Brief Report

DOI: 10.5582/ddt.2026.01030

Associations between wound exudate amino acid profiles and microbial dissimilarity in wound and peri-wound skin in healing wounds

Mao Kunimitsu[1], Makoto Tsunoda[2], Tomoko Akase[3], Mikio Kinoshita[4], Gojiro Nakagami[1,5,*]

[1] Department of Gerontological Nursing/Wound Care Management, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;
[2] International Christian University, Tokyo, Japan;
[3] Department of Biological Science and Nursing, Graduate School of Medicine, Yokohama City University, Kanagawa, Japan;
[4] TOWN Home Care Clinic, Tokyo, Japan;
[5] Global Nursing Research Center, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

SUMMARY: Dysbiotic wound microbiota, which is dissimilar to that of the peri-wound skin, delays healing in hard-to-heal wounds. However, rapid and practical methods for detecting wound dysbiosis are lacking and the characteristics of the wound environment in which dysbiosis occurs remain unclear. Consequently, microbiota-targeted care to prevent delayed healing has not yet been established. This study investigated the association between amino acid profiles in wound exudates and the degree of microbial dissimilarity between the wound and peri-wound skin microbiota. Nine wounds from eight patients receiving home care were analyzed. The concentrations of 18 amino acids were measured using high-performance liquid chromatography, and the microbiota were detected using 16S rRNA gene amplicon sequencing. The microbial dissimilarity was assessed using the weighted UniFrac dissimilarity index, and correlations between the relative abundances of amino acids, amino acid ratios, and microbial dissimilarities were evaluated using Spearman's rank correlation. Most of the wounds were in the healing phase. The relative abundance of arginine showed a strong correlation with microbial dissimilarity ($\rho = -0.80$, $p = 0.01$). Additionally, eight amino acid ratios (arginine/asparagine and arginine/tyrosine) were significantly correlated with microbial dissimilarity. These findings support the development of point-of-care tools for assessing wound microbiota and improving wound management.

Keywords: Commensal skin microbiota, wound microbiota, hard-to-heal wounds, wound fluid, wound healing

1. Introduction

Wound infection is characterized by bacterial proliferation within the wound bed, resulting in tissue damage and disruption of the healing process (*1*). Such infections impose a significant economic burden on healthcare systems, reduce patient quality of life (*2*), and increase the risk of mortality (*3*). The relationship between the host and bacteria in wounds is continuous and progresses through various stages of microbial presence, from contamination to colonization, local infection (covert and overt), and eventually to spreading and systemic infection (*4*). In particular, covert local wound infections are difficult to detect, as delayed healing often occurs before the appearance of overt signs and symptoms, resulting in delayed initiation of appropriate treatment. Therefore, early prevention of covert local wound infections is essential to promote timely wound healing.

The wound microbiota composition is associated with wound healing outcomes (*5*). In particular, dysbiosis, defined as the formation of wound microbiota that differs markedly from the patient's own peri-wound skin commensal microbiota, has been implicated in the development of covert local wound infections (*6*). Excessive inflammation and delayed wound healing have been observed in animal models that mimic dysbiotic wound microbiota without overt signs of infection. Furthermore, compared to wounds colonized by commensal skin microbiota, these models showed a reduced number of Forkhead box P3-positive cells, a marker of regulatory T cells (Tregs) with anti-inflammatory functions (*7*). Conversely, even when commensal skin microbiota are colonized, delayed healing is observed if Treg induction is suppressed (*8*). These findings suggest that dysbiosis induces covert

local wound infections by impairing Treg-mediated immune tolerance. Accordingly, the wound and peri-wound skin microbiota must be assessed in daily care, and interventions aimed at preventing or correcting dysbiosis must be implemented based on the assessment results. However, next-generation sequencing (NGS), which is commonly used to identify microbiota, is time-consuming and costly, making it impractical as a point-of-care diagnostic tool. Moreover, the characteristics of the wound environment during dysbiosis remain unclear.

Host-related factors, such as immune status, underlying diseases, and local wound characteristics, play major roles in the formation of wound microbiota (*9*). In the present study, we focused on amino acids in wound exudates as key components of the wound environment. Amino acids serve as essential nutrients for bacterial growth (*10*) and are known to vary according to wound conditions (*11-13*). Therefore, the amino acid profile of wound exudate may be associated with the composition of the wound microbiota. In the present study, we tested this hypothesis using wound exudates and microbiota samples obtained from patients with hard-to-heal wounds. Specifically, amino acid profiles were analyzed using high-performance liquid chromatography (HPLC), and the wound and peri-wound skin microbiota were identified using NGS. We then investigated the association between amino acid profiles and microbial dissimilarity in the wound and peri-wound skin in detail.

