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Effects of 308 nm excimer light treatment on the skin microbiome of atopic dermatitis patients

Running Head: Effects of excimer light on the microbiome of AD

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Abbreviations: AD, atopic dermatitis; HC, healthy controls; NB, narrowband; UVB, ultraviolet B; UV, ultraviolet; EASI, Eczema Area and Severity Index; TEWL, transepidermal water loss; SC, stratum corneum; rRNA, ribosomal RNA; ITS, internal transcribed spacer; PCR, polymerase chain reaction; SD, standard deviation; pre NL, pre-untreated non-lesional skin; post NL, post-untreated non-lesional skin; pre L, pre-treated lesional skin; post L, post-treated lesional skin; *S. aureus*, *Staphylococcus aureus*; *M. globosa*, *Malassezia globosa*.

ABSTRACT

Background: The skin microbiome has been implicated in the pathophysiology of atopic dermatitis (AD). Although 308 nm excimer light treatment is an effective phototherapy for AD, its effects on the skin microbiome currently remain unclear. Therefore, we investigated the effects of the excimer light treatment on the skin bacterial and fungal microbiome of lesional skin of AD.

Methods: Swab samples were collected from 11 healthy controls (HC), non-lesional and lesional skin of 11 AD patients. The excimer light treatment was administered to the lesional skin. The composition of the skin microbiome, the clinical score and skin barrier function of the lesional skin were examined before and after the treatment. The composition of the skin microbiome was determined by sequencing bacterial 16S and fungal ITS regions.

Results: The excimer light treatment significantly changed the composition of the bacterial microbiome in the lesional skin of AD, as well as improved the clinical score and skin barrier function. The treatment increased the relative abundance of the phylum Cyanobacteria and decreased that of the phylum Bacteroidetes in lesional skin. At the species level, the treatment significantly decreased the relative abundance of *Staphylococcus aureus* (*S. aureus*) in lesional skin. There was also a significant correlation between the reduction of *S. aureus* and improvement of the clinical outcomes.

Conclusion: Our findings suggest that alterations of the skin microbiome with excimer light treatment, specifically the decrease of the abundance of *S. aureus*, are partly involved in the improvement of AD lesions.

Keywords: Atopic dermatitis, 308 nm excimer light, Skin microbiome, *Staphylococcus aureus*

1. INTRODUCTION

Atopic dermatitis (AD) is a chronic and itchy inflammatory skin disease that is influenced by genetic and environmental factors following skin barrier dysfunction.[1,2] Besides these factors, the skin microbiome, the community of microorganisms colonizing the skin, is regarded as playing a role in the pathophysiology of AD.[3] Healthy skin is inhabited by large numbers of microorganisms in the skin microbiome.[4] In AD, however, *Staphylococcus aureus* (*S. aureus*) colonizes the lesional skin, and flare-ups are often associated with *S. aureus* infection.[5] Furthermore, the altered skin fungal microbiome has been associated with compromised skin immunities.[6] In particular, it is documented that *Malassezia* yeasts may contribute to the pathogenesis of AD.[7] Therefore, dysbiosis in the bacterial and fungal microbiome is suggested to aggravate AD.

Clinically, phototherapy with narrowband (NB) ultraviolet B (UVB) is recommended for moderate to severe AD, offering several benefits including being a steroid-sparing and long-lasting treatment.[8] 308 nm excimer light treatment is an effective monochromatic UVB therapy for AD.[9,10]

The advantages of monochromatic excimer light treatment over NB-UVB are as follows: (i) it has a shorter irradiation time; (ii) it only irradiates localized areas, which reduces the risk of malignancy; and (iii) fewer sessions are required per week, which is beneficial for patients.[11] However, the effects of 308 nm excimer light irradiation on the skin microbiome have not yet been clarified.

Therefore, the purpose of this study is to investigate the effects of 308 nm excimer light treatment on the skin bacterial and fungal microbiome of the lesional skin of AD patients by comparing it with that of non-lesional skin and healthy controls (HC).

