Pilot study on novel skin care method by augmentation with *Staphylococcus epidermidis*, an autologous skin microbe – A blinded randomized clinical trial

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ABSTRACT

Background and objective: *Staphylococcus epidermidis* is an autologous bacterium that is beneficial to skin health. Our goal was to develop a novel, personalized basic cosmetic that exploits this characteristic.
Methods: We conducted a double-blinded, randomized clinical trial on augmentation with *S. epidermidis* as a pilot study, in which *S. epidermidis* was collected from the subject, cultured for proliferation, and then continuously applied to the subject's own face before sleep twice per week for four weeks in order to increase colonization levels.
Results: The results showed that this treatment increased the lipid content of the skin and suppressed water evaporation, thereby markedly improving skin moisture retention. Moreover, augmentation with *S. epidermidis* maintained a low acidic condition on the skin surface. The low risk of undesirable effects induced by augmentation with *S. epidermidis* was also confirmed by measuring erythema and melanin levels.
Conclusions: These results may serve as a driving force to accelerate the development of novel, personalized basic cosmetics.

1. Introduction

Most women strongly desire new basic cosmetics with longer-lasting skin care effects. However, it is very difficult to find basic cosmetics that optimally suit personal needs. The use of *Staphylococcus epidermidis* has recently attracted attention in the development of basic cosmetics for individual skin conditions, as *S. epidermidis* is a well-known beneficial bacterium that participates in the maintenance of skin health [1–3]. Metabolic products of *S. epidermidis*, including glycerin and organic acids, improve skin moisture retention, maintain a low acidic condition on the skin surface, and improve rough skin texture [4–6]. Moreover, the anti-microbial bio-substances produced by *S. epidermidis* suppress the colonization of *Staphylococcus aureus*, which is an important pathogen responsible for a wide variety of conditions ranging from subclinical inflammation to severe infections causing pneumonia, endocarditis and septicemia [7–10]. The skin anti-aging properties of *S. epidermidis* have garnered significant attention, as superoxide dismutase produced by *S. epidermidis* is a known destroyer of reactive oxygen species [11]. Thus, numerous basic cosmetics that facilitate the growth of *S. epidermidis* on the skin surface have been developed to exploit the skin care benefits induced by *S. epidermidis*. However, the stimulating effects of these basic cosmetics on the colonization of *S. epidermidis* are often insufficient because of the differences in the skin characteristics of individuals and the gradual depletion of active substances in basic cosmetics induced by the metabolism of the skin microbiota itself [12].

In order to resolve the problems with basic cosmetics and to provide a novel skin care method, we hypothesized that an

intentional and substantial increase in *S. epidermidis* on the skin would boost the levels of these beneficial products and improve skin health. In the present study, we constructed a novel skin care method based on augmentation with *S. epidermidis* using the following five steps: (i) collection of skin microbiota from the subject's forehead skin; (ii) isolation of autologous *S. epidermidis* from skin microbiota based on the genetic analysis; (iii) culture for proliferation and lyophilization of isolated *S. epidermidis*; (iv) mixture of lyophilized *S. epidermidis* with a gel; and (v) continuous application of the individualized *S. epidermidis* to the face to increase the colonization (Fig. 1A). A clinical trial then evaluated the skin care effects of augmentation with *S. epidermidis* (Fig. 1B). Here, we report several findings to further the development of novel, personalized basic cosmetics that exploit the attractive characteristics of *S. epidermidis*.

2. Materials and methods

2.1. Materials

Trypticase soybean (TS) broth was purchased from Becton, Dickinson & Company (Franklin Lakes, NJ). Skim milk and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Snow Brand Milk Products Co., Ltd. (Sapporo, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. All other reagents used in this study were high-quality, analytical-grade materials.

2.2. Collection of individual skin microbiota samples

Skin microbiota samples were collected by the Dekio method, as described previously [13]. Briefly, the open end of a 4.9 cm^2 sterile plastic cylinder was manually placed on the subjects' forehead skin. The skin within the enclosed area was scrubbed for 20 s using a sterile swab moistened with phosphate-buffered saline (PBS) at a pH of 7.4. The tip of the swab was then broken against the wall of a glass tube containing PBS (1 mL), and the tube was immediately capped and shaken to suspend the bacteria ("skin microbiota sample").

2.3. Collection of individual substance samples

Individual samples for analyzing the substances on the forehead skin, such as glycerin and organic acids, were also collected from another area on the forehead skin using PBS (1 mL) containing 0.05% Tween-20 ("substance sample"). Skin areas used to collect substance samples were precisely recorded, and the same areas were used as sampling areas to evaluate the skin care effects of augmentation with *S. epidermidis*.

