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Active Ingredients

fensebiome™

peptide

Reconnect with your origins for healthier skin

Strengthening the double barrier function

Metagenomics analysis on urban skin

Restoring sensitive skin

TECHNICAL REPORT (TR)

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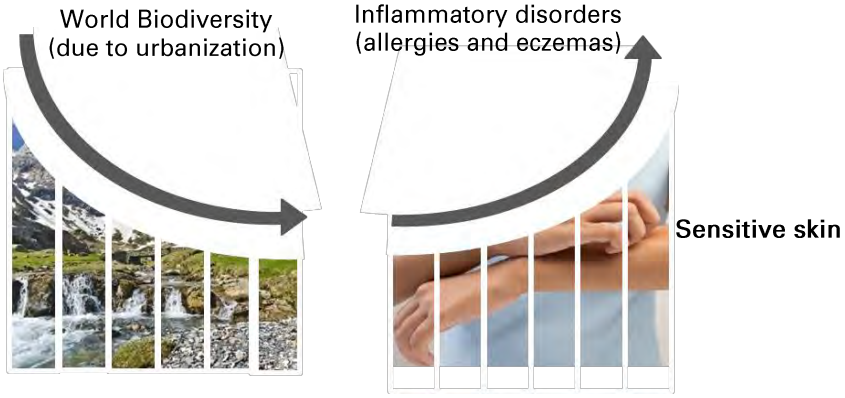
CONTENT

MODERNIZATION, URBAN SKIN AND BEYOND

Discomfort, fragility, dehydration, scaliness and redness are all characteristics of **sensitive skin that is lacking an effective defense system**. The **intolerance** of sensitive skin to many **external stimuli**, mostly resulting from a defective barrier function, has **increased** over the past few years especially in the most urbanized regions. In addition, an ongoing **decline in the biodiversity of species** may suggest a correlation between these megatrends (biodiversity loss and skin sensitivity increase) [1].



The **massive growth of urbanization** that came along with the **Industrial Revolution** in the 18th century became a turning point in the relationship of humans with their environment, **reducing human interaction with nature**. This important moment also represented a relevant trigger in the prevalence of inflammatory disorders, such as allergies and eczemas. From this evidence, it can be inferred that the **reduced exposure to nature** may be related to the **increase in the risk of inflammatory skin conditions**.

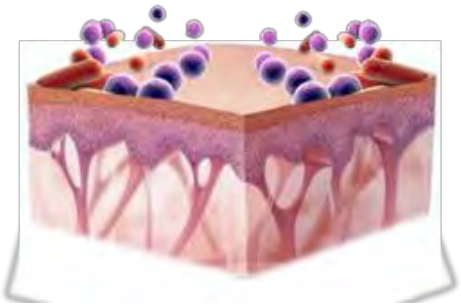
Why was the skin of our ancestors, who were in closer contact with nature, more resilient?

In 2009, a scientific group discovered an isolated **Yanomami Amerindian hunter-gatherer village** in the Amazon jungle, whose members did not have any previous contact with urban or modern lifestyles. This discovery provided the opportunity to travel back in time to understand the skin characteristics of our ancestors before modernization. The research **compared** the Yanomami's skin microbial genome, also known as the **microbiome, to that of individuals living in the United States** with a lower connection with nature and higher antimicrobial practices such as the use of antibiotics, hyper-cleanliness and birth by caesarean section.

Surprisingly, the research showed that the **Yanomami's bacterial diversity was twice that of the Americans** and that their **skin was enriched in organic acids, amino acids, vitamins and methane bacterial pathways**, all associated with a **healthier and reinforced skin**. These findings suggest that exposure to modern lifestyles may reduce the microbiota richness, making the skin more prone to sensitivity and discomfort [2]. The study demonstrated the **negative impact that modernization has had on the skin**, while uncovering the important often underappreciated bacterial universe that resides on our skin. The results also suggest the need for skin care to reconnect with the origins to recover the protective characteristics of our ancestors' skin.

Alterations in the cutaneous microbiota due to urbanization could contribute to an increase in skin sensitivity.

The cutaneous **microbiota** can provide **vital functions to the skin**, such as host protection against pathogens, barrier function improvement, modulation of the skin immune system and skin nutrition. The **microbiota-skin communication** helps obtain a microbial **balance** that is linked to a more **protected healthy skin**. Disruption of this ecological balance, also termed **dysbiosis, can result in several skin disorders**. One example is the overrepresentation of the bacterial Staphylococcaceae family, which has been linked to skin conditions such as atopic dermatitis, acne and rosacea [3].



Keeping the important role of the skin microbiota in mind, the concept of cutaneous barrier function gained a new approach. The understanding of the **skin's own defense system** evolved from being formed not only by the most traditional physical barrier made up of **lipids and corneocytes**, but also by a **living ecosystem** considered as the **second protective shield** of the skin.

