Spermidine-induced recovery of human dermal structure and barrier function by skin microbiome

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An unbalanced microbial ecosystem on the human skin is closely related to skin diseases and has been associated with inflammation and immune responses. However, little is known about the role of the skin microbiome on skin aging. Here, we report that the Streptococcus species improved the skin structure and barrier function, thereby contributing to anti-aging. Metagenomic analyses showed the abundance of Streptococcus in younger individuals or those having more elastic skin. Particularly, we isolated Streptococcus pneumoniae, Streptococcus infantis, and Streptococcus thermophilus from the face of young individuals. Treatment with secretions of S. pneumoniae and S. infantis induced the expression of genes associated with the formation of skin structure and the skin barrier function in human skin cells. The application of culture supernatant including Streptococcal secretions on human skin showed marked improvements on skin phenotypes such as elasticity, hydration, and desquamation. Gene Ontology analysis revealed overlaps in spermidine biosynthetic and glycogen biosynthetic processes. Streptococcus-secreted spermidine contributed to the recovery of skin structure and barrier function through the upregulation of collagen and lipid synthesis in aged cells. Overall, our data suggest the role of skin microbiome into anti-aging and clinical applications.
The human skin has a multi-layered structure composed of various cell types, fibers, lipids, and other components. It is the primary organ to protect the body from the external environment. The skin surface maintains acidic, desiccated, and aerobic environments, whereas sebaceous follicles maintain an anaerobic and lipid-rich environment. Furthermore, the human skin is populated by various microorganisms including fungi, viruses, archaea, and bacteria, which feed on sebum, lipids, and keratin. The most abundant microorganisms are bacteria, which produce several metabolites and affect the proliferation and immune responses of the skin. Numerous non-pathogenic bacteria living on the skin have been studied through culture isolation methods, and their characteristics have been identified by molecular analysis. Recently, high-throughput deep sequencing of the bacterial 16S ribosomal RNA (rRNA) has enabled researchers to analyze the skin microbiome (skin microbiome means bacteria living on the skin or genomic information of them) and to investigate the link between skin health and the skin microbiome. Many studies revealed that the marked changes in the skin microbiome can alter skin conditions and cause diseases such as acne, psoriasis, and atopic dermatitis. Skin aging is caused by a combination of intrinsic factors, such as changes in hormones, cellular metabolism, and immune responses, and external factors, such as exposure to pollutants and ultraviolet rays. They induce major physiological and physical changes in the skin, such as sagging, wrinkles, dryness, and low elasticity. Changes in the lipid composition, sebaceous secretion, pH, and hydration of the skin during aging can alter the skin microbiome composition which is colonized at birth.

The effects of the gut microbiome on skin health were also reported. The absorption of probiotics through the intestinal tract has been shown to improve skin health, known as gut–skin axis. However, these studies have only looked at the indirect role of the microbiome in improving skin conditions; direct evidence on the impact of the skin microbiome on skin health should be evaluated.

Here, we sought to investigate the direct link between skin health and skin microbiome in terms of aging. We designed a stepwise study to identify the components of the skin microbiome and their roles in controlling skin conditions. We observed that Streptococcus colonies are enriched in the facial skin of young Korean women (20–29 years old) and demonstrated their roles in the improvement of skin health through metagenomic analysis, in vitro assays using human skin cells, clinical analysis, and genomic analysis with validations (metabolic and cellular analyses). Our findings suggest the potential of the Streptococcus species to rejuvenate aged skin, thereby providing insights on the possible applications of the skin microbiome in clinical practice.

**Results**

**Metagenomic profiling reveals specific microbes related to facial skin aging.** To examine the relationship between age and facial skin microbiome, we enrolled 52 healthy Korean women participants (Supplementary Table 1). They were divided into two groups: old (40–53 years old) and young (20–29 years old). The age limit was set to 54 years old to minimize age-related changes in sebum, moisture, and cell proliferation, which could lead to microbial bias due to marked alterations in skin microbiome.