2 METHODS

2.1 Study participants and sample collection

Eleven Japanese patients with moderate to severe AD were enrolled between September and October 2018 at the outpatient clinic of the Department of Dermatology, Juntendo University Urayasu Hospital, Japan. AD was diagnosed according to the criteria of Hanifin and Rajka, which include the typical morphology and distribution of eczematous lesions, chronicity of the disease, and a personal or family history of atopy.[12] Inclusion criteria for AD patients were an age of 20–50 years and the presence of eczematous lesions on the upper arm at enrollment. Eleven HC were also recruited as a negative control group, consisting of subjects without a history of AD or other skin diseases. Exclusion criteria for study subjects were as follows: pregnancy, known malignancy, active systemic diseases, the use of topical or systematic antibiotics or antimyctotics, systemic anti-inflammatory or immunomodulating treatment, and intensive ultraviolet (UV) exposure 4 weeks prior to study enrollment.

During the study, the study participants did not receive any systemic treatment. Furthermore, the participants were instructed not to apply any topical agent to the upper arm. Topical application was restricted to moisturizing cream and betamethasone valerate 0.12% for other AD lesions that were not targeted in this study. Neither showering nor bathing was permitted for 12 hours before sample collection. Skin swab samples were obtained from HC and the non-lesional and lesional skin of AD by swabbing the skin in a 5×5 cm area with HydraFlock swabs (Puritan Medical Products, Guilford, ME, USA), which were premoistened with sterilized saline. Swabs were stored at 4°C until DNA extraction.

This study was conducted according to the Declaration of Helsinki Principals, and

approved by the Ethical Committee of Juntendo University Urayasu Hospital. The purpose and procedures of the study were explained in detail, and written informed consent was obtained from participants.

2.2 Phototherapy protocol with 308 nm excimer light

The excimer light treatment was administered to the lesional skin on the upper arm of AD patients using TheraBeam UV308 mini excimer light (Ushio Inc., Tokyo, Japan). The initial dose of irradiation was based on the minimal erythema dose and skin type, and subsequent doses were determined based on the response to the treatment. The lesional skin of AD was treated with excimer light once a week for two months. The non-lesional skin of AD was not treated (untreated) with excimer light. Thereafter, swab samples were collected from the post-treated lesional skin and post-untreated non-lesional skin of AD, which were the same sampling sites as pre-treated lesional skin and pre-untreated non-lesional skin.

2.3 Assessment of the severity of AD and clinical score of lesional skin

The severity of AD was assessed using the Eczema Area and Severity Index (EASI) score at enrollment. The EASI score is an index of overall eczema with the quantification of erythema, papulation, excoriation, and lichenification on each part of the body (head, arms, trunk, and legs). The severity of AD based on the EASI score is categorized as follows: 0 = clear; 0.1 to 1.0 = almost clear; 1.1 to 7.0 = mild; 7.1 to 21.0 = moderate; 21.1 to 50.0 = severe; 50.1 to 72.0 = very severe.[13]

The clinical score of lesional skin on the upper arm was evaluated with the local severity score according to 4 symptoms: erythema, edema/papulation, excoriation, and

lichenification. Each symptom was graded from 0 to 3 (none, 0; mild, 1; moderate, 2; severe, 3). The local severity score was defined as the sum of the individual scores and ranged from 0 to 12.

2.4 Assessment of skin barrier function

To assess the skin barrier function of subjects, transepidermal water loss (TEWL) and stratum corneum (SC) hydration were measured. TEWL reflects the function of the epidermal permeability barrier by measuring vaporized water through the SC. TEWL increases when epidermal barrier structures are disrupted.[14] SC hydration is an indicator of skin dryness and reflects the water content of the SC.[15] TEWL and SC hydration were evaluated using VapoMeter and MoistureMeterSC (Delfin Technologies Ltd., Kuopio, Finland), respectively, at skin swabbing sites on the lesional and non-lesional skin of AD and HC, according to the manufacturer's instructions.

2.5 Bacterial 16S ribosomal RNA (rRNA) and fungal internal transcribed spacer (ITS) sequencing

DNA was extracted from swab samples with the QIAamp UCP Pathogen Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, including a mechanical pre-lysis step, followed by a spin protocol.

