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Cold atmospheric plasma and skin wound healing: influence on microbial diversity and composition

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Abstract

Background Skin wound healing presents a complex challenge, often compounded by the risk of infection. Cold atmospheric plasma (CAP) emerged as a novel therapeutic for reducing bacterial load and expediting wound healing. However, its effect on the wound microbiome remained unclear. This study aimed to characterize the microbiome of different types of wounds and determine whether CAP influenced microbial diversity.

Methods Twenty-five patients (ten with acute, fifteen with chronic skin wounds) and ten healthy controls were enrolled. CAP was tailored to individual clinical conditions. Skin samples were collected before and after CAP, and microbiota composition was determined by 16 S ribosomal RNA sequencing.

Results Microbial communities differed between acute and chronic groups. CAP could accelerate wound healing. However, it did not change microbial α and β-diversity in acute wounds. In chronic wounds, α-diversity indices, including the chao and ACE, were significantly increased, and a significant clustering was observed in post-CAP group. In addition, CAP led to higher abundance of *Staphylococcus*, lower levels of *Proteobacteria* and *Pseudomonas* in chronic wounds.

Conclusions This study provided novel insights into the impact of CAP on skin wound microbiota. Further research was required to ascertain causality between microbiota and CAP and to develop personalized CAP treatment strategies.

Keywords Skin wound, Cold atmospheric plasma, Skin Microbiome

Background

Skin wounds represent unnatural breaks, tears, or defects resulting from thermal/physical trauma or underlying pathologies, such as drug rashes, psoriasis, eczema, or diabetes [1, 2]. These wounds can be classified as acute or chronic according to their repair process. Acute wounds typically heal within 8 to12 weeks with minimal scarring, while chronic wounds tend to recur and take longer than 12 weeks to heal [3, 4]. Treatment strategies vary depending on their primary disease, with healing often challenging due to underlying physiological conditions. Moreover, impaired wound healing can exacerbate

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underlying diseases or pose life-threatening risks to patients [5].

Microbiomes play a vital role in both maintaining human health and causing disease [6]. A variety of microorganisms, including bacteria, fungi, viruses, and arthropods, colonize the skin and contribute to immune regulation, pathogen defense, metabolite decomposition, and maintenance of the host barrier [6–8]. Recent studies have shown that the skin microbiome significantly impacts wound healing, although these studies have reached contradictory conclusions [9]. According to Canesso MC. et al., wound closure and epithelization were accelerated, and wound leukocyte profiles were markedly altered in mice lacking commensal skin microbes, suggesting a potential impairment of wound healing by commensal bacteria [10]. Conversely, oral vancomycin administration delayed wound healing and decreased bacterial density near wounds, possibly through inhibiting interleukin-17 (IL-17) production and regenerating islet-derived protein-III gamma (RegIIIγ) [11]. Despite these discrepancies, it is widely accepted that the microbiome plays a multifaceted role in wound healing, requiring a balance among different types of microbes. Antimicrobial treatments aimed at reducing bacterial burden may inadvertently deplete beneficial bacteria, warranting exploration of alternative strategies, especially given concerns about antibiotic resistance [9].

Cold atmospheric plasma (CAP) is an ionized gas containing ions, electrons, electrical emissions, optical emissions, radicals, and bioactive molecules, generated through gas excitation by electron impacts at mild temperatures and atmospheric pressures [12, 13]. CAP has demonstrated potential in promoting wound healing and tissue regeneration [14]. Its application in treating skin wounds is attributed to its ability to enhance blood coagulation, facilitate wound healing, and eradicate pathogens without harming normal cells [15]. In a standardized acute wound healing model, CAP was proven to increase capillary blood flow and oxygen saturation in cutaneous tissue [16]. Gao J.et al. reported CAP's efficacy in improving or expediting wound healing in various conditions, including pyoderma gangrenosum, traumatic wounds, giant genital warts by laser treatment, chronic eczema, and skin lesions of diabetic foot [17]. Besides, CAP has been observed to influence human skin microorganisms such as Staphylococcus aureus and Aspergillus flavus [18, 19]. Despite its proven therapeutic effect on skin wounds, the detailed mechanism of CAP action, especially its impact on the cutaneous microbiome in acute and chronic skin wounds, remains poorly understood. This study aimed to describe the cutaneous microbiome of patients with acute and chronic skin wounds, analyze skin wound microbial diversity, and determine the effects of CAP treatment on the cutaneous microbiome.

Methods

Participants

Ten patients with acute skin wounds and fifteen with chronic skin wounds were recruited from the dermatology department of the Second Hospital of Anhui Medical University between February 2021 and March 2022. Ten healthy adults served as controls. Inclusion criteria were as follows: (1) Acute skin wounds present for less than two weeks, including trauma, burns or scalds, eczema, zoster, or laser therapy wounds. (2) Chronic skin wounds present for more than four weeks, including diabetic wounds, eczema, chronic ulcers, skin infections, drug rash, and vasculitis wounds. (3) Healthy controls randomly selected from individuals undergoing routine health screening. Exclusion criteria included patients receiving systemic antibiotic treatment within 14 days prior to the study, local antibiotic treatment, immunosuppressive agents, corticosteroid therapy, pregnant patients, lactating women, or patients with cognitive impairment or mental disease. Informed consent forms were signed by all subjects. The study was approved by the Ethical Committee of the Second Hospital, Anhui Medical University, and conformed to the Helsinki Declaration.

CAP treatment methods

The CAP device, designed by the Institute of Plasma Physics, Chinese Academy of Sciences, comprised a dielectric barrier discharge generator with a copper needle as the high-voltage electrode. The device operated at 200 V input voltage, 150 W power, and 0.5 mA electric current. Before treatment, the operator applied the electrode piece to the patient's skin as a grounding electrode. Plasma was generated when the device was positioned 0.5 cm from the skin surface, and each lesion was treated for 2 ~ 5 min, 2–3 times a week. Detailed CAP device and treatment methods were as described in our previous study [17].

Skin sample collection

Skin samples were collected to analyze the composition of the skin wound microbiota, following a modified protocol from a previous report [20]. The wound area was cleansed with sterile normal saline solution to eliminate transient contamination. A sterile cotton swab presoaked with sterile normal saline was attached to the patient's wound area and then applied repeatedly on the skin lesions for over 30 s (an average of 8 to10 times/cm^2). The cotton swab was transferred into a sterile tube and stored at -80 °C until further analysis. Samples from healthy controls were collected from the anterior tibia of the lower limbs, covering an area of 4×4 cm^2.

DNA extraction and 16 S ribosome RNA sequencing

Bacterial genomic DNA from the collected swabs was extracted using a genomic DNA purification kit following the manufacturer's instructions (Omega Biotek, USA). The V3 ~ V4 hypervariable region of the 16 S rRNA gene was amplified by polymerase chain reaction (PCR) as in a previous report [21]. PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter, USA), and library construction and quality analysis were performed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). Suitable libraries were sequenced on the HiSeq platform (BGI, Shenzhen, China).

