Original Article

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Factors related to the composition and diversity of wound microbiota investigated using culture-independent molecular methods: a scoping review

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SUMMARY All open wounds are often colonized by commensal microbes as a loss of skin can provide a ready portal of entry for microorganisms. Although the wound microbiota is known to be associated with wound infection and with delayed healing, the factors related to the formations of wound microbiota contributing to such poor clinical outcomes are not clear and have not led to effective infection prevention interventions. This review aimed to scope the factors related to the composition and diversity of wound microbiota that have been investigated using culture-independent molecular methods. Original articles on wound microbiota published from January 1986 to February 2020 were included in this review. Thirty-one articles met the inclusion criteria and were grouped according to wound types: chronic, acute, and animal model wounds. The factors identified were categorized according to patient characteristics, wound characteristics, treatment, and sampling. Although some studies reported the effect size of the factors, the values were small. No studies elucidated the mechanism of wound microbiota formation. The results of this scoping review highlight that the factors associated with the diversity of wound microbiota are poorly understood and that further studies are needed.

Keywords microbiota, biodiversity, sequence analysis, whole genome sequencing, wound infection

1. Introduction

A wound involves an interruption to the structure and function of fundamental skin tissue (*1*). Wounds result from a variety of mechanisms, such as surgical intervention, injury, extrinsic factors, and underlying conditions, and they are often classified as a result of their underlying cause into acute wounds, such as surgical wounds and burns, and chronic wounds, such as leg ulcers, diabetic foot ulcers (DFUs), and pressure ulcers (*2*). Wounds are exposed to external bacteria from the skin defect, and bacteria colonized on the wound bed assemble into a microbiota. Wound microbiota causes wound infection, which increases financial burdens on patients and the healthcare system and consequently increases mortality (*3-5*). For the development of infection, three elements are necessary: infectious host, source of infection, and route of transmission. Thus, interventions to improve host immunity (*e.g.* nutritional management and treatment for the underlying disease), to reduce the bioburden on the wound bed (*e.g.* wound cleansing and debridement), and to break the route of transmission (*e.g.* using wound dressing and disinfection of peri-wound skin) have been implemented for patients with wounds (*6-9*). However, despite these preventative measures taken, wound infections continue, and new approaches to wound infection prevention are needed.

More than 100 trillion symbiotic microorganisms, including bacteria, archaea, viruses, and eukaryotic microbes, live on and within the human body (*10*), and ensuring wound sterility is not possible. In infection control, the culture method has been used to assess the bacterial bioburden; however, this method underestimates the bacterial bioburden in a wound because most microorganisms circulating in the environment are not easily cultured (*11*). Given this, a growing number of studies have investigated wound

microbiota using culture-independent methods. Those previous studies have reported that wound microbiota is associated both with wound healing and wound infection (*12,13*). However, the factors related to the formations of wound microbiota contributing to such poor clinical outcomes are not clear. Thus, effective interventions targeting wound microbiota have also not been established. This scoping review aimed to identify which factors are related to the composition and diversity of wound microbiota in studies that used culture-independent molecular methods. A better understanding of the factors related to the diversity and composition of wound microbiota may lead to innovative preventive wound infection strategies, such as intervening in those factors to inhibit an adverse wound microbiota formation or alter it in a more positive direction. Furthermore, the results of this review are likely to be useful for researchers who study wound microbiota in helping to determine the direction of future research.

2. Materials and Methods

2.1. Protocol and registration

A review protocol has not been published. We used the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews checklist to guide this review (*14*).

2.2. Eligibility criteria

For this study, culture-independent molecular methods were defined as methods for identifying microbiota based on direct analysis of DNA without any culturing step. We limited our search to research articles published from January 1, 1986, to February 17, 2020 because the earliest device using a culture-independent molecular method was developed in 1986 (*15*). The languages of publications were restricted to English and Japanese. We included studies that involved participants of any age who had been described as having wounds in any setting, including acute care, aged care, and at home. Studies using animal wound models were also included because such experiments are required to identify the function of the researched microbiota. We included original research in this scoping review. Literature reviews, meta-analysis, practice guidelines, editorials, case studies, letters, conference notes/abstracts, posters, and oral presentations were excluded.