After DNA extraction, seven bacterial 16S hypervariable regions were amplified with two primer sets: one for amplifying the V2-4-8 hypervariable regions and the other for amplifying the V3-6, 7-9 hypervariable regions. The primers were supplied with the Ion 16S™ Metagenomics Kit (Thermo Fisher Scientific, Grand Island, NY, USA) and used according to the manufacturer's instructions. The fungal ITS primers used were as follows

(forward and reverse, respectively); ITS1, 5'-GGAAGTAAAAGTCGTAACAAGG-3' and 5'-GCTGCGTTCTTCATCGATGC-3'; ITS2, 5'-GCATCGATGAAGAACGCAGC-3' and 5'-TCCTCCGCTTATTGATATGC-3'. The polymerase chain reaction (PCR) was performed using the PrimeSTAR GXL DNA Polymerase (Takara, Shiga, Japan) according to the manufacturer's instructions. PCR products were confirmed using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with DNA 1000 Kit (Agilent Technologies). The amplified fragments were then bar-coded and sequenced on the Ion PGM sequencer system (Thermo Fisher Scientific) using 318 chips with the Ion PGM Hi-Q View Sequencing Kit (Thermo Fisher Scientific).

2.6 Bioinformatics analysis

The determined 16S rRNA, ITS1, and ITS2 sequences were subjected to homology searches for a BLAST analysis against the NCBI 16S Microbial database (National Center for Biotechnology Information, Bethesda, MD) and rdp_classifier_2.12 with the fungalits_unite database, respectively, and then classified using Metagenome@KIN (World Fusion, Tokyo, Japan).

2.7 Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Comparisons were performed using paired *t*-tests or Welch's unpaired *t*-tests where appropriate. To investigate the correlation between percent change of *S. aureus* and that of local severity score or TEWL or SC hydration, Pearson's correlation tests were performed. Statistical analyses were conducted using Prism 7 software (Graphpad Software Inc., La Jolla, CA, USA). In all analyses, $p < 0.05$ was regarded as statistically significant.

3 RESULTS

3.1 Characteristics of study subjects

The characteristics of AD patients and HC are shown in Table 1. The mean ages of AD and HC were 29.9 ± 8.55 and 33 ± 5.75 years, respectively. The EASI score of AD patients ranged between 9.6 and 36; 5 patients had moderate AD and 6 had severe AD. The local severity score of lesional skin before the excimer light treatment (pre-treated) was 6.09 ± 0.83. The cumulative UV dose that AD patients received on lesional skin was 2.33 ± 0.61 J/cm^2 at the end of the 2-month treatment period. The characteristics as well as skin barrier function of each subject are found in Table S1.

3.2 Effects of the excimer light treatment on the clinical score and skin barrier function of the lesional skin of AD

The local severity score of lesional skin significantly decreased after the excimer light treatment from 6.09 ± 0.83 to 3.64 ± 1.03 (Fig. 1a). TEWL was higher in the pre-treated lesional skin (pre L) of AD than in HC; however, the excimer light treatment significantly decreased TEWL in post-treated lesional skin (post L) (Fig. 1b). SC hydration was significantly lower in the pre-treated lesional skin (pre L) of AD than HC, and the excimer light treatment significantly increased SC hydration in the post-treated lesional skin (pre L) of AD (Fig. 1c). These results suggest that the excimer light treatment significantly improved the clinical score and skin barrier function of the lesional skin of AD.

3.3 Analysis of the skin bacterial microbiome at the phylum level

The relative abundance of the bacterial phyla of all swab samples is shown in Figure 2a-e. Distribution of bacterial phyla of each sample is found in Table S2a. Five dominating phyla

were identified: Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria. The relative abundance of Cyanobacteria was lower in the pre-untreated non-lesional skin (pre NL) and pre-treated lesional skin (pre L) than in HC (Fig. 2f). After the excimer light treatment, the relative abundance of Cyanobacteria significantly increased, while Bacteroidetes significantly decreased in the post-treated lesional skin (post L) (Fig. 2 f, g).

