Clinical, Cosmetic and Investigational Dermatology

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CASE SERIES

Plasma Treatment – Results of Skin Microbiome Analysis

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Purpose: Skin health is deeply linked with the balance of resident bacteria, and a disturbance in this balance can readily cause skin problems. Sterilization with plasma treatment offers potential to improve symptoms in patients with acne vulgaris or atopic dermatitis. The aim of this study was to examine the antibacterial effect of plasma and analyze the correlation between changes in the balance of resident skin bacteria and clinical symptoms following plasma treatment in patients with acne vulgaris or atopic dermatitis.

Patients and Methods: Five patients each with acne vulgaris or atopic dermatitis of moderate severity were treated with plasma irradiation of their face (including lesions) using a nitrogen plasma device called NeoGen Plasma Skin Regeneration. Changes in the resident flora were assessed before and after irradiation using next-generation sequencing.

Results: At 24 hours, the proportion of Propionibacterium acnes decreased from 31.2% to 16.2%, and Staphylococcus epidermidis increased from 10.8% to 11.7%; in patients with atopic dermatitis, Staphylococcus aureus decreased from 4.2% to 2.1%. Across all 10 patients, there was a significant increase in Simpson's Index (P=0.045), indicating improvement of the microbiome balance of resident bacteria.

Conclusion: Plasma treatment had a sterilization effect and improved microbiome balance. Larger studies are therefore warranted.

Keywords: plasma treatment, neogen PSR, resident skin bacteria, acne vulgaris, atopic dermatitis

Introduction

With temperature increases, the state of matter changes from solid to liquid to gas, and molecular movement simultaneously becomes more active. Application of further energy to matter in a gaseous state ionizes the atoms by releasing electrons. This state is called plasma, and is more highly activated and has higher energy than the gaseous state. Given that plasma was initially generated at very high temperatures, its application was limited to waste disposal and nuclear fusion. However, in the early 1990s, a sterilization technique utilizing low-temperature plasma of hydrogen peroxide was put to practical use and is now applicable to various fields involved in the handling of heat-sensitive materials – for example, food processing, the living body, and agriculture.

In recent years, applications in healthcare have been of particular interest, and are being actively researched and developed. These include sterilization by low-temperature plasma,[1] hemostasis, surface treatment, sterilization by ultrasonic plasma, and teeth whitening. Since the plasma state is unstable due to the free movement of atomic nuclei, which release ions and electrons, it can easily bind to other matter. This enables the elimination of cuticles and waste on the skin surface by adsorption to plasma and vaporization.

The following effects have been reported in vivo and in vitro:[2]

1. Skin disinfection and sterilization
2. High transdermal effect (drug delivery)
3. Promotion of heat energy turnover
4. Anti-inflammatory effect
5. Improvement of blood flow
6. Promotion of apoptosis

Received: 11 June 2024
Accepted: 28 April 2025
Published: 26 May 2025

Skin health is deeply linked to the balance of resident bacteria, and hence a disturbance in this balance can cause skin problems.[3,4] Based on the properties listed above, plasma has significant potential for the treatment of various skin diseases, including atopic dermatitis, acne vulgaris, acne scars, and skin tumors, as well as for aesthetic rejuvenation.[5–13]

For example, acne vulgaris is a multifactorial chronic inflammatory disease associated with enhanced sebaceous secretion, hormonal abnormality, dyskeratosis, and inflammation. It is very common in Japan, with an average age of onset of 13 years and a prevalence of almost 60% among young people of secondary school and university age.[14] The condition is caused by Propionibacterium acnes, which releases Christie–Atkins–Munch-Petersen (CAMP) factor, inducing inflammation of hair follicles.[15] It is commonly treated using topical formulations such as adapalene, benzoyl peroxide, and antibacterial agents, but is often refractory due to dermatitis and bacterial resistance. In addition, these treatments are sometimes associated with side effects when used long-term.

Atopic dermatitis primarily causes itchy eczema, characterized by repeated exacerbations and remissions. Exacerbations have been associated with Staphylococcus aureus.[16] In Japan, prevalence estimates range up to 32% in infants and 15% in school-age children; the estimated prevalence is lower among adults, but remains ≥ 8% in those below 40 years, and the proportion of severe cases is higher among young adults compared with children.[17] Atopic dermatitis is commonly treated using topical steroids and tacrolimus ointments, and other options have recently become available, including JAK inhibitors and biologics. However, common side effects include dermatitis, skin irritation, and worsening of acne.

Thus, there remains a need for novel therapies for both acne vulgaris and atopic dermatitis that are more effective and safe than current options. Plasma treatment is particularly promising because it is minimally invasive, combines antimicrobial and tissue repair effects, has few side effects, and avoids the problem of antibiotic-resistant bacteria.