Microbiome and bioinformatics

Raw sequencing data were filtered into clean data and assembled using FLASH software (http://www.cbcb.umd.edu/software/flash). Tags were clustered into operational taxonomic units (OTUs) using USEARCH software (v7.0.1090), with a sequence similarity threshold of 97% assigned to a single OTU, as performed by the "pick_de_novo_otus.py" command in QIIME2 (version: 2023.2) [22]. The 16 S rRNA database of SILVA (version 138.1) was used to assign taxonomy [23]. Singletons (OTUs with only one associated read) and low-read OTUs were excluded by DADA2 (Divisive Amplicon Denoising Algorithm) in QIIME2 [24]. The OTUs and clean data of each sample were listed in Table S1 and S2. Alpha diversity was assessed using Chao1, Sobs, Ace, Shannon, and Simpson indices. Beta diversity of the population was assessed using weighted and unweighted UniFrac distances, along with principal coordinate analysis (PCoA). Nonmetric multidimensional scaling (NMDS) analysis was also performed [25]. Least discriminant analysis effect size (LEfSe) and Meta stats were employed to examine the variations in the relative abundance of phylum and genus. Additionally, *p* value and q value were calculated to assess significance of differences between groups [26].

Statistical analysis

All data were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 26.0 software (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used for normally distributed variables, while non-parametric tests (Kruskal-Wallis test for multiple pairwise comparison and Mann-Whitney test for pairwise comparison) were applied for non-normally distributed data. Chi-square test was used for categorical variables. Statistically significant was defined as $P < 0.05$.

Results

Demographics and clinical characteristics of study population

The demographics and clinical characteristics of the participants were summarized in Table 1 and Table S3. There were no significant differences in age and sex among the groups. Patients in the chronic skin wound group had significantly longer wound present time compared to those in the acute skin wound group ($p < 0.001$). Most participants in the acute group presented with vasculitis, scalds, herpes zoster, and condyloma lesions treated with laser, while in the chronic skin wound group, most patients underwent treatment for eczema, chronic ulcers, skin infections, and wart lesions with liquid nitrogen. Following CAP treatment, 60% of patients in the acute skin wound group showed improvement, and 40% were

Table 1 Clinical characteristics of participants

Characteristics	Healthy control ($n = 10$)	Acute skin wound ($n = 10$)	Chronic skin wound ($n = 15$)	*P* value
Age (years), mean ± SD	44.6 ± 18.31	54.7 ± 18.74	57.87 ± 20.35	0.691[a]
Female, *n* (%)	5 (50.0)	5 (50.0)	10 (66.7)	0.615[c]
Wound existing time (days), mean ± SD	0	7.3 ± 2.75	> 44.0 ± 41.20	< 0.001[b]
Wound type, *n* (%)				
Asculitis	0	2 (20.0)	2 (13.3)	-
Scald	0	2 (20.0)	0	-
Herpes zoster	0	2 (20.0)	0	-
Condyloma lesion by lazer	0	2 (20.0)	0	-
Trauma	0	1 (10.0)	0	-
Eczema	0	1 (10.0)	4 (26.7)	-
Diabetic foot	0	0	1 (6.6)	-
Chronic ulcer	0	0	4 (26.7)	-
Skin infection	0	0	2 (13.3)	-
Wart lesion by liquid nitrogen	0	0	2 (13.3)	-
Results				
Improvement, *n* (%)	NA	6 (60.0)	13 (86.7)	0.019[b]
Cure, *n* (%)	NA	4 (40.0)	1 (6.6)	

a. one way ANOVA; b.Mann-Whitney Test; c. Chi-squre test. *P* values were presented as the acute skin wound group versus the chronic skin wound group

cured, whereas in the chronic skin group, 86.7% showed improvement, and 6.6% were cured ($p = 0.019$) (Table 1).

Skin microbiota characteristics in patients with acute and chronic skin wounds

To explore differences in skin microbiota between skin wound patients and healthy controls, 16 S rRNA amplicon sequencing was conducted to analyze bacterial composition at the skin wound sites in 10 patients with acute skin wounds (J1), 15 patients with chronic skin wounds (M1), and 10 adult healthy controls (C), without any treatment. Alpha diversity indices, including Sobs, Chao, Ace, and Shannon, were significantly lower, while the Simpson index was higher in the J1 and M1 groups compared to the control group (Fig. 1A-E, Table S4). However, no significant difference was observed between the J1 and the M1 groups, indicating a significant reduction in species richness and diversity of bacteria in skin wounds, with no significant differences in microbial changes between acute and chronic skin wound groups. To compare the diversity of species between samples, a beta diversity analysis was conducted. PCoA analysis showed that different clusters formed by acute and chronic participants compared to healthy groups

