Skin Microbiome-Based Personalized Cosmetics: The Next Generation of Skincare

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Abstract:

Skin microbiome is a community of microorganisms living on the skin that play a critical role in skin health. It acts as a protective barrier against environmental factors such as temperature, ultraviolet radiation, and physical friction. We have researched the relationship between skin microbiome and skin conditions. We have published numerous papers on the changes in skin microbiome according to skin condition. In the next step, we investigated the possibility of using a skin microbiome for skin diagnosis. We found that it is possible to diagnose and improve skin conditions by controlling the skin microbiome.

We developed a new diagnostic platform by integrating questionnaire data, clinical results, and microbiome analysis of 1,000 subjects. The results of individual microbiome analysis showed that human skin types can be newly classified into 12 categories. This is the first case of classifying skin types from the perspective of microbiome, which fundamentally determines skin condition, rather than skin types based on skin phenomenology. We also selected key microbiomes that play a vital role in the skin and developed 12 solutions based on them. The company also completed cell biology

research and clinical validation to improve skin conditions. In this study, we attempted to clinically validate personalized cosmetics for skin types using optimized microbiome solutions for 12 skin types.

To verify the effectiveness of the solutions, we conducted a clinical study of 319 subjects aged 10 to 70 to confirm the skin improvement effects of the solutions for 12 skin types. The subjects were matched to one of the 12 skin types, and each type was matched to an individual solution (essence formulation). The subjects applied the customized essence for 2 or 4 weeks and evaluated 10 items, including moisturization, exfoliation, and anti-aging. The clinical results showed that the skin of most subjects improved, especially in the areas of moisturization, elasticity, and skin texture. Additionally, the skin types of 16 to 40% of the subjects improved in the direction of increasing tone/elasticity and oil/moisture content, resulting in an ideal skin condition.

This study is the first to develop and confirm personalized cosmetics for skin types by combining skin-derived microbiome cultivation and materialization technologies. The results of the study indirectly prove that the distribution state of the microbiome is directly linked to the maintenance of skin health.

In particular, our solutions are composed of substrates that focus on the microbial flora living on the skin. These substrates play the role of prebiotics, supplying nutrients to the microbes and allowing them to produce postbiotics. Therefore, even though our solutions do not contain live bacteria, they can have the effect of transplanting healthy skin microbiomes to the customer (SMT, Skin Microbiome Translation). This suggests that the solutions can help maintain a healthy skin microbiome and skin condition. (In actual efficacy evaluation under the same conditions, the effectiveness was similar to that of live bacteria, with approximately 60 to 70% of the similarity.)

We intend to present a new paradigm for future-oriented cosmetic skincare by integrating microbiology, IT, and AI technologies based on the research results on skin microbiomes. We also intend to continue to develop and realize advanced personalized cosmetics technology from a new perspective to realize the beauty of humanity.

Keywords: skin microbiome, personalized cosmetics, skin condition improvement, SMT

Introduction

The human microbiome is a population of microorganisms that live in symbiosis with the human body, including bacteria, fungi, archaea, protists, and other invisible organisms.[1–4] The microbiome has a significant effect on human health. The skin microbiome is linked to skin health. For instance, it is understood that the immune system is programmed in infants, that it is involved in the metabolism of liver and bile acids, which are involved in energy metabolism and food digestion, and that it is involved in the hormonal regulation of the HPA axis and the production of neurotransmitters such as serotonin and dopamine.[5–8] Furthermore, the microbiota of different body parts varies considerably. A notable example is the skin microbiome, which comprises microorganisms adapted to thrive in the skin environment and are intimately associated with skin health. The skin microbiome plays a crucial role in maintaining the integrity of the skin by forming a physical defense against pathogen invasion. Consequently, disruption of the skin microbiome can lead to the development of skin diseases caused by external epidermal pathogens, such as psoriasis and atopic dermatitis.[3,9] The skin microbiome modulates the gut microbiome and immune responses against more potent epidermal pathogens.[10] This suggests a link between skin health and skin microbiome, underscoring the need to study the skin microbiome. We previously developed a skin diagnostic algorithm based on skin microbiome analysis and data from 1,000 Koreans.[11]