In our clinic, we have introduced a nitrogen plasma device called NeoGen Plasma Skin Regeneration (PSR) (Energist Ltd, Swansea, United Kingdom). The technology works by converting nitrogen gas to pure nitrogen plasma, which emerges from the handpiece in pulses and delivers controlled heating without creating an open wound. We have used NeoGen PSR for various skin diseases, including acne vulgaris, acne scarring, and atopic dermatitis – as well as lichen pilaris and chloasma, and aesthetic indications like pores, wrinkles, pigmentation, and texture improvement.

Plasma treatment is a noninvasive method for enhancing skin turnover, and has a variety of potential mechanisms of action.[7,8,12,18] Among various possible effects that may contribute to the improvement of clinical symptoms in patients with acne vulgaris or atopic dermatitis, we have focused on the sterilization effect of plasma.

The aim of the present study was to evaluate the impact of the antimicrobial action of plasma therapy on the balance of indigenous skin bacteria and its relationship with clinical symptoms. This was assessed using next-generation sequencing from lesions in patients with each disease before and after plasma irradiation.

Methods

Study Overview

Five patients with acne vulgaris and 5 with atopic dermatitis were included, each with moderate disease severity according to guidelines from the Japanese Dermatological Association. The study was conducted between June and July 2018. The Medical Ethics Committee of Non-Profit Organization Health Institute Research of Skin granted ethical approval to publish case details (No. IB202404-01). It was confirmed with patients that the details of any images, videos, or recordings could be published. All patients provided written informed consent for publication.

Procedures

One week before irradiation, patients with acne vulgaris discontinued topical and oral medications with antibacterial and anti-inflammatory effects. Similarly, those with atopic dermatitis discontinued topical medications with an anti-inflammatory effect (including topical steroids). Intake of foods that could affect the composition of resident bacteria, such as natto (fermented soybeans) and yogurt, was also prohibited for 1 week before irradiation.

On the morning of the day of plasma irradiation, a skin swab sample of resident bacteria was collected from the sampling region, which was defined as a circle with a 3 cm diameter in the cheek and 1 cm apart from the lower eyelid and the nasal ala, as determined using a specific scale. The patient then washed their face.

Full-face irradiation was performed using NeoGen PSR at an intensity of 0.8 J/cm^2, followed by iontophoresis of tranexamic acid. Twenty-four hours after irradiation, a second sample of resident bacteria was collected from the same region as previously.

Samples were analyzed using the next-generation sequencer, S-KIN (metagenome analysis, World Fusion Co. Ltd., Tokyo, Japan). Specifically, for the microbiome analysis, DNA samples were fragmented and amplified to read out the V2, 3, 4, 5, 6, 7, 8, and 9 regions of the 16S rRNA gene by Ion PGM sequencer, while eliminating from the analysis any bases of poor quality or lead lengths of ≤ 120 base pairs. Total bacterial counts were determined by real-time polymerase chain reaction (PCR) with a Takara Bacteria Quantitative PCR Kit.

Statistical Methods

For mean bacterial counts (Staphylococcus epidermidis, Staphylococcus aureus, and Propionibacterium acnes) before and 24 hours after irradiation, 95% confidence intervals (CI) were calculated. Changes in Simpson's Index (a diversity index of resident skin bacteria) over the same time period were assessed using a paired *t*-test. Analyses were performed using the S-KIN software.

Results

Changes in the primary microbiome were analyzed over time for 24 hours after plasma irradiation using S-KIN metagenome analysis. The proportion of Propionibacterium acnes decreased from 31.2% (95% CI: 8.8, 53.5) to 16.2% (95% CI: 4.3, 28.2) (Figure 1), and for Staphylococcus epidermidis, it increased from 10.8% (95% CI: 2.3, 19.4) to 11.7% (95% CI: 3.7, 19.7) (Figure 2). Staphylococcus aureus was assessed in the 5 patients with atopic dermatitis, and was found to have decreased from 4.2% (95% CI: –1.7, 10.1) to 2.1% (95% CI: –1.2, 5.3) (Figure 3).

Four case examples are provided in Figures 4–7. Case 1 was a 31-year-old woman with acne vulgaris who had pustules and papulae on the whole of her forehead, cheeks, and jaw. She showed improvement after irradiation without new symptoms (Figure 4A). Within 24 hours, the proportion of Propionibacterium acnes in the total bacterial count decreased from 5% to 4% (Figure 4B).

Case 2 was a 23-year-old woman with acne vulgaris who had pustules and papulae on the whole of her forehead and cheeks. She showed improvement after irradiation (Figure 5A). After 24 hours, the proportion of Propionibacterium acnes markedly decreased from 74% to 36% (Figure 5B).