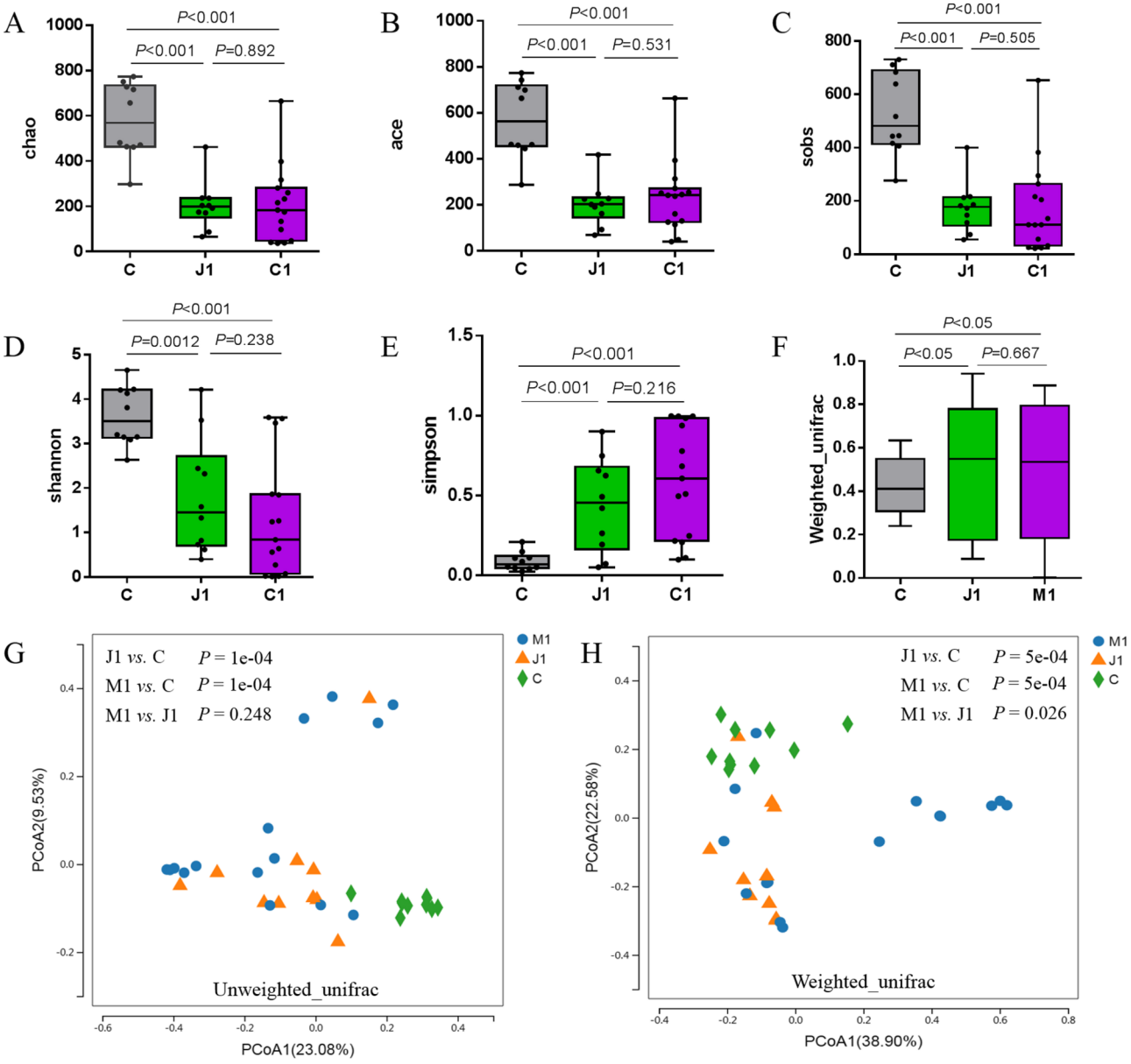

Fig. 1 Community richness and diversity of skin microbiome in patients with acute and chronic skin wounds. Alpha-diversity indices of (**A**) chao, (**B**) ace, (**C**) soba, (**D**) Shannon, and (**E**) simpson. (**F**) Box plot of β-diversity among different groups. (**G**) PCoA analysis based on unweighted UniFrac distance. (**H**) PCoA analysis based on weighted UniFrac distance

($P < 0.05$, Fig. 1F-H). In addition, PCoA analysis demonstrated differences in beta-diversity between the acute and chronic groups, suggesting significant community differences among the groups ($P < 0.05$, Fig. 1H).

Skin microbiota composition in patients with acute and chronic skin wounds

A total of 949, 124, and 298 unique OTUs were obtained in the Control (C), acute (J1), and chronic skin (M1) groups, respectively (Fig. 2A-B). Patients with acute or chronic skin wounds exhibited markedly different skin microbial species compared to the control group. In the J1 group, the relative abundance of *Firmicutes* and *Fusobacteria* increased, while *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Candidatus_Saccharibacteria* decreased compared to the control group (Fig. 2C-D, Table S5). Furthermore, the M1 group showed reduced levels of *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Deinococcus_Thermus*, *Fusobacteria*, *Planctomycetes*, and *Candidatus_Saccharibacteria*, with elevated *Verrucomicrobia* compared to the control group (Fig. 2C-D, Table S5). At the genus level, the J1 group had higher abundance of *Staphylococcus* and *Enterococcus*, and lower levels of *Corynebacterium* and *Enhydrobacter* compared

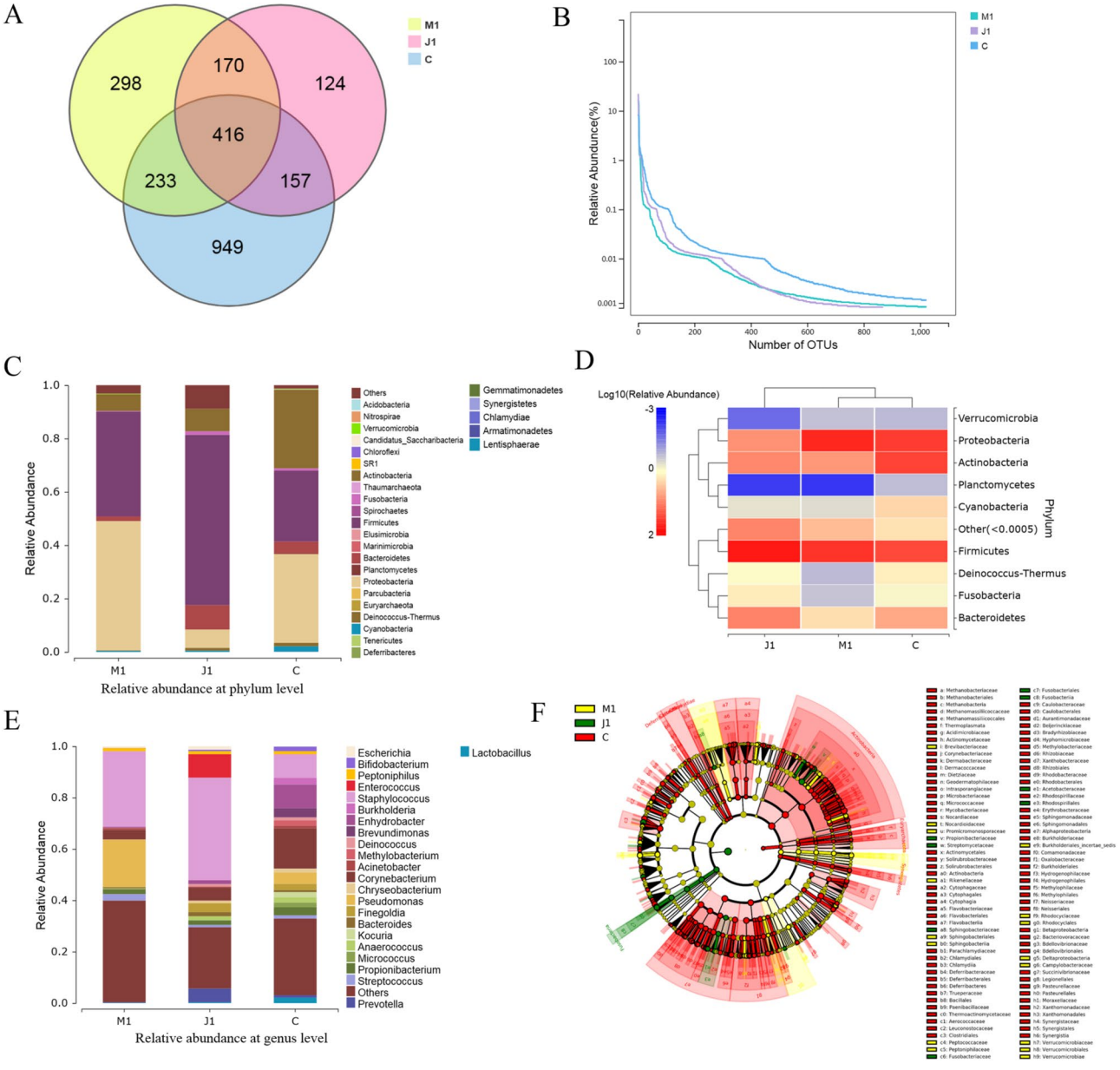

Fig. 2 Taxonomic distribution of skin microbiota in patients with skin wounds. (**A**) Venn diagram of the OTUs. (**B**) OTU rank abundance curves of each group. (**C**) Phylum-level taxonomic composition. (**D**) Community heatmap analysis at the phylum level. (**E**) Genus-level taxonomic composition. (**F**) Cladogram plot of LEfSe analysis

to the control group (Fig. 2E-F, Table S6-7). Similarly, the M1 group showed higher levels of *Staphylococcus* and *Pseudomonas*, and lower *Corynebacterium* and *Enhydrobacter* compared to the control group (Fig. 2E-F, Table S6-7).