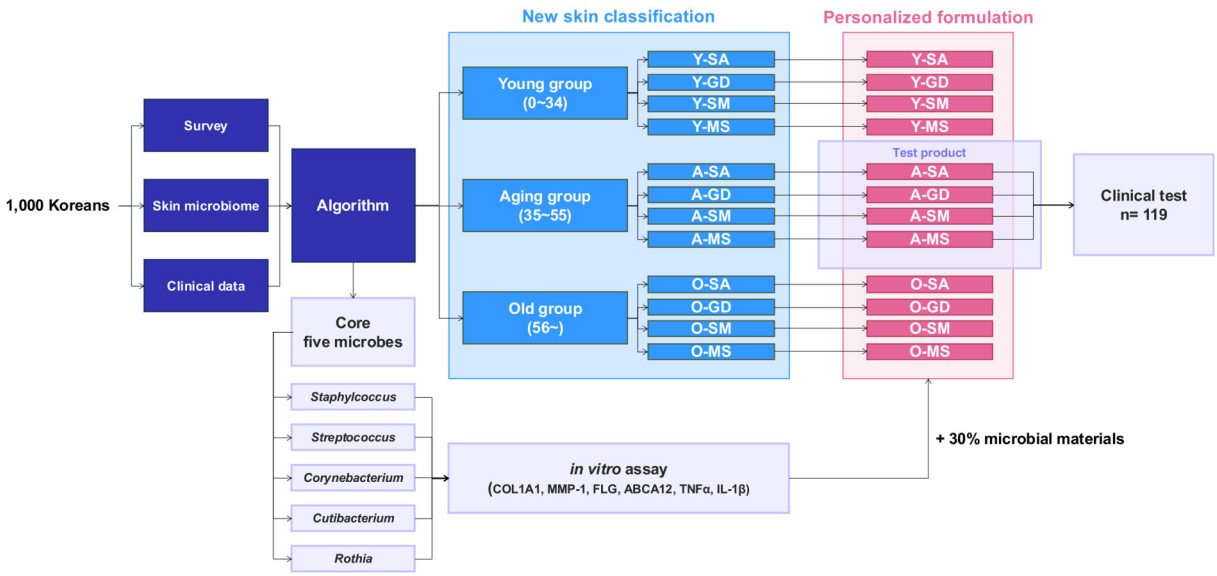
For developing the algorithm, we collected questionnaire surveys, skin microbiome, and clinical evaluation data from approximately 1000 Korean subjects. First, we collected information on self-assessment and lifestyle based on questionnaire results. Subsequently, we studied the correlation between these data and their skin microbiome and selected the 30 questions that were the most representative of the skin. We then correlated the skin microbiome with clinical data to identify the key microbes that influenced skin clinical indicators. Based on the results of the correlation analysis, we developed an algorithm that can predict skin conditions based on a questionnaire alone and identified 12 new skin types.[11] Specifically, we identified three age groups in which measures of clinical aging and changes in the microbiome change rapidly (young group: 0–34 years, aging group: 35–55 years, and old group: 56+ years). Aging was not age-dependent until the age of 25–34 (we called it the ‘gray zone’); however, aging starts in the aging group (after the age of 35 years). The age groups were further divided into quadrants within the same group based on clinical markers and microbiome data, resulting

in 12 new skin types. Specifically, the new skin types were divided into four quadrants based on the skin moisture/sebum index (M/S) and skin tone/elasticity (T/E) index, and each was labeled as follows: SA type: High M/S, T/E; SM type: Low M/S, High T/E; GD type: High M/S, Low T/E; MS type: Low M/S, T/E.

In addition, we determined the correlation between the skin microbiome distribution and clinical indicators and subsequently identified five microbes that could represent skin conditions. This indicates that our algorithm could facilitate the discovery of key skin microorganisms, the development of raw materials, the diagnosis of skin conditions, and the formulation of products based on scientific big data. This suggests the possibility of microbiome-based personalized cosmetics diverging from conventional customized cosmetics.

In this study, we aimed to confirm the efficacy of five key microorganisms and the improvement of skin health by using personalized products tailored for different skin types based on the algorithm results. First, we evaluated the skin improvement efficacy of core microbial ingredients. Subsequently, we assessed skin parameters to evaluate skin health improvement and skin type changes after using the personalized formulations.

Figure 1: Flow diagram of the study



Materials and Methods

1. Microbial isolation & culture

To develop skin-beneficial microbiome ingredients, five bacteria (*Staphylococcus, Streptococcus, Corynebacterium, Cutibacterium, Rothia*) that were suggested by the algorithm based on a previous study were isolated from facial skin samples.[11] The collection of human facial skin samples in this study was approved by the Institutional Review Board (IRB Protocol Number: HBABN01-210217-HR-0181-01) of the H&BIO Corporation R&D CENTER (H&BIO, South Korea). The isolated bacteria were stored at the COSMAX Microbiome Bank (CMB, COSMAXBTI Inc., Seongnam, South Korea). A single colony of each bacterium was picked from the plate and grown overnight in a skin-mimetic broth (SMB). The SMB consisted of 1.06 g of glucose, 0.2 g of rhamnose, 0.013 g of fructose, 0.05 g of MgSO4, 2.5 g of K_2HPO_4, 5 g of NaCl, and 1 g of yeast extract in a liter of distilled water. The overnight culture was then diluted 1:100 in fresh SMB medium (50 mL), transferred into a 250-mL Erlenmeyer flask, and incubated at 30°C on a shaker (160 rpm) for 48 h in the dark. After incubation, the culture was filtered using a syringe filter (0.45-μm pore size; Minisart, Sartorius, Germany). All culture filtrates were prepared in triplicates. [12]

2. Cell culture & treatment

The human dermal fibroblast cell line Hs68 was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Hs68 cells were seeded at a density of 3.5×10^5 in a 6-well plate and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 1% antibiotic antimycotic (HyClone Laboratories, Logan, UT, USA) and 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT, USA) for 24 hours in an incubator at 37°C and 5% CO_2. Immortalized human keratinocytes (HaCaT) were also obtained from the ATCC. The cells were cultured in DMEM media supplemented with 10% FBS and incubated for 24 h in an incubator at 37°C and 5% CO_2.