2.3. Information sources

To identify potentially relevant documents, the following bibliographic databases were searched: PubMed and the Cumulative Index to Nursing & Allied Health Literature. Additionally, we searched the Japan Medical Abstracts Society (JAMAS) database to collect articles in Japanese. The search strategies were developed through team discussion. The final search results were exported to Mendeley and duplicates were removed prior to screening by two researchers.

2.4. Search

The search was performed using a combination of search terms, including "burns" OR "open fractures " OR "lacerations" OR "surgical wound" OR "penetrating wounds" OR "abrasion" OR "pressure ulcer" OR "pressure injury" OR "leg ulcer" OR "diabetic foot" OR "varicose ulcer" OR "traumatic wound" OR "acute wound" OR "chronic wound" AND "microbiota" OR "microbiome". In the JAMAS database, we used the same combination of keywords in Japanese.

2.5. Selection of sources of evidence

Potentially relevant literature was imported into Rayyan for screening (*16*). Titles and abstracts were screened by two researchers (MK and YK) independently, and those that clearly did not fit the inclusion criteria were excluded. Potentially eligible full-text articles were screened for inclusion by two independent reviewers (MK and YK) according to the inclusion criteria. Disagreements on study selection were resolved through discussion.

2.6. Data charting process

Table S1 (online data: *http://www.ddtjournal.com/action/getSupplementalData.php?ID=73*) provides an overview of all included manuscripts. A data-charting form was developed by one author (MK) to determine which variables to extract. The form captured the relevant information concerning a study's characteristics and the specific factors found to be related to the composition and diversity of wound microbiota. To assess the factors found to be related, the composition and diversity of wound microbiota were evaluated based on the relative abundance of bacteria and according to the index of alpha and beta diversity, respectively. Alpha diversity is species richness within a single microbiota and Beta diversity shows the differences in the microbiota between different environments (*17*). Data were extracted by a single author (MK) and verified by co-authors (YK and GN). Discrepancies in the extracted data were resolved through discussion between the three authors.

2.7. Data items

We extracted the following data from eligible literature identified in our search: the study attributes: author (s), publication year, country, and title; study objectives; study design; study population: sample size, human

or animal, wound type, and sample type; the method used to obtain the microbiota data: analysis techniques, sequencer, and the region of bacterial DNA; outcome measures: alpha and beta diversity indices; factors related to the composition and diversity of wound microbiota.

2.8. Synthesis of results

We grouped the studies according to wound types and presented them in three tables: chronic wounds (Table S2) (online data: *http://www.ddtjournal.com/action/getSupplementalData.php?ID=73*), acute wounds (Table 1), and animal models (Table 2). The factors related to the composition and diversity of wound microbiota were summarized for each study along with the study attributes, objective, study design, populations, and analysis techniques. Where we identified a study that had investigated the effect on wound microbiota diversity in relation to certain factors, we summarized the indicators of the effect size.

3. Results

3.1. Selection of the sources of evidence

Figure 1 shows a flow chart of the study selection process. We identified 792 records through the database searches. Following de-duplication, 743 titles and abstracts were screened for eligibility. Of these, 53 studies were retained for full-text screening and 22 failed to meet the inclusion criteria. Of the excluded full texts, 12 studies (54.5%) did not investigate factors related to the composition and diversity of wound microbiota. Five studies (22.7%) did not meet the inclusion criteria in relation to publication type. Three studies (13.6%) investigated microbiome samples collected from non-wound sites. One study (4.5%) did not use culture-independent molecular methods. One study (4.5%) investigated certain factors but found no significant differences between the groups. The remaining 31 studies met our eligibility criteria.

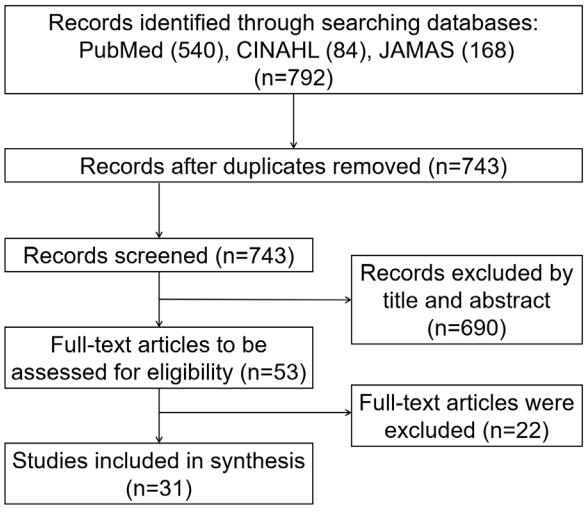

Figure 1. Flow diagram of the study selection process. CINAHL, the Cumulative Index to Nursing & Allied Health Literature; JAMAS, the Japan Medical Abstracts Society database.