2.4. Culture analysis

Culture analysis was performed as described previously [13]. Briefly, 10^{-1}, 10^{-3} and 10^{-5} dilutions of individual skin microbiota samples were plated on TS agar medium, and were cultured aerobically at $37\,^{\circ}\text{C}$ for 48 h. Based on polymerase chain reaction (PCR) analysis, as described below, all colonies with the morphological features of *S. epidermidis* on each agar plate were identified. The colonies formed by *S. epidermidis* were counted to calculate the number of cells per milliliter (and per 4.9 cm^2 of skin surface).

2.5. PCR analysis

Part of the α-subunit of the ribonucleotide reductase region in the *S. epidermidis* genome was amplified by PCR using KOD FX Neo

polymerase (TOYOBO Co., Ltd., Osaka, Japan), in accordance with the manufacturer's protocol. The sequences of primers corresponding to the DNA target sequences were: 5′-ATCAAAAAGT-TGGCGAACCTTTTCA-3′ (forward) and 5′-CAAAAGAGCGTGGAGAA AAGTATCA-3′ (reverse) [14]. PCR was performed at an initial denaturation at $94\,^{\circ}\text{C}$ for 2 min, 40 cycles of denaturation ($98\,^{\circ}\text{C}$ for 10 s), annealing ($60\,^{\circ}\text{C}$ for 30 s) and extension ($68\,^{\circ}\text{C}$ for 30 s), and a final extension at $68\,^{\circ}\text{C}$ for 10 min. PCR products were electro-phoresed on 1.8% agarose gels at a constant voltage of 100 V for 40 min and were visualized by ethidium bromide staining.

2.6. Preparation of individual S. epidermidis samples

One colony identified as *S. epidermidis* was cultured in TS broth at $37\,^{\circ}\text{C}$ for 48 h. After centrifugation at $10,000 \times g$ for 5 min, the supernatant was discarded, and the resulting precipitate was washed three times with 10% skim milk solution (1 mL). Finally, the bacterial concentration was adjusted with the same solution to 1.36×10^9 cells/mL using a Bacterial Counter DU-AA01NP-H (Panasonic Healthcare Co., Ltd., Tokyo, Japan) [15,16]. The resuspension of *S. epidermidis* was then lyophilized in a sterilized sample ("*S. epidermidis* sample").

2.7. Application of individual S. epidermidis sample to skin surface
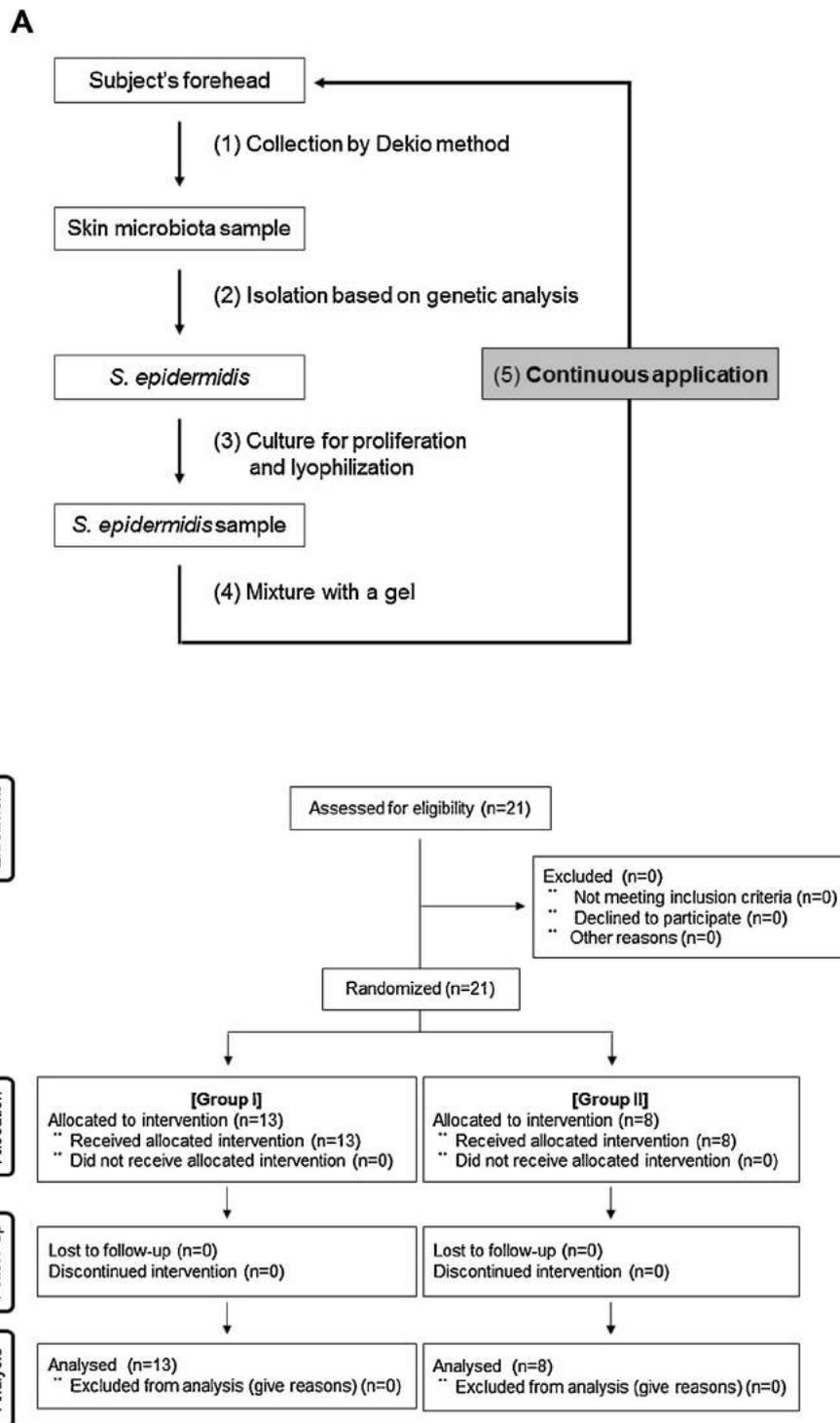
For each subject, individual *S. epidermidis* samples were added to a facial gel (2.0 g, HOURIN Co., Ltd., Chikuzen, Japan) containing primarily water and minimal minerals, and were uniformly mixed for 30 s. The subject gently applied the resulting mixture of the individual *S. epidermidis* sample on her face for 30 s.