3. Gene expression analysis

1) COL1A1 and MMP-1

For collagen type I alpha 1 chain (COL1A1) and matrix metalloproteinase-1 (MMP-1) assay in UV-irradiated condition, the Hs68's medium was then removed, Dulbecco's phosphate-buffered saline (DPBS; Gibco, USA) was added, and the cells were irradiated with or without 12 mJ/cm^2 of UVB

(wavelength 290–320 nm, maximum peak 311 nm). After UVB irradiation, DPBS was removed and replaced with FBS-free DMEM media. The *Streptococcus* culture filtrates (1% (w/w)) were treated and further incubated for 24 h. A sample treated with UV light but no culture filtrate treatment was used as the negative control. [13–15]

2) FLG and ABCA12

To HaCaT cells, *Staphylococcus* and *Cutibacterium* culture filtrates (1% (w/w)) were added and further incubated for 24 h. The expression of filaggrin (FLG) and keratinocyte lipid transporter (ABCA12), factors related to skin barrier strengthening and moisturizing, was measured using PCR with the aforementioned conditions, except that primers for FLG and ABCA12 were used. [16,17]

3) TNFα and IL1β

HaCaT cells were cultured in DMEM media supplemented with 10% FBS and incubated for 24 h in an incubator at 37°C and 5% CO_2. The medium was changed every 3–4 days and the cells were subcultured when they overgrew. Cells were seeded at a density of 5×10^5/well and washed with phosphate-buffered saline (PBS) after 24 h of incubation. The cell medium was replaced with FBS-free DMEM, and 10 ng/mL polyinosinic–polycytidylic acid (Poly I:C) and 10 ng/mL interleukin 4 (IL-4) were added to induce inflammation. *Rothia* culture filtrate (1% (w/w)) was added to the cells and incubated for 4 h. The gene expression of tumor necrosis factor-alpha (TNFα) and interleukin-1β (IL1β) was analyzed using PCR with the aforementioned conditions, except those primers for TNFα and IL1β were used.[18,19]

4. Melanin content assay

To analyze the anti-melanogenic effects of *Corynebacterium* culture filtrate, a melanin content assay was performed. Murine melanoma cells (B16F10) were obtained from ATCC. The cells were suspended in 2 mL of DMEM media supplemented with 10% FBS, inoculated into 6-well plates at a density of 1×10^6 cells/well, and incubated at 37°C and 5% CO_2 for 24 h until 40–50% of the cells adhere to the bottom of the wells. Subsequently, they were treated with α-Melanocyte-stimulating hormone (α-MSH) at a concentration of 100 nM to induce melanogenesis. Subsequently, the culture filtrate (1% (w/w)) was added to the cells and incubated for 3 d. The cells were then harvested and centrifuged at 1,000 rpm for 10 min to obtain the supernatant. The supernatant was evaluated for melanin secretion inhibition by measuring the absorbance at 490 nm in triplicate using a microplate

reader (Victor 3). Percentage melanin secretion was calculated using the following equation: arbutin (100 ppm, Sigma Aldrich) was used as a positive control.[20]

$$\text{Melanin contents (\%)} = \frac{\text{Average absorbance of each sample}}{\text{Average absorbance of α-MSH treatment}} \times 100(\%)$$

5. RNA isolation and real-time PCR

Total RNA was isolated from cells using TRIzol reagent according to the manufacturer's instruction (TaKaRa, Shiga, Japan). cDNA was synthesized from 1 μg of total RNA using Reverse Transcription Premix (Elpis-biotech, Daejeon, Korea) under the following reaction conditions: cDNA synthesis at 45°C for 45 min and heat inactivation 95°C for 5 min. Gene expression signals were quantified with real-time PCR, and the data were analyzed using StepOne Plus™ system software (Applied Biosystems, Foster City, CA, USA). Real-time PCR amplification reactions were performed using SYBR Green PCR Master Mix with premixed ROX (Applied Biosystems, Foster City, CA, USA). Total RNA was isolated from cells using TRIzol reagent according to the manufacturer's instruction (TaKaRa, Shiga, Japan). cDNA was synthesized from 1 μg of total RNA using Reverse Transcription Premix (Elpis-biotech, Daejeon, Korea) under the following reaction conditions: 45°C for 45 min and 95°C for 5 min. Gene expression signals were quantified with real-time PCR, and the data were analyzed using StepOne Plus™ system software (Applied Biosystems, Foster City, CA, USA). Real-time PCR amplification reactions were performed using SYBR Green PCR Master Mix with premixed ROX (Applied Biosystems, Foster City, CA, USA). The following primer pairs (Bioneer, Daejeon, Korea) were used in the reactions performed in an ABI 7300 following the manufacturer's instructions. The reaction conditions were as follows: initiation at 50 °C for 2 min and 95 °C for 10 min, followed by cycling at 95 °C for 10 s and 60 °C for 1 min for 40 cycles. β-actin was used as an internal control.