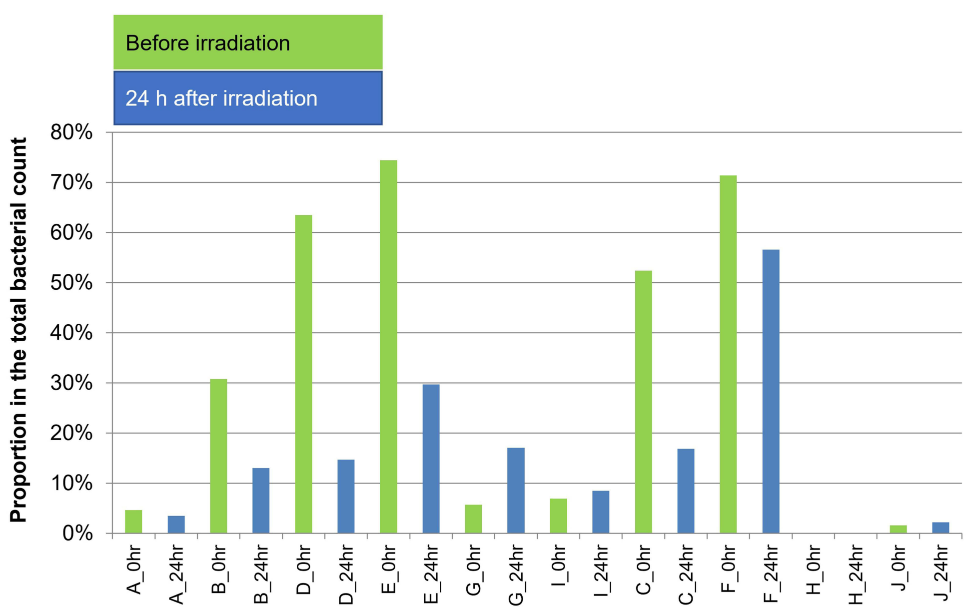

Figure 1 Changes in primary bacterial species over time: Propionibacterium acnes. The figure shows results from swab samples before irradiation and at 24 hours after irradiation.

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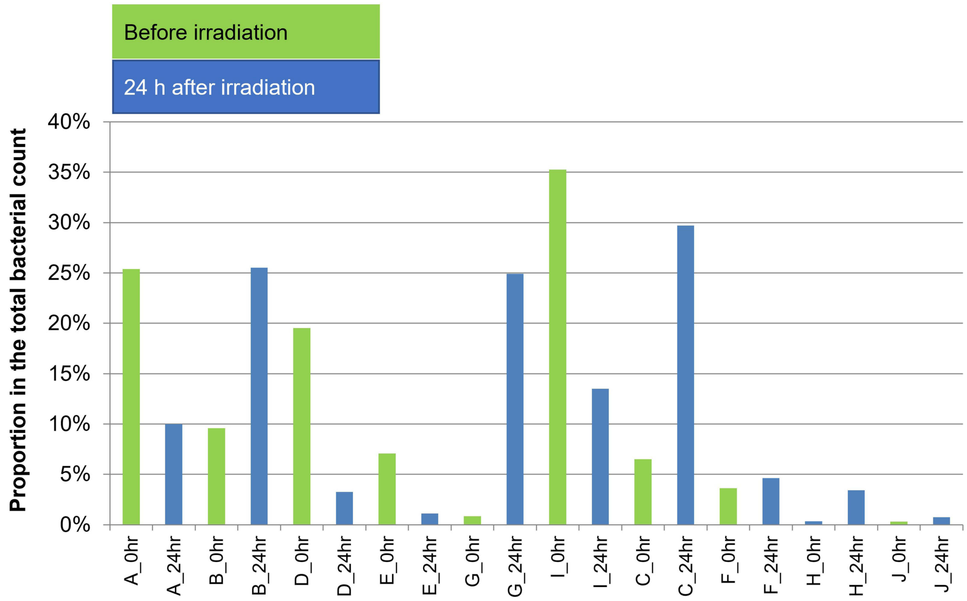

Figure 2 Changes in primary bacterial species over time: Staphylococcus epidermidis. The figure shows results from swab samples before irradiation and at 24 hours after irradiation.