2. Materials and Methods

2.1. Ethical considerations

The study protocol was approved by the ethics committee of the Graduate School of Medicine, The University of Tokyo (Approval No. 2022182NI-(3)) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their enrollment in the study.

2.2. Study design and participants

This prospective cohort study was conducted between January and March 2023. Participants were recruited from a home care clinic specializing in wound management that provides services in the Kanagawa and Tokyo prefectures, Japan. The inclusion criteria were as follows: individuals aged 20 years or above; those with hard-to-heal wounds extending into the subcutaneous tissue; and patients who received home care from the clinic and were followed up with a second visit 2 weeks later. Patients whose wounds were deemed difficult to swab according to the attending physician were excluded. Following baseline data collection, patients were followed-up until their next scheduled visit to assess their wound healing status. A complete enumeration method was used, which included all patients with hard-to-heal wounds who underwent home visits to the clinic during the study period.

2.3. Sample collection

To assess the microbiota, samples were collected by a trained wound researcher using flocked swabs (Puritan, Guilford, ME, USA) pre-soaked in sterilized saline containing 0.1% Tween-20 (Nacalai Tesque, Kyoto, Japan). To minimize contamination, both the wound bed and peri-wound skin were cleansed with a skin cleanser before sampling. Wound microbiota samples were obtained by swabbing a 1 cm^2 area at the center of the wound bed using Levine's technique (*14*). Peri-wound skin microbiota samples were collected from the surrounding skin on the cranial side of the wound (the area not covered by the wound dressing) using the Z-stroke technique, with the area swabbed twice for consistency (*15*). All swab samples were stored at −80°C until DNA extraction to preserve microbial integrity.

For amino acid analysis of the wound exudate, additional wound swab samples were collected using the same Levine technique, with flocked swabs soaked in 80 μL of sterilized saline (*16*). Exudate was extracted by centrifuging the swabs at 3,000× *g* for 1 min, and the supernatant was stored at −80°C until analysis.

2.4. Data collection

Wound healing status was evaluated by comparing the total DESIGN scores at baseline and follow-up (*17*). Wounds were classified as "deteriorated" if the DESIGN total score remained unchanged or increased, indicating worsening of the wound, and as "healing" if the total score decreased compared to baseline. Trained nurses independently evaluated the scores.

Wound images were captured using a digital camera (RX100II; Sony Corporation, Tokyo, Japan) combined with a color calibration chart (CASMATCH; Bear Medic Co., Tokyo, Japan) to ensure standardized image analysis. Patient demographic data (age, sex, physical function level, and underlying diseases) and wound-related data (type, location, duration, and treatment) were extracted from medical records.

2.5. Microbiome analysis

Bacterial DNA was extracted from swab samples using the QIAamp DNA Mini Kit (Qiagen N. V., Venlo, Netherlands) following previously described methods (*18*). The 16S rRNA gene amplicon sequencing was performed to characterize the wound and peri-wound skin microbiota. Polymerase chain reaction

amplification was conducted using a 16S Barcoding Kit 1-24 [SQK-16S024; Oxford Nanopore Technologies (ONT), Oxford, UK] targeting the near-full-length bacterial 16S rRNA gene. Sequencing was performed using a MinION portable sequencer (ONT) equipped with an FLO-MIN106D flow cell (ONT). Basecalling was conducted using the EPI2ME online platform (ONT), and microbial diversity analysis was performed using QIIME 2.

Microbiota composition was evaluated based on relative abundance, representing the proportion of each bacterium within the sample. To assess the microbial dissimilarity between the wound and peri-wound skin of the same individual, the weighted UniFrac dissimilarity index was calculated. This index ranged from 0 to 1, with higher values indicating greater dissimilarity in the microbiota.

2.6. Amino acid analysis

In this study, 18 amino acids were examined in the wound exudate. Sample preparation and HPLC analysis were performed according to previously described methods (*19*). The relative abundance of amino acids, defined as the ratio of their concentration to the total amino acid content, was calculated to evaluate the amino acid composition. In addition, the ratio of concentrations (amino acid ratio) was calculated for all combinations of the 18 amino acids.

2.7. Statistical analysis

Continuous variables are presented as median [interquartile range (IQR)], and categorical variables are presented as the number of cases (%). The Spearman's rank correlation coefficient was used to evaluate the association between the weighted UniFrac dissimilarity index in the wound and peri-wound skin microbiota and the relative abundance of individual amino acids and their ratios. To evaluate the influence of the repeated observation from one wound, a sensitivity analysis was performed by excluding one of the repeated measurements and recalculating the Spearman correlation coefficients using one observation per wound. All statistical analyses were performed using the EZR software (*20*). Statistical significance was set at $p < 0.05$.