Table I. Table of primer sequences

Gene name	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)
COL1A1	CTCGAGGTGGACACCACCCT	CAGCTGGATGGCCACATCGG
MMP-1	CGAATTTGCCGACAGAGATGA	GTCCCTGAACAGCCCAGTACTT

FLG	AGTGCACTCAGGGGGCTCACA	CCGGCTTGGCCGTAATGTGT
ABCA12	ACAGGAATGGCCTTCATCAC	AACATGGTGCCCTGAGAAAC
TNFa	CTCTTCTGCCTGCTGCACTTTG′	ATGGGCTACAGGCTTGTCACTC
IL1β	GTCATTCGCTCCCACATTCT	ACTTCTTGCCCCCTTTGAAT
β-actin	GGCCATCTCTTGCTCGAAGT	GACACCTTCAACACCCCAGC

6. Development of personalized formulations containing microbial materials

In-vitro analysis was conducted to determine the changes in targeted gene expression of the culture filtrates compared to the control. Subsequently, personalized material combinations and formulations for the 12 skin types were developed based on the algorithm. Each formulation varied in the composition of the five culture filtrates, with each type approximately 30% or more of the test product for the clinical trial. Each culture filtrate contained postbiotics from each bacterium and passed three toxicity tests: bacterial reverse mutation test (Ames test), eye irritation (animal replacement test), and skin irritation (animal replacement test) (Biotoxtech, Cheongju, South Korea), and was certified as microbiome-friendly by an external organization (Kind to Biome, Stockholm, Sweden). Clinical safety assessments of these microbial materials did not reveal the presence of subjective symptoms or objective signs associated with any adverse reactions during or after testing. (HBABN01-220404-HR-E0069-01).

7. Clinical study

A double-blind, randomized, placebo-controlled clinical trial was conducted at the Korea Biomedical Research Institute in South Korea to assess skin improvement and safety of the test (IRB No.: E-2023-010-01). The investigator fully explained the purpose and method of the study and expected adverse skin events to the participants. All participants provided written informed consent for using the test product for 1–4 weeks.

Based on the findings of previous studies (based on data from 1,000 Koreans), the study population was selected as women aged 35–55 years, who are at the end of the ‘gray zone’ and have entered the 'beginning of aging' stage, where managing signs of aging is essential.[11] A total of 119 participants were recruited for this study (average age: 46.76±5.21, minimum age: 38, maximum age: 55), and the

recruited participants were classified based on skin types using the algorithm developed in a previous study.[11] The clinical test products were determined by the subject's skin type, each containing specific microbiome materials tailored to improve specific skin conditions. To analyze the improvement effect on each skin type in detail, we measured several skin parameters related to each product's target benefits (Table II). Up to 15 clinical parameters were evaluated, including skin moisture, elasticity, and non-comedogenicity, and at least 20 participants were evaluated for each measurement. The participants applied an appropriate amount of the test product to the designated test area twice a day (morning and evening) and allowed it to absorb. Safety assessments included observation of subjective symptoms or objective signs associated with any adverse reactions that occurred during or after testing.

Table II. Study design by test product

Test product	Duration	Measurement
A-SA	1 time	24-hour Moisture persistence
		Moisture content in 10 layers of stratum corneum
		Skin temperature (Cooling)
	1 day	Improving barriers & calming to external (chemical) stimuli
	2 weeks	Five types of wrinkles (eye, under eyes, nasolabial folds, mouth, neck)
		Skin moisture
		Transepidermal water loss (TEWL)
		Skin keratinization
		Skin elasticity
		Skin lifting
		Skin density
A-GD	1 time	Skin keratinization
		24-hour moisture persistence
		Moisture content in 10 layers of stratum corneum
	2 weeks	Improving barriers & calming to external (chemical) stimuli
A-SM	2 weeks	Five types of wrinkles (eye, under eyes, nasolabial folds, mouth, neck)
		Skin tone (brightness)
		Skin pores

		Skin moisture
		Skin elasticity
		Skin lifting
		Skin density
A-MS	1 time	24-hour Moisture persistence
		Moisture content in 10 layers of stratum corneum
	2 weeks	Improving barriers & calming to external (chemical) stimuli
	2 weeks and 4 weeks	Five types of wrinkles (eye, under eyes, nasolabial folds, mouth, neck)
		Skin tone (brightness)
		Skin pores
		Skin moisture
		Skin elasticity
		Skin lifting
		Skin density

8. Test product information

The 'A-SA' product, which targets individuals with balanced moisture-sebum and tone-elasticity, was composed of a light hydrating ingredient (*Staphylococcus*) and a soothing ingredient (*Rothia*) to maintain skin health. The 'A-GD' product, which targets individuals with high moisture/sebum but low tone/elasticity, was composed of an elasticity-inducing ingredient (*Streptococcus*) and a moisturizing ingredient (*Cutibacterium*) to address skin thinning associated with accelerated aging. The ‘A-SM’ product, which targets individuals with skin conditions similar to those of the A-SA product, but with lower moisture/sebum levels, was composed of a higher content of a hydrating ingredient (*Staphylococcus*), while 'A-MS', which targets individuals with moisture-, sebum-, tone- and elasticity-deficiency, was composed of an elasticity-inducing ingredient (*Streptococcus*) to restore the compromised skin barrier, a moisturizing ingredient (*Cutibacterium*), and a hydrating ingredient (*Staphylococcus*) to restore moisture/sebum (Table I).