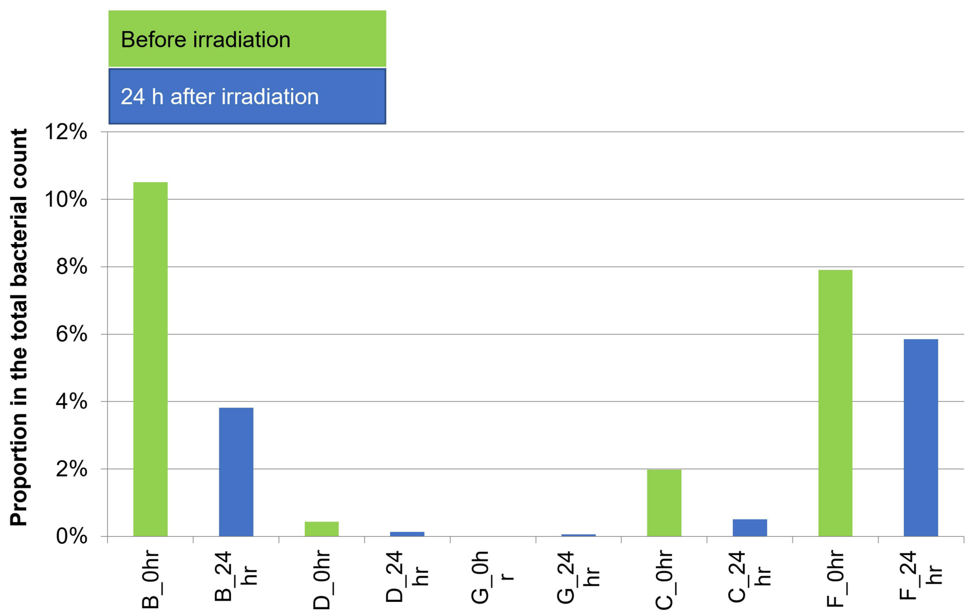

Figure 3 Changes in primary bacterial species over time in patients with atopic dermatitis: Staphylococcus aureus. The figure shows results from swab samples before irradiation and at 24 hours after irradiation.

Case 3 was a 36-year-old man who had had atopic dermatitis since childhood. Following irradiation, he showed improvement of diffuse erythema on the whole face, as well as smoother skin (Figure 6A). Within 24 hours, the proportion of Staphylococcus aureus decreased from 11% to 4%, and the proportion of Staphylococcus epidermidis increased from 10% to 26% (Figure 6B).

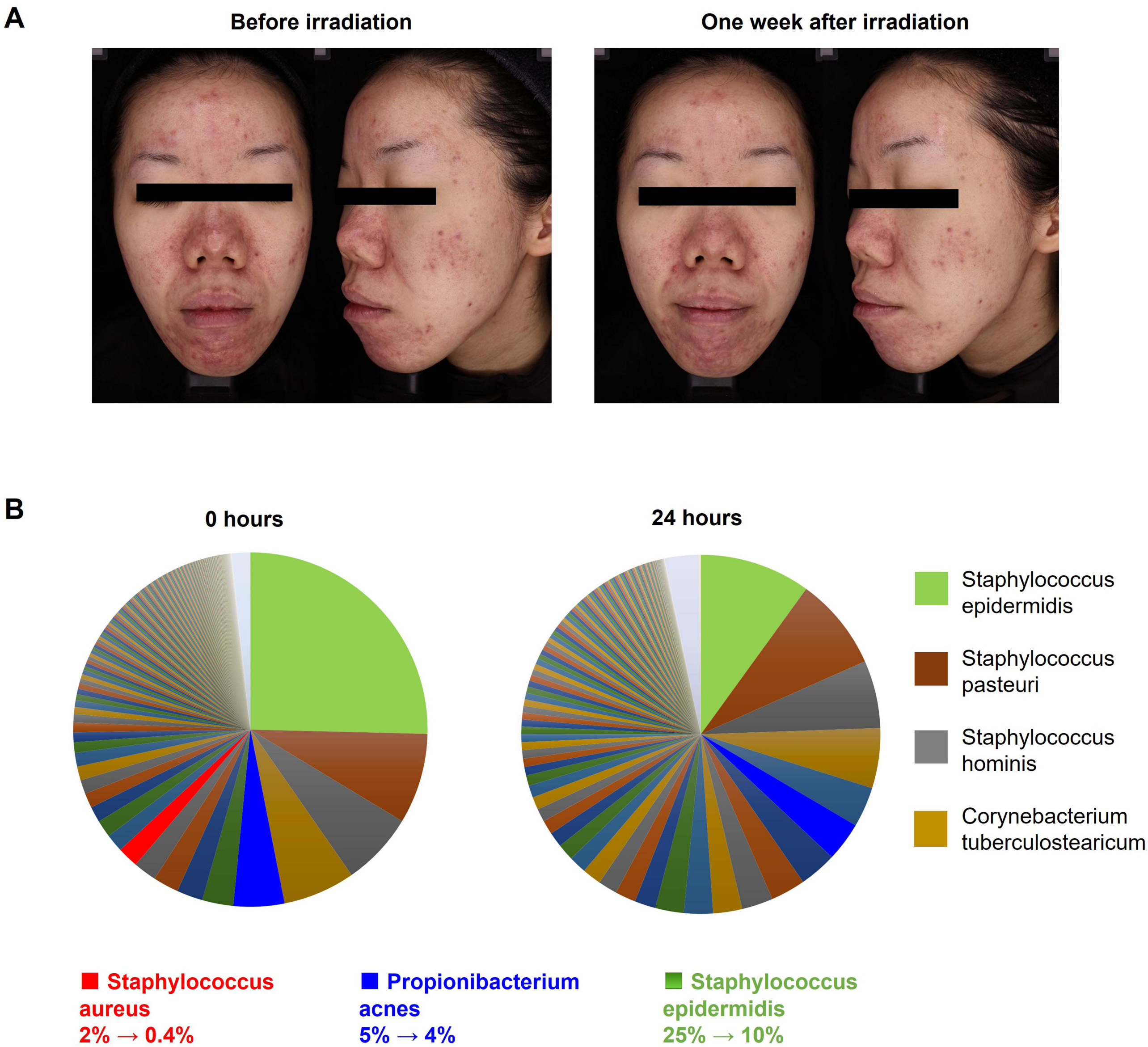

Figure 4 A 31-year-old woman with acne vulgaris. (**A**) Shows the patient before and 1 week after treatment. (**B**) Shows changes in all bacterial species at 0 and 24 hours.