3.2. Characteristics of the sources of evidence

The characteristics of the included studies are presented in Tables (Table S2 (online data: *http://www.ddtjournal.com/action/getSupplementalData.php?ID=73*), Table 1, and Table 2), along with data relevant to the scoping review question. All the studies were written in English and published between 2008 and 2020, and no Japanese papers were included.

3.3. Synthesis of the results of studies on chronic wounds

We included 25 studies (Table S2, online data: *http://www.ddtjournal.com/action/getSupplementalData.php?ID=73*): 13 from the United States; three each from the United Kingdom and Australia; two studies from India, and one each from Korea, Denmark, China, and Canada. All the included studies except one were prospective cohort studies (58%) or cross-sectional studies (38%).

In the 10 studies investigating patient characteristics, diabetes mellitus was investigated as a factor related to microbial diversity in wounds in seven studies. Indicators used included a diagnosis of diabetes mellitus (*18,19*), hemoglobin A1c (HbA1c) levels (*11,20-23*), and the duration of diabetes mellitus (*20*). Among them, both HbA1c levels and the duration of a patient's diabetes mellitus correlated with the alpha diversity index (dominance, $p = 0.0174$; diversity, $p = 0.0168$, the correlation coefficient was not shown) (*20*). Bacteria shown to be associated with indicators of diabetes mellitus included *Streptococcaceae* (*18*), *Curvibacter* sp. (*19*), *Bacteroidetes*, *Peptoniphilus*, *Streptococcus* (*22*), and *Streptococcus* species (*21*). Followed diabetes mellitus, sex was the second most commonly reported factor, which was considered in three studies. The dominant bacteria in the female samples included *Clostridiales* (*24*), *Burkholderia*, and *Proteus* (*25*). *Actinomycetale*s was dominant in the males' wound samples (*24*). Moreover, analysis using the beta diversity index showed that different wound microbiota formed in males and females (*23*). Age was considered in two studies. The wounds of patients aged < 65 years contained more bacteria types than patients aged > 65 years and the dominant bacterium was also different (< 65 years, *Clostridiales*; > 65 years, *Actinomycetales*) (*24*). Analysis using permutational multivariate analysis of variance showed statistical significance for age ($R^2 = 0.0454$, $p = 0.0001$) (*23*). Other factors identified

Table 1. Details on studies of acute wounds included in the review

Study	Design	Sample size	Sample type	Wound type	Analysis techniques	Region of DNA or primer	Alpha diversity index	Beta diversity index	Factors related to microbiota (diversity result)
Hannigan *et al*., (2014) (*43*)	Prospective	74 samples (30 patients)	Swab	Open fractures	16S rRNA sequencing by MiSeq	V4	PD, Observed OTUs	Bray-Curtis	Injury mechanism, location, severity, complication
Romano-Bertrand *et al*., (2015) (*45*)	Prospective	25 patients	Swab	Incision sites	TTGE	27F, 1492R	N/A	N/A	Antisepsis
Bartow-McKenney *et al*., (2018) (*44*)	Prospective	208 samples (52 patients)	Swab	Open fractures	16S rRNA sequencing	V1-V3	PD	Weighted UniFrac	Injury mechanism, sampling time-points

TTGE, temporal temperature gel electrophoresis; PD, Faith's phylogenetic diversity index; OTU, operational taxonomic unit; rRNA, ribosomal RNA.