Table III. Information of test products

Test product	Skin condition	Used microbiome materials (I.N.C.I.)	Target efficacy

A-SA	High M/S index High T/E index	*Staphylococcus Epidermidis* ferment filtrate	Hydration
		Rothia Kristinae ferment extract filtrate	Soothing (anti-inflammatory)
A-GD	High M/S index Low T/E index	*Streptococcus Pneumoniae* ferment extract filtrate	Elasticity (Skin barrier repair)
		Streptococcus Mitis ferment extract filtrate	
		Streptococcus Infantis ferment extract filtrate	
		Cutibacterium Acnes ferment filtrate	Moisturizing
A-SM	Low M/S index High T/E index	*Staphylococcus Epidermidis* ferment filtrate	Hydration
		Rothia Kristinae ferment extract filtrate	Soothing (anti-inflammatory)
A-MS	Low M/S index Low T/E index	*Staphylococcus Epidermidis* ferment filtrate	Hydration
		Streptococcus Pneumoniae ferment extract filtrate	Elasticity (Skin barrier repair)
		Streptococcus Mitis ferment extract filtrate	
		Streptococcus Infantis ferment extract filtrate	
		Cutibacterium Acnes ferment filtrate	Moisturizing

4. Statistical analysis

Each experimental trial was conducted a minimum of three times, and each trial was performed in triplicates. The results are presented as the mean ± standard deviation. In vitro cell assay results were analyzed by a two-tailed Student's t-test using SPSS version 25.0 (SPSS Inc.). Differences were considered significant at $p < 0.05$ and $p < 0.01$.

Results

We evaluated the efficacy of microbial culture filtrates on various skin parameters. Treatment with 1% *Streptococcus* culture filtrate increased the mRNA expression of COL1A1 by approximately 151% compared to that in the UV treatment group (Figure 2). This indicates that *Streptococcus* culture filtrate restored COL1A1 mRNA expression in UV-damaged cells to normal levels. After *Streptococcus* culture filtrate treatment, the mRNA expression of MMP-1 was reduced to 54% compared to that in UV-damaged cells (482%).

Treatment with 1% *Staphylococcus* culture filtrate increased FLG mRNA expression by 161% compared to the untreated group, and 1% *Cutibacterium* culture filtrate increased ABCA12 mRNA expression by 291% compared to the untreated group (Figure 3). Treatment with 1% *Rothia* culture filtrate reduced the gene expression of IL1β and TNFα, which are known pro-inflammatory factors, compared to cells treated with Poly IC 10 ug/mL + IL-4 10 ng/mL. Furthermore, it decreased expression of IL1β from 187% to 138% and that of TNFα from 582% to 416%.

We also compared the amount of melanin after treatment with 10% *Corynebacterium* culture filtrate and the vehicle control (Figure 4). The vehicle control treated with α-MSH increased the amount of melanin by 208% compared with the untreated control. However, after treatment with *Corynebacterium* culture filtrate, the amount of melanin decreased to 68.4%, whereas arbutin treatment at the same concentration decreased it to 84.5%, indicating that the *Corynebacterium* culture filtrate inhibited melanin production more effectively than arbutin.

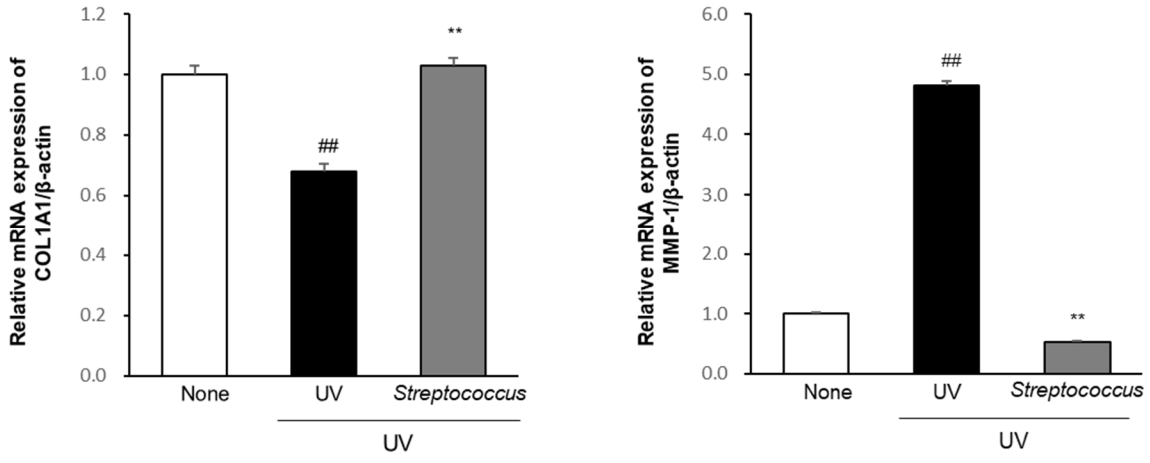


Figure 2. Effect of *Streptococcus* culture filtrate on the mRNA expression levels of COL1A1 and MMP-1 in Hs68 cells. #p<0.05 vs. None, ##p<0.01 vs. None, ###p<0.005 vs. None, *p < 0.05 vs. UV, **p < 0.01 vs. UV.