Case 4 was a 44-year-old woman with atopic dermatitis since childhood who had used topical steroids for a long time, which induced a complication of cataract. After irradiation, the clinical symptoms improved not only for erythema and dryness but also for skin texture, resulting in smoother skin (Figure 7A). Within 24 hours, the proportion of Staphylococcus aureus decreased from 8% to 6% (Figure 7B).

Across the 10 included patients, analysis of bacterial counts using real-time PCR showed decreases in all three bacterial species at 24 hours after plasma irradiation (Table 1). There were particularly meaningful reductions in Propionibacterium acnes and Staphylococcus aureus. Furthermore, Simpson's Index – a diversity index of resident skin bacteria – significantly increased within 24 hours (P=0.045), indicating improvement of the microbiome balance of resident bacteria (Table 2).

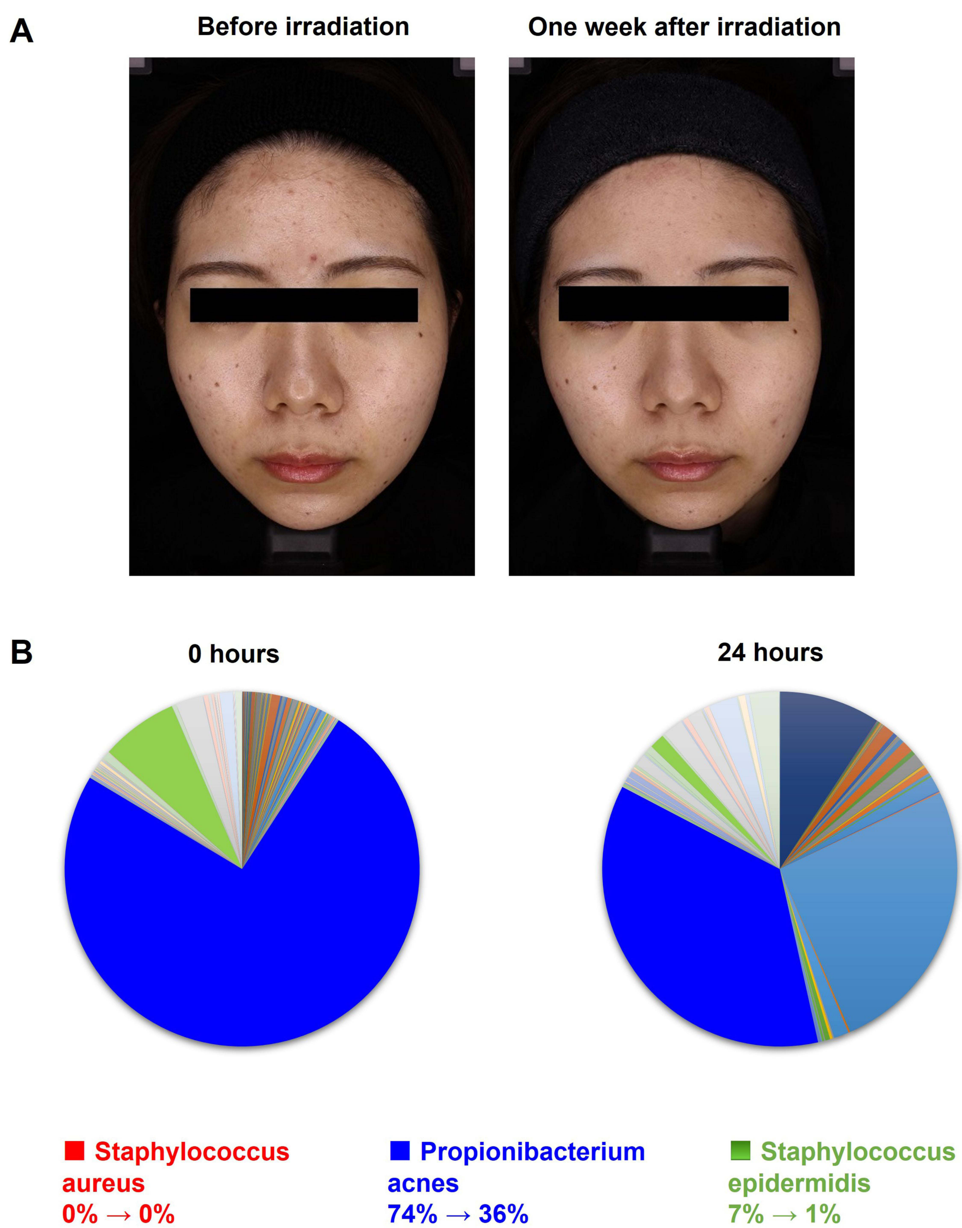

Figure 5 A 23-year-old woman with acne vulgaris. (**A**) Shows the patient before and 1 week after treatment. (**B**) Shows changes in all bacterial species at 0 and 24 hours.