3.4 Analysis of *Staphylococcus* species

A previous study reported that *S. aureus* was isolated in 80-100% of AD patients.[16] Therefore, this study investigated the effects of the excimer light treatment on *Staphylococcus* species in AD. Seven *Staphylococcus* species were identified, as shown in Figure 3a-e. Distribution of *Staphylococcus* species of each sample is found in Table S2b. In HC, *Staphylococcus epidermidis* was the most abundant species (Fig. 3a). *S. aureus* was frequently detected in each AD group (pre NL, post NL, pre L, and post L), and every AD group had a significantly higher abundance of *S. aureus* than HC (Fig. 3f). These results suggest that *S. aure*us is abundant in AD skin. *S. aureus* accounted for 1.47 ± 2.28% in HC (Fig. 3f). In contrast, *S. aureus* accounted for 28.2 ± 21% in pre-treated lesional skin (pre L) (Fig. 3f). After the excimer light treatment, the relative abundance of *S. aureus* significantly decreased to 11.7 ± 14.4% in post-treated lesional skin (post L) (Fig. 3f). In correlation analysis, there was a significant correlation between percent reduction of S. aureus and that of local severity score in the lesional skin (Fig. 4a). Furthermore, there was a significant correlation between the reduction of S. aureus and that of TEWL and the increase of SC hydration (Fig. 4b, c).

3.5 Analysis of the skin fungal microbiome at the phylum level

The relative abundance of the fungal phyla of all swab samples is shown in Figure S1a-e. Distribution of fungal phyla of each sample is found in Table S3a. Two major fungal phyla, Ascomycota and Basidiomycota, were identified. In HC, Basidiomycota was the dominant phylum (Fig. S1a). Pre-treated lesional skin (pre L) had a significantly higher abundance of Ascomycota than HC (Fig. S1f). However, there was no significant change in the composition of the fungal phyla in lesional skin after the excimer light treatment (Fig. S1d-f).

3.6 Analysis of *Malassezia* species

Malassezia species have been suggested to contribute to skin inflammation and flares of AD.[3] Thus, *Malassezia* species were focused on in the fungal analysis. Nine *Malassezia* species were identified from swab samples (Fig. S2a-e). Distribution of *Malassezia* species of each sample is found in Table S3b. In HC, *Malassezia restricta* was the most abundant species (Fig. S2a). The second most abundant species in HC was *Malassezia globosa* (*M. globosa*), accounting for 15.5 ± 23.1% (Fig. S2a, f). In AD, *M. globosa* was the most dominant species, accounting for 55 ± 32.8% in pre-untreated non-lesional skin (pre NL) and 55.8 ± 36.2% in pre-treated lesional skin (pre L) (Fig. S2f). The relative abundance of *M. globosa* was higher in AD than in HC (Fig. S2f). However, no significant changes were observed in the composition of *Malassezia* species in lesional skin after the excimer light treatment (Fig. S2d-f).

4 DISCUSSION

In this study, 308 nm excimer light treatment demonstrated significant changes in the composition of the bacterial microbiome in the lesional skin of AD, along with improvement of the clinical score and skin barrier function.

Monochromatic excimer light has the following benefits over NB-UVB for AD patients: a shorter irradiation time and fewer treatment sessions for the targeted lesional skin, limiting unnecessary exposure to UV light.[11,17] The therapeutic potential of manipulating the skin microbiome in AD has been suggested in the previous study.[18]

We hereby investigated the effects of 308 nm excimer light on the skin microbiome of AD. At the bacterial phylum level, two remarkable alterations were observed in lesional skin after the excimer light treatment: an increase in the relative abundance of Cyanobacteria and a decrease in that of Bacteroidetes. HC had more abundance of Cyanobacteria than the lesional skin of AD, and the relative abundance of Cyanobacteria in lesional skin significantly increased following the excimer light treatment. Cyanobacteria, also known as blue-green algae, are photosynthetic bacteria and distributed in moist habitats.[19] Healthy skin has more water content in SC than AD skin, which may be why higher abundance of Cyanobacteria in HC than the lesional skin of AD was observed.