3. Results and Discussion

Eight patients (eight wounds) were included in this study. Among the eight wounds, one wound was sampled at two time points separated by two weeks. Consequently, the analysis included nine observations derived from eight wounds. Patient and wound characteristics are summarized in Table 1. The median age of the participants was 74.5 years, and the median wound duration was 10 months. The DESIGN scores at baseline and two weeks later are shown in Table 2. The median change in the total DESIGN score over 2 weeks was −3 points (IQR: −4–−3). In eight out of nine cases (88.9%), the score decreased, and in the remaining case, the score remained unchanged. Thus, this cohort predominantly comprised wounds in the process of healing.

The composition of the wound and peri-wound skin microbiota is shown in Figure 1. The bacterial genus with the highest median relative abundance in the wound samples was *Staphylococcus* [median 2.8% (IQR: 0.7–64.4)]. In the peri-wound skin, *Staphylococcus* [median 36.6% (IQR: 6.7–61.5)] was also the predominant bacterial genus. The median weighted UniFrac dissimilarity index in the wound and peri-wound skin was 0.27 (IQR: 0.22–0.35). Similar findings have been reported in a previous study of pressure injuries showing a healing trend, in which both the weighted UniFrac dissimilarity index and the predominant bacterial genera were comparable to those observed in the present study (*6*). Together, these findings suggest that the microbiota observed in this cohort may represent a wound environment undergoing healing rather than dysbiosis.

Table 1. Characteristics of participants and wounds (n = 8)

	n or median	% or IQR
Age	74.5	(68.8–80.0)
Sex (male)	4	(50.0)
Degree of independence		
Rank A (Independent, but may show occasional forgetfulness)	2	(25.0)
Rank B (Needs supervision or help with some daily tasks)	3	(37.5)
Rank C (Completely dependent on others for daily care)	3	(37.5)
Disease		
Cardiovascular diseases	4	(50.0)
Endocrine disorders	5	(62.5)
Nervous diseases	2	(25.0)
Gastrointestinal diseases	5	(62.5)
Respiratory diseases	2	(25.0)
Braden scale	18.5	(10.0–20.8)
Treatment		
Steroid drug	3	(37.5)
Dialysis	2	(25.0)
Wound area		
Sacrum	2	(25.0)
Lower leg	2	(25.0)
Toe	2	(25.0)
Foot	1	(12.5)
Ischium	1	(12.5)
Wound type		
Pressure injury	3	(37.5)
Amputation wound	2	(25.0)
Venous leg ulcer	1	(12.5)
Arterial ulcer	1	(12.5)
Skin ulcer in rheumatoid arthritis	1	(12.5)
Duration (month)	10	(4.5–18.0)

IQR, interquartile range.

Correlation coefficients between the relative abundance of each amino acid and microbial dissimilarity were calculated (Figure 2A). Among the 18 amino acids, arginine showed a significant strong negative correlation ($\rho = -0.80$, $p = 0.01$). A higher relative abundance of arginine in the wound exudate was associated with increased microbial similarity between the wound and peri-wound skin. The biological significance of this association is supported by previous reports demonstrating that higher arginine concentrations are associated with skin microbiota profiles more closely resembling those of healthy individuals, even in patients with atopic dermatitis, in whom the pathogenic bacterium *Staphylococcus aureus* typically predominates (*21*). Furthermore, in non-inflammatory healthy skin, microbial composition has been linked to metabolites in the arginine biosynthesis pathway, suggesting that bacterial-derived arginine contributes to skin health (*22*). On the other hand, in infected chronic wounds, the concentrations of citrulline, ornithine, and arginase in the wound exudate were significantly higher than those in non-infected chronic wounds, despite the lack of a significant difference in arginine levels (*12*). Therefore, identifying biomarkers of microbial dissimilarity in infected wounds may require the examination of arginine as well as arginine metabolism.