Table 2. Details on animal studies included in the review

Study	Design	Sample size	Sample type	Wound type	Analysis techniques	Region of DNA or primer	Alpha diversity index	Beta diversity index	Factors related to microbiota (diversity result)
Grice *et al*., (2010) (*46*)	Animal experiments	20 mice	Swab	6-mm full thickness excisional wound	16S rRNA sequencing by ABI 3730xl sequencer	27F, 1492R	Shannon	N/A	Sampling time-points, DM
Kim *et al*., (2019) (*47*)	Animal experiments	37 samples (37 mice)	Swab	7-mm full thickness skin excision wound	16S rRNA sequencing by MiSeq	ITS-1507F, ITS-23SR, 129F, 1492R	Shannon	N/A	Level of oxidative stress
Sanjar *et al*., (2019) (*48*)	Animal experiments	24 samples (24 rats)	Biopsy	Deep partial-thickness burn	16S rRNA sequencing by MiSeq	V3-V4	Chao1, Shannon, PD, Simpson, Observed OTUs	Bray–Curtis, Unweighted UniFrac	Sampling time-points

DM, diabetes mellitus; OTU, operational taxonomic unit; PD, Faith's phylogenetic diversity index; rRNA, ribosomal RNA.

included serum C-reactive protein levels (*21*), white blood cell counts (*21*), autoimmune disease (*26*), disease course (*24*), and end-stage renal disease (*22*), all of which were reported in a single study. However, in one large study of 2,963 chronic wounds, no differences were found in microbial diversity for sex, age, ethnicity, and the presence of diabetes mellitus (*27*).

In total, 17 studies compared wound characteristics. The factor reported in most studies was the healing outcome (9/17). Wounds were classified based on whether a patient's wound had healed at 6 weeks (*28*), 7 weeks (*29*), 8 weeks (*30*), 12 weeks (*21,31,32*), and 6 months (*33*); whether the wound area had been found to have enlarged at the next visit (*26*), and whether there was a 50% wound size reduction after 4 weeks (*23*). In non-healing wounds, *Anaerococcus* (*29*), *Actinomycetales* (*28*), *Bacteroidales* (*28,32*), *Pseudomonas* (*26*), and *Ascomycota* (*30*) were reported as the dominant bacteria. Furthermore, *Staphylococcus aureus* 10757 was found only in non-healing wounds (*31*), whereas *Staphylococcus* (*29*), *Gammaproteobacteria*, and *Pseudomonadacea* (*28*) were reported as the dominant bacteria in healing wounds. Regarding the index of diversity, beta biodiversity was significantly lower within the healing wounds than within the non-healing wounds (*32*). Non-healing wounds were less likely to transition away to other clusters of microbiota compared with healing wounds (*21,33*). Additionally, a traditional linear model showed a negative association between healing time and changes in microbiota between baseline and the next follow-up visit (2 weeks' time) ($R^2 = 0.16$, $p < 0.0001$) (*21*). Wound duration was the second most frequently reported factor in relation to wound characteristics, which was reported in four studies. Beta diversity was shown to significantly differ in terms of wound duration (*23*). Longer duration wounds were associated with a greater relative abundance of *Proteobacteria* (*34,35*), and shorter duration wounds were associated with a greater relative abundance of *Firmicutes* (*34*). Furthermore, wound duration was negatively correlated with a relative abundance of *Staphylococcus* ($\rho = –0.30$; $p = 0.0333$), and positively correlated with the number of operational taxonomic units (OTUs) ($\rho = 0.41$; $p = 0.0022$), the Shannon diversity index ($\rho = 0.32$; $p = 0.020$), and a relative abundance of *Proteobacteria* ($\rho = 0.38$; $p = 0.0059$) (*11*). Additionally, higher frequencies of DFUs containing obligate anaerobes were correlated with a longer duration ($p = 0.03$), but the correlation coefficient was not shown (*34*). Ulcer depth and wound infection were reported in three studies, respectively. Ulcer depth was negatively associated with a relative abundance of *Staphylococcus* ($\rho = –0.47$; $p = 0.0005$) and positively associated with a relative abundance of anaerobic bacteria ($\rho = 0.33$; $p = 0.0182$) (*11*), as well as anaerobe levels (*21*). In addition, a study using whole shotgun metagenome sequencing shows differences in microbiota function by ulcer depth (*31*). Regarding wound infection, samples from infected wounds exhibited lower microbial diversity than those from uninfected wounds (*33*), and diversity depended on the severity of the infection (*34*). However, the mycobiome diversity in specimens of an infection-related complication was significantly higher (*30*). Surface area, tissue oxygenation, wound location, and ulcer severity was reported in two studies, respectively. In terms of DFU surface areas, a weak but significant positive correlation was found with OTU richness ($\rho = 0.27$; $p = 0.051$) (*11*). For tissue oxygenation, it was correlated with alpha diversity (the number of observed OTUs, $\rho = –0.258$, $p = 0.046$; phylogenetic distance, $\rho = –0.295$, $p = 0.022$, respectively) (*30*) and microbial functional profiles (*31*). In terms of ulcer location, differences in alpha diversity were reported between DFUs on the forefoot and the hindfoot (*30*) and between non-healing wounds located on a foot or leg and others (*19*), respectively. The severity of ulcers was assessed using the Wagner classification in both studies. *Firmicutes* in tissue samples were more abundant in the grade 0-2 group whereas *Bacteroidetes*, *Prevotella*, *Peptoniphilus*, *Porphyromonas*, and *Dialister* were more abundant in the grade 3-5 group (*22*). The DFUs in the grade 5 group were relatively more diverse than in the other wound grades (*25*). Other factors included recurrent ulcers (*20*), metabolite concentrations in wounds (*36*), and slough (*34*), all of which were reported in a single study. In particular, metabolite concentrations strongly correlated with a relative abundance of bacteria ($\rho > 0.700$, $p < 0.05$) (*36*).