We first identified and quantified the overall facial microbial composition in relation to age as described previously through 16S rRNA sequencing. At the genus level, only Streptococcus were more abundant in the young group compared with the old (Fig. 1a, b), and no significant differences were observed for other genera (Supplementary Fig. 2b). In particular, Streptococcus infantis was significantly enriched, as well as some unclassified species of Streptococcus but to a lesser extent (Fig. 1c). The five most abundant phyla were present in both the young and old groups (Supplementary Fig. 1). The lack of age-related stratification was confirmed by microbial compositional distance measurements (Supplementary Fig. 2a). Based on the metagenomic analysis, we hypothesized that S. infantis or some other unclassified Streptococcus species could be closely related to facial skin aging.

**Abundance of Streptococcus is associated with facial biophysical properties.** To confirm Streptococcus as a potential major microbe that determines skin aging, we evaluated the biophysical properties of the skin. Because elasticity is an important indicator of facial skin condition and is associated with aging, we investigated the abundance of Streptococcus in participants with varying facial elasticity. After measuring elasticity from 0 to 1, we split the elasticity index into low (0–0.3), normal (0.4–0.6), and high (0.7–1). Most of the participants with high elasticity belonged to the young group (Fig. 1d). After plotting the relative abundances of Streptococcus against the elasticity-to-age ratio, we obtained a positive correlation as seen in Fig. 1e. The microbial compositional distances did not differ significantly in participants with low or high elasticity (Supplementary Fig. 3a). However, consistent with the age-metagenomic profiling results, Streptococcus and S. infantis were significantly more abundant in the high-elasticity group than in the low-elasticity group (Supplementary Fig. 3b). A comparison of other variables, such as skin appearance and skin moisture, revealed no dependence with microbial distances (Supplementary Fig. 4a, c, respectively). Specifically, Streptococcus and S. infantis were abundant in participants with clear skin surfaces based on appearance (Supplementary Fig. 4b). However, skin moisture did not seem to have a part (Supplementary Fig. 4d). These results indicate the potential of Streptococcus to improve facial skin elasticity.

Streptococcal secretions upregulated genes related to skin structure and barrier. A previous study revealed that a pyrimidine compound from a bacterial strain, EPI-7T, had an anti-aging effect on human dermal fibroblasts (HDFs). Therefore, we hypothesized that the abundant Streptococcus facial colonies could secrete compounds related to skin aging. First, we isolated Streptococcus pneumoniae, Streptococcus infantis, Streptococcus infantis, and Streptococcus thermophilus from the facial skin of young female participants. To demonstrate the functional roles of the secretions from Streptococcus, we treated primary HDFs and human epidermal keratinocytes (HEKs) with the supernatant derived from the Streptococcus culture (St solution) from the said four strains. Prior to the analysis, we ensured that all four St solutions were not toxic to the two cell types (Supplementary Fig. 5a), whereas St solutions could encourage the proliferation of skin cells (Supplementary Fig. 5b). We then treated HDFs and HEKs with up to 10% of the St solution in growth medium to determine the optimal concentration for subsequent experiments (Supplementary Fig. 5d), and 10% was chosen as the optimal dose.