Proper cosmetic care of the skin's double barrier should be viewed as a tool to holistically improve urban exposed skin by reducing hyperreactivity, fragility, dehydration and scaliness.

THE SKIN'S DOUBLE BARRIER, MICROBIOTA AND EPIDERMIS

One of the most important vital functions of the skin microbiota is its contribution to the role of the skin as a **protective barrier** and this can occur through different mechanisms.

Bacterial competition

Through **direct competition** for space and nutrients, the beneficial bacteria can cause the exclusion of potentially harmful microbes from the skin surface. For instance, *Staphylococcus epidermidis* inhibits colonization by *Staphylococcus aureus* through nutrient and space competition and the production of antimicrobial peptides [4].

Immune response

The skin acts as an **immunological barrier**, which is vital for its proper defense. This **innate immunity** depends on the reciprocal interaction of the microbiota with keratinocytes. It helps enhance skin immunity, allowing the recognition of pathogens and prevent their invasion.

• Innate immune response

As part of this defense, keratinocytes continuously monitor the presence of microorganisms in the skin through pattern recognition receptors, such as **Toll-like receptors (TLRs)** and the **nucleotide oligomerization domain (NOD)-like receptors (NLRs)**. These receptors recognize molecules associated with pathogenic bacteria. TLR are predominantly expressed on the surface of keratinocytes, and NLR are intracellular receptors. The different subcellular localization of these receptors and the broad array of molecules they can recognize allows the skin to sense a large number of pathogens and develop an adequate response.

Upon recognition of pathogens, the receptors are activated, interact with intracellular adaptor proteins, such as MYD88, and initiate **intracellular signaling pathways** that converge in transcription factors, such as nuclear factor-kappaB (NF-κB) or interferon regulatory factors (IRFs). This results in the expression of soluble factors like **cytokines and antimicrobial effectors** that mediate the immune response. The presence of beneficial bacteria, sensed by keratinocytes via TLRs, allows amplification of the skin's immune defense against pathogens through an increased expression of antimicrobial peptides [5, 6].

• Immune tolerance

Bacterial species that inhabit the skin allow this pro-inflammatory response to cease, preventing a potential skin overreaction. Research shows that certain Gammaproteobacteria, which are found on the skin of people in close contact with natural areas, are able to offer an **immunomodulatory balance** that helps **prevent an inappropriate inflammation**. These microorganisms are known to induce interleukin IL-10 and other anti-inflammatory molecules in cells that help balance the immune response offering an essential tolerance to the skin [7, 8].

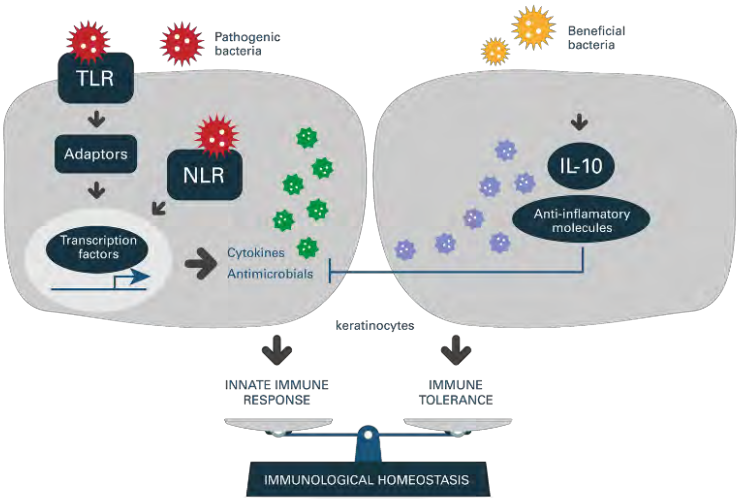


Fig. 1. Maintenance of the skin immunological homeostasis activated by the cutaneous microbiota.

Epidermal barrier integrity

The **mechanical wall** that resides on the epidermis is key to provide an efficient **permeability barrier** against excessive loss of water and electrolytes. It also **protects** the organism from **harmful elements of the environment.** Two of its main players are the **intercellular junctions** found in the stratum granulosum, and the **lipid barrier** in the stratum corneum.

Different types of **intercellular junctions** are important to maintain integrity of the epidermis. In the stratum granulosum, **tight junctions** (TJs) between adjacent keratinocytes help **prevent free passage of solutes and water** through the paracellular space between cells. TJs are formed by transmembrane proteins with extracellular domains that join directly to those of adjacent cells. Major types of these membrane proteins are occludin, claudins, and junction adhesion molecules (JAMs). These components interact with a complex array of scaffolding proteins inside the cell, for instance the tight junction plaque proteins (TJPs) that serve as links with the actin cytoskeleton [9].