Treatment interventions altered chronic wound microbiota. Four studies reported on antibiotic therapy. Antibiotic-treated and untreated wound microbiota had significantly different composition (*18,19*) and alpha diversity (*30*). Multiple logistic regression showed that antibiotic use was associated with a 41% reduction in risk of *Streptococcus* colonization ($p = 0.009$, the odds ratio was not shown) (*19*). Furthermore, both complications and antibiotics use contributed to bacterial community disruption, although the larger effect was noted for antibiotics use (*21*). Additionally, the composition of the microbiota altered in treatments other than antibiotic therapy. A modification of the wound microbiota was observed in samples following an angioplasty procedure (*37*), debridement (*31,37*), and in the use of cadexomer iodine (*i.e.* antiseptics) (*38*) and traditional Chinese medicine (*24*).

The sampling region and sampling time-points were reported as factors associated with sampling in three studies (*36,39,40*) and one study (*41*), respectively. Although the microbiota in separate samples collected from the same wound differed in diversity (*40*), the microbiota in samples collected from different sites within the same wound (*39*), from the superior and inferior sections of the wound (*36*), and at different time points (*41*) were similar. Most variations between

the samples could be explained in terms of the individuals involved (*23*), although one study reported a high level of dissimilarity within individuals (*30*). Additionally, one study reported that different wound types demonstrated different microbial composition and diversity (*42*), whereas another study found no differences in terms of wound types and patient characteristics (*27*).

3.4. Synthesis of the results of studies on acute wounds

Three prospective cohort studies investigated acute wounds (Table 1); two studies from the United States (*43,44*) and one from France (*45*). Factors regarding the wound characteristics, treatment, and sampling were reported.

Two studies (*43,44*) investigated open fracture wounds. The change in the relative abundance of *Corynebacterium* and unclassified *Enterobacteriaceae* between the first and second visit time points was significantly different for penetrating and blunt wounds ($p = 0.006$ and $p = 0.038$, respectively). Besides, location, severity, and complications have been reported as factors associated with diversity (*43*). Moreover, significant differences in microbial communities were found according to the mechanism of injury ($p < 0.05$), and the wound microbiota in penetrating wounds was more similar to the adjacent skin microbiota over time. (*44*). The third study investigated surgical wounds. Antisepsis was considered as a factor and the trend of microbiota dynamics in wounds observed after antisepsis showed a decrease of *Firmicutes* and *Actinobacteria* and an increase of *Proteobacteria* (*45*).

3.5. Synthesis of the results of studies on acute wounds

Three studies used animals for investigating wound microbiota (Table 2) and all three studies had been performed by groups of researchers in the United States (*46-48*). Factors regarding patient characteristics (*i.e.*, animal characteristics), wound characteristics, and sampling were reported.

In one study, a 6-mm full-thickness excisional wound was created in mice with Leprdb mutations (db/db) and age-matched nondiabetic heterozygous littermates (db/+) (*46*). After wounding on day 3, both db/db and db/+ wounds showed a significant decrease in diversity as compared with day 1 ($p = 1.1 \times 10^{-4}$ and 0.024, respectively). The OTU diversity of the db/+ wounds was significantly greater than the db/db on day 7 ($p = 0.026$). In another study, a 7-mm full-thickness skin excision wound was created in db/db$^{-/-}$ mice, and higher doses of antioxidant inhibitors were applied to create increasing levels of oxidative stress (*47*). The level of oxidative stress significantly contributed to a difference in the Shannon diversity index ($p < 0.0001$). Diversity across time was also significantly related ($p = 0.0198$). The third study used a mouse burn model (*48*). A deep partial-thickness burn was created in mice comprising a 10% burn of the total body surface area. In the burn wound phyla profile, the abundance of *Actinobacteria* increased from 4.86% on post-wounding day 1 to 22.9% on post-wounding day 11, whereas the abundance of *Proteobacteria* declined from 39.8% on post-wounding day 1 to 16.8% on post-wounding day 11.