To assess the effects of the St solutions on skin aging, we analyzed the expression levels of genes involved in the dermis structure. The solutions from S. pneumoniae and S. infantis significantly increased the expressions of collagen type I alpha 1 chain (COL1A1) and collagen type III alpha 1 chain (COL3A1) (Fig. 2a), which are two major components of the extracellular matrix. The major dermal elastic-fiber genes, elastin (ELN) and fibrillin 1 (FBN1) were also considerably induced by the St solutions in HDFs (Fig. 2b). S. pneumoniae culture medium
exhibited the most potent effect on skin structural components. Multi-dimensional model of the skin layer also showed a thicker epidermal layer after St solution treatment in Polyinosinic: polycytidylic acid (Poly I:C)-induced damaged skin model (Fig. 2e, f). Additionally, treatment with the St solutions increased the expression levels of desmocollin 2 (DSC2) and filaggrin (FLG), which are responsible for skin barrier function (Fig. 2c). A similar trend was observed for glucosylceramidase beta (GBA) and ATP-binding cassette subfamily A member 12 (ABCA12), which enable the syntheses of lamellar body and ceramide to form the lipid barrier (Fig. 2d). Next, we evaluated the effect of the St solutions on skin lipid synthesis and the consequent maintenance of a healthy barrier in HEKs. The solutions from *S. pneumoniae*, *S. infantis 1*, and *S. infantis 2* increased lipid accumulation, and this was confirmed by the gene expression profiles for lipid synthesis, which involved DSC2, FLG, GBA, and ABCA12 (Fig. 2g, h). The beneficial outcomes were not obtained following treatment with *S. thermophilus* and other skin microbiome culture medium (Supplementary Fig. 5c, e).

**Streptococcal secretions improved the physiology of human skin.** To confirm whether Streptococcal secretions are clinically effective on human skin, we applied the St solutions on both cheeks of healthy female participants who do not have any skin disease (Fig. 3a and Supplementary Table 2). Phenotype changes were measured on cheeks applied with the control solution and 30% St solution (10% *S. pneumoniae*, 10% *S. infantis 1*, and 10% *S. infantis 2*) at day 0 (day of application) and 28 days after application. After 28 days, significant improvements in various skin phenotypes were observed in cheeks treated with the St solution. Consistent with the increased expressions in the collagen and elastin genes of our in vitro assay, skin elasticity increased significantly from 0.60 ± 0.07 to 0.67 ± 0.07 (mean ± standard deviation; difference 0.06 ± 0.04 or 12.1%; Fig. 3b, right), and the extent of which was significantly higher than those in control (Fig. 3b, right), which changed from 0.601 ± 0.07 to 0.61 ± 0.07 (difference 0.01 ± 0.03; Supplementary Fig. 6a). Skin elasticity was maintained at baseline values during the test period in the control-treated cheek. The effectiveness of the St solution on skin moisturization was confirmed by measuring trans-epidermal...
**Fig. 2** *Streptococcus* growth media improve skin cells with various phenotypes. **a–d** Relative mRNA expression levels of: **a** collagen-associated genes important for elasticity in HDFs, **b** elastic-fiber-associated genes in HDFs, **c** tight-junction-associated genes important for skin barrier function and moisture in HEKs, and **d** lipid barrier-associated genes in HEKs. HDFs human dermal fibroblast, HEKs human epithelial keratinocytes. Expression values are relative to control cells and represent the mean ± S.E. Three technical replicates were done. **e** Micrographs of a skin cell layer in the control and after treatment with Poly I:C, Poly I:C and supernatant of *S. pneumoniae*, Poly I:C and supernatant of *S. infantis 1*, and Poly I:C and supernatant of *S. infantis 2*. 1 μg/mL of Poly I:C was used in each treatment. Arrow line indicates the thickness of the epidermal layer. **f** Corresponding plot of the thickness of skin cell layer, n = 6. **g** Nile red staining of HEKs treated with different *Streptococcus*-cultured media. **h** Plot of area of lipid accumulations, n = 7. The Student’s two-tailed t-test was used to calculate statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001. ns non-significant. Scale bar corresponds to 100 μm. Control is a non-treated condition.
water loss (TEWL) and horny layer moisture content. TEWL indicates the moisturizing ability and skin barrier function. In the St solution-treated cheeks, the TEWL score declined from $19.90 \pm 4.23$ to $17.44 \pm 3.88 \text{ g/h/m}^2$ (difference $-2.45 \pm 3.30$ or $11.3\%$ reduction) after 28 days (Fig. 3c, left), whereas that in the control did not decrease significantly (difference $-0.17 \pm 2.82$ or $0.21\%$ reduction; Fig. 3c right and Supplementary Fig. 6b). Unsurprisingly, skin hydration in the St solution-treated group was significantly higher after 28 days ($66.39 \pm 7.73 \text{ A.U}$) compared with the baseline ($52.40 \pm 0.38 \text{ A.U}$; Fig. 3d left). Even though skin hydration was improved also with control solution, augmenting from $51.83 \pm 8.75 \text{ A.U}$ to $57.97 \pm 7.68 \text{ A.U}$.