At the bacterial species level, we observed a marked reduction in the relative abundance of *S. aureus* in the lesional skin after the excimer light treatment. Furthermore, we observed a significant correlation between the reduction of *S. aureus* and improvement of clinical outcomes. In a previous study, NB-UVB treatment significantly decreased *S. aureus* in the skin of AD and control patients with vitiligo as well as its production of toxins.[4] Another study reported that 308 nm excimer light irradiation exerts

antipruritic effects through the induction of epidermal nerve degeneration and reduces scratching behavior in dry skin model mice.[20] Healing of the lesion impairs the attachment of *S. aureus* in AD.[21]

The overgrowth of *S. aureus* correlates with disease severity and eczematous flares in AD.[22,23] *S. aureus* downregulates terminal differentiation proteins, such as filaggrin and loricrin, which lead to skin barrier disruption.[24] Severe AD had more *S. aureus* than moderate AD, and the relative abundance of the bacteria markedly decreased in lesional skin after the excimer light treatment. Therefore, our results suggest that the reduction of the relative abundance of *S. aureus* with the excimer light treatment partly contributed to the improvement of barrier function in lesional skin. *S. aureus* was previously reported to adhere to atopic corneocytes[25,26], which may explain why *S. aureus* was abundant not only in the lesional skin of AD, but also in the non-lesional skin of AD.

The fungal microbiome has been suggested to play a role in aggravating AD.[27] In our study, 308 nm excimer light treatment did not markedly affect the skin fungal microbiome of lesional skin. Fungi possess various types of melanins, which are resistant to UV light.[28-30] Topical treatments have been applied to alter the fungal microbiome of AD.[31,32]

There are limitations to the study. The excimer light treatment was not administered to the non-lesional skin of AD and HC. However, our aim was to investigate the effects of the excimer light treatment on the skin microbiome in lesional skin.

In conclusion, 308 nm excimer light treatment significantly changed the composition of the bacterial microbiome in the lesional skin of AD, as well as improved the clinical outcomes. There was a significant correlation between the reduction of *S. aureus* and improvement of the clinical outcomes. Our findings suggest that alterations of the skin microbiome with excimer light treatment, specifically the decrease of the abundance of *S.*

aureus, are partly involved in the improvement of AD lesions.

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FIGURE LEGENDS

Table 1. Characteristics of AD patients and healthy controls.

Data are shown as the mean ± SD. N. D., not done; HC, healthy controls; AD, atopic dermatitis.

Figure 1. Clinical effects of 308 nm excimer light treatment on the lesional skin of AD.

(a) Local severity score of lesional skin before and after the excimer light treatment. (b) TEWL of HC and untreated non-lesional and treated lesional skin of AD. (c) SC hydration of HC and untreated non-lesional and treated lesional skin of AD.

TEWL, transepidermal water loss; SC, stratum corneum; pre NL, pre-untreated non-lesional skin; post NL, post-untreated non-lesional skin; pre L, pre-treated lesional skin; post L, post-treated lesional skin; Untreated, not treated with 308 nm excimer light; Treated, treated with 308 nm excimer light. Statistical analyses were performed with paired or Welch's unpaired *t*-tests. **p*<0.05; ***p*<0.01; ****p*<0.001.

Figure 2. Analysis of bacterial phyla in HC and the non-lesional and lesional skin of AD.

(a) Composition of bacterial phyla in HC samples. (b-e) Composition of bacterial phyla in AD samples. Samples of (b) pre-untreated non-lesional skin, (c) post-untreated non-lesional skin, (d) pre-treated lesional skin, and (e) post-treated lesional skin. (f, g) Statistical analysis of the relative abundance of (f) Cyanobacteria and (g) Bacteroidetes. Statistical analyses were performed with paired or Welch's unpaired *t*-tests. **p*<0.05; ***p*<0.01.

Figure 3. Analysis of *Staphylococcus* species in HC and the non-lesional and lesional skin of AD.

(a) Composition of *Staphylococcus* species in HC samples. (b-e) Composition of *Staphylococcus* species in AD samples. Samples of (b) pre-untreated non-lesional skin, (c) post-untreated non-lesional skin, (d) pre-treated lesional skin, and (e) post-treated lesional skin. (f) Statistical analysis of the relative abundance of *S. aureus*. Statistical analyses were performed with paired or Welch's unpaired *t*-tests. *S. aureus*, *Staphylococcus aureus*; **p*<0.05; #*p*<0.05 *vs* HC.