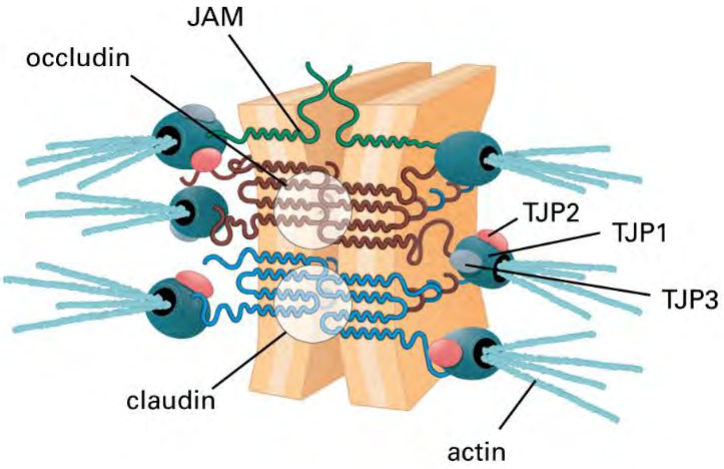


Fig. 2. Structure of tight junctions.

The microbiota can also contribute to strengthening the skin barrier from a more physical point of view. The interaction of the microbiota with the epidermis is able to **enhance TJs functions in keratinocytes via the activation of TLRs signaling**. This would help reinforce the barrier function of the skin and prevent further invasion of harmful microorganisms [10].

The **lipid barrier** is mainly composed of ceramides, cholesterol and fatty acids. **Ceramides** are sphingolipids composed of sphingosine and fatty acids, and are vital to the **organization and functioning of the barrier**. Each ceramide type can have a varying chain length, which has an impact on the permeability and function of the lipid membrane. Specifically, ceramides with **long hydrophobic chains** are essential for the formation of tightly packed impermeable lipid lamellae, which are required for a **proper barrier function** [11].

The microbiota can also contribute to preserve the cutaneous lipid barrier, through its implication in biological **pathways involved in the synthesis of lipids, fatty acids and sphingolipids**, such as ceramides, in the stratum corneum [12].

An impaired defensive function is observed in **sensitive skin**, characterized by an **abnormal microbial colonization**, an **impaired barrier function** and an **aberrant lipid organization** [13].

In order to minimize such alterations and maintain a proper skin condition, the cosmetic application of **prebiotics and probiotics** on the skin becomes a powerful alternative.

Crosstalk between microbiota and skin cells modulates the defensive response of the skin.

SKIN CARE FOCUSED ON THE MICROBIOTA

The terms **prebiotic** and **probiotic** have recently stepped out from their conventional use in foods to be given new promising applications in the skin care field. The cosmetic industry is joining this **currently growing trend** to deliver innovative **microbiota-inspired products** that aim to preserve the important bacteria-host homeostasis for a healthy and good-looking skin.

Probiotics correspond to **"live microorganisms,** which, when administered in adequate amounts, confer a **health effect** on the host" [14]. The benefits of **probiotics** on the skin condition are multiple, including effects such as skin **rejuvenating, antioxidant, hydrating, anti-inflammatory** and **healing,** transepidermal water loss **(TEWL) prevention, defense** against **pathogens, microbiota balance, barrier function** and **immune response** improvement and acne and atopic eczema reduction [15].

Prebiotics fall into the category of **"non-digestible substances** that provide a beneficial physiological effect on the host by selectively stimulating the **favorable growth** of **indigenous bacteria"** [14]. The effects of **prebiotics** are related to creating favorable conditions on the skin (e.g. hydration, presence of nutrients and lipids, modulation of bacterial metabolic pathways) that **promote the growth of the beneficial bacteria**.

Prebiotic and probiotic-based cosmetic products represent innovative solutions to protect the skin from undesired bacterial colonization and to restore the barrier function and immunologic balance for a maintained **healthy state of the skin.**

In order to determine the benefits that prebiotics and probiotics provide to the skin, several powerful **omics platforms** can be used. **Metagenomics**, which is the study of the genetic content of microbial communities, can be leveraged to obtain a stronger understanding of the microbiome changes in the skin. Other techniques such as **transcriptomics**, consisting of the study of the complete set of RNA molecules in one cell or organism, can help determine the modulation of genes related to the immune response and epidermal barrier integrity. Finally, the epidermal lipids profile can be determined by means of **lipidomics**. By putting these different omics platforms together, a **holistic approach towards a double barrier function** efficacy can be obtained.

Through the trend of microbiota-inspired products, a prebiotic approach for a probiotic-like effect was developed.