CAP affects microbial taxonomic profile in patients with acute skin wounds

Microbial community characteristics and composition of acute wounds were analyzed post-CAP-treatment. There were 275 and 1162 unique OTUs in the pre-CAP treatment (J1) and post-CAP treatment (J2) groups, respectively (Figure S1A). A paired comparison of the J1 and J2 groups did not show any significant differences in α-diversity (Fig. 3A-E, Table S8). Moreover, β-diversity analysis via PCoA and NMDS revealed no separation between these groups, indicating no significant difference in structural diversity of the skin microbiota between the J1 and the J2 groups (Fig. 3F-H). UPGMA clustering tree results confirmed those of PCoA and NMDS (Figure S2).

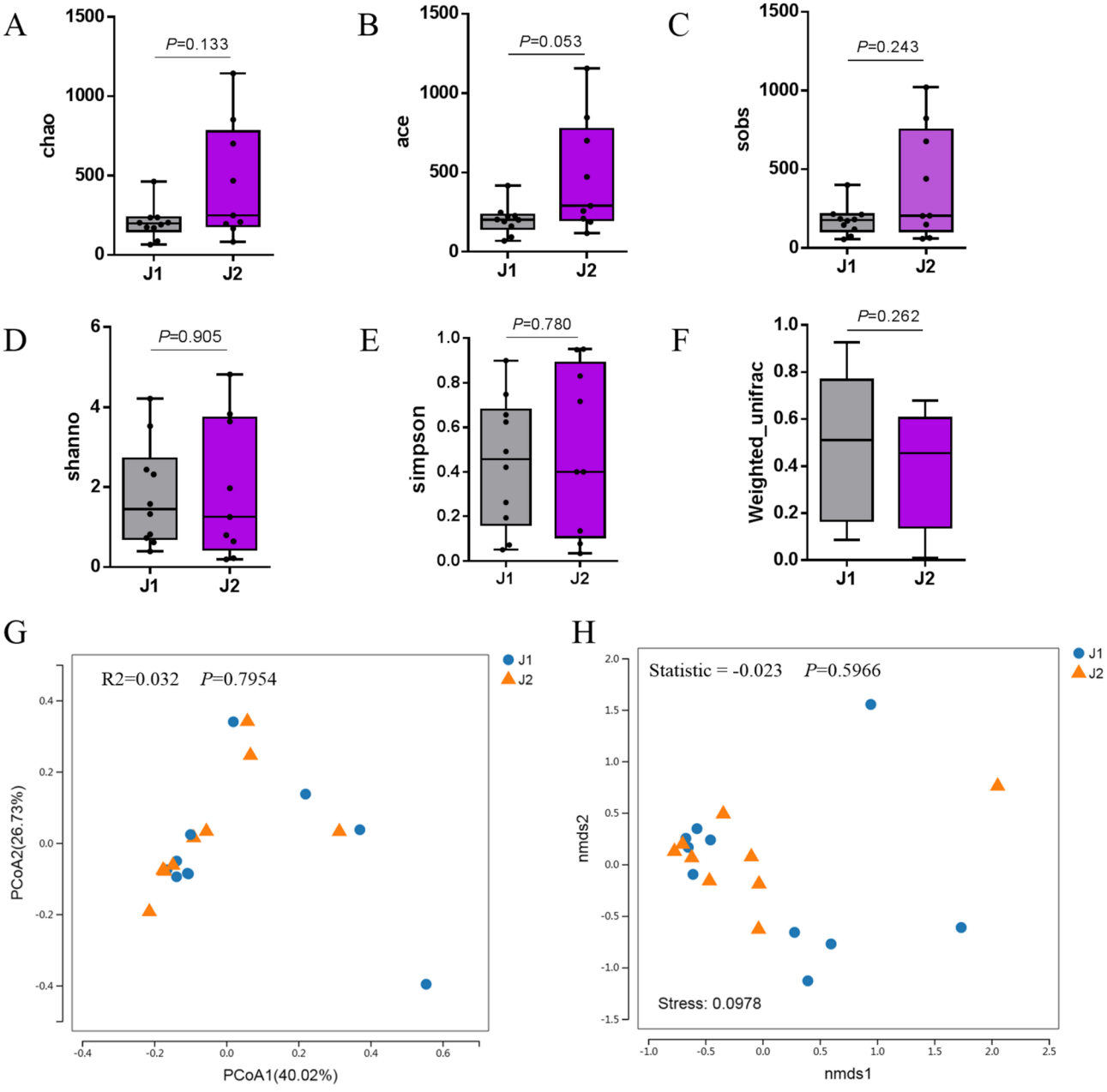

Fig. 3 Community richness and diversity of skin microbiome in patients with acute skin wounds before and after CAP treatment. (**A-E**) α-diversity analysis in community richness and diversity. (**F**) Box plot of β-diversity among different groups. (**G**) PCoA (weighted_unifrac) and (**H**) NMDS analysis on OTU level between J1 and J2 groups

At the phylum level, *Firmicutes* accounted for 63.82% in the J1 group and 67.72% in the J2 group, *Actinobacteria* for 8.39% and 13.04%, and *Proteobacteria* for 6.93% and 7.45%, respectively. (Fig. 4A, Table S9). At the genus level, *Staphylococcu* accounted for 39.91% in the J1 group and 49.48% in the J2 group, *Enterococcus* for 9.14% and 0.04% ($P < 0.05$), and *Prevotella* for 5.36% and 1.23%, respectively (Fig. 4B, Table S10), indicating an increase in *Streptococcus* and a decrease in *Enterococcus* post-CAP treatment in the acute skin wound group. In addition, there was a greater abundance of *Massilia* and *Methylobacterium* genera, and *Oxalobacteraceae* and *Methylobacteriacea* families after CAP treatment (Fig. 4C-D).

CAP alters bacterial diversity in patients with chronic skin wounds

In the cohort of patients with chronic skin wounds, a total of 498 and 905 unique OTUs were observed in the pre-CAP treatment (M1) and post-CAP treatment (M2) groups, respectively (Figure S1B). Regarding α-diversity, the chao and ace indices of the M2 group were significantly increased compare to the M1 group (Fig. 5A-B, Table S11). PCoA and NMDS analysis demonstrated no distinct separation based on weighted UniFrac (Fig. 5F, H and I). However, significant clustering based on Bray-Curtis dissimilarity indicated a change in the structural diversity of chronic skin wounds after CAP treatment (unweighted UniFrac, $P = 0.0092$, Fig. 5G and J). UPGMA clustering tree results were consistent with PCoA and NMDS results (Figure S3).