### Fig. 3 St solutions clinically improve skin phenotypes.

| Type       | Day 0 | Day 28 | Difference | P-value |
|------------|-------|--------|------------|---------|
| Elasticity | 0.6   | 0.74   | 0.14       | 0.011   |
| TEWL       | 19.90 | 17.44  | -2.45      | 0.0015  |
| Moisture   | 52.40 | 66.39  | 13.99      | 0.005   |
| Desquamation | 51.83 | 57.97  | 6.14      | 0.005   |

The statistical calculation for paired comparison was done using the Wilcoxon signed-rank test, whereas inter-comparison was done using the Wilcoxon–Mann–Whitney test. A.U arbitrary unit, DSC diffuse scattering correction, DI desquamation index.
Genomic characteristics and biological pathways are triggered by *Streptococcus*. To provide a better insight into the genomic and functional characteristics of *Streptococcus*, we analyzed their entire genomes. Consistent with the results of molecular and cellular level assays, *S. infantis 1* and *S. infantis 2* displayed the closest genomic distance, followed by *S. pneumoniae*, whereas *S. thermophilus* showed a low genomic similarity (Fig. 4a). We thus split them into two groups: *S. pneumoniae*, *S. infantis 1*, and *S. infantis 2* in one; *S. thermophilus* in the other. We annotated the genomic fragments using whole-genome analysis, compared their cluster of orthologous groups (COGs) for selecting common genes (Supplementary Fig. 7a, b), and identified their functional roles (Fig. 4b). *S. pneumoniae* was enriched for the following gene ontology (GO) terms: maltodextrin transport, purine ribonucleotide interconversion, and glyceraldehyde-3-phosphate metabolic process; *S. infantis 1*, glyceraldehyde-3-phosphate metabolic process, purine ribonucleotide interconversion, and glycoprotein biosynthetic process; *S. infantis 2*, glyceraldehyde-3-phosphate metabolic process, polyol metabolic process, and alditol metabolic process; and *S. thermophilus*, histidine biosynthetic process, triketocarboxylic acid metabolic process, and arginine biosynthetic process. To investigate the biological processes of *Streptococcus* candidates to human skin, we sought all common biosynthetic processes in *S. pneumoniae*, *S. infantis 1*, and *S. infantis 2*. The overlapping GO terms were the spermidine biosynthetic process and glycogen biosynthetic process.