FENSEBIOME™ *peptide*, RECONNECT WITH YOUR ORIGINS FOR HEALTHIER SKIN

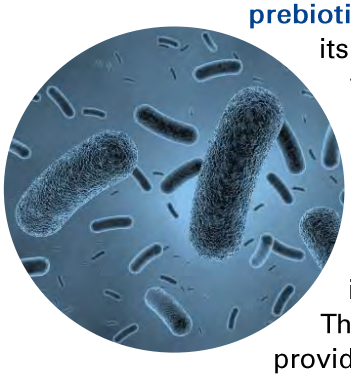
FENSEBIOME™ *peptide* is a heptapeptide intended to strengthen vulnerable urban skin, by promoting microbiota balance, diversity and an increase in beneficial bacteria, associated with a healthier skin in higher contact with nature. The ingredient helps reinforce the double cutaneous barrier function and prevent dehydration.

Omics platforms were used to evaluate the efficacy of the ingredient. Using a **metagenomics** analysis on volunteers, who were exposed to urban conditions, the ingredient showed to **increase the microbial diversity** and to favor the presence of **beneficial bacteria** on the skin. Furthermore, it also helped modulate **bacterial functional pathways** with potential benefits for the skin. Other *in vivo* efficacy tests on female urban volunteers, showed that the peptide helped **reinforce skin cell cohesion** and reduce TEWL levels after damage, suggesting a **protective effect of the skin barrier.**

In addition, fluorescence microscopy was used to demonstrate that FENSEBIOME™ *peptide* helped to promote the adhesion of beneficial bacteria to keratinocytes. The use of **transcriptomics** suggested a certain ability of the peptide to stimulate the cutaneous **immune response** (through pathogen recognition pathways) to keep the skin ready in case of any potential invasion. Components in charge of strengthening the physical barrier integrity were also evaluated by means of **transcriptomics and lipidomics**, obtaining an enhancement in **tight junctions** genes and **long-chain ceramides**, contributing to a well-preserved barrier.

When evaluated on skin models, the ingredient appeared to improve the functionality of the epidermal barrier through a **reduced permeability**, while **preserving the stratum corneum and granulosum integrity** that helps prevent alterations by aggressors.

FENSEBIOME™ *peptide* falls into a **prebiotic approach** due to its ability to stimulate the growth of Moraxellaceae bacteria with anti-inflammatory effects that can improve the skin's immune tolerance. The ingredient also provides **probiotic-like effects** to the skin, by modulating skin microbiota balance with a reduction in pathogen adhesion and by reinforcing the immune and physical barrier function for a healthy-looking skin. The peptide also represents a **way to reconnect with nature**, the inseparable part of **our origins**, since it helps increase cutaneous bacterial diversity and favors the beneficial bacteria on the skin, which is a marker of the healthy and protected skin of populations in closer contact with nature.



FENSEBIOME™ *peptide* helps strengthen the double barrier function for a reinforced skin resembling that of our ancestors.

fensebiome™ *peptide*

IN VIVO EFFICACY

Study of the skin microbiome

The ability of FENSEBIOME™ *peptide* to modulate the skin microbiome was assessed on volunteers by means of a **metagenomics study** of their skin microbiota.

A panel of 21 female volunteers between 18 and 59 years old from the metropolitan area of Barcelona, Spain, applied a cream containing 1% FENSEBIOME™ *peptide solution* on the cubital fossa of one arm and a placebo cream on the other, twice a day for 7 days.

Samples of the skin microbiota were obtained before and after the treatment using swabs moistened with a solution that helps improve the adhesion of skin microbiota. Then, the bacterial DNA from the swabs was extracted and purified.

Changes in the microbiome were assessed by metagenomics. The method used for bacterial identification corresponded to 16S rRNA sequencing, which focuses on the analysis of ribosomal RNA. 16S rRNA gene is commonly used in the identification of bacterial microorganisms because it is present in almost all bacteria, it has been highly conserved in nature and it is large enough for informatic purposes. This gene also contains nine hypervariable regions (V1-V9) that show considerable sequence diversity to differentiate between closely related bacteria.

All the data was analyzed by three different bioinformatic analysis, which provided information on diversity, composition and functional profile of the skin microbiome.

Diversity

The characterization of microbial species diversity was determined by the Shannon index from the analysis of the DNA sequencing results of the V3 region of the 16S rRNA gene. The Shannon index takes into account the richness, or the amount of taxonomic groups, and the evenness, or the proportion of these bacterial types. A low Shannon index value indicates a low microbial diversity, which is commonly associated with a less healthy skin [16].