At the phylum level, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* were the three main taxonomic components, accounting for 39.32%, 48.47%, and 6.27% in the M1 group, and 56.42%, 18.21%, and 11.57% in the M2 group, respectively (Fig. 6A, Table S12). At the genus level, the abundance of *Staphylococcus* accounted for 29.33% in the M1 group, while it increased to 46.82% in the M2 group. Conversely, *Pseudomonas* accounted for 18.28% in the M1 group, and decreased to 3.17% after CAP treatment (Fig. 6B, Table S13). Cladogrma and LEfSe analyses revealed that the abundance of 103 species increased following CAP treatment (Fig. 6C, Table S14). The top five species with the highest fold increase in abundance were *Deinococcus* genus, *Deinococcaceae* family, *Deinococcales* and *Fusobacteriales* orders, and *Fusobacteriia* class. Conversely, the abundance of *d_Bacteria*, *g_Parvimonas*, *g_Roseomonas*, *g_Comamonas*, and

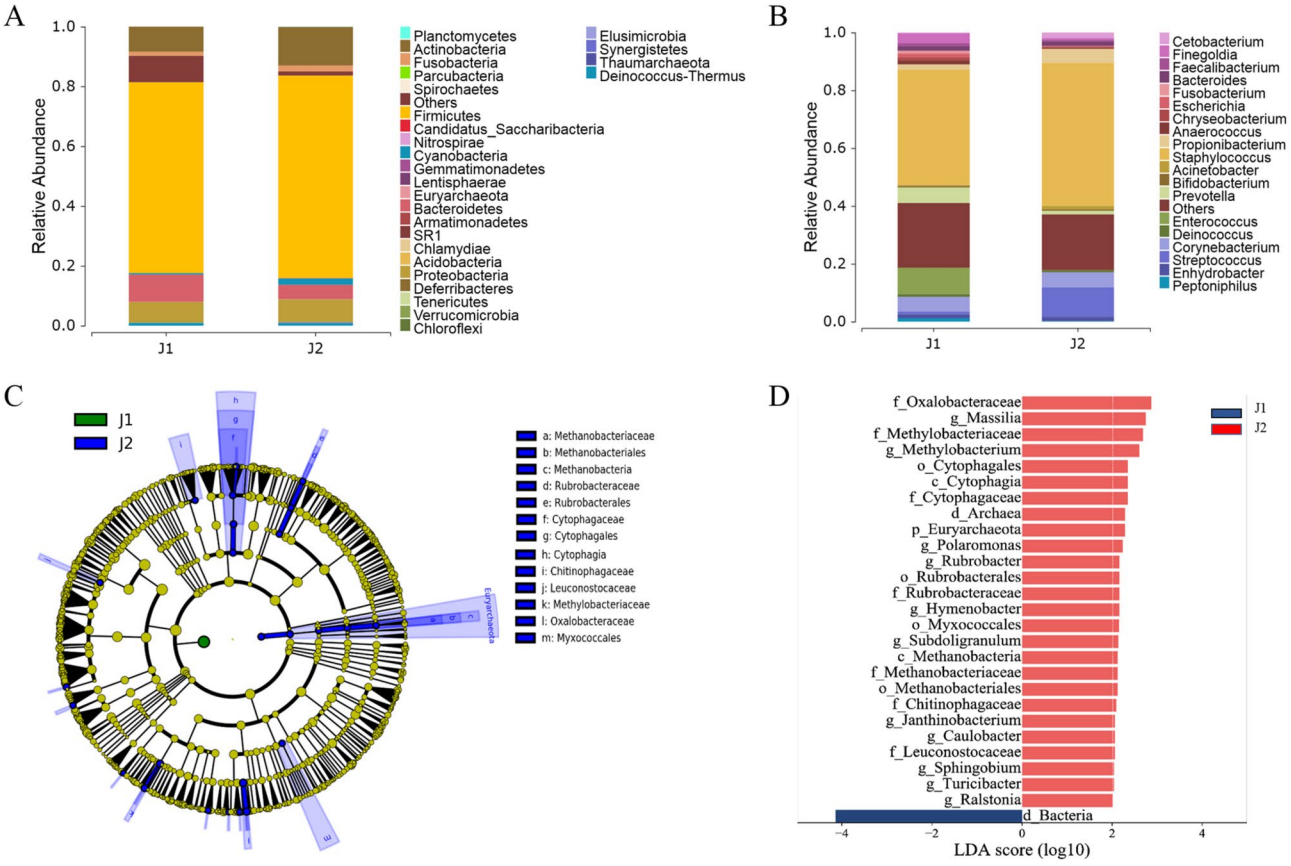

Fig. 4 Relative abundance and LEfSe analysis before and after CAP treatment in acute skin wound. Relative abundance of bacteria communities at (**A**) phylum level and (**B**) genus level. (**C**) Taxonomic cladogram generated from LEfSe analysis. (**D**) Linear discriminant analysis (LDA) histogram