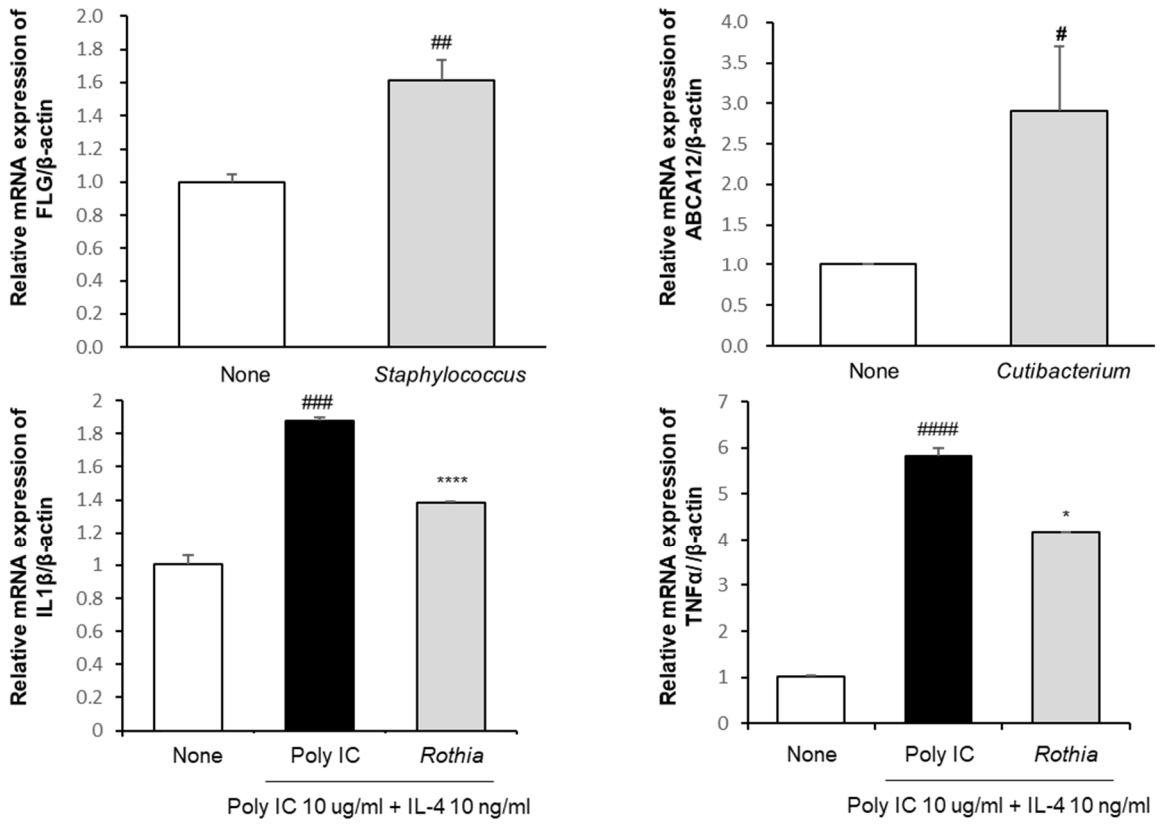


Figure 3. Effect of *Staphylococcus*, *Cutibacterium,* and *Rothia* culture filtrates on the mRNA expression levels of the FLG, ABCA12, IL1β, and TNFα in HaCaT cells. #p<0.05 vs. None, ##p<0.01 vs. None, ###p<0.005 vs. None, *p < 0.05 vs. Inducer, **p < 0.01 vs. Inducer, ***p < 0.001 vs. Inducer, ****p < 0.0001 vs. Inducer.