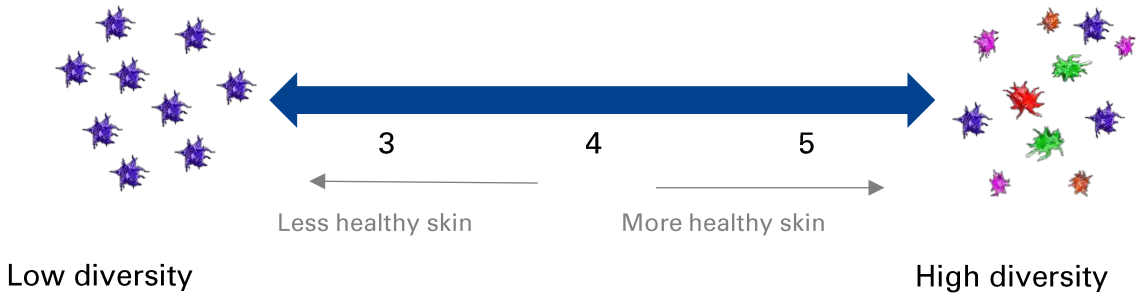


Fig. 3. Representation of Shannon index values according to microbial diversity.

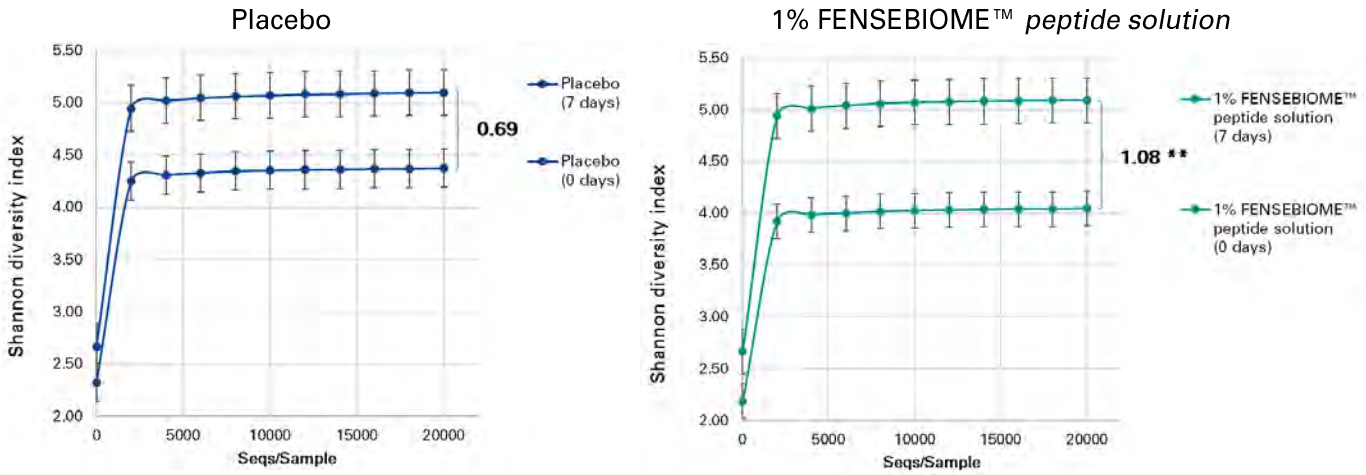


Fig. 4. Curves representing the mean Shannon index values from all the volunteers before and after each treatment (vs initial time: **p<0.01).

After the active treatment, the **Shannon index value** showed a **higher increment** compared to placebo, indicating an **enhanced microbial diversity**.
A high microbial diversity, related to a healthy skin, has been detected in the skin of people in close contact with nature.

FENSEBIOME™ *peptide* helps increase the diversity of skin bacteria for a rewilding effect that leads to a better skin health.

Composition

In order to identify the microbial composition, the relative abundance at different taxonomic levels was evaluated from the sequencing results of seven hypervariable regions of the 16S rRNA gene. Results of the microbiota composition analysis are presented at the phylum and family levels.

- PHYLUM

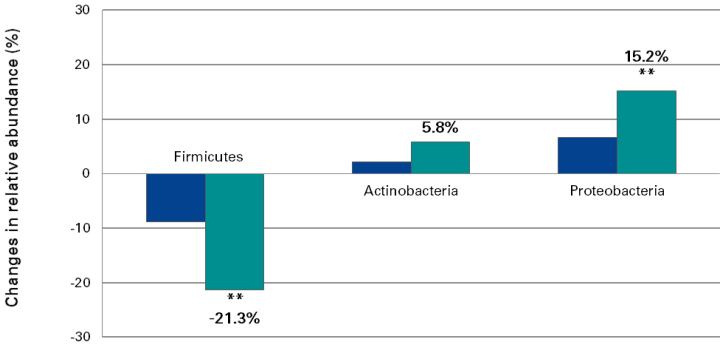


Fig. 5. Variation in the relative abundance at phylum level after 7 days of treatment (vs initial time: **p<0.01; vs placebo: *p<0.05 (Firmicutes and Proteobacteria)).