Correlations between the ratios of all possible pairs among the 18 amino acids and the weighted UniFrac dissimilarity index were examined (Figure 2B). Significant correlations with microbial dissimilarity were observed for the following amino acid ratios (median [IQR]): arginine/histidine [$\rho = -0.76$, $p = 0.04$;

Table 2. DESIGN score (n = 9)

Components	Score	Baseline	Two weeks later
Depth	D3	5 (55.6)	5 (55.6)
	D4	4 (44.4)	4 (44.4)
Exudate	e1	0 (0.0)	2 (22.2)
	e2	9 (100.0)	7 (77.8)
Size	s1	3 (33.3)	3 (33.3)
	s2	2 (22.2)	2 (22.2)
	s3	1 (11.1)	2 (22.2)
	s4	1 (11.1)	1 (11.1)
	s5	1 (11.1)	0 (0.0)
	S6	1 (11.1)	1 (11.1)
Infection/inflammation	i0	2 (22.2)	5 (55.6)
	i1	4 (44.4)	4 (44.4)
	I2	3 (33.3)	0 (0.0)
Granulation	g1	0 (0.0)	5 (55.6)
	g2	4 (44.4)	4 (44.4)
	G3	3 (33.3)	0 (0.0)
	G4	2 (22.2)	0 (0.0)
Necrotic tissue	n0	0 (0.00)	3 (33.3)
	N1	8 (88.9)	6 (66.7)
	N2	1 (11.1)	0 (0.0)
Pocket	p0	9 (100.0)	9 (100.0)
Total score		9 (8–13)	7 (6–8)

The number of wounds (%) per score is shown for each component of the DESIGN tool. The total score is also shown as the median (interquartile range) at each time point. Of the eight subjects, one patient (one wound) could be followed a total of two times every 2 weeks.

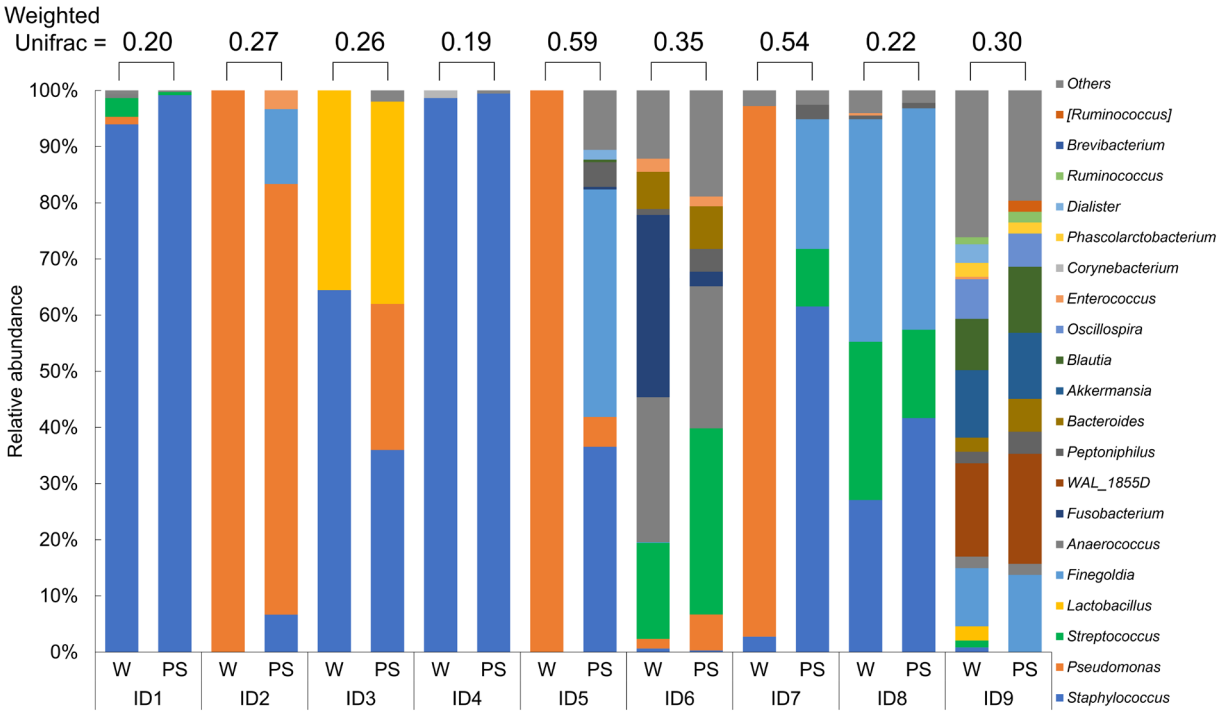
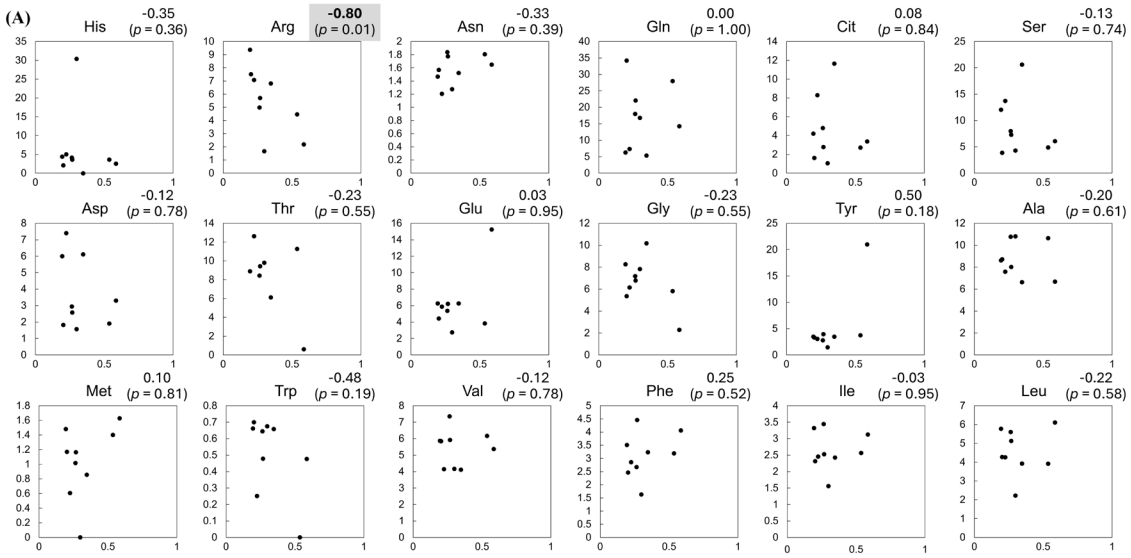