Spermidine recovered reduced gene expressions in aged skin cells. We selected spermidine as a potential molecule contributing to skin improvements according to previous studies. Spermidine biosynthetic process was also enriched in *S. pneumoniae*, *S. infantis 1*, and *S. infantis 2*. Prior to cell-based assays, we confirmed the existence of spermidine in the St solutions using mass picking and calibration curve. As expected, the concentration of spermidine was higher in *S. pneumoniae* (635.3 μg/mL), *S. infantis 1* (361.3 μg/mL), and *S. infantis 2* (1026.9 μg/mL) compared to the media (3.3 μg/mL) (Fig. 4c and Supplementary Fig. 8). To investigate the role of spermidine in skin aging, we used aged cells (see Methods for details) and observed the molecular changes induced by spermidine. We confirmed that treatment with spermidine reduced the senescence-associated β-galactosidase (SA-β-gal) in aged HDFs (Fig. 4d and Supplementary Fig. 9). Spermidine also increased the COL1A1 and COL3A1 levels compared with non-treated aged cells (Fig. 4e). Interestingly, the mRNA level of COL3A1 was higher than those of young cells. We found that spermidine induced lipid synthesis with increased mRNA levels of ABCA12 (Fig. 4f, g). We also observed similar outputs from the recovery of aging induced by UV which is a major external factor of skin aging. UV-aged HDFs secreted greater levels of SA-β-gal than the control (Fig. 4h). Further, UV-aged skin cells expressed lower levels of COL1A1, ELN, and FBN1 genes compared with control cells. Interestingly, spermidine recovered the reduced expressions of these genes (Fig. 4i). Furthermore, upon the examination of the regulatory sequences in promoters of genes upregulated upon spermidine treatment, one of the sequence motifs corresponded to the TWIST1 motif, suggesting that spermidine may act as a transcriptional activator (Fig. 4i). Additionally, we discovered that *S. pneumoniae* and *S. infantis*, the effective skin microbiome in this study, exhibited greater growth in presence of spermidine compared with the non-spermidine conditions, whereas *S. thermophilus* showed the opposite growth trend (Supplementary Fig. 10).

**Discussion**

The facial skin microbiome formed at birth changes gradually with aging, and any imbalance in their composition is closely related to skin diseases, such as atop dermatitis, psoriasis, and acne.

UV-aging induced by UV which is a major external factor of skin aging. UV-aged HDFs secreted greater levels of SA-β-gal than the control (Fig. 4h). Further, UV-aged skin cells expressed lower levels of COL1A1, ELN, and FBN1 genes compared with control cells. Interestingly, spermidine recovered the reduced expressions of these genes (Fig. 4i). Furthermore, upon the examination of the regulatory sequences in promoters of genes upregulated upon spermidine treatment, one of the sequence motifs corresponded to the TWIST1 motif, suggesting that spermidine may act as a transcriptional activator (Fig. 4i). Additionally, we discovered that *S. pneumoniae* and *S. infantis*, the effective skin microbiome in this study, exhibited greater growth in presence of spermidine compared with the non-spermidine conditions, whereas *S. thermophilus* showed the opposite growth trend (Supplementary Fig. 10).

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skin well-being observed in in vitro screening could explain the mechanism underlying these skin improvements\(^*\). Additionally, the recovery of the damaged skin layer and increased lipid synthesis of skin cells supported the effects of Streptococcal secretions to improve skin structure and the skin barrier function\(^{49-51}\). Then, we demonstrated those improvements in human skin layers showing consequent outcomes.

The importance of microbial secretions emphasized at the start-line of our study needed a genomic approach to identify possible microbial secretions\(^{52-54}\). The genomic distances of \(S.\ pneumoniae\), \(S.\ infantis\) 1, and \(S.\ infantis\) 2 suggested that the effective Streptococcus candidates shared similar genomic characteristics, which was further confirmed by having common biosynthetic pathways, such as those for spermidine and...
glycogen. Based on previous studies, we extrapolated that spermidine could be related to skin rejuvenation30–32. Spermidine is a key precursor in adipogenesis and lipid synthesis30, as well as associated with cell viability and autophagy55,56. Our analysis showed spermidine can increase the expression of collagen and elastin, as well as the synthesis of lipids in aged skins based on previous researches34,43–46,49–51. The increased accumulation of lipid in aged cells treated with spermidine confirmed the role of Streptococcal secretions in lipid synthesis and in strengthening the skin barrier function57,58. Based on the motif analysis of the upregulated genes, spermidine activates the transcriptional activities of genes related to skin improvement59,60.

The increased abundances of Streptococcus colonies at the genus level, particularly that of S. infantis, were also observed. Spermidine in the St solution positively affected the growths of S. pneumoniae and S. infantis and this observation suggested that improved skin conditions could positively feedback to themselves through increments of the beneficial skin microbes. In short, our stepwise analysis and findings show that a colonized skin microbiome could improve skin conditions by producing beneficial secretions and suggest that the potential skin microbiome as therapeutic and clinical applications.