Discussion

Applying heat to a gas at thousands of degrees Celsius ionizes the molecules into positive ions and electrons that can move freely; this is plasma, considered as a fourth state of matter. In recent years, there has been significant interest in potential applications within the healthcare field. Since the 1990s, low-temperature sterilization using various types of plasma has been vigorously studied, for example based on low-pressure discharge plasma[19–29] and atmospheric pressure discharge plasma.[30–35] The principle of plasma sterilization is that highly reactive radicals created by the collision of high-energy electrons with atoms and molecules in plasma chemically react with cells to kill bacteria. In addition, bacterial death may be caused by damage to cellular DNA from ultraviolet rays emitted from atoms excited by the high-energy electrons, as well as the direct collision with cells of discharged particles, such as electrons and ions.

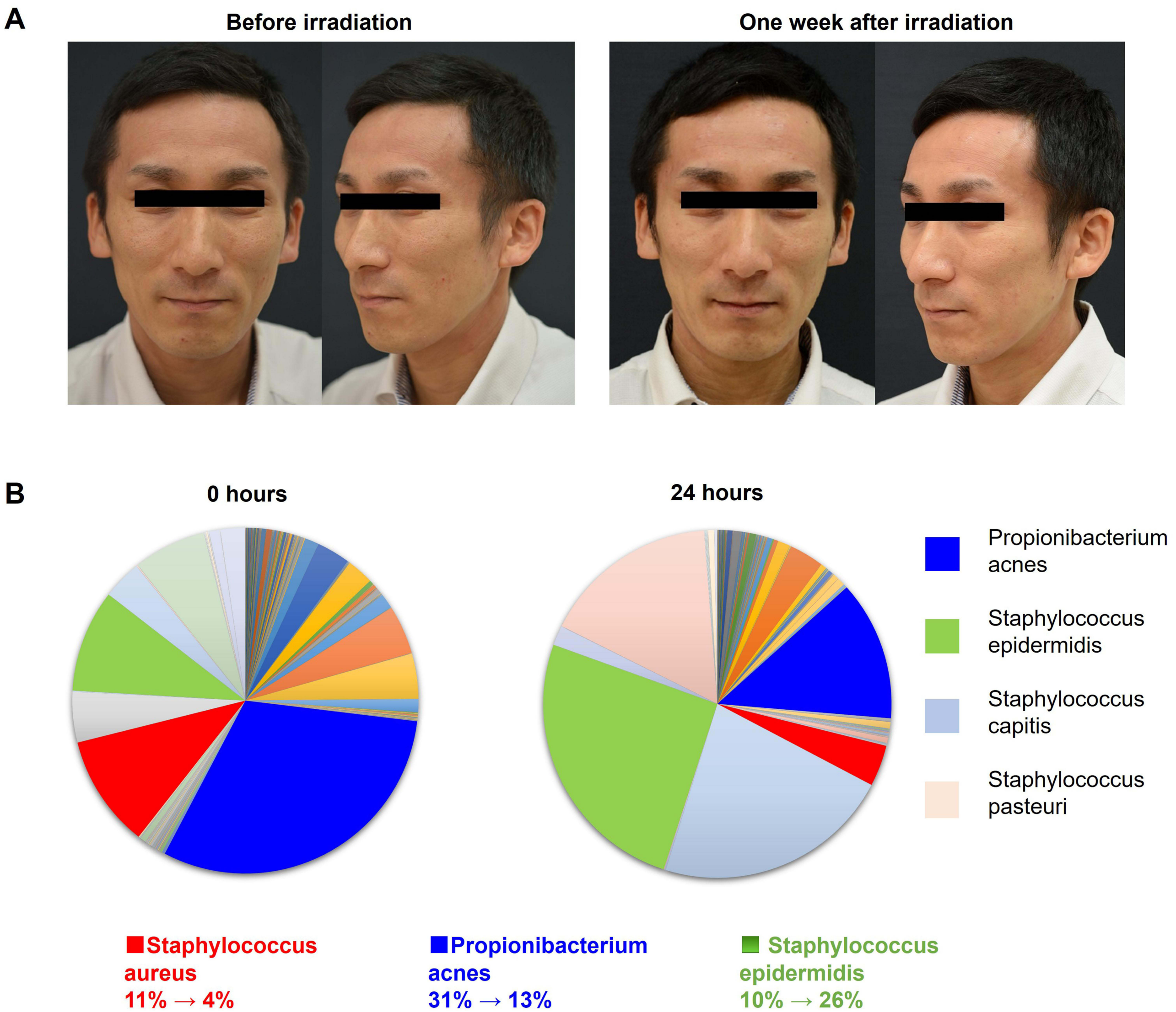

Figure 6 A 36-year-old man with atopic dermatitis. (**A**) Shows the patient before and 1 week after treatment. (**B**) Shows changes in all bacterial species at 0 and 24 hours.