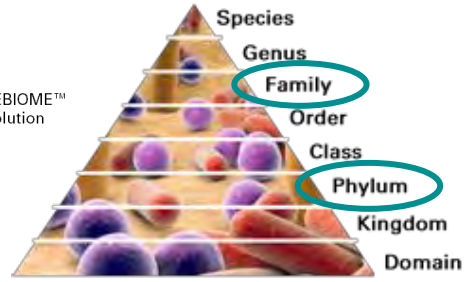


Fig. 6. Representation of levels of bacterial classification.

FENSEBIOME™ *peptide* helped increase the relative abundance of the beneficial bacterial phylum of **Proteobacteria (15.2%)** and reduce the **Firmicutes** phylum **(21.3%)**, achieving a balance of the microbiota for a healthy skin.

Favoring the balance of the microbiota to reinforce the bacterial protective shield of the skin.

- FAMILY

From the above significant results, the relative abundance at family level was also evaluated, being only the corresponding most significant results represented.

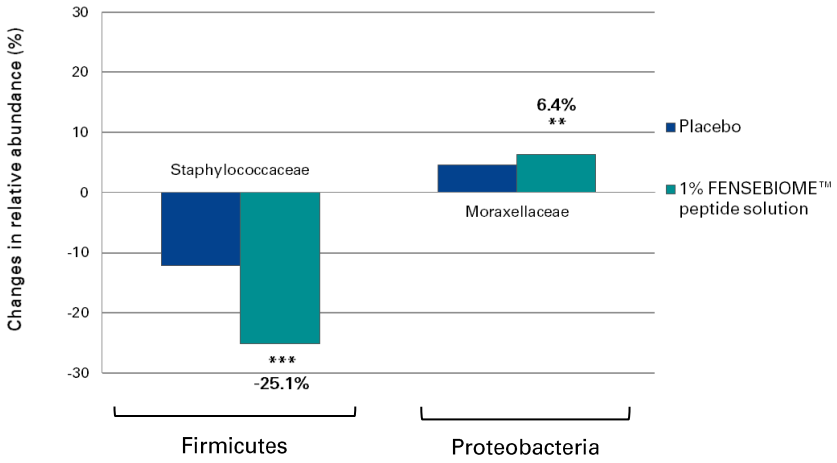


Fig. 6. Changes in the relative abundance at family level at the end of the treatment (vs initial time: **p<0.01, ***p<0.001; vs. placebo: *p<0.05).

FENSEBIOME™ *peptide*, showing a prebiotic approach, helped significantly **increase** the **relative abundance of Moraxellaceae (6.4%),** that belongs to the gammaproteobacterial class, demonstrated to present potential **anti-inflammatory properties** related to immunological homeostasis and which has been found on the skin of populations in close contact with nature. The ingredient also showed a **reduction in Staphylococcaceae (25.1%),** which is mostly associated with skin conditions such as atopic dermatitis and psoriasis.

Increase in anti-inflammatory bacteria found on the skin of people in close interaction with natural areas.

Microbiome functional profile

The microbial communities on the skin were studied by a computational approach that uses data from the V3 region of the 16S rRNA gene, together with a database of reference genomes. A predictive microbial functional profile was obtained and represented by KEGG pathway mapping (level 3).