In this present study, we have some limitations. In addition to our insight into microbial activity with spermidine, unknown interactions between Streptococcus and other bacteria on the human skin could have suppressed the growth of the competitive bacteria for Streptococcus candidates, such as the inhibition of S. aureus by Bacillus subtilis and Staphylococcus epidermidis61,62. We also have the restricted sex limit to parlay our investigation into the broad applications. Hence, we anticipate that further studies focusing on these details establishing links to skin health and skin microbiome.

**Methods**

**Microbial sample collection and preparation.** Microbial community samples from the faces of 26 old and 26 young participants were collected using sterilized tape (Elizabeth pack; Cell Lab, Republic of Korea). The tape was then dipped into a Tape (Elizabeth pack; Cell Lab, Republic of Korea). The tape was then dipped into a saline moistened with n-Butanol and then dipped into a peroxide solution to remove any excess moisture. Finally, the tape was rinsed with deionized water and air-dried before being stored at −80°C.

**16S rRNA PCR amplification and sequencing.** The V3–V4 region of the bacterial 16S rRNA gene was amplified according to the Illumina 16S Metagenomic Sequencing Library Preparation guide (Illumina, San Diego, CA, USA) using the following primers with an added adapter overhang sequence: forward, 5′-TCGTCGGGCTCGGAGATGTGTATAAGAGACAG-3′; reverse, 5′-GTCTCTTACGGGCTACCTTGTACACTCTTGTGTTTATGGCTCTATGGTC-3′. PCR reactions were performed in a total volume of 25 μl containing 2 μl of genomic DNA (10 ng/μl), 0.5 μl of each primer (10 μM), 12.5 μl of 2× KAPA HiFi HotStart Ready Mix (Kapa Biosystems, Wilmington, MA, USA), and 9.5 μl of distilled water. PCR conditions were as follows: initial denaturation at 95°C for 3 min; 25 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. The PCR products were purified with AMPure XP Beads (Beckman Coulter, Brea, CA, USA) according to the manufacturer’s protocol. For the sequencing libraries, the Illumina adapter sequences were removed using Cutadapt version 2.0.6. The trimmed sequences were processed using QIME2 version 2019.7. Briefly, the reads were assigned to each sample using the ‘qime taxon’ program with a unique taxon name. The reads were then used to calculate the taxonomic structure of the microbial communities using QIME2. A consensus method implemented in DADA2 was used. Beta diversities and weighted UniFrac metrics. Similarity among the groups was evaluated using LEfSe (Linear discriminant effect size analysis) with LEfSe software. Statistical plots and calculations were generated in R studio with the ggplot2 package.38

**Metagenomic analysis.** The quality of the raw sequence reads was analyzed using FastQC64. Illumina adapter sequences of the paired-end reads were removed using Cutadapt version 2.0.6. Then, the trimmed sequences were processed using QIME2 version 2019.7. Briefly, the reads were assigned to each sample according to a unique index; pairs of reads from the original DNA fragments were merged using an import tool in QIME2.68 Quality control and trimming were performed using Trimmomatic. The quality control for the Illumina sequencing libraries was performed using the Agilent Technologies 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Libraries were normalized and pooled for sequencing on the MiSeq platform (Illumina) by 2 × 250 bp paired-end sequencing, following standard Illumina sequencing protocols.

**Isolation and identification of Streptococcus from the face.** Sterilized water was used to wash the faces of the participants and was then spread on solid Tryptic Soy Agar (TSA) medium. Single colonies were collected and incubated in liquid TSB medium at 37°C for 24 h in stationary culture. Each sample was centrifuged at 6000 rpm for 30 min; the pellet was collected, and microbial DNA was extracted with a Quick-DNA™ Fungal/Bacterial Miniprep Kit according to the manufacturer’s instructions. DNA purity and quantity were estimated using a NanoDrop One Spectrophotometer (Thermo Scientific, Waltham, MA, USA).