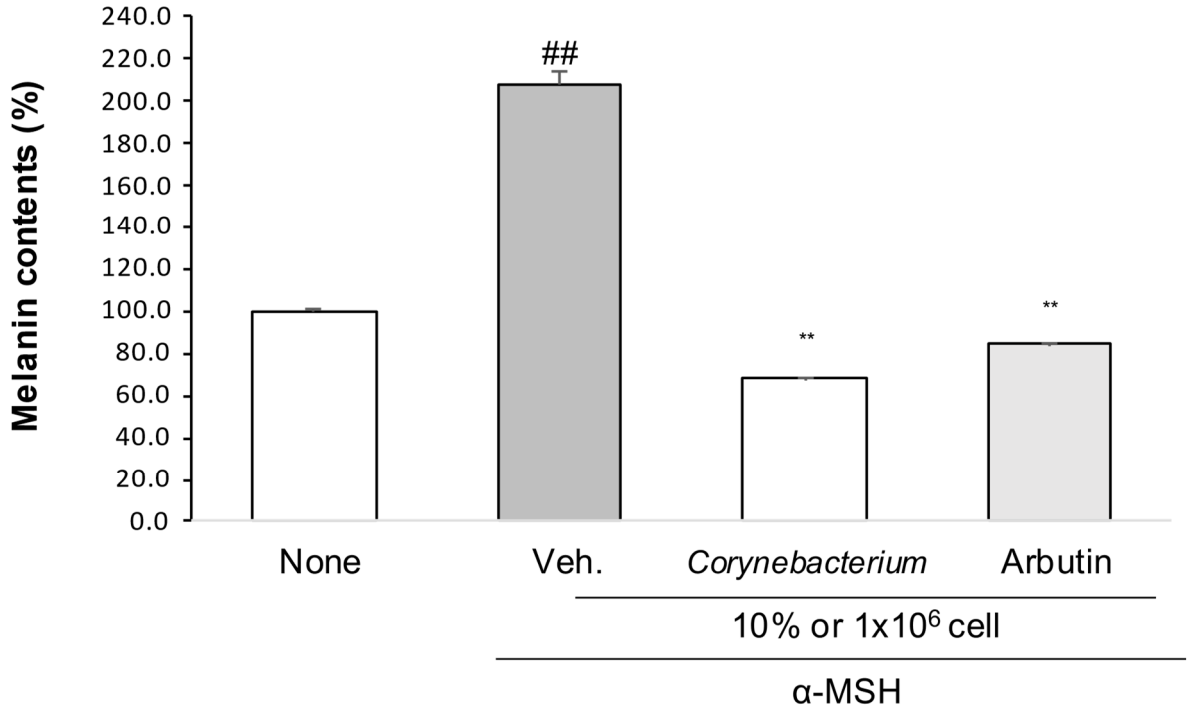


Figure 4. Effect of *Corynebacterium* culture filtrate on the melanin content in B16F10 cells. $^{\#}p<0.05$ vs. None, $^{\#\#}p<0.01$ vs. None, $^{*}p < 0.05$ vs. Vehicle control $^{**}p < 0.01$ vs. Vehicle control.

In terms of clinical study, the four test products assessed in this study led to significant improvements in all skin parameters related to aging and skin health (Table IV, p-value<0.05).

With the use case of ‘A-SA’ product, the hydrating (*Staphylococcus*) and soothing (*Rothia*) ingredients improved skin hydration and anti-inflammation. Furthermore, elasticity-inducing ingredients help maintain skin health. In 'A-GD', elasticity-inducing (*Streptococcus*) and moisturizing (*Cutibacterium*) ingredients improved the skin barrier, improving skin moisture and irritation. 'A-SM' helped improve various aging-related items by moisturizing the skin owing to moisturizing (*Staphylococcus*) ingredients compared to 'A-SA'. 'A-MS' helped improve the skin through comprehensive positive effects such as skin barrier function restoration, providing soothing relief, enhancing elasticity, and improving moisture retention through the most effective elasticity-inducing and barrier repair (*Streptococcus*), moisturizing (*Cutibacterium*), and hydrating (*Staphylococcus*) ingredients.

Table IV. Measurement improvement rate by test product

Test product	Measurement		Improvement rate
A-SA	24-hour moisture persistence		30.09%
	Moisture content in 10 layers of stratum corneum		95.18%
	Skin temperature (Cooling)		12.68%
	Improving barriers & calming to external (chemical) stimuli	Transepidermal water loss (TEWL)	57.19%
		Skin redness (soothing)	56.62%
	Five types of wrinkles (eye, under eyes, nasolabial folds, mouth, neck)		4.69%~17.11%
	Skin moisture		1.95%
	Transepidermal water loss (TEWL)		4.56%
	Skin keratinization		32.77%
	Skin elasticity		0.71%
	Skin lifting		2.87%
	Skin density		5.65%
A-GD	Skin keratinization		76.74%
	24-hour moisture persistence		28.21%
	Moisture content in 10 layers of stratum corneum		111.26%
	Improving barriers & calming to external (chemical) stimuli	Transepidermal water loss (TEWL)	75.09%
		Skin redness (soothing)	34.14%
A-SM	Five types of wrinkles (eye, under eyes, nasolabial folds, mouth, neck)		7.14%~10.34%
	Skin tone (brightness)		0.40%
	Skin pores		21.45%
	Skin moisture		2.90%
	Skin elasticity		0.49%
	Skin lifting		2.11%
	Skin density		26.22%
A-MS	24-hour moisture persistence		35.01%
	Moisture content in 10 layers of stratum corneum		111.26%
	Improving barriers & calming to external (chemical) stimuli	Transepidermal water loss (TEWL)	75.48%
		Skin redness (soothing)	34.56%
	Five types of wrinkles (eye, under eyes, nasolabial folds, mouth, neck)		6.06%~16.46%
	Skin tone (brightness) (2, 4 weeks)		0.86%, 1.51%
	Skin moisture (2, 4 weeks)		1.85%, 2.75%

	Transepidermal water loss (TEWL) (2, 4 weeks)	2.13%, 3.42%
	Skin keratinization (2, 4 weeks)	17.82%, 33.53%
	Skin elasticity (2, 4 weeks)	1.05%, 2.32%
	Skin lifting (2, 4 weeks)	2.22%, 4.41%

