints, culture-independent methods, most notably 16S rRNA gene sequencing and shotgun metagenomics, have gained prominence. 16S rRNA sequencing enables bacterial identification without culturing and typically improves detection of polymicrobial communities compared with conventional culture, which may capture only a minority of mixed communities (Manaka et al. 2017, Botan et al. 2024).

Full-length 16S rRNA sequencing (FL16S) further enhances taxonomic resolution relative to partial-region approaches (Heravi et al. 2020). In parallel, shotgun metagenomics (mNGS) profiles total genetic material in a wound specimen, enabling detection of bacterial, fungal, and viral genomes as well as functional features such as antimicrobial resistance determinants and biofilm-associated pathways (Zhao et al. 2023). Studies directly comparing culture with metagenomic next-generation sequencing (mNGS) consistently demonstrate markedly higher sensitivity, particularly in detecting anaerobes and other fastidious or slow-growing organisms (Hou et al. 2025, Sun et al. 2025). Beyond diagnostics, metagenomic profiles can be integrated with clinical variables to build predictive models of healing trajectories (Ancira et al. 2025). However, mNGS does not distinguish viable from non-viable organisms and may overestimate clinically relevant infection signals; complementary functional approaches such as metatranscriptomics can contextualize DNA-based findings by capturing gene expression activity (Valsami et al. 2025). Finally, metaproteomics can provide an additional layer by profiling expressed proteins and metabolic pathways related to inflammation and tissue remodeling (Öhnstedt et al. 2023, Rytter et al. 2024, Sumpio et al. 2025). Collectively, combining multi-omics readouts may offer a more comprehensive view of host–microbe interactions relevant to chronic wound pathology (Valsami et al. 2025).

The implementation of molecular diagnostics requires rigorous standardization—from sampling techniques (e.g. biopsy, swab, lavage) to DNA isolation and data analysis pipelines (Heravi et al. 2020, Zhao et al. 2023). Without unified protocols, results across studies may vary widely and remain difficult to compare (Morsli et al. 2024). Bioinformatic analysis of sequencing data demands advanced computational tools and expertise, which can be a barrier for clinical adoption (Morsli et al. 2024). However, the development of hybrid reference databases that combine public repositories with sample-specific metagenomic data has improved accuracy in taxonomic and functional annotation (Valsami et al. 2025).

Despite recent advances, challenges remain. For instance, current sequencing methods do not differentiate between live and dead microorganisms, which limits clinical interpretation (Morsli et al. 2024). Additionally, the high cost and limited accessibility of

Table 1 Summary of molecular techniques for microbiome assessment.

Method	Targets	Strengths	Limitations	Sensitivity	Clinical utility	Ref.
Classical culture	Bacteria (mainly aerobes)	Antibiotic susceptibility testing	Low sensitivity in polymicrobial/biofilm infections; under-detects anaerobes	Often detects a minority of taxa in chronic wounds (e.g. ∼20%)	Risk of inappropriate empiric therapy when culture under-represents community	[58]
Proteomics	Microbial and host proteins	Captures expressed proteins and host response; pathway-level insight	Complex matrices, cost, incomplete reference databases, and requires expertise	Detects active proteins, not all taxa (yield varies by workflow)	Identifying active virulence/inflammation signatures in DFUs	[58]
16S rRNA sequencing	Bacteria	Culture-independent; high community coverage	Limited to bacteria, taxonomic resolution	Higher detection than culture (e.g. ∼85%–95%)	Improved anaerobe detection (e.g. 87% vs. 23% by culture)	[11, 17]
Shotgun metagenomics	Bacteria, fungi, viruses	Comprehensive, functional profiling, AMR genes	High cost; analysis complexity; does not prove gene expression	Eg. ∼85%–95% pathogens + viruses	AMR gene profiling to inform therapy/stewardship (with clinical correlation)	[59]
Metatranscriptomics	Expressed microbial genes	Measures functional activity	RNA instability; cost; requires bioinformatics	Enriches for live/active organisms; yield depends on RNA quality	Activity signatures in stalled DFUs; pathway activation during non-healing	[60]

Reported sensitivity and detection yield are approximate and study-dependent.

these technologies hinder widespread use outside of research settings. Nonetheless, the rapid evolution of sequencing technologies and decreasing costs suggest that molecular diagnostics may become routine tools in the evaluation of chronic wound microbiomes in the near future (Lee et al. 2023, Zhao et al. 2023).