2.8. Clinical trial

Design. As shown in Protocol S1, the experimental protocol in the clinical trial was approved by the Ethics Review Committee of Nagasaki International University (Approval No. 11 and 13). The clinical trial in this study was conducted in accordance with the Declaration of Helsinki. Although the registration of the clinical trial was delayed because we limited the disclosure of information on the augmentation of *S. epidermidis* through the clinical trial to apply for a patent, all related protocols of the clinical study were registered at the UMIN Clinical Trials Registry UMIN000012829. Each subject provided written informed consent and agreed to the policy of no concomitant use of other skin care soaps or cosmetics during the trial period. Moreover, they were advised not to wash or touch their own forehead skin (sampling area) for at least 8 h prior to the commencement of sample collection and measurement of skin conditions. The first subject was recruited on February 13, 2012 at Nagasaki International University, and follow-up was completed on June 30, 2012.

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Subjects and interventions. Subjects who did not have atopic dermatitis, serious dry skin or other dermatologic diseases were randomized in a double-blind manner and divided into two groups by RS (Fig. 1B). Before the clinical trial, *S. epidermidis* samples from all subjects were prepared by the above-mentioned techniques. In Group I, subjects applied their individualized *S. epidermidis* samples, while subjects in Group II applied a lyophilized powder of skim milk without their individual *S. epidermidis* (placebo). The application of the individualized *S. epidermidis* sample to the skin surface was performed twice per week for four weeks ("first term"). This treatment was performed 30 min before sleeping. Subsequently, after the first term, the subjects in Group II applied their individualized *S. epidermidis*

A

Subject's forehead

(1) Collection by Dekio method

Skin microbiota sample

(2) Isolation based on genetic analysis

S. epidermidis

(3) Culture for proliferation and lyophilization

S. epidermidis sample

(4) Mixture with a gel

(5) **Continuous application**

B

Enrollment

Assessed for eligibility (n=21)

Excluded (n=0)
" Not meeting inclusion criteria (n=0)
" Declined to participate (n=0)
" Other reasons (n=0)

Randomized (n=21)

Allocation

[Group I]
Allocated to intervention (n=13)
" Received allocated intervention (n=13)
" Did not receive allocated intervention (n=0)

[Group II]
Allocated to intervention (n=8)
" Received allocated intervention (n=8)
" Did not receive allocated intervention (n=0)

Follow-Up

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Analysis

Analysed (n=13)
" Excluded from analysis (give reasons) (n=0)

Analysed (n=8)
" Excluded from analysis (give reasons) (n=0)

Fig. 1. (A) Scheme of augmentation with *S. epidermidis* by continuous application of individual samples and (B) CONSORT flow diagram. In the present study, a novel skin care method based on augmentation with *S. epidermidis* was devised, in which autologous *S. epidermidis* was collected from the subject's forehead skin, cultured for proliferation, and then continuously applied to the subject's own face to increase colonization levels. The major advantage augmentation with *S. epidermidis* is that it leads to a specific increase in only *S. epidermidis*. In the clinical trial, the skin care effects of augmentation with *S. epidermidis* are shown in Figs. 4–7.

samples for four weeks, but Group I switched to the skim milk powder ("second term"). At the start and end of the clinical trial, substance samples were collected and several skin parameters (water content, water evaporation, lipid content, pH, erythema and melanin levels) indicative of skin health were measured in all subjects using a Cutometer MPA580 (Courage + Khazaka electronic GmbH, Cologne, Germany) [17–20].