Figure 1. Wound and peri-wound skin microbiota composition. The top 20 bacterial genera present in the microbiota are shown. Bacterial genera with lower relative abundances were grouped under the category "Others." The relative abundance is represented in the vertical axis. Weighted UniFrac dissimilarity index values are shown at the top of the graph. W, wound samples; PS, peri-wound skin samples.



(B)

ρ	His	Arg	Asn	Gln	Cit	Ser	Asp	Thr	Glu	Gly	Tyr	Ala	Met	Trp	Val	Phe	Ile	Leu
His	-	**-0.76**	0.19	0.14	-0.17	-0.43	-0.50	-0.31	-0.10	-0.62	0.21	0.14	0.17	-0.31	0.10	0.29	0.00	0.55
Arg	0.43	-	**0.83**	0.57	0.60	0.37	0.32	-0.05	**0.72**	0.55	**0.85**	0.63	0.42	0.30	0.55	**0.80**	**0.73**	0.48
Asn	-0.35	**-0.83**	-	0.05	-0.07	-0.13	-0.28	-0.38	-0.07	-0.48	0.03	-0.45	0.02	-0.37	**-0.77**	0.17	-0.42	-0.38
Gln	-0.30	-0.57	-0.05	-	-0.07	-0.13	-0.02	-0.47	0.18	-0.27	0.27	-0.17	-0.08	-0.17	-0.20	0.00	-0.12	-0.18
Cit	-0.03	-0.60	0.07	0.07	-	-0.22	-0.47	-0.27	0.08	-0.32	0.20	-0.15	0.07	-0.30	0.07	0.02	0.08	0.02
Ser	0.17	-0.37	0.13	0.13	0.22	-	-0.17	-0.08	0.27	-0.22	0.35	0.00	0.05	-0.15	0.07	0.43	0.20	0.18
Asp	0.22	-0.32	0.28	0.02	0.47	0.17	-	-0.05	0.35	0.02	0.50	0.17	0.22	-0.22	0.13	0.25	0.12	0.08
Thr	0.08	0.05	0.38	0.47	0.27	0.08	0.05	-	0.10	0.25	0.32	0.27	0.15	0.30	0.13	0.33	0.20	0.10
Glu	-0.10	**-0.72**	0.07	-0.18	-0.08	-0.27	-0.35	-0.10	-	0.18	0.62	-0.17	-0.23	-0.52	-0.17	0.00	-0.37	-0.40
Gly	0.33	-0.55	0.48	0.27	0.32	0.22	-0.02	-0.25	-0.18	-	0.35	0.50	0.17	-0.02	0.28	0.37	0.12	-0.17
Tyr	-0.37	**-0.85**	-0.03	-0.27	-0.20	-0.35	-0.50	-0.32	-0.62	-0.35	-	-0.27	-0.43	-0.48	-0.48	-0.35	-0.63	**-0.82**
Ala	-0.32	-0.63	0.45	0.17	0.15	0.00	-0.17	-0.27	0.17	-0.50	0.27	-	0.05	-0.08	0.07	0.33	-0.02	0.00
Met	-0.45	-0.62	-0.07	0.05	-0.17	-0.17	-0.36	-0.21	0.21	-0.29	0.45	-0.14	-	-0.45	-0.33	0.10	-0.48	-0.24
Trp	-0.17	-0.69	0.17	-0.12	0.10	-0.10	-0.02	-0.67	0.33	-0.24	0.29	-0.21	0.00	-	-0.21	0.00	0.05	-0.12
Val	-0.27	-0.55	**0.77**	0.20	-0.07	-0.07	-0.13	-0.13	0.17	-0.28	0.48	-0.07	0.25	-0.08	-	0.40	0.05	-0.03
Phe	-0.42	**-0.80**	-0.17	0.00	-0.02	-0.43	-0.25	-0.33	0.00	-0.37	0.35	-0.33	-0.12	-0.25	-0.40	-	-0.60	-0.47
Ile	-0.20	**-0.73**	0.42	0.12	-0.08	-0.20	-0.12	-0.20	0.37	-0.12	0.63	0.02	0.33	-0.30	-0.05	0.60	-	-0.23
Leu	-0.23	-0.48	0.38	0.18	-0.02	-0.18	-0.08	-0.10	0.40	0.17	**0.82**	0.00	0.15	-0.18	0.03	0.47	0.23	-