In dermal diseases, plasma treatments may be used for skin disinfection and sterilization, transdermal effects (drug delivery), promotion of heat energy turnover, anti-inflammatory effects, blood flow improvement, promotion of apoptosis (as well as a cancer cell reduction[1]), skin rejuvenation,[7,8,13] and scar treatment.[36–38] In the optically damaged layer produced by plasma irradiation, the epidermis remains intact and forms a thin crust that acts as a natural dressing to promote healing of heat injury in the dermis and lower layers.

In our practice, we use the nitrogen gas plasma device, NeoGen PSR, to achieve good results in various indications, including atopic dermatitis, acne vulgaris, acne scarring, post-inflammatory pigmentation, and wrinkle treatment. Treatment benefit may be induced by the combined effects described above.

In the present study, we focused on the correlation between plasma sterilization effects and improvements in skin symptoms, and observed changes in the facial microbiome before and after irradiation – analyzed by next-generation sequencing and clinical findings. The bacterial component of acne development is familiar within the dermatologic field. However, in recent years, abnormal balances of the microbiome constituting resident flora have been linked with the development of other common concerns, such as allergy and autoimmune diseases.[3,4]

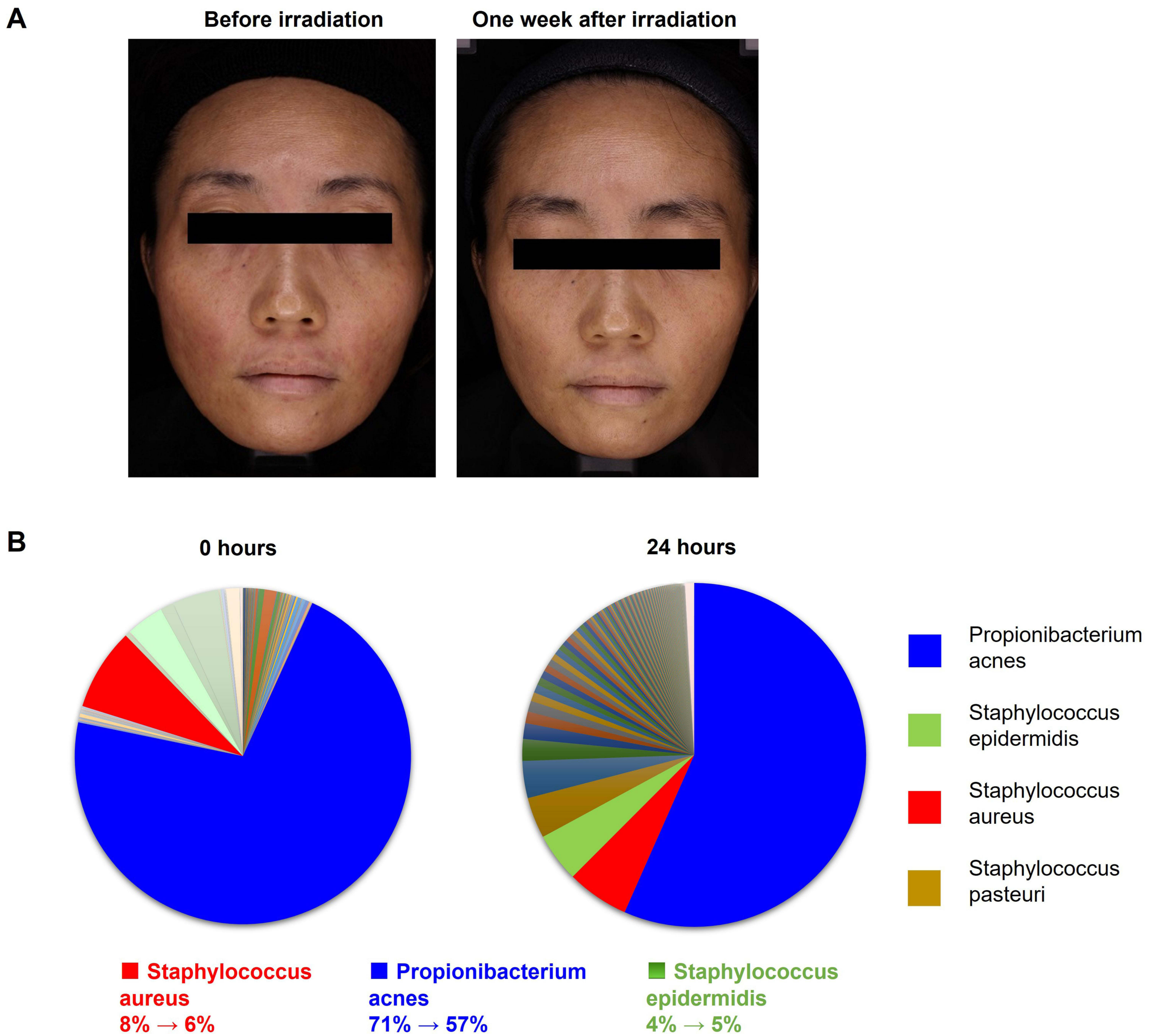

Figure 7 A 44-year-old woman with atopic dermatitis. (**A**) Shows the patient before and 1 week after treatment. (**B**) Shows changes in all bacterial species at 0 and 24 hours.

Resident bacteria on skin include Staphylococcus epidermidis, Propionibacterium acnes, and Staphylococcus aureus.[16] Staphylococcus epidermidis is found on the surface of the epidermis and pores;[39] it is considered to feed on sweat (alkaline) and sebum to produce glycerin and fatty acids,[40] keep the skin mildly acidic,[41] avoid proliferation of Staphylococcus aureus by producing antibacterial peptides, and maintain skin barrier function.[42]

Table 1 Changes in Bacterial Counts Before and After Irradiation as Determined by Real-Time PCR

Bacteria Name	Mean Diversity Index at 0 h	Mean Diversity Index at 24 h
Staphylococcus epidermidis	1769	1452
Propionibacterium acnes	6271	1274
Staphylococcus aureus	544	200

Abbreviation: PCR, polymerase chain reaction.

Table 2 Diversity Index of Resident Skin Bacteria

	Mean Diversity Index at 0 h	Mean Diversity Index at 24 h	P value (0 h vs 24 h; Paired *t*-test)
Simpson's Index	0.734	0.845	0.045

Propionibacterium acnes is an anaerobic bacterium found in the pores and sebaceous glands, and may feed on sebum to produce propionic acid and fatty acids, keep the skin mildly acidic, and inhibit the proliferation of highly pathogenic bacteria.[43] It is generally considered to be a key cause of acne vulgaris. Excess proliferation of Propionibacterium acnes may result from increased sebum secretion and plugged pores as a result of this or of dyskeratosis.

Staphylococcus aureus is found on the skin surface and pores,[44,45] and is considered to induce dermatitis by proliferation on alkaline skin.[46]