2.9. Detection of glycerin and organic acids on skin surface

In order to examine the production of glycerin and low acidic substances by *S. epidermidis* on the forehead skin, we performed gas chromatography–mass spectroscopy (GC–MS) and ion-exclusion high-performance liquid chromatography (HPLC), respectively. Part of the collected substance samples (500 μL) was

transferred to a glass tube. To remove Tween-20, an equivalent amount of chloroform was added, and the mixture was centrifuged at $3,000 \times g$ for 10 min. The water layers were transferred to other glass tubes and were washed four times with chloroform, followed by final centrifugation at $12,000 \times g$ for 10 min. Aliquots of supernatants were used to detect glycerin and organic acids.

After drying by evaporation, supernatants (400 μL) were reacted with BSTFA (1 mL) at 60 °C for 10 min to detect glycerin using a GC–MS system [21,22]. After the resulting reactants were cooled and centrifuged at $3,000 \times g$ for 10 min, the acquired supernatants (1 μL) were injected into a Varian GC–MS system (Santa Clara, CA) composed of a 450-GC, a VF-5 ms (30 m × 0.25 mm id, 0.25 μm film thickness) and a 240-MS ion trap mass spectrometer. Oven temperature was maintained at 70 °C for 5 min and was programmed from 70 °C to 120 °C at a speed of 5 °C/min, and then to 240 °C at a speed of 10 °C/min. Temperatures in the injector and the detector were maintained at 240 °C. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min and the ion trap was scanned from m/z 35 to 400 in full scan mode. Peak assignments were based on retention times and mass spectra.

Organic acids in the supernatants were separated using a Waters HPLC system (Milford, MA) equipped with a 600E multisolvent delivery system and a 431 conductivity detector using an organic acid column (7.8 mm × 300 mm, Waters). In addition, 5 mM p-toluenesulfonic acid solution and the same solution containing 20 mM Bis–Tris and 100 μM ethylenediaminetetraacetic acid were used as a mobile phase and a buffer solution, respectively. Supernatants (100 μL) of the substance samples treated with chloroform were injected into the HPLC system. Detection of organic solvents was performed at a constant flow rate of 0.8 mL/min and column temperature was adjusted at 40 °C. Peak assignments were based on retention times.

2.10. Statistical analysis

All data are presented as means ± standard error. Statistical significance was evaluated using Student's t-test for paired comparisons with $p < 0.05$ being considered to indicate statistical significance.

3. Results and discussion

3.1. Subjects

Twenty-one subjects were enrolled and completed this clinical trial. As shown in Fig. 1B and Table 1, there were 21 subjects (100% female) aged 22 to 57 years, with a mean age of 39.00 ± 2.59. Subjects were randomized in a double-blind manner and were divided into Groups I ($n = 13$) and II ($n = 8$).

3.2. Isolation of autologous S. epidermidis and preparation of S. epidermidis samples

Before the clinical trial, PCR analysis of all colonies with the morphological features of S. epidermidis that formed on TS agar medium was performed in order to isolate S. epidermidis colonies from skin microbiota samples. On PCR analysis using DNA from the 21 subjects' colonies as templates, DNA fragments corresponding to sharp bands of approximately 124 bp were obtained. This suggests that we were able to isolate the autologous S. epidermidis from all subjects' forehead skin. Therefore, one of the colonies identified as composed of S. epidermidis was cultured in TS broth, and was then used to prepare individual S. epidermidis samples. The results of agarose gel electrophoresis based on these 21 selected colonies are shown in Fig. 2.

Table 1
Baseline subjects characteristics.

| Characteristics	Group I ($n = 13$)	Group II ($n = 8$)
Continuous application		
Staphylococcus epidermidis	+	–
Age (years)		
Means ± SE	36.54 ± 3.81	43.00 ± 2.46
Median	40.0	43.0
Sex – n (%)		
Female	13 (100)	8 (100)
Male	0 (0)	0 (0)
Skin diseases		
Atopic dermatitis	–	–
Serious dry skin	–	–
Other dermatologic disease	–	–

Twenty-one subjects were randomized in a double-blind manner and divided into two groups by RS. In Group I, subjects applied their individualized S. epidermidis samples. Subjects in Group II applied a lyophilized powder of skim milk without their individual S. epidermidis (placebo).