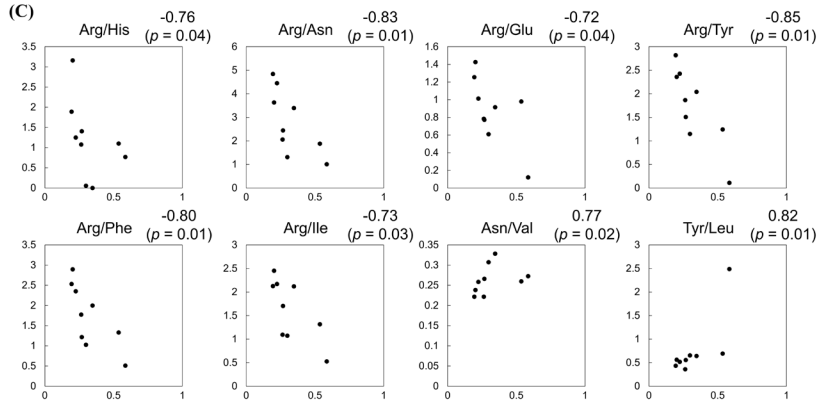


Figure 2. Associations between wound exudate amino acid profiles and microbial dissimilarity. (A) Scatter plots of the relative abundance of amino acids and the weighted UniFrac dissimilarity index. Data for each sample are plotted with the relative abundance of amino acids on the vertical axis and the weighted UniFrac dissimilarity index value on the horizontal axis. The numbers shown in the upper right of each scatter plot represent Spearman's rank correlation coefficients (p-value). Among the 18 amino acids, only arginine showed a statistically significant correlation ($\rho = -0.80$, $p = 0.01$). **(B)** Summary of Spearman's rank correlation coefficients between amino acid ratios derived from the 18 amino acids in wound exudate and the weighted UniFrac dissimilarity index. Amino acid ratios showing statistically significant correlations are highlighted in bold within shaded grey cells. Each amino acid ratio was calculated by dividing the concentration of the amino acid listed in the columns by that listed in the rows. For histidine (His), methionine (Met), and tryptophan (Trp), some wounds had concentrations of 0 mM, resulting in different correlation coefficients depending on whether these amino acids were used as the numerator or denominator of the ratio. **(C)** Scatter plots of amino acid ratios and microbial dissimilarities. The data for each sample are plotted with the amino acid ratio on the vertical axis and the weighted UniFrac dissimilarity index value on the horizontal axis. The numbers shown in the upper right of each scatter plot represent the correlation coefficients (p-value). Correlation coefficients shown in this figure are based on the primary analysis including nine observations derived from eight wounds. His, histidine; Arg, arginine; Asn, asparagine; Gln, glutamine; Cit, citrulline; Ser, serine; Asp, aspartic acid; Thr, threonine; Glu, glutamic acid; Gly, glycine; Tyr, tyrosine; Ala, alanine; Met, methionine; Trp, tryptophan; Val, valine; Phe, phenylalanine; Ile, isoleucine; Leu, leucine.