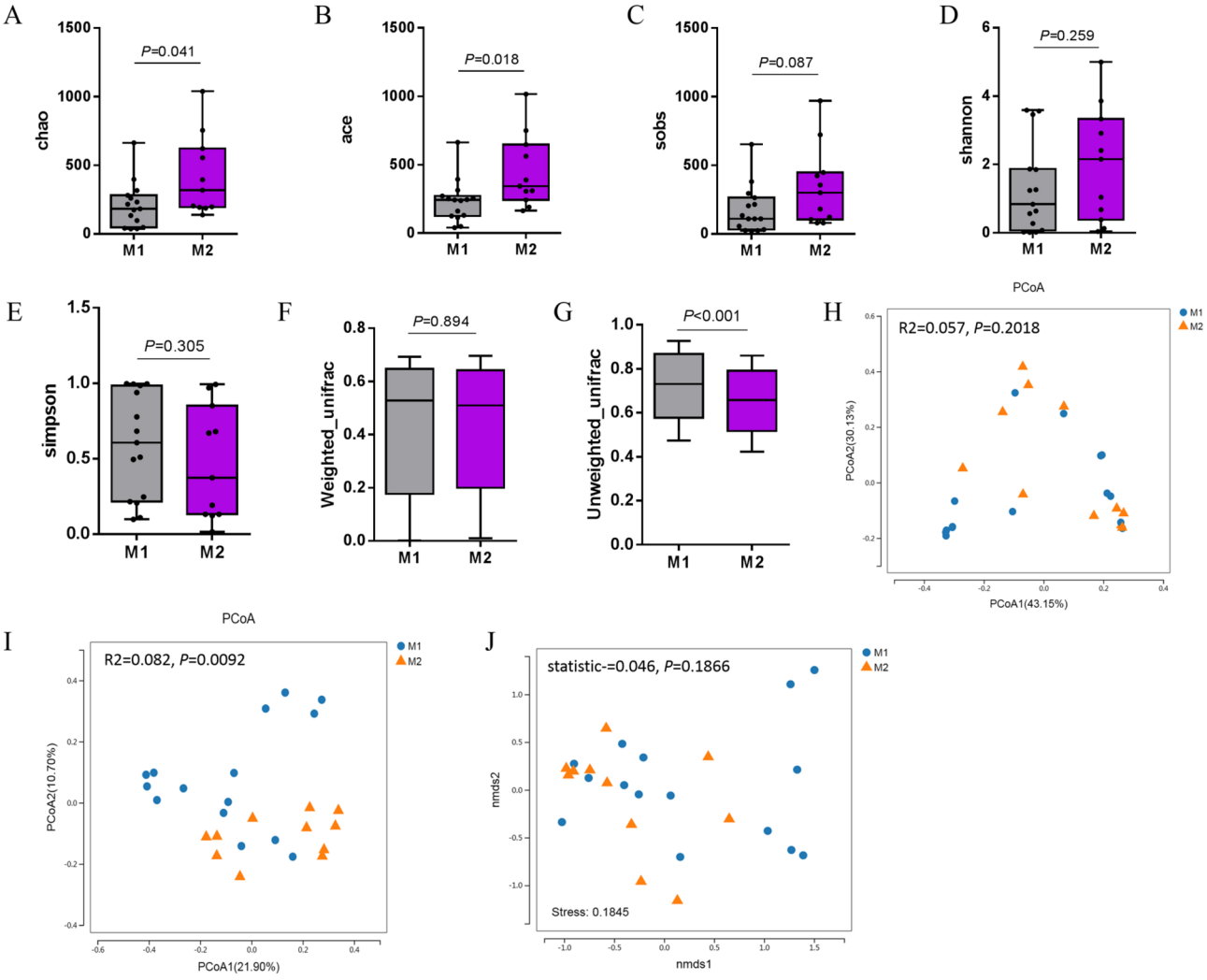

Fig. 5 Community richness and diversity of skin microbiome in patients with chronic skin wounds before and after CAP treatment. (**A-E**) α-diversity analysis in community richness and diversity. (**F**) Box plot of β-diversity (weighted_unifrac). (**G**) Box plot of β-diversity (unweighted_unifrac). (**H**) PCoA analysis (weighted_unifrac). (**I**) PCoA analysis (unweighted_unifrac). (**J**) NMDS analysis on OTU level

g_Brevundimonas was lower in the M2 group compared to the M1 group (Fig. 6D, Table S14).

Discussion

The skin microbiota plays a crucial role in maintaining a healthy cutaneous system [9]. As part of cutaneous wound healing, commensal microbiota interact with various types of cells involved in regulating immune response and restoring barrier function [27]. In this study, we characterized microbial changes in skin wound healing of different types and provided valuable insights into the impact or response to CAP treatment. We observed significant alterations in both α-diversity and β-diversity among individuals with acute and chronic skin wounds compared to healthy controls.

Alpha diversity indices, including Sobs, Chao, Ace, Shannon, and Simpson, are critical in assessing the richness and evenness of microbial communities, providing insights into the complexity and stability of ecosystems such as the skin microbiota. The Chao and Ace indices are used to estimate species richness, while the Shannon and Sobs indices are used to estimate species abundance and evenness. The Simpson index emphasizes the dominance of particular species within a community. Additionally, a significant difference was found in the richness and diversity of the cutaneous microbiome between acute and chronic wounds. Furthermore, our study found that chronic skin wounds exhibited significantly increased microbial richness and diversity after CAP treatment. However, microbial richness and diversity in acute skin wounds were not affected by CAP therapy, and no clear correlation was identified between specific microbial changes and CAP efficacy.

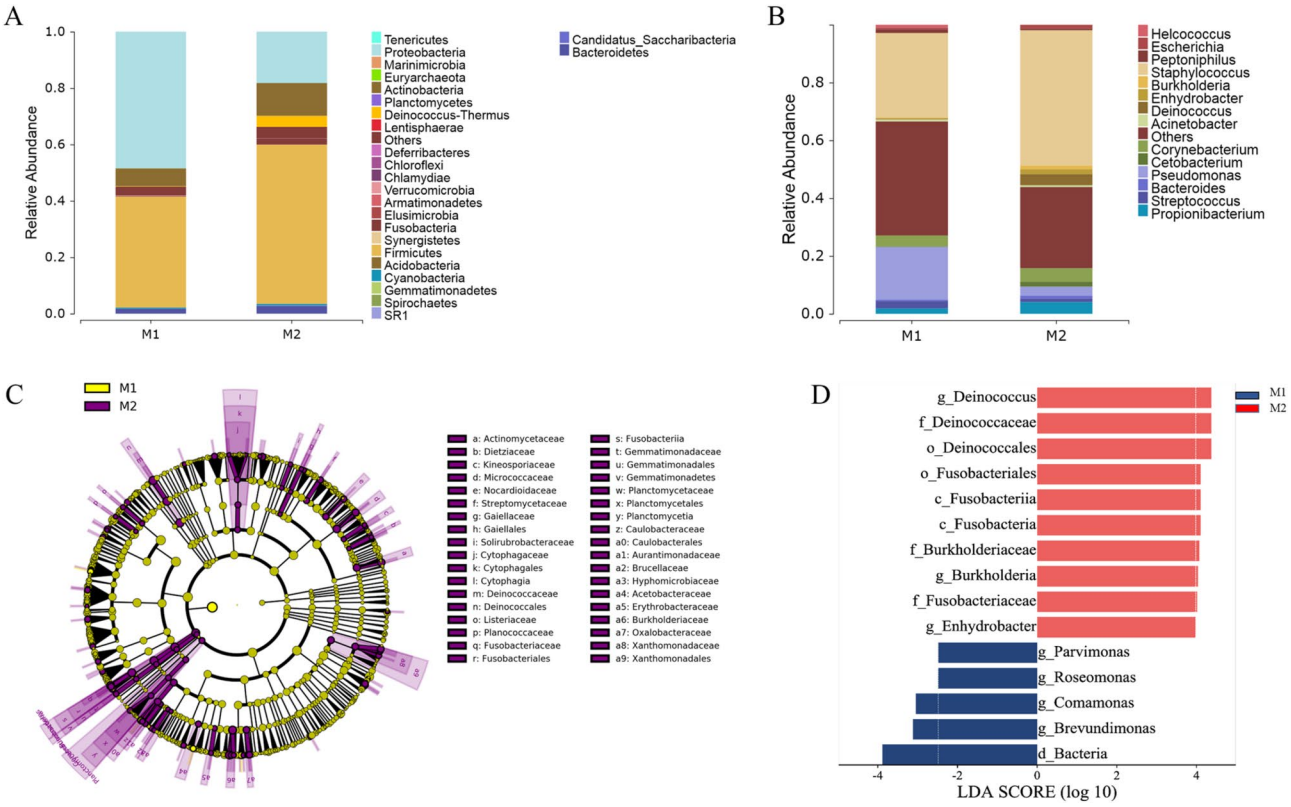

Fig. 6 Relative abundance and LEfSe analysis before and after CAP treatment in the patients with chronic skin wounds. Relative abundance of bacteria communities at (**A**) phylum level and (**B**) genus level. (**C**) Taxonomic cladogram generated from LEfSe analysis. (**D**) Linear discriminant analysis (LDA) histogram