3.3. Application of S. epidermidis samples to skin

In the clinical trial conducted to evaluate the skin care effects obtained from the application of S. epidermidis to skin, the number of S. epidermidis cells detected on the forehead skin was first examined by culture and PCR analysis. The time taken to detect the relative mean number of S. epidermidis from the forehead skin is indicated in Fig. 3. During the first term, the relative mean number of S. epidermidis in Group I significantly increased more than tenfold with the continuous application of individualized S. epidermidis samples twice a week, as compared with that before the clinical trial. The amount of S. epidermidis detected from the subjects in Group II also greatly increased in the second term, whereas the relative mean number of S. epidermidis was approximately constant with the application of the skim milk powder during the first term. Therefore, continuous application of
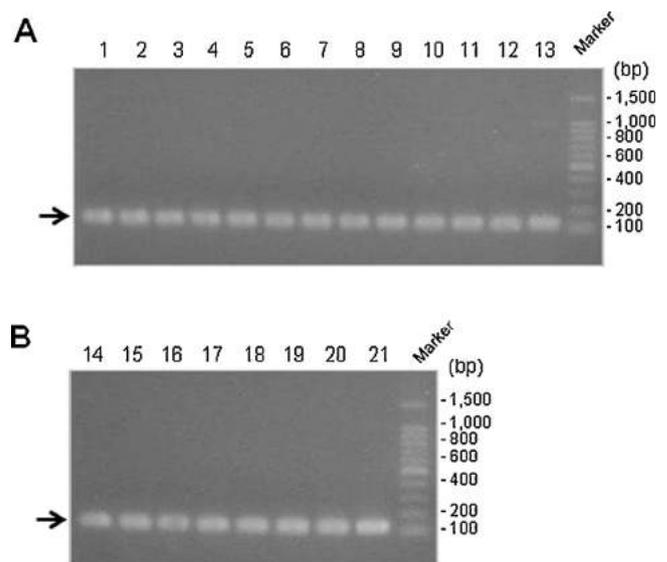
Fig. 2. PCR analysis to confirm S. epidermidis isolated from individual skin microbiota samples collected from subjects' forehead skin in (A) Groups I and (B) II. PCR was performed as described in the text. The numbers (1–21) in the figure represent each subject. Subjects were divided into Groups I (No. 1–13) and II (No. 14–21) for the clinical trial. The arrows indicate the detection position (124 bp) of the DNA fragment amplified using the S. epidermidis genome under experimental conditions. Electrophoresis was performed on 1.8% agarose gels at a constant voltage of 100 V for 40 min and PCR products were stained with ethidium bromide.
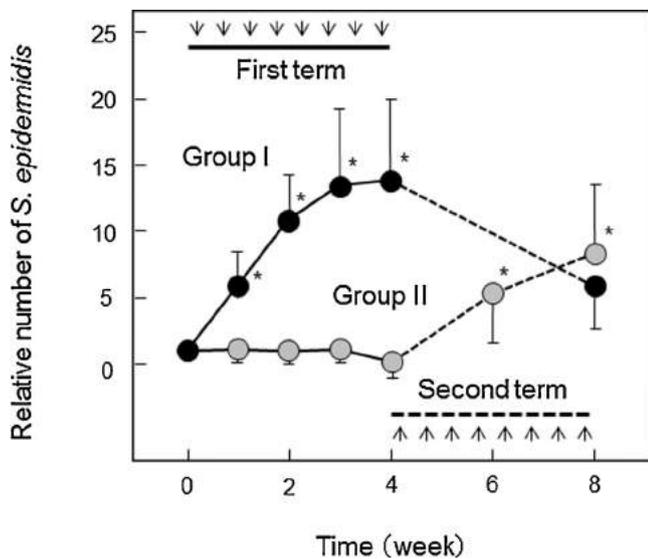
Fig. 3. Effects of continuous application of individual *S. epidermidis* sample to subjects' own skin on the number of detected *S. epidermidis*. In Groups I (*n* = 13) and II (*n* = 8), the continuous application of individualized *S. epidermidis* samples to each subject's own skin was performed before bedtime twice per a week for four weeks in the first (solid line) and second (dashed line) terms, respectively, as described in the text. The number of *S. epidermidis* detected on the subjects' forehead skin before the clinical trial was taken as 1.0, and data for Groups I (black line) and II (gray line) are presented as means ± standard error. Data for Group I showed significant differences from Group II with $^*p < 0.05$. Arrows indicate the duration of application of individual *S. epidermidis* samples.

individualized *S. epidermidis* samples was effective for promoting the robust colonization of *S. epidermidis*. In addition, in the second term, the interruption in application of *S. epidermidis* gradually reduced the number of *S. epidermidis* detected on the subjects in Group I. Continuous application may also be important for maintaining the augmentation levels of *S. epidermidis*.