Figure 4. Correlation between percent change of *S. aureus* and that of the clinical score and skin barrier function in the lesional skin.

Correlation analysis between percent change of *S. aureus* and that of (a) local severity score and (b) TEWL and (c) SC hydration. Statistical analyses were performed with Pearson's correlation tests.

Table S1. Characteristics and skin barrier function of each subject.

N. D., not done.

Table S2. Distribution of the skin bacterial microbiome.

Distribution of (a) bacterial phyla and (b) *Staphylococcus* species of each sample. Numerals indicate a percentage.

Table S3. Distribution of the skin fungal microbiome.

Distribution of (a) fungal phyla and (b) *Malassezia* species of each sample.

Figure S1. Analysis of fungal phyla in HC and the non-lesional and lesional skin of AD.

(a) Composition of fungal phyla in HC samples. (b-e) Composition of fungal phyla in AD samples. Samples of (b) pre-untreated non-lesional skin, (c) post-untreated non-lesional skin, (d) pre-treated lesional skin, and (e) post-treated lesional skin. (f) Statistical analysis of the relative abundance of Ascomycota.

Statistical analyses were performed with paired or Welch's unpaired *t*-tests. **p*<0.05.

Figure S2. Analysis of *Malassezia* species in HC and the non-lesional and lesional skin of AD.

(a) Composition of *Malassezia* species in HC samples. (b-e) Composition of *Malassezia* species in AD samples. Samples of (b) pre-untreated non-lesional skin, (c) post-untreated non-lesional skin, (d) pre-treated lesional skin, and (e) post-treated lesional skin. (f) Statistical analysis of the relative abundance of *M. globosa*. Statistical analyses were performed with paired or Welch's unpaired *t*-tests. *M. globosa*, *Malassezia globosa*. **p*<0.05; ***p*<0.01.

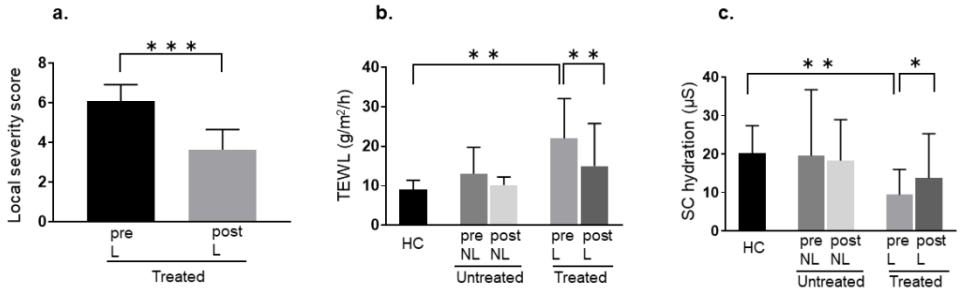
Table 1

Characteristics of AD patients and healthy controls.

	AD (n = 11)	**HC (n = 11)**
Age, years	29.9 ± 8.55	33 ± 5.75
Male: female ratio	3:8	6:5
EASI score	20.8 ± 7.27	N.D.
Severity of AD (moderate: severe ratio)	5:6	N.D.
Local severity score of pre-treated lesional skin	6.09 ± 0.83	N.D.
Cumulative UV dose, joules	2.33 ± 0.61	N.D.