Regarding bacteria, the most abundant living microorganisms on healthy human skin are affiliated with three phyla: *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, which collectively accounted for approximately 90% of the skin microbiota [28, 29]. In this study, these three phyla were found to account for 89.32% of the abundance of *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, consistent with previous studies [30, 31]. However, in the acute skin wound group, the level of *Firmicutes* increased to 63.82%, while *Proteobacteria* and *Actinobacteria* decreased to 6.93% and 8.39%, respectively. In contrast, in the chronic wound group, the levels of *Proteobacteria* and *Firmicutes* increased to 48.46% and 39.32%, respectively, while the level of *Actinobacteria* decreased to 6.27%. Overall, skin wounds exhibited a higher abundance of *Firmicutes* and a lower abundance of *Actinobacteria* compared to healthy skin. Moreover, the abundance of *Proteobacteria* was higher in chronic wounds and lower in acute wounds, a change consistent with the microbiome observed in diabetic foot ulcers and wounds of long duration [32].

16 S rRNA sequencing has been used for several years to uncover the characteristics of skin microbiome, although modest progress has been made investigating correlations between microbiota alterations and wound outcomes [27]. *Staphylococcaceae* and *Pseudomonadaceae* have been reported as the predominant families regardless of wound types and sampling methods [32–37]. Besides, Wolcott RD, et al. claimed that *Pseudomonas* and *Staphylococcus* are the most common genera found in 2963 chronic wound samples with various etiologies [38]. In the present study, *Staphylococcus* and *Pseudomonas* were also identified as the most common genera, with *Staphylococcus_petrasii* and *Pseudomonas_aeruginosa* being the predominant species in chronic skin wounds. Considering the lack of microbiome data for acute skin wounds, we conducted microbiome analysis based on 16s rRNA sequencing. *Staphylococcus* and *Enterococcus* were identified as the two main genera, with *Staphylococcus_petrasii* and *Enterococcus_saccharolyticus* being the prominent species in acute skin wounds, respectively. These findings suggested that the composition and structure of the microbiome in skin wounds were altered, with *Staphylococcus_petrasii* being the main species in skin wounds of any type. *Pseudomonas_aeruginosa* was typically colonized in chronic skin wounds, while *Enterococcus_saccharolyticus* was colonized in acute skin wounds.

Microbial infection plays a significant role in wound healing, a complex process influenced by various factors

and pathophysiological states of wounds [39]. Accumulating studies have highlighted the efficacy of CAP in sterilization, potentially offering a novel approach to combatting infections caused by multi-drug resistant ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*) and complex biofilm [40]. Our previous research demonstrated CAP's sterilization effects on skin wound refractory bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (unpublished data). Modic et al. [41] compared the bactericidal efficacy of CAP against both Gram-positive and Gram-negative pathogens under two distinct discharge conditions that generate active species, thereby confirming its antimicrobial potential. Although CAP treatment did not alter α- and β-diversity in acute skin wounds, PCoA analysis hinted at distinct tendencies between pre- and post-CAP treatment groups. Following CAP treatment, the microbiome composition of acute skin wounds was restored to resemble that of the normal group, with *Firmicutes*, *Actinobacteria*, and *Proteobacteria* emerging as the predominant microbial taxa. Notably, there was a significant increase in the abundance of the genera *Massilia* and *Methylobacterium*, as well as the families *Oxalobacteraceae* and *Methylobacteriaceae*, and the species *Streptococcus anginosus*, all of which are components of the normal microbial flora [41]. Conversely, *Enterococcus_saccharolyticus* species significantly reduced after CAP treatment, indicating a shift in the microbial taxonomic profile of acute skin wounds.

In recent years, the skin microbiota associated with chronic wounds has received significant attention, with researchers investigating microbiota-based mechanisms to prevent or manage skin disorders and impaired wound healing [6]. In this research, patients with chronic skin wounds exhibited higher α-diversity (chao and ACE) in the post-CAP group compared to the pre-CAP group, with significant differences in β-diversity between the paired groups.

Comparable to the healthy control and acute skin wound groups, Firmicutes, Proteobacteria, and Actinobacteria were identified as the predominant taxonomic components in chronic wounds, both pre- or post-CAP treatment. A total of 103 species, including members of the genera *Deinococcus* and *Parvimonas*, families *Deinococcaceae*, orders *Deinococcales* and *Fusobacteriales*, and class *Fusobacteriia*, exhibited a significant increase in abundance following CAP treatment. Conversely, the abundance of several bacterial taxa, including *Parvimonas*, *Roseomonas*, *Comamonas*, and *Brevundimonas*, was notably reduced in the post-CAP group. These findings suggest that CAP treatment altered the diversity and composition of the skin microbiome in chronic wounds.

Conclusion

This study represents the first investigation into the impact of CAP treatment on the skin microbiome, comparing microbial diversity among various types of wounds and healthy controls. Our findings revealed reduced diversity in patients with both acute and chronic skin wounds, suggesting a potential association between CAP and the skin microbiome. While the database is limited and exploratory in nature, it underscores the importance of conducting large-scale mechanistic studies to elucidate the regulatory effects of CAP on the skin microbiome and its implications for wound healing.

Abbreviations

CAP	Cold atmospheric plasma
PCR	Polymerase chain reaction
PCoA	Principal coordinate analysis
NMDS	Nonmetric multidimensional scaling
LEfSe	Least discriminant analysis effect size
SD	Standard deviation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03965-x.

Supplementary Material 1

Supplementary Material 2

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Author contributions

Conceptualization, Chunjun Yang; methodology, Ruidi Gao and Houyu He; software, Ruidi Gao; validation, Xingyu Yang and W.W.; formal analysis, Ruidi Gao and Houyu He; investigation, Ruidi Gao and Jing Gao; resources, Ruidi Gao and Wei Wang; data curation, Ruidi Gao, Houyu He and Xingyu Yang; writing—original draft preparation, Ruidi Gao and Chunjun Yang; writing—review and editing, Chunjun Yang; visualization, Ruidi Gao Houyu He and Xingyu Yang; supervision, Chunjun Yang Chunjun Yang and Jing Gao; project administration, Chunjun Yang; funding acquisition, Chunjun Yang; All authors have read and agreed to the published version of the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files. The datasets used in the present study are available in the SRA in NCBI repository under accession number PRJNA1014787.