1.17 (1.00–1.53)], arginine/asparagine [ρ = −0.83, p = 0.01; 2.44 (1.88–3.63)], arginine/glutamic acid [ρ = −0.72, p = 0.04; 0.91 (0.77–1.01)], arginine/tyrosine [ρ = −0.85, p = 0.01; 1.87 (1.24–2.36)], arginine/phenylalanine [ρ = −0.80, p = 0.01; 1.77 (1.22–2.53)], arginine/isoleucine [ρ = −0.73, p = 0.03; 1.70 (1.09–2.13)], asparagine/valine [ρ = −0.77, p = 0.02; 0.26 (0.24–0.27)], and tyrosine/leucine [ρ = 0.82, p = 0.01; 0.56 (0.52–0.66)] (Figure 2C). These findings suggest that specific amino acids and their concentration ratios in wound exudates may serve as potential biomarkers reflecting the degree of microbial similarity between the wound and peri-wound skin in healing wounds. Currently, microbiota identification is primarily performed using NGS techniques based on bacterial DNA sequencing (*23*). However, the substantial cost and time required for this method render it unsuitable for point-of-care testing, thereby limiting the integration of microbiota data into clinical decision-making for wound management. Therefore, future validation studies are warranted to establish these amino acids as clinically applicable biomarkers by evaluating their discriminative ability through receiver operating characteristic analysis and determining appropriate cutoff values based on sensitivity and specificity.

To evaluate the influence of the repeated observation, a sensitivity analysis was performed by excluding one of the repeated measurements and recalculating the Spearman correlation coefficients using one observation per wound (n = 8). In this analysis, the correlation between arginine abundance and microbial dissimilarity was attenuated (ρ = −0.60, p = 0.13) and was no longer statistically significant. In contrast, all eight amino acid ratios that were significantly associated with microbial dissimilarity in the primary analysis remained statistically significant. These findings suggest that amino acid ratios may represent more robust indicators of microbial dissimilarity than individual amino acids.

Among the significant amino acid ratios, the asparagine/valine and tyrosine/leucine ratios were low in most samples and exhibited a narrow range. In contrast, amino acid ratios with arginine as the numerator were generally higher. Together with the findings on arginine abundance, these results suggest that an arginine-rich wound environment may be associated with the formation or maintenance of a wound microbiota similar to that of the commensal skin microbiota.

Beyond its potential role as a microbiota-related biomarker, arginine may also influence the local immune environment. Arginine plays a role in immune tolerance mediated by Tregs. Tregs express arginase 2, which metabolizes arginine to enhance their suppressive capacity (*24*). These findings suggest that an arginine-rich microenvironment allows Tregs to function more effectively. Therefore, experimental studies using animal models are warranted to investigate the effects of arginine supplementation on wound microbiota and its local interaction with Tregs.

In conclusion, this study demonstrated that specific amino acids and their concentration ratios in wound exudates, particularly arginine, are associated with microbial similarity between the wound and peri-wound skin in wounds showing a trend toward healing. These findings suggest the potential utility of amino acid profiles as surrogate markers of wound microbiota dysbiosis. Given the limitations of the current sequencing-based microbiota analyses in clinical practice, amino acid–based biomarkers may serve as feasible alternatives for point-of-care wound assessment. Further studies involving larger cohorts and patients with delayed-healing wounds are warranted to validate their clinical applicability and specificity.

Acknowledgements

We would like to thank Editage (*www.editage.jp*) for English language editing. During the preparation of this work the author used ChatGPT (OpenAI) for the sole purpose of clarifying language and correcting grammar. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Funding: This study was supported by grants from JSPS KAKENHI (JP22J01636 and JP24K20272).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Edwards R, Harding KG. Bacteria and wound healing. Curr Opin Infect Dis. 2004; 17:91-96.
2. Sharma A, Shankar R, Yadav AK, Pratap A, Ansari MA, Srivastava V. Burden of chronic nonhealing wounds: An overview of the worldwide humanistic and economic burden to the healthcare system. Int J Low Extrem Wounds. 2024; 25:371-378.
3. Ding X, Tang Q, Xu Z, Xu Y, Zhang H, Zheng D, Wang S, Tan Q, Maitz J, Maitz PK, Yin S, Wang Y, Chen J. Challenges and innovations in treating chronic and acute wound infections: from basic science to clinical practice. Burn Trauma. 2022; 10:tkac014.
4. Haesler E, Swanson T, Ousey K, Carville K. Clinical indicators of wound infection and biofilm: Reaching international consensus. J Wound Care. 2019; 28:S4-12.
5. Misic AM, Gardner SE, Grice EA. The wound microbiome: modern approaches to examining the role of microorganisms in impaired chronic wound healing. Adv wound care. 2014; 3:502-510.
6. Kunimitsu M, Nakagami G, Kitamura A, Minematsu T, Koudounas S, Ogai K, Sugama J, Takada C, Yeo S, Sanada H. Relationship between healing status and microbial dissimilarity in wound and peri-wound skin in pressure injuries. J Tissue Viability. 2023; 32:144-150.
7. Kunimitsu M, Nakagami G, Minematsu T, Koudounas S,