Discussion

In this study, we evaluated the efficacy of five types of microbes that play a key role in skin health, as discovered through big data analysis of 1000 Koreans. Subsequently, *in vitro* cellular efficacy data of the targeted efficacy of each of the five microbes was obtained. At the mRNA expression level, we found that *Staphylococcus*, a microbe with hydrating properties, increased FLG expression; *Streptococcus*, a microbe that improves elasticity, increased COL1A1 and decreased MMP-1 expression; *Corynebacterium*, a brightening microbe, decreased melanin levels; *Cutibacterium*, a moisturizing microbe, increased ABCA12 levels; and *Rothia*, an anti-inflammatory microbe, decreased IL1β and TNFα expression with statistical significance. To elucidate the functional aspects of the gene, the *Staphylococcus* culture filtrate treatment was observed to increase the mRNA expression of FLG in comparison to the untreated group. FLG is the human gene encoding profilaggrin and filaggrin, and loss-of-function mutations in FLG are known to cause dry, scaly skin (in severe cases, ichthyosis vulgaris).[21] Following the treatment of UV-damaged keratinocytes with *Streptococcus* culture filtrate, the mRNA expression of COL1A1 was restored to normal levels, while the mRNA expression of MMP-1 was decreased. The gene for COL1A1 is a component of type 1 collagen, which fulfills numerous roles within the skin, including maintaining its elasticity and firmness.[14] In contrast, MMP-1 is a pivotal enzyme that facilitates the degradation of type I collagen, thereby contributing to the breakdown of skin elasticity.[22] The ABCA12 gene plays a role in transporting lipids, including ceramides, to form an extracellular lipid layer in the stratum corneum of the epidermis.[17,23] The increased mRNA expression of ABCA12 observed in the treated group compared to the untreated group suggests that *Cutibacterium* culture filtrate has a positive effect on ceramide transport for skin health. The inflammatory process is significantly influenced by the interleukin-1β (IL1β) and tumor necrosis factor-α (TNF-α). These cytokines are considered the most important proinflammatory cytokines due to their potent proinflammatory actions and ability to promote the secretion of various inflammatory mediators. [24] In

HaCats that were inflamed by Poly IC and IL-4 Inducer, treatment with *Rothia* culture filtrate resulted in statistically significant decreases in IL1β and TNF-α levels, indicating a reduction in inflammatory activity. The synthesis and storage of melanin occurs within the cellular organelles of melanocytes, known as melanosomes.[25] The melanogenesis pathway is known to be influenced by various pathways and hormones. Therefore, it is postulated that *Corynebacterium* culture filtrate may interfere with some of the melanin production mechanisms in B16F10 cells, whose melanogenesis is induced by a-MSH, resulting in a melanin inhibitory effect similar to arbutin.

Although not presented in this paper, postbiotics that are expected to play a role in mediating the targeted effects of each microbe were identified by analyzing the culture filtrate, thus indirectly proving the efficacy of each microbe at the metabolite level, in addition to cellular efficacy evaluation. This demonstrated the accuracy of our algorithm in identifying core microbes. We successfully verified the individual efficacy of the five microbial ingredients. For clinical evaluation, we also included 30% of these microbial ingredients in the test products and provided test products that matched the skin type of the subjects diagnosed by our algorithm.

We confirmed that A-SA, A-GD, A-SM, and A-MS products, which were customized for women aged 35–55 years who had entered the 'beginning of aging' stage and were categorized according to their skin condition in terms of moisture/sebum and tone/elasticity, showed significant improvements in each targeted benefits. Each product was appropriately prescribed for the targeted skin condition and proved effective in improving the skin when used once or consistently, depending on the participant’s skin condition. Based on the positive results of this human clinical trial, it is recommended that consumers choose a customized product suitable for their current skin condition and use it consistently.

Although not presented in this paper, when skin type was diagnosed according to the algorithm for 119 people in the aging group, we found that certain skin types (GD and SA types) were more prevalent. This is representative of the skin types of Koreans (and East Asians in general) according to age and suggests that intensive research and improvement measures are needed for these prevalent skin types.

Conversely, it is evident that the recruitment of subjects with non-prevalent skin types is necessary to achieve higher statistical significance than previously observed. Furthermore, the development of clinical markers and microbiome analyses that can more accurately categorize prevalent skin types is

essential. Finally, additional research is required to refine our algorithms and prescriptions, including the re-sampling of skin from subjects with low clinical improvement to assess the effects of long-term use.

In conclusion, the findings of this study have wide implications in the development of cosmetics aimed at anti-aging and skin improvement by categorizing skin into 12 different conditions through algorithms based on big data. Our findings may offer crucial data a insights for future product development and improvement.

Conclusion

We verified clinical skin improvement through the use of a personalized formulation of five core microbial postbiotics. An average change in skin type of more than 30% was observed across age groups. This is the first study to integrate three dimensions of heterogeneous data (questionnaire, genomic (microbiome), and clinical data) to develop a skin diagnosis algorithm, create diagnosis-based personalized formulations, and confirm clinical improvement based on the skin diagnosis results. These results suggest that the use of core microbial postbiotics corresponding to the skin microbiome, distinct from existing botanical- and chemical-based ingredients, has significant skin improvement effects and may represent a paradigm shift in the cosmetic industry. In a future study, we plan on using artificial intelligence prediction technology based on microbiome data to improve the accuracy of the current algorithm and develop various skin prediction models. This approach is expected to contribute to the rapid recovery and homeostasis of human skin health.

Acknowledgments

Ethical approval

This research approved by Ethical Committee of the institution.

Institutional Review Board Statement: All procedures in this work were carried out following the principles expressed in the Declaration of Helsinki and have been approved by KBI (Korea Biomedical research Institute) (IRB No.: E-2023-010-01).

Funding information

This work was supported by project COSMAX and COSMAX BTI center of Korea Cosmetic Industry Institute, Republic of Korea.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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