A number of basic cosmetics contain oligosaccharides to facilitate the colonization of *S. epidermidis*; however, they also have the undesirable property of promoting the growth of *S. aureus* [12]. In contrast, the continuous application of individualized *S. epidermidis* samples has the advantage of leading to only a specific increase in *S. epidermidis*. Considering that approximately $1.0 \times 10^{3-4}$ cells/cm^2 of *S. epidermidis* typically live on the skin surface, it is a concern that the augmentation of *S. epidermidis* may more easily allow invasion of this bacteria into the body via the mouth or wounds. However, in general, *S. epidermidis* infections are restricted to those with compromised immune function or patients with indwelling catheters who receive various medical treatments [23,24]. In addition, we previously presented clear evidence supporting the low toxicity of *S. epidermidis* in two toxicity studies focused on its administration in mice [25]. We performed acute and subacute toxicity studies using 1.3×10^{12} and 1.3×10^{11} cells/dose/day *S. epidermidis* and demonstrated that no mice died or exhibited significant disorders during the trial period. The influence of augmentation with *S. epidermidis* on other bacteria contributing to skin health remains largely unknown; we must first understand the interactions and networks of augmented *S. epidermidis* with those of other bacteria microbiologically.

3.4. Improvement of skin moisture retention by augmentation with S. epidermidis

Several results showing the relationship between augmentation with *S. epidermidis* and the improvement of skin condition were obtained in this clinical trial. First, application of

S. epidermidis on the skin surface greatly influenced the retention of skin moisture; water content in the skin of subjects in Group I increased by approximately 1.4 times, as compared with the contents before clinical trial (Fig. 4A). In Group I, augmentation
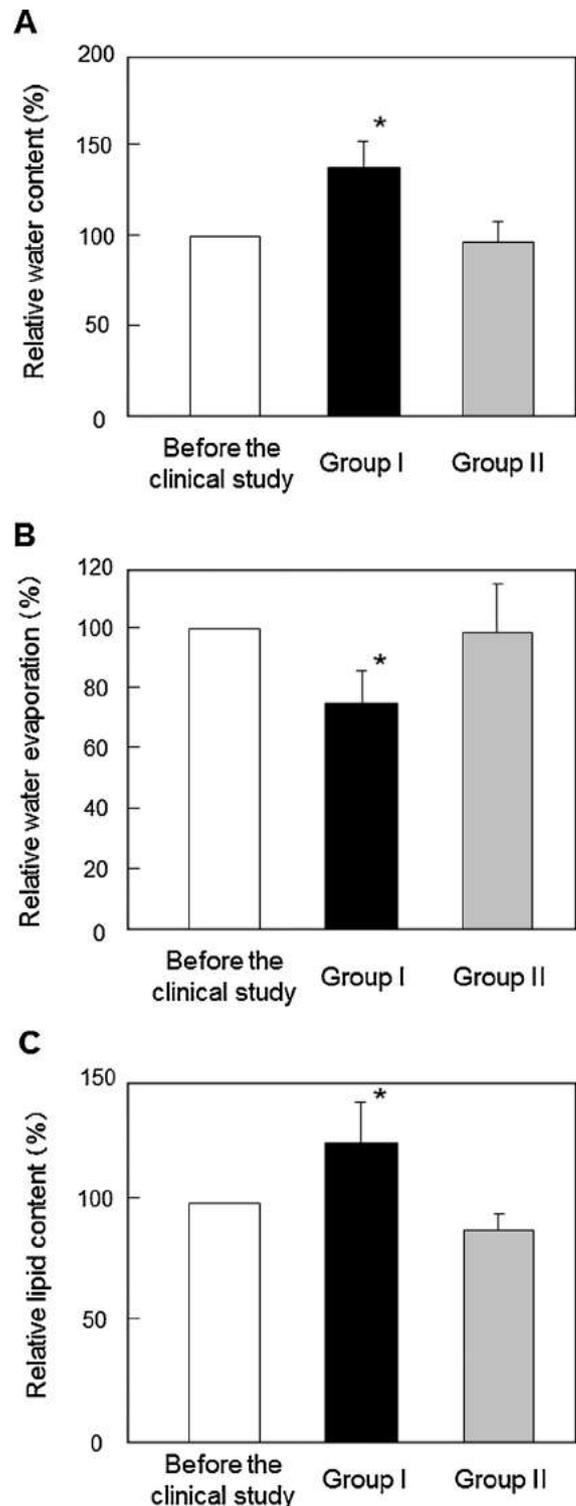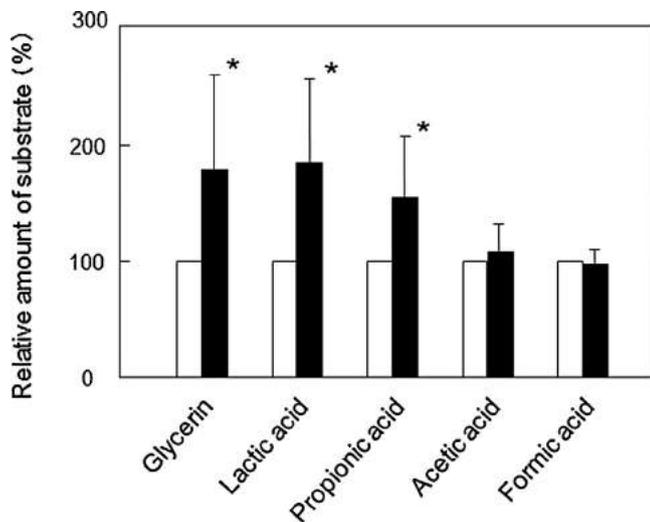