Sanada H. An *in vivo* critically colonised wound model with dysbiotic wound microbiota. Int Wound J. 2023; 20:648-658.
8. Kunimitsu M, Minematsu T, Koudounas S, Sanada H, Nakagami G. Relationship between dysbiotic wound microbiota and critical colonization: Involvement of FOXP3-positive cells in rats. Ann Plast Surg. 2024; 93:617-23.
9. Kunimitsu M, Kataoka Y, Nakagami G, Weller CD, Sanada H. Factors related to the composition and diversity of wound microbiota investigated using culture-independent molecular methods: a scoping review. Drug Discov Ther. 2021; 15:78-86.
10. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids. 2009; 37:1-17.
11. Berger MM, Binz P, Roux C, Charrière M, Scaletta C, Raffoul W, Applegate LA, Pantet O. Exudative glutamine losses contribute to high needs after burn injury. J Parenter Enter Nutr. 2022; 46:782-788.
12. Debats IBJG, Booi D, Deutz NEP, Buurman WA, Boeckx WD, van der Hulst RRWJ. Infected chronic wounds show different local and systemic arginine conversion compared with acute wounds. J Surg Res. 2006; 134:205-214.
13. Iizaka S, Sanada H, Minematsu T, Oba M, Nakagami G, Koyanagi H, Nagase T, Konya C, Sugama J. Do nutritional markers in wound fluid reflect pressure ulcer status? Wound Repair Regen. 2010; 18:31-37.
14. Levine NS, Lindberg RB, Mason AD, Pruitt BA. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. J Trauma. 1976; 16:89-94.
15. Rushing J. Obtaining a wound culture specimen. Nursing (Lond). 2007; 37:18.
16. Kunimitsu M, Nakagami G, Tunoda M, Akase T, Oe M. Investigation of the applicability of a sampling method of wound exudate using swabs for the amino acid analysis. J Nurs Sci Eng. 2023; 11:47-56.
17. Sanada, H, Moriguchi T, Miyachi Y, Ohura T, Nakajo T, Tokunaga K, Fukui M, Sugama J, Kitagawa A. Reliability and validity of DESIGN, a tool that classifies pressure ulcer severity and monitors healing. J Wound Care. 2004; 13:13-18.
18. Kunimitsu M, Nakagami G, Kitamura A, Minematsu T, Mugita Y, Ogai K, Sugama J, Aoki M, Takada C, Sanada H. Dissemination of microbiota between wounds and the beds of patients with pressure injuries: A cross-sectional study. Wound Pract Res. 2021; 29:70-76.
19. Tsunoda M, Ishida Y, Kunimitsu M. Amino acid analysis in rat wound exudate by high-performance liquid chromatography-fluorescence detection. Chromatography. 2024; 45:31-34.
20. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013; 48:452-458.
21. Tay ASL, Li C, Nandi T, *et al*. Atopic dermatitis microbiomes stratify into ecologic dermotypes enabling microbial virulence and disease severity. J Allergy Clin Immunol. 2021; 147:1329-1340.
22. Leung MHY, Tong X, Shen Z, Du S, Bastien P, Appenzeller BMR, Betts RJ, Mezzache S, Bourokba N, Cavusoglu N, Aguilar L, Misra N, Clavaud C, Lee PKH. Skin microbiome differentiates into distinct cutotypes with unique metabolic functions upon exposure to polycyclic aromatic hydrocarbons. Microbiome. 2023; 11:124.
23. Hodkinson BP, Grice EA. Next-generation sequencing: a review of technologies and tools for wound microbiome research. Adv Wound Care. 2015; 4:50-58.
24. Lowe MM, Boothby I, Clancy S, *et al*. Regulatory T cells use arginase 2 to enhance their metabolic fitness in tissues. JCI Insight. 2019; 4:e129756.

Received May 7, 2026; Revised June 16, 2026; Accepted June 17, 2026.

**Address correspondence to:*
Gojiro Nakagami, The University of Tokyo, Faculty of Medicine Building 5-304, 7-3-1, Hongo, Bunkyo-Ku, Tokyo 113-0033, Japan.
E-mail: gojiron@g.ecc.u-tokyo.ac.jp

Released online in J-STAGE as advance publication June 23, 2026.