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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 antimicrobial 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

Abbreviations: TS, trypticase soybean; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; PBS, phosphate-buffered saline at pH 7.4; PCR, polymerase chain reaction; GC–MS, gas chromatography–mass spectroscopy; HPLC, high performance liquid chromatography.

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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 °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′-ATCAAAAAGTTGGCGAACCTTTTCA-3′ (forward) and 5′-CAAAAGAGCGTGGAGAAAAGTATCA-3′ (reverse) [14]. PCR was performed at an initial denaturation at 94 °C for 2 min, 40 cycles of denaturation (98 °C for 10 s), annealing (60 °C for 30 s) and extension (68 °C for 30 s), and a final extension at 68 °C for 10 min. PCR products were electrophoresed 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 °C for 48 h. After centrifugation at 10,000 × *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*