Fig. 4. Influence of augmentation with *S. epidermidis* on (A) water content, (B) water evaporation and (C) lipid content on skin surface. At the start and end of the clinical trial, water content, water evaporation and lipid content of the 21 subjects' forehead skin were measured using a Cutometer MPA580. Data for Groups I (*n* = 13) and II (*n* = 8) shown in bar graphs are presented as means ± standard error, and significant differences from the values before the clinical trial (*n* = 21) are indicated by $^*p < 0.05$. Values before the clinical trial were taken as 100%.

with S. epidermidis on the skin surface also induced the suppression of relative water evaporation and increased relative lipid content (Fig. 4B and C). Therefore, we hypothesized that the moisture retention of skin improved as follows: (i) lipid content first increased on the skin surface via the metabolism of S. epidermidis; (ii) water evaporation on the skin surface was subsequently suppressed by the coating effect based on a membrane formed by lipid substances; and (iii) subsequently, the skin's water content improved. To develop basic cosmetics that exploit the skin care benefits induced by S. epidermidis, it is important to demonstrate relationship between S. epidermidis augmentation and moisture retention on a molecular level.

As it is necessary to increase the lipid content on the skin surface to improve the moisture retention, detection of lipid substances supplied by S. epidermidis is important. It is known that glycerin is produced by epidermal cells and autologous skin microbiota to maintain skin moisture; reports have shown that sebaceous-gland-derived glycerol is a major contributor to stratum corneum hydration [26]. The production of extracellular lipase (EC 3.1.1.3) to hydrolyze sebum cutaneum is a general property of S. epidermidis and the lipase gene has been identified in this bacterium [27]. In fact, glycerin on the skin surface was detected as a lipid candidate by GC–MS analysis, and the amount of glycerin was significantly increased by augmentation with S. epidermidis (Fig. 5). Glycerin, expanded by the continuous application of individual S. epidermidis samples, may be able to support additionally both the protection of skin surface from dryness and the reconstruction of the skin barrier.

3.5. Maintenance of low acidic condition on skin surface by augmentation with S. epidermidis

Augmentation with S. epidermidis helped to maintain a low acidic condition on the skin surface. Namely, the pH in Group I shifted from 5.47 to 5.04 during the trial period, whereas the pH on

Fig. 5. Influence of augmentation of S. epidermidis on glycerin and organic acids on the skin surface. On GC–MS analysis, individual S. epidermidis samples were used for detecting glycerin after removal of Tween-20 and reaction with BSTFA. Separation and detection conditions were as described in the text. Peak assignments were based on retention times and mass spectra. On ion-exclusion HPLC analysis, individual S. epidermidis samples were used for detecting the organic acids after removal of Tween-20. Separation and detection conditions were as described in the text. Peak assignments were based on retention times. Data before the clinical trial (white bar) and that at the end of the clinical trial (black bar) from subjects in Group I (n = 13) are presented as means ± standard error, and significant differences from the values before the clinical trial are indicated by *p < 0.05. Values before the clinical trial were taken as 100%.
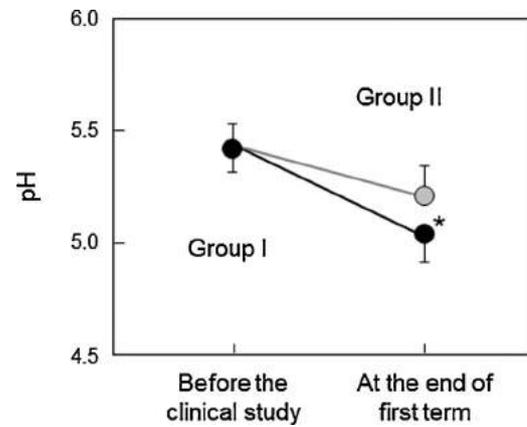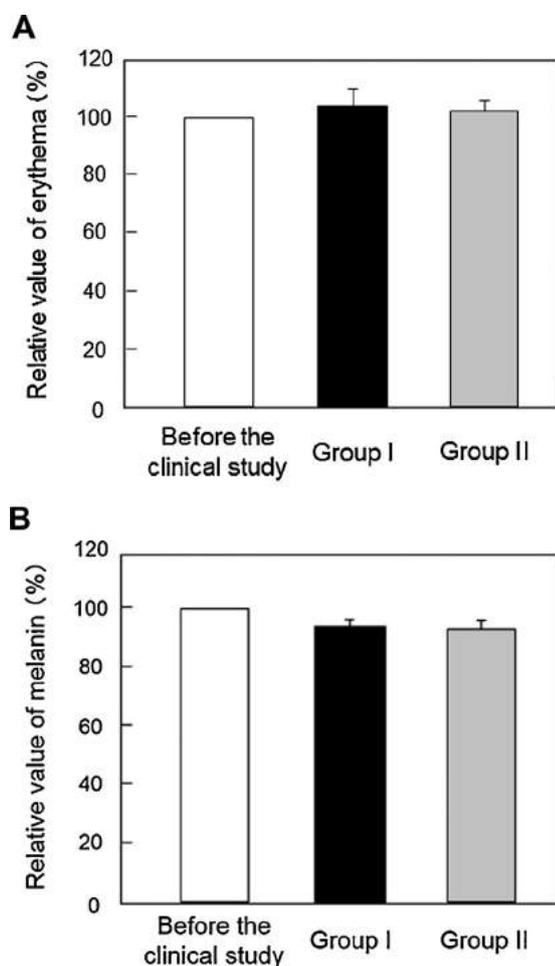