**HDFs and HEKs.** HDFs and HEKs were purchased from the National Cell Culture Centre (NCC) and were used as positive controls for the experiments. HDFs were cultured in Fibroblast Growth Medium 2 with supplementMix and Keratinocyte Growth Medium 2 with
supplementMix, respectively (PromoCell). For St solution treatment, the cells were seeded at 80% confluence into 6-well plates and incubated in an atmosphere of 5% CO₂ at 37 °C. After 24 h, the cells were washed once with phosphate-buffered saline (PBS) and 10% of conditioned medium was added to the cells together with supplement-free medium, followed by a 24 h incubation.

**Cell viability assay**. HDFs and HEKs were seeded in 48-well plates and incubated for 24 h in 1 mL of the complete medium; 10% of the *Streptococcus* culture supernatant was added to the cells; they were incubated for another 72 h. After washing the cells once with PBS, 5-dimethyltryazol (2-5)-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well, followed by a 4-h incubation. Then, the medium was discarded, and dimethyl sulfoxide was added to dissolve the formazan crystals. Optical density was measured at 570 nm using a microplate reader and was normalized relative to the untreated control.

**RNA isolation and real-time PCR**. Total RNA was isolated from cells using TRIzol reagent according to the manufacturer’s instructions (TaKaRa, Shiga, Japan). cDNA was synthesized from 1 μg of total RNA using Reverse Transcription Premix (Elsips-biotech, Daejeon, Republic of Korea) under the following reaction conditions: 45 °C for 45 min and 95 °C for 5 min. Gene expression was quantified by real-time PCR, and the data were analyzed using StepOnePlus™ software (Applied Biosystems). Real-time PCR amplification reactions were performed using SYBR Green PCR Master Mix with premixed ROX (Applied Biosystems).

The following primer pairs (Bioneer, Daejeon, Korea) were used in the reactions inside an ABI 7300 cycler following the manufacturer’s protocol: β-actin (forward, 5′-GGCCATCATCTTGGCTGAAGT-3′; reverse, 5′-GACACCCTTCAACA CCGCAGC-3′); COL1A1 (forward, 5′-GGAGGGCCAAGACGAAGACATC-3′; reverse, 5′-GACATGACGTCCAGTCGACAC-3′); COL1A2 (forward, 5′-TGAGGATGTGGTCGACGAAA-3′; reverse, 5′-ACAGCCTTGGTGTGGTACAA-3′); ELN (forward, 5′-CACCCTTCTCTTGTGATACTCA-3′; reverse, 5′-CCAAGGAGGGGCTCACA-3′); FLG (forward, 5′-AGTGTGAATGATTTGGTGACGGGGTTCCT-3′; reverse, 5′-TGGAGTTGTTGTCGACGAAA-3′); FBN1 (forward, 5′-AGTTGTCAGACGAAGCCAGGG-3′; reverse, 5′-GATTTGGTGACGGGGTTCCT-3′); DSC2 (forward, 5′-AGTGCACTCAGGGGGCTCACA-3′; reverse, 5′-CGCGCTTGCGGATGATATGT-3′); ABCA12 (forward, 5′-GACTAGAACGAGTGGATGAGT-3′; reverse, 5′-AATGTCAGACGAAGCCAGGG-3′); and Uf (forward, 5′-GATGTTGTTGTCGACGAAA-3′; reverse, 5′-GTTCAGGCGGTAAGTCCAGTA-3′); FLG (forward, 5′-AGTGTAATGATTTGGTGACGGGGTTCCT-3′; reverse, 5′-GAGGGCCAAGACGAAGACATC-3′).

The reactions were set 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.