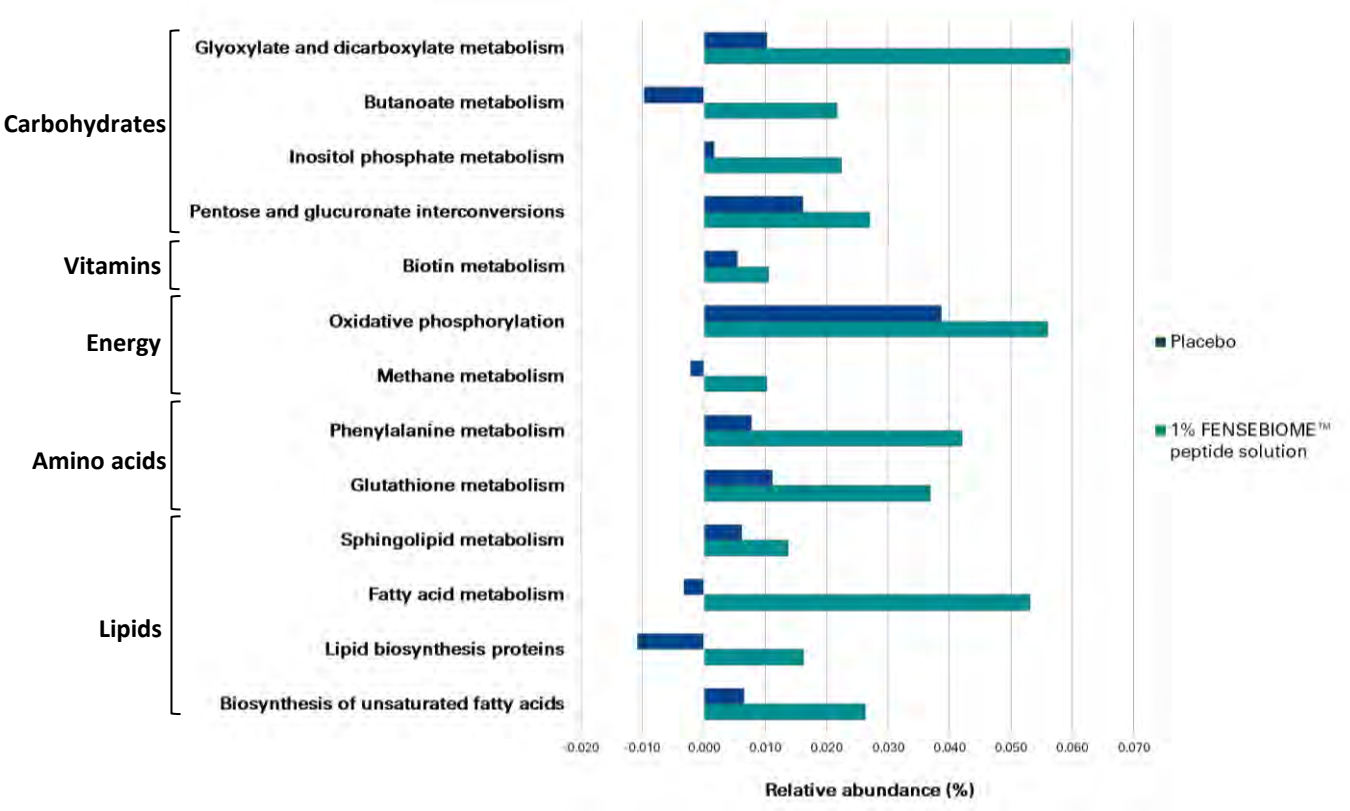


Fig. 7. KEGG predictive metabolic pathways of skin bacteria.

The active ingredient helped **modulate bacterial metabolic pathways** related to energy, lipids, amino acids, carbohydrates and vitamins, which have been detected in the skin of uncontacted populations.

Enhancement in bacterial metabolic pathways that could promote the external supply of nutrients to the skin.

Enhanced skin cell cohesion

The implication of FENSEBIOME™ *peptide* on the cohesion of corneocytes was assessed by evaluating skin desquamation through a tape stripping technique.

A panel of 18 female volunteers between 26-50 years old, from the metropolitan area of Barcelona, Spain, who presented dry skin on the legs applied a cream containing 1% FENSEBIOME™ *peptide solution* on one shin and a placebo cream on the other shin, twice a day for 7 days.

Before and after the treatment, samples of the stratum corneum of both legs were obtained by tape stripping. The samples were then stained with fluorescein, and fluorescence levels, representing the corneocytes attached to the tape, were quantified. Further visualization of the corneocytes was performed by means of fluorescence microscopy.

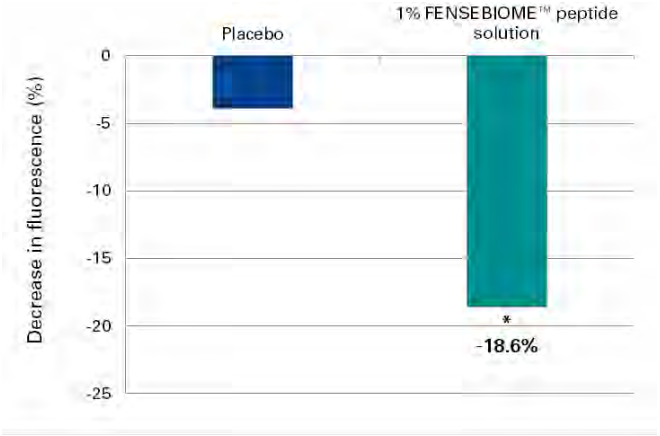


Fig. 8. Fluorescence levels, representing the corneocytes that were detached from the skin, after 7 days of treatment (vs initial time: *p<0.05).