Fig. 6. Influence of augmentation with S. epidermidis on pH on subjects' forehead skin. At the start and end of the first term in the clinical trial, the pH of the subjects' forehead skin in Groups I (n = 13) and II (n = 8) was measured using a Cutometer MPA580. Data for Groups I (black line) and II (gray line) are shown as means ± standard error, and significant differences from the values before the clinical trial are indicated by *p < 0.05.

the skin surface of subjects in Group II was largely constant (Fig. 6). In the ion-exclusion HPLC system analysis of the substrate samples in Group I, some organic acids increased with continuous application of individualized S. epidermidis samples among the detected and annotated organic acids (Fig. 5). The amount of lactic and propionic acids increased by approximately 1.9 and 1.6 times, respectively, as compared to that before the clinical trial.

It is known that lactate acts as a humectant, which absorbs water in its interaction with skin [28]; this suggests that lactates with high hygroscopicity that are converted from lactic acid supplied by the augmented S. epidermidis contribute to improving moisture retention and skin barrier function. In addition, as skin already tends to maintain a low acidic condition, it may be appropriate that the lactic acid and propionic acid from augmented S. epidermidis metabolism supports the acidic condition of skin. As skin needs to be under low acidic conditions for the synthesis of ceramides from sphingomyelin and glucosylceramide [29,30], we also need to examine the relationship between augmentation with S. epidermidis and ceramide synthesis.

3.6. Low possibility of side effects induced by augmentation of S. epidermidis

At the start and end of the clinical trial, erythema and melanin levels, which reflect the degree of inflammation in the skin and the influence on melanocytes, were measured. No significant changes in erythema and melanin values in the skin of the subjects were observed, owing to the augmentation of S. epidermidis (Fig. 7A and B). It was reported that lipoteichoic acid produced by S. epidermidis serves to modulate inflammation through a TLR-crosstalk phenomenon between TLR2 and TLR3 [31], and the augmentation of S. epidermidis may inactivate cytokines, hormones and chemical mediators that are released from epidermal cells to stimulate melanocytes [32–35]. These results indicate that there is a small risk of undesirable effects (side effects) by augmentation with S. epidermidis under the experimental conditions in this clinical trial and suggest that novel personalized skin care products that exploit the physiological functions of S. epidermidis can be safely developed. In addition, we were concerned that the subjects may feel undesirable skin stickiness or reflection, but no subjects stopped the clinical trial because of increased lipid contents on the skin (Fig. 4C).

Fig. 7. Effects of augmentation with *S. epidermidis* on (A) erythema and (B) melanin. At the start and end of the first term in the clinical trial, erythema and melanin in the subjects' forehead skin in Groups I (*n* = 13) and II (*n* = 8) were measured using a Cutometer MPA580. Data for Groups I (*n* = 13) and II (*n* = 8) shown in bar graphs are presented as means ± standard error. Values before the clinical trial were taken as 100%.

As noted above, our skin care method using cultured *S. epidermidis*, augmentation with *S. epidermidis*, clearly differs from other basic cosmetics containing compounds that replenish moisturizing factors in the skin or increase the amount of *S. epidermidis*. Our findings regarding augmentation with *S. epidermidis* may serve as a driving force to accelerate the development of a novel, personalized basic cosmetic that can provide long-lasting beneficial skin-care effects.

Supporting information

Protocol S1. Clinical trial protocol approved by Ethics Review Committee of Nagasaki International University.

Competing interests

SM and RM are employees of BIOGENOMICS Co., Ltd. HH is an employer of same company. BIOGENOMICS Co., Ltd. provided support in the form of salaries for authors SM, RM, and HH, but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The specific roles of these authors are articulated in the 'author contributions' section.

Based on the data acquired in this study, we have applied for a patent (Beauty treatment method, skin care composition, bacterial cell, and dried bacterial cell, Patent No. JP2014-177489). This will not alter the authors' adherence to all journal policies on sharing of data and materials.

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