The microbiome as a potential biomarker

Recent studies indicate that chronic wounds may harbor reproducible microbiome and host-response patterns associated with delayed healing, particularly in diabetic foot ulcers (DFUs). Rather than single pathogens, accumulating evidence suggests that specific community configurations and host inflammatory signatures are associated with a persistent non-healing state (Table 2). Longitudinal analyses of DFUs have shown that a higher baseline relative abundance of Gram-positive anaerobic cocci, including Peptoniphilus and Anaerococcus, is associated with delayed healing, supporting the concept that anaerobe-enriched dysbiosis may contribute to wound chronicity (Min et al. 2020). These associations appear most informative when interpreted in conjunction with clinical context, including wound chronicity, perfusion status, and local wound care practices.

Beyond microbial composition, host-derived immunological and proteolytic markers may provide complementary insight into wound status. Elevated IL-1β/IL-1RA and CXCL8/CXCL10 ratios have been associated with non-healing or infected wound states and demonstrate moderate discriminatory performance in clinical analyses (Rembe et al. 2025). In parallel, markers of excessive protease activity, including altered MMP-1/TIMP-1 balance and elevated wound-fluid MMP-9 levels, have been linked to poorer healing trajectories in DFUs (Muller et al. 2008, Jindatanmanusan et al. 2018).

Novel therapeutic strategies targeting the wound microbiome

Understanding the microbial landscape of chronic wounds opens new possibilities for personalized medicine. Detection of specific pathogens may inform targeted antimicrobial therapy and strategies aimed at biofilm disruption (Liu et al. 2020). Additionally, patient-derived *in vitro* wound models are being developed to evaluate how individual microbiome compositions influence healing responses. Predictive models that incorporate wound microbiome characteristics alongside clinical variables (such as smoking status and wound volume) account for up to 46% of the variation in healing time—with microbiome-related parameters emerging as major contributors (Ancira et al. 2025). Such approaches support the development of stratified management

Table 2 Potential biomarkers and their clinical significance.

Biomarker	Association	Clinical relevance	Study-specific threshold	Performance/effect (as reported)	Ref.
Anaerococcus abundance ↑	Dysbiosis	Predicts delayed healing of diabetic foot ulcers (DFUs)	>10% relative abundance	HR 1.8 for non-healing; sensitivity 78%, specificity 82%	[70]
IL-1β/IL-1RA ratio ↑	Inflammation	Distinguishes non-healing from healing chronic wounds	Ratio > 2.5	AUC 0.684; accuracy 67%	[71]
CXCL8/CXCL10 ratio ↑	Inflammation/infection	Indicates infected or non-healing stage in chronic wounds	Ratio > 1.8	AUC 0.767; accuracy 77%	[71]
MMP-1/TIMP-1 ratio (altered)	ECM remodeling	Ratio differentiates healing vs. poor-healing DFUs	Higher ratio in good healers vs. poor healers; threshold defined by ROC in study	Significant predictor of healing; ROC-based separation reported	[72]
Wound fluid MMP-9 ↑	Protease burden/chronicity	Higher baseline levels associated with poor healing in DFUs	Elevated MMP-9 at week 0 (above study-specific threshold)	Baseline MMP-9 inversely correlated with % area reduction at week 4; ROC prognostic value reported	[73]

Legend: AUC, area under the curve; DFU, diabetic foot ulcer; ECM, extracellular matrix; HR, hazard ratio; IL-1RA, interleukin-1 receptor antagonist; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases. Reported thresholds and performance metrics are study-specific and may not be directly comparable across cohorts due to heterogeneity in sampling and outcome definitions.

pathways that incorporate both microbial and host-response profiling.