Declarations

Ethics approval and consent to participate
This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This work was supported by the Ethics Committee of The second Hospital of Anhui Medical University (YX2021-029(F1)). Informed consent was obtained from all patients.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. White RA. Management of specific skin wounds. Veterinary Clin North Am Small Anim Pract. 2006;36:895–912.
2. Fu C, Chen J, Lu J, et al. Roles of inflammation factors in melanogenesis (Review). Mol Med Rep. 2020;21:1421–30.
3. Mir M, Ali MN, Barakullah A, et al. Synthetic polymeric biomaterials for wound healing: a review. Prog Biomater. 2018;7:1–21.
4. Shen S, Chen X, Shen Z, Chen H. Marine polysaccharides for wound dressings application: an overview. Pharmaceutics. 2021;13.
5. Fountain K, Blackett T, Butler H et al. Fatal exudative dermatitis in Island populations of red squirrels (Sciurus vulgaris): spillover of a virulent Staphylococcus aureus clone (ST49) from reservoir hosts. Microb Genomics. 2021;7.
6. Harris-Tryon TA, Grice EA. Microbiota and maintenance of skin barrier function. Science. 2022;376:940–5.
7. Grice EA. The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease. Semin Cutan Med Surg. 2014;33:98–103.
8. De Pessemier B, Grine L, Debaere M et al. Gut-Skin axis: current knowledge of the interrelationship between microbial dysbiosis and skin conditions. Microorganisms. 2021;9.
9. Johnson TR, Gomez BI, McIntyre MK et al. The cutaneous Microbiome and wounds: new molecular targets to promote wound healing. Int J Mol Sci. 2018;19.
10. Canesso MC, Vieira AT, Castro TB, et al. Skin wound healing is accelerated and scarless in the absence of commensal microbiota. J Immunol. 2014;193:5171–80.
11. Zhang M, Jiang Z, Li D, et al. Oral antibiotic treatment induces skin microbiota dysbiosis and influences wound healing. Microb Ecol. 2015;69:415–21.
12. Isbary G, Shimizu T, Li YF, et al. Cold atmospheric plasma devices for medical issues. Expert Rev Med Dev. 2013;10:367–77.
13. Stratmann B, Costea TC, Nolte C, et al. Effect of cold atmospheric plasma therapy vs standard therapy placebo on wound healing in patients with diabetic foot ulcers: A randomized clinical trial. JAMA Netw Open. 2020;3:e2010411.
14. He R, Li Q, Shen W, et al. The efficacy and safety of cold atmospheric plasma as a novel therapy for diabetic wound in vitro and in vivo. Int Wound J. 2020;17:851–63.
15. Daeschlein G, Scholz S, Ahmed R, et al. Skin decontamination by low-temperature atmospheric pressure plasma jet and dielectric barrier discharge plasma. J Hosp Infect. 2012;81:177–83.
16. Matzkeit N, Schulz L, Schleusser S, et al. Cold atmospheric plasma improves cutaneous microcirculation in standardized acute wounds: results of a controlled, prospective cohort study. Microvasc Res. 2021;138:104211.
17. Gao J, Wang L, Xia C, et al. Cold atmospheric plasma promotes different types of superficial skin erosion wounds healing. Int Wound J. 2019;16:1103–11.
18. Kondeti V, Phan CQ, Wende K, et al. Long-lived and short-lived reactive species produced by a cold atmospheric pressure plasma jet for the inactivation of Pseudomonas aeruginosa and Staphylococcus aureus. Free Radic Biol Med. 2018;124:275–87.
19. Simoncicova J, Kalinakova B, Kovacik D, et al. Cold plasma treatment triggers antioxidative defense system and induces changes in hyphal surface and subcellular structures of Aspergillus flavus. Appl Microbiol Biotechnol. 2018;102:6647–58.
20. Nagase S, Ogai K, Urai T, et al. Distinct skin Microbiome and skin physiological functions between bedridden older patients and healthy people: A Single-Center study in Japan. Front Med. 2020;7:101.
21. Castelino M, Eyre S, Moat J, et al. Optimisation of methods for bacterial skin Microbiome investigation: primer selection and comparison of the 454 versus miseq platform. BMC Microbiol. 2017;17:23.
22. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible Microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.
23. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590–6.
24. Liang B, Zhang K, Wang LY, et al. Different diversity and distribution of archaeal community in the aqueous and oil phases of production fluid from High-Temperature petroleum reservoirs. Front Microbiol. 2018;9:841.
25. Xiao S, Zhang G, Jiang C, et al. Deciphering gut microbiota dysbiosis and corresponding genetic and metabolic dysregulation in psoriasis patients using metagenomics sequencing. Front Cell Infect Microbiol. 2021;11:605825.
26. Li JM, Yu R, Zhang LP, et al. Dietary fructose-induced gut dysbiosis promotes mouse hippocampal neuroinflammation: a benefit of short-chain fatty acids. Microbiome. 2019;7:98.
27. Tomic-Canic M, Burgess JL, O'Neill KE, Strbo N, Pastar I. Skin microbiota and its interplay with wound healing. Am J Clin Dermatol. 2020;21:36–43.
28. Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci USA. 2007;104:2927–32.
29. Grice EA, Kong HH, Conlan S, et al. Topographical and Temporal diversity of the human skin Microbiome. Science. 2009;324:1190–2.
30. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci USA. 2008;105:17994–9.
31. Egert M, Simmering R. The microbiota of the human skin. Adv Exp Med Biol. 2016;902:61–81.
32. Gardner SE, Hillis SL, Heilmann K, Segre JA, Grice EA. The neuropathic diabetic foot ulcer Microbiome is associated with clinical factors. Diabetes. 2013;62:923–30.
33. 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;3:502–10.
34. Price LB, Liu CM, Frankel YM, et al. Macroscale Spatial variation in chronic wound microbiota: a cross-sectional study. Wound Repair Regeneration: Official Publication Wound Healing Soc [and] Eur Tissue Repair Soc. 2011;19:80–8.
35. Price LB, Liu CM, Melendez JH, et al. Community analysis of chronic wound bacteria using 16S rRNA gene-based pyrosequencing: impact of diabetes and antibiotics on chronic wound microbiota. PLoS ONE. 2009;4:e6462.
36. Wolcott RD, Gontcharova V, Sun Y, Dowd SE. Evaluation of the bacterial diversity among and within individual venous leg ulcers using bacterial tag-encoded FLX and titanium amplicon pyrosequencing and metagenomic approaches. BMC Microbiol. 2009;9:226.
37. Frank DN, Wysocki A, Specht-Glick DD, et al. Microbial diversity in chronic open wounds. Wound Repair Regeneration: Official Publication Wound Healing Soc [and] Eur Tissue Repair Soc. 2009;17:163–72.
38. Wolcott RD, Hanson JD, Rees EJ et al. Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. wound repair and regeneration: official publication of the wound healing society [and] the European tissue repair society. 2016;24:163–74.
39. Negut I, Grumezescu V, Grumezescu AM. Treatment strategies for infected wounds. Molecules. 2018;23.
40. Flynn PB, Higginbotham S, Alshraiedeh NH, et al. Bactericidal efficacy of atmospheric pressure non-thermal plasma (APNTP) against the ESKAPE pathogens. Int J Antimicrob Agents. 2015;46:101–7.
41. Modic M, McLeod NP, Sutton JM, Walsh JL. Cold atmospheric pressure plasma elimination of clinically important single- and mixed-species biofilms. Int J Antimicrob Agents. 2017;49:375–8.

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