4. Discussion

We conducted a scoping review to investigate wound microbiota in studies using culture-independent molecular methods. We included 31 papers, and consequently the factors obtained through the review were categorized in terms of patient characteristics, wound characteristics, treatment, and sampling.

To our knowledge, this scoping review is the first to summarize the factors related to microbiota across chronic and chronic wounds. Previous reviews focusing on wound microbiota have mainly targeted chronic wounds (*12,13*). In contrast, this review was not limited to chronic wounds, but also included studies on acute wounds and animal experimental studies. This approach allowed for a clearer picture of the current state of microbiota research on wounds to emerge. First, there is a paucity of studies investigating factors related to acute wound microbiota, and only three were included in this review. Future studies are needed to investigate the microbiota of acute wounds for more effective treatment. Second, it was found that there were factors related to the diversity of wound microbiota that were common in chronic wounds, acute wounds, and wounds created in animals. Sampling time-points were the factor obtained in all the wound group types and wound microbiota changes during the healing process. Furthermore, a difference in microbiota diversity was observed between animals with diabetes mellitus and controls, similar to the results of the clinical studies. Based on the results revealed in the clinical data, further research is required to elucidate phenomena occurring within wound microbiota (*e.g.*, mechanisms of microbiota formation and host interactions) using animal models.

This scoping review showed that factors related to the composition and diversity of wound microbiota could be categorized into the patient and wound characteristics, treatment, and sampling. Diabetes mellitus, autoimmune disease, and end-stage renal disease are known as factors associated with a patient's immunity (*49,50*). Factors of wound characteristics, such as wound area and depth, are related to the bacterial bioburden and the treatment factor included the removal of bacteria in the wound, such as through antimicrobial treatment and debridement. These results suggest that current research on wound microbiota is largely focused on the infectious host or the source of infection, with little focus on the route of infection.

Therefore, future investigations of wound microbiota should investigate the relevant factors according to the route of infection.

Several studies reported the impact of factors on wound microbiota diversity. However, these studies did not report indicators in relation to the effect size (*e.g.*, correlation coefficients and odds ratios) or showed limited values, and a strong correlation was only found concerning metabolite concentrations. Given this situation, it is likely that the relevant factors associated with the diversity of the wound microbiota have not been fully investigated. Also, many of the studies included in this review were cross-sectional, and the causal relationship between factors and the diversity of microbiota could not be determined. For example, it is unclear whether metabolite concentrations increased due to the production of bacteria or whether the presence of metabolites facilitated the growth of specific bacteria. Thus, further research is needed to investigate the causal relationship between the identified factors and microbiota diversity, as well as the varying specific effects of these factors.

For this scoping review, we used a database containing articles in Japanese. However, no documents in Japanese were included for full-text screening. Healthcare systems differ globally, and it is also possible that relevant factors in relation to patients' backgrounds and the causes of wound development may differ between countries or ethnicities. Thus, more studies are needed in Japan to investigate the microbiota of wounds as well as comparative studies across countries.

Our scoping review had some limitations. The quality of the studies identified was not assessed systematically in this scoping literature review. Furthermore, the studies frequently had small sample sizes or did not describe the wound type in detail. However, the healing process differs depending on the depth of the wound, that is, whether there has been partial or full thickness loss of dermal tissue. However, no studies specified wound depth, and the influence of different factors within the same or differing wound depths was not clear. This review also included studies that used different methods and devices to identify microbiota. Additionally, even if the same next-generation sequencer had been used across some studies, the target region may have differed. Caution should be exercised in comparing the results obtained using these different methods.

In conclusion, our scoping review that factors related to the diversity and composition of wound microbiota included patient characteristics, wound characteristics, treatment, and sampling. Further research is needed to implement these results in wound infection prevention.

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