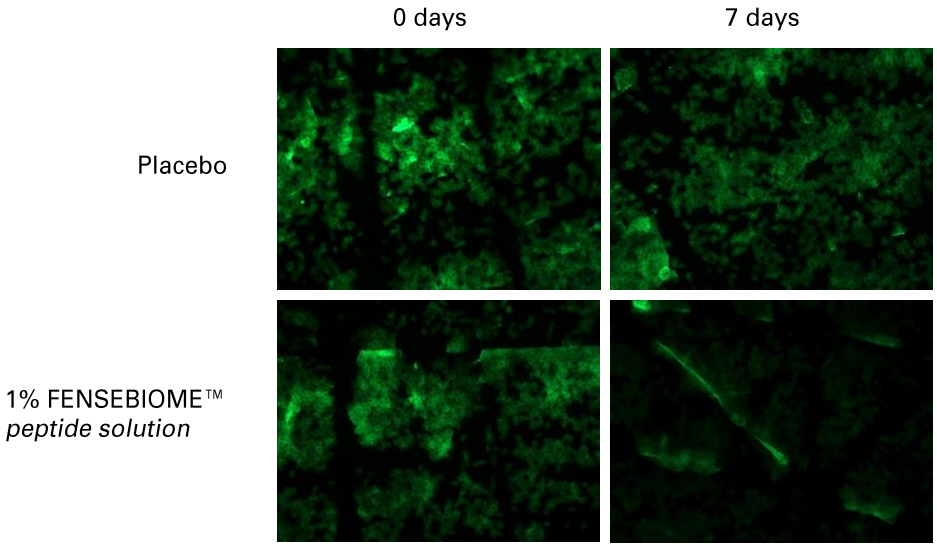


Fig. 9. Fluorescence microscopy images obtained from different strips before and after each treatment.

The active treatment **decreased sloughed corneocytes** by **18.6%**, showing a better skin cell cohesion for an improved barrier function.

Reduced skin scaliness, suggesting the ability to restore sensitive skin.

Barrier protection effect

The ability of FENSEBIOME™ *peptide* to improve skin barrier protection was evaluated by means of a Tewameter® TM300 through measurements of the transepidermal water loss (TEWL).

A group of 20 female volunteers between 22 and 45 years old from Oporto, Portugal, applied a cream containing 1% FENSEBIOME™ *peptide solution* and a placebo cream the forearm, twice a day for 7 days.

TEWL values were obtained after 7 days of product application. Then, a solution with 1% sodium lauryl sulfate was applied under occlusion on each test site and it was kept in contact with the skin for 24 hours. TEWL values were obtained again after 48 hours of patch removal. TEWL decrease versus damage was finally calculated.

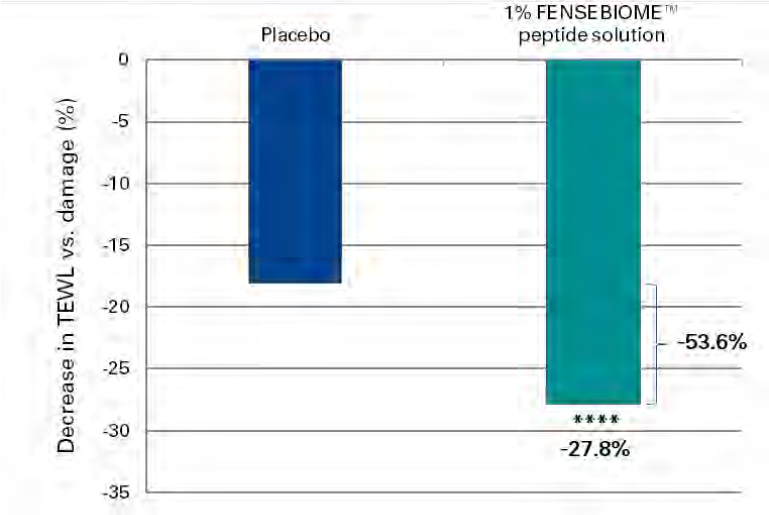


Fig. 10. TEWL decrease vs. damage after different treatments (vs initial time: ****p<0.0001).

FENSEBIOME™ *peptide* helped **reduce the TEWL levels,** when applied before inducing irritation and evaluated 48 hours after damage, with a decrease of **27.8%.**

Better protective effect on the physical barrier to prevent dehydration.

IN VITRO EFFICACY

Promoting the adhesion of beneficial skin microbiota

The ability of FENSEBIOME™ *peptide* to influence the presence of different microorganisms that constitute the skin microbiota was assessed by measuring their resulting adhesion to skin cells.

Human keratinocytes were pre-incubated for 24 hours with 50 µg/mL FENSEBIOME™ *peptide* or with culture medium as a control.

Then, the epidermal cells were exposed to a mixture of *S. epidermidis* labelled with green fluorescence and inactivated red-fluorescently labeled *S. aureus*. After 30 minutes, the cells were washed to remove non-adhered bacteria and cell nuclei were stained with blue fluorescence.

The adhesion of each type of bacteria to the keratinocytes was determined by fluorescence microscopy.