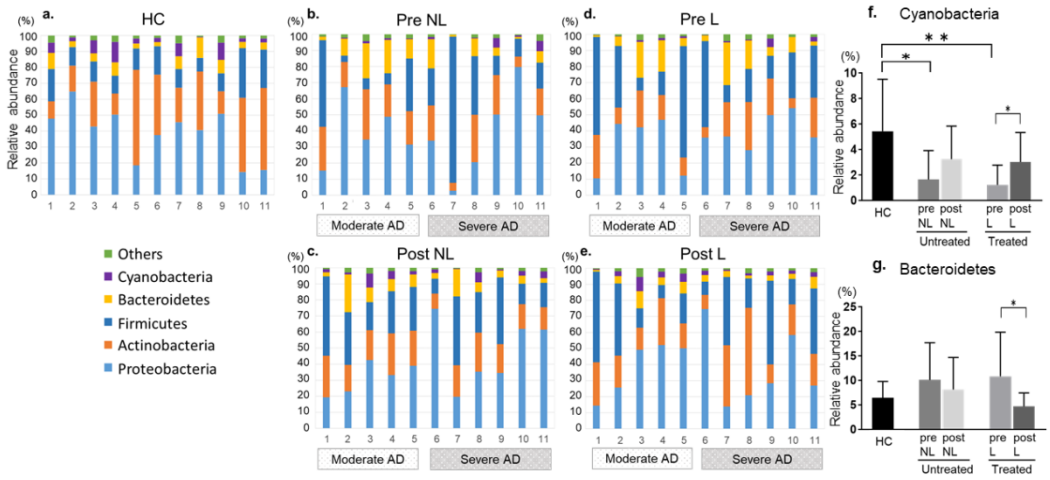
Data are shown as the mean ± SD. HC, healthy controls; AD, atopic dermatitis; N. D., not done.

Figure 1



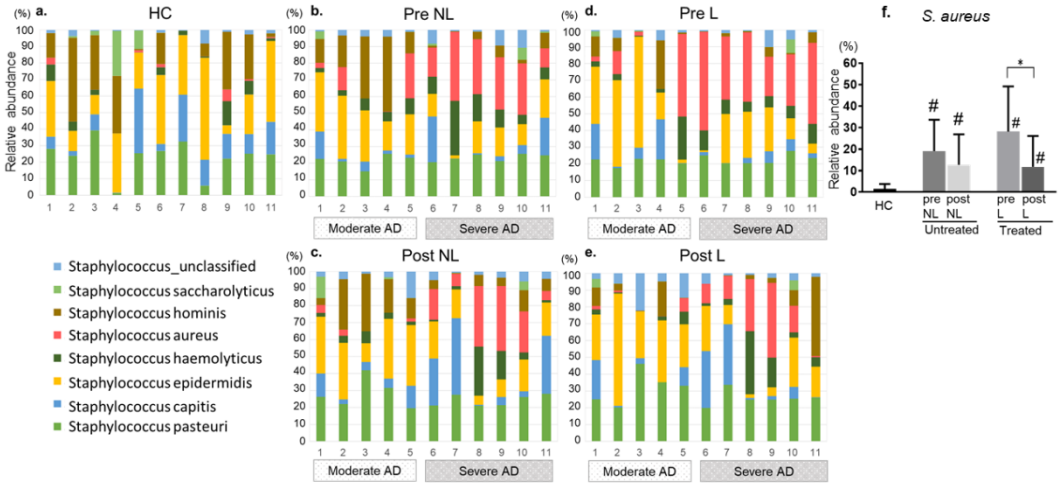
phpp_12531_f1.tif

Figure 2.



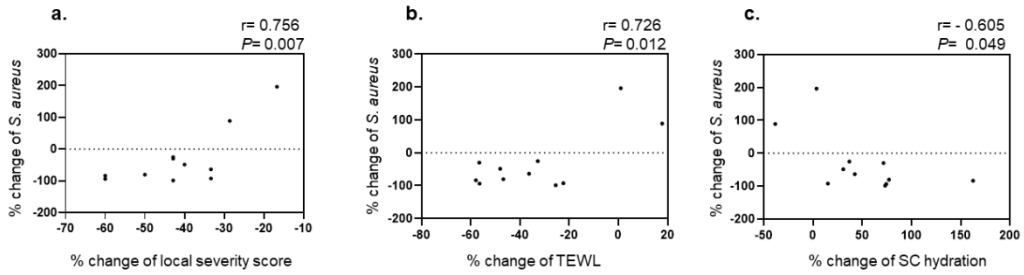
phpp_12531_f2.tif

Figure 3.



phpp_12531_f3.tif

Figure 4



phpp_12531_f4.tif