**Nile red staining for neutral lipids**. HEKs were seeded in 6-well plates. After 24 h, 10% of the *Streptococcus* culture supernatant was added to the cells, and these were incubated for another 24 h. After washing, a stock solution of Nile red (1 mg/mL) in acetone was prepared and stored at −20 °C away from light. A fresh staining solution was made by adding 1 μL of the stock solution to 1 mL of PBS and then 500-μL aliquots of the mixture were added to each well. After 10 min at room temperature in the dark, the cells were examined using a fluorescence microscope (Axio Observer Z1; Carl Zeiss, Jena, Germany). Normal human keratinocytes and normal human fibroblasts, was cultured. The tissue was transferred to 6-well plates and cultured overnight in DMEM (MatTek Co., MA, USA), containing 5 μg/mL gentamicin B (MatTek Co., MA, USA, 0.25 μg/mL amphotericin B (MatTek Co., MA, USA), and other growth factors, in a 5% CO₂ atmosphere at 37 °C. To induce aging, 3D skin models were treated with poly I:C (1 μg/mL) and incubated at 37 °C for 24 h. After washing, the stock solution of Nile red (1 mg/mL) in acetone was prepared and stored at −20 °C away from light. A fresh staining solution was made by adding 1 μL of the stock solution to 1 mL of PBS and then 500-μL aliquots of the mixture were added to each well. After 10 min at room temperature in the dark, the cells were examined using a fluorescence microscope (Axio Observer Z1; Carl Zeiss, Jena, Germany). Normal human 3D skin model culture and treatment. A normal human 3D skin model at full-thickness (Epiderm-FT; MatTek Co., Ashland, MA, USA) was purchased from PromoCell (Heidelberg, Germany) and cultured similarly (see Cell culture and St treatment). To stabilize the dose of UVB irradiation, the UVB irradiation machine (CL-1000 ultraviolet crosslinker) was pre-warmed for 30 min. Normal human HDF cells were then irradiated with UVB (20 mJ/cm²) for 2 s twice a day. After 2 days, the UV-induced aged skin cells were treated with spermidine. Both aged skin cells and UV-induced aged skin cells were treated with spermidine (Sigma, StL, USA). Motif enrichment analysis in the promoters of the upregulated genes was performed by Pscan using the default option 81.

**Measurement of growth rate**. Each *Streptococcus* species was activated in 10 mL of TSB medium at 37 °C while shaking at 200 rpm for 24 h. Then, 10 μL of pre-cultured bacterial cells were subsequently inoculated in 10 mL of TSB broth and 10 mL of TSB supplemented with spermidine (300 μg/mL) to compare the growth of bacterial cultures. Optical density (OD₅₇₆) was measured by a microplate reader (SPECTRA MAX 190, POWER LAB) every hour for 28 h.

**Statistics and reproducibility**. Wilcoxon–Mann–Whitney test was used to calculate the significant differences for non-parametric data. Wilcoxon signed-rank test was used for paired comparison. Pearson correlation was used for correlation analysis. The Student’s two-tailed t-test was used in the in vitro cell assay analysis. The power test of the clinical tests was done using 0.7 power. Statistical analyses were performed using R studio or Prism (GraphPad).
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Author contributions
G.K. analyzed the 16S rRNA sequences and whole-genomic sequences, prepared the figures, and wrote the manuscript. Both M.K. (Misun Kim and Minji Kim) performed the qPCR experiment and prepared the 3D scanning model. C.P. analyzed the 16S rRNA sequences. Y.M. and S.K. (Sujeong Kim) edited the manuscript. D.-H.L. performed the aged-skin cell assay. H.Y., S.K. (Seunghyun Kang), and J.L. performed the in vitro cell assay. Y.-G.L. and N.-I.B. analyzed the streptococcal product of the supernatant. Y.K., W.-W.C., and C.L. revised the manuscript. K.W.Y., D.-G.L., and H.P. designed and supervised all experiments and analyses.

Competing interests
The authors declare no competing interests.

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