In recent years, gene decoding technology has advanced as a result of next-generation sequencing, which enables exhaustive investigation of the microbiome through analysis of bacterial 16S rRNA. A microbiome analysis in atopic dermatitis found that the composition of the skin flora changed dramatically, with a significant increase of Staphylococcus aureus.[47]

In our study of a nitrogen gas plasma treatment device in patients with acne vulgaris or atopic dermatitis, counts of Propionibacterium acnes and Staphylococcus aureus decreased while Staphylococcus epidermidis increased in some cases. Furthermore, there was a recovery in the diversity of resident skin flora. Case studies showed that patients experienced parallel improvements in symptoms. The reasons for this may have been decreases in causal bacteria (Propionibacterium acnes inducing inflammation in acne vulgaris, and Staphylococcus aureus in atopic dermatitis), as well as improvements in the balance of resident skin bacteria.

We must acknowledge the limitations of the present study. In particular, the sample size was small and the follow-up period lasted only a few days. Nonetheless, the positive results achieved provide a rationale for larger multicenter studies of longer duration, which will be needed to conclusively demonstrate the impact of plasma treatment in these patients. Additional research is also required to explore the utility of combining it with other standard treatments (eg topical steroids, antibacterials, and biologics).

In the long-term, plasma treatment may offer the possibility of regulating indigenous skin bacteria to a more balanced composition and promoting the restoration of skin barrier function. Further studies are needed to assess such changes in barrier function, as well as the immune response and the process of recovery of the indigenous microbiota. These would provide a more definitive assessment of the safety and efficacy of plasma treatment. Another important research area will be to analyze how persistent changes in specific skin microbiota affect the risk of recurrence of inflammatory diseases.

Conclusion

In this pilot study in 10 patients with acne vulgaris or atopic dermatitis, plasma treatment not only had a sterilization effect but also improved the microbiome balance of resident skin bacteria. This has potential to physically and physiologically improve skin barrier function by regenerating the keratinous layer, resulting in the improvement of skin symptoms in acne vulgaris and atopic dermatitis. Larger studies are therefore warranted.

Consent for Publication

We have confirmed with the patients that the details of any images, videos, recordings, etc can be published. Patients' informed consent for publication of their case details and images was obtained in written form. The Medical Ethics Committee of Non-Profit Organization Health Institute Research of Skin has granted ethical approval for this study to publish case details (No. IB202404-01).

Disclosure

The author reports no conflicts of interest in this work.

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