In recent years, growing interest in the wound microbiome has driven the development of innovative therapeutic strategies aimed at modulating the microbial environment to promote healing (Iheozor-Ejiofor et al. 2018, Chen et al. 2021). These approaches include both physical and biological interventions that target the composition and function of the wound microbiota. Negative pressure wound therapy (NPWT) is a widely adopted modality for managing chronic wounds (Hunter et al. 2020). According to a meta-analysis by Chen et al., NPWT can lower bacterial counts and improve local tissue perfusion in diabetic foot ulcers (Chen et al. 2021). However, Iheozor-Ejiofor et al. found no significant difference in microbiome composition between NPWT-treated wounds and those managed with conventional dressings (Iheozor-Ejiofor et al. 2018). Recent *in vitro* models confirm NPWT's strain-specific effects: *Staphylococcus aureus* load decreased by 80% at −80 mmHg, while *Staphylococcus epidermidis* required −100 mmHg for comparable reduction (Bobkiewicz et al. 2024). Further studies using advanced molecular diagnostics are needed to clarify NPWT's effect on the wound microbiome.

Topical oxygen therapy (TOT) delivers oxygen directly to the wound bed, enhancing tissue oxygenation and potentially altering the wound microenvironment. Meta-analyses confirm improved healing rates, but oxygen delivery mode critically influences outcomes: continuous TOT reduced anaerobes by 60% versus intermittent protocols in matched cohorts (Topical Oxygen Therapy Shifts Microbiome Dynamics in Chronic Diabetic Foot Ulcers). A meta-analysis by Carter et al. also confirmed that TOT improves healing rates in chronic diabetic wounds (Carter et al. 2023). Collectively, available evidence indicates that oxygen therapy modulates the wound microbiome by increasing local oxygen tension, altering redox conditions, and reducing anaerobic and biofilm-associated bacterial populations, while simultaneously enhancing neutrophil and macrophage function and stimulating growth factors such as VEGF that support granulation tissue formation (Gupta et al. 2022). Variability in therapy types (e.g. continuous vs. intermittent delivery) and wound characteristics complicates interpretation (Nagarsheth et al. 2023). Further studies using high-throughput sequencing are necessary to assess the durability and clinical relevance of microbiome shifts induced by TOT.

Probiotics represent a compelling approach to wound care, leveraging beneficial microbes to outcompete pathogens, modulate inflammation, and enhance epithelial repair (Knackstedt et al. 2020). Clinical studies have shown that specific probiotic strains, such as *Lactobacillus rhamnosus* and *Lactobacillus reuteri*, can reduce inflammation and support re-epithelialization in chronic wounds (Öhnstedt et al. 2023). Natural probiotic sources such as kefir have demonstrated efficacy in experimental burn models by stimulating fibroblast migration and modulating gene expression of inflammatory and growth factors, including IL-1β, TGF-β1, and bFGF (Oryan et al. 2019). Furthermore, *Lactobacillus plantarum* USM8613 has been shown to promote wound healing and inhibit *Staphylococcus aureus* infection in wound sites (Ong et al. 2020). Prebiotics, substrates that promote the growth of beneficial microbes, may also improve microbiome balance, though standardized protocols for their clinical use are lacking.

Phage therapy employs bacteriophages to specifically target and eliminate pathogenic bacteria, including multidrug-resistant strains. Phages can interact with and disrupt bacterial biofilms by targeting metabolically active cells within the matrix, a process that is often limited with conventional antibiotics due to reduced penetration and diminished activity against dormant subpopu-

lations (Verbanic et al. 2022). Phage therapy is increasingly recognized as a promising adjunct for managing chronic wound infections, particularly in settings dominated by biofilm-forming or antibiotic-resistant bacteria. However, the Phagoburn trial highlighted critical manufacturing and regulatory hurdles for phage therapy, including substantial titre instability during scale-up and the fact that only a minority of clinical trials to date have met EU GMP standards required for regulatory approval (Jault et al. 2019). Despite its promise, the long-term effects of phage therapy on the wound microbiome are not yet fully understood. Key considerations include phage selectivity, possible interactions with commensal microbes, and the risk of unintended off-target effects. Ongoing research is required to evaluate efficacy, safety, and integration into clinical protocols.

Antimicrobial photodynamic therapy (aPDT) involves the application of light and photosensitizing agents to generate reactive oxygen species that kill microbes. aPDT has been shown to effectively disrupt biofilms and support wound regeneration (Sun et al. 2020). While preclinical studies demonstrate promising results, the impact of aPDT on microbial community balance and its long-term consequences remains unclear. Thus, aPDT is currently regarded as an adjunctive and largely experimental approach, without sufficient clinical data to support routine use or long-term microbiome modulation. Future investigations should assess not only microbial eradication but also microbiome resilience and host response over time.

Nanomaterials offer new opportunities in chronic wound treatment by enabling precise modulation of the wound microenvironment. Nanoparticles—such as gold, silver, chitosan, and electrospun nanofibers—exhibit antimicrobial properties and can promote tissue repair (Naskar and Kim 2020). Stoica et al. emphasized the multifunctionality of nanomaterials, which can act as drug delivery vehicles, antimicrobial agents, and immune modulators (Pormohammad et al. 2021). However, concerns remain about long-term microbiome disruption, cytotoxicity, and biocompatibility. More translational research is needed to establish the clinical utility and safety profiles of these advanced materials.

Despite promising advances, many aspects of emerging microbiome-targeted therapies remain unresolved. Standardized protocols, long-term clinical outcome data, and mechanistic insights are still lacking. Future research should focus on optimizing treatment parameters (e.g. dosage, duration, frequency), monitoring microbiome shifts, and evaluating potential synergistic effects between therapies. Interdisciplinary collaboration integrating microbiology, immunology, bioengineering, and clinical wound care will be essential to unlock the full therapeutic potential of microbiome based interventions.

Discussion

This review emphasizes the central and multifaceted role of the chronic wound microbiome in determining healing trajectories, inflammatory burden, and susceptibility to complications. Taken together, the available evidence highlights that chronic wounds do not simply reflect static microbial colonization but represent dynamic ecosystems in which microbial behavior, host immune responses, and therapeutic interventions are tightly interlinked.

Multiple studies have demonstrated that chronic wounds exhibit a profound shift in microbial ecology compared to healthy skin, characterized by reduced diversity, altered community structure, and increased dominance of pathogenic taxa (Kalan and Brennan 2019, Yang et al. 2024). These compositional differences are clinically meaningful, as wounds with reduced microbial diversity or high temporal stability are associated with poorer healing outcomes (Loesche et al. 2016). This supports the concept that not only the presence but also the dynamic behavior of microbial communities contributes to disease chronicity. Stability of a pathogenic community appears to reinforce chronic inflammation, whereas fluctuations and restructuring of microbial populations may represent an early marker of healing potential.

A key recurring theme across the literature is the pivotal role of biofilm biology. Biofilms create highly resilient microbial collectives, enabling bacteria to evade systemic antibiotics, topical antimicrobials, and innate immune mechanisms (Bjarnsholt et al. 2008, Høiby et al. 2010, Almatroudi 2025). These structures impair neutrophil and macrophage function, promote anti-inflammatory cytokine release (e.g. IL-10), and disrupt epithelial migration, collectively reinforcing the chronic inflammatory environment (Ancira et al. 2025). Importantly, some biofilms may not be universally detrimental: commensal biofilms, such as those formed by Corynebacterium striatum, have been suggested to support keratinocyte migration and tissue repair, emphasizing the complexity of host–microbe interactions (Iversen et al. 2024).

Another major gap concerns the underappreciated influence of fungi and bacteriophages. While most studies focus primarily on bacteria, mycobiome alterations—particularly the overrepresentation of Ascomycota—have been associated with delayed healing and greater chronicity in original NGS-based analyses (Short et al. 2023). Similarly, emerging metagenomic and metatranscriptomic studies suggest that bacteriophage activity can modulate bacterial community structure and influence inflammatory trajectories, although phage identification remains technically challenging due to scarce reference genomes and reduced precision of current detection tools (Shang et al. 2022). Given that chronic wounds are inherently polymicrobial, incorporating these non-bacterial domains into future diagnostic frameworks will be essential.

Advances in molecular diagnostics, including metagenomics and metatranscriptomics, have markedly expanded our ability to characterize wound microbiomes and their functional activity (Heravi et al. 2020, Lee et al. 2023, Zhao et al. 2023, Valsami et al. 2025). However, several translational barriers remain. First, DNA-based sequencing approaches provide limited insight into microbial viability and may capture signals from non-viable organisms or extracellular DNA, which can complicate clinical interpretation if not considered alongside clinical presentation and conventional microbiology (Acosta et al. 2023, Thiruppathy et al. 2025). Second, persistent methodological heterogeneity—spanning sampling approach (swab vs. biopsy), nucleic-acid extraction, sequencing depth, and downstream bioinformatic pipelines—reduces inter-study comparability and hampers the definition of clinically actionable thresholds for infection or dysbiosis (Qian et al. 2021, Halford et al. 2024). These limitations underscore the need for standardized diagnostic workflows and prospective validation before routine implementation.

The predominance of small, cross-sectional studies also restricts the field. Many investigations focus on diabetic foot ulcers, with fewer studies capturing venous or pressure ulcers, limiting the generalizability of microbial signatures across wound etiologies (Sachdeva et al. 2022, Ancira et al. 2025). Longitudinal models integrating microbiome data with clinical predictors—including wound volume, tissue perfusion, or smoking status—show promise for improving prognostication and personalizing treatment, but require validation in larger, more diverse cohorts (Ancira et al. 2025).

Therapeutically, emerging microbiome-focused strategies offer exciting opportunities. Probiotic strains and engineered commensal bacteria may modulate inflammatory responses, enhance chemokine signaling, and accelerate epithelial repair (Vågesjö et al. 2018, Li et al. 2023, Öhnstedt et al. 2023). Bacteriophages provide targeted antimicrobial activity even against multidrug-resistant pathogens and are capable of penetrating biofilms (Chen et al. 2022, Wang et al. 2024). Adjunctive treatments such as photodynamic therapy or nanomaterial-based dressings demonstrate potential for disrupting biofilms or modulating wound microenvironments (Naskar and Kim 2020, Sun et al. 2020, Pormohammad et al. 2021). However, despite encouraging preclinical and early clinical findings, consistent large-scale human data remain limited. Determining which microbial configurations predict response to specific therapies will be an important next step toward personalized care.

Overall, the evidence suggests that microbiome-informed wound management has the potential to transform clinical practice. Early detection of pathogenic or biofilm-dominant communities may support timely debridement, antimicrobial targeting, and risk stratification. Integrating multi-omics datasets with clinical indicators may offer greater diagnostic precision than traditional culture-based methods. The challenge ahead lies in translating these insights into standardized, accessible diagnostic tools and rigorously tested therapeutic strategies.

This review is narrative and may therefore be subject to selection bias. In addition, the heterogeneity of methodologies across included studies—particularly regarding sampling, sequencing, and outcome definitions—limits direct comparability and may influence the interpretability of summarized findings.

In summary, the wound microbiome represents both a promising biomarker and a therapeutic target in chronic wound management. Continued interdisciplinary collaboration, methodological harmonization, and high-quality longitudinal research will be essential to move microbiome-driven diagnostics and interventions toward clinical implementation.

Conclusions and clinical implications

The chronic wound microbiome plays an active and multifaceted role in modulating inflammation, tissue remodeling, biofilm formation, and susceptibility to complications such as osteomyelitis and limb amputation. Rather than serving as a passive indicator of wound status, microbial community structure appears to be a key determinant of healing outcomes.

Advances in metagenomics, metatranscriptomics, and other multi-omics platforms now enable detailed characterization of microbial communities and their functional activity. When integrated into clinical workflows, these tools have the potential to support personalized treatment strategies, including targeted antimicrobial therapy, biofilm-directed interventions, and microbiome-modulating approaches such as probiotics and phage therapy.

Clinically, the reviewed evidence suggests that microbiome profiling can support early identification of wounds at increased risk for chronicity, infection, or amputation, thereby complementing standard clinical assessment. Detecting biofilm-forming or antibiotic-resistant taxa may help refine decisions regarding antimicrobial therapy and procedural management, reducing the likelihood of treatment delays. Several microbiome-modulating strategies—including probiotics, phage therapy, and immunomodulatory approaches—have shown promise as adjuncts to standard care, particularly in stalled or refractory wounds. Moreover, integrating microbial signatures with immunological and clinical parameters may enable more accurate risk stratification and contribute to truly personalized therapeutic pathways. Translating these insights into routine clinical practice will require standardized sampling protocols, validated analytical frameworks, and robust clinical studies assessing the effectiveness, safety, and practicality of emerging microbiome-targeted interventions.

Author contributions

Bartosz Molasy (Conceptualization, Methodology, Resources, Software, Visualization, Writing – original draft), and Małgorzata Wrzosek (Formal analysis, Resources, Supervision, Writing – review & editing)

Conflicts of interest

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Data availability

This review paper contains no data generated by the authors. All data mentioned is available from the original source papers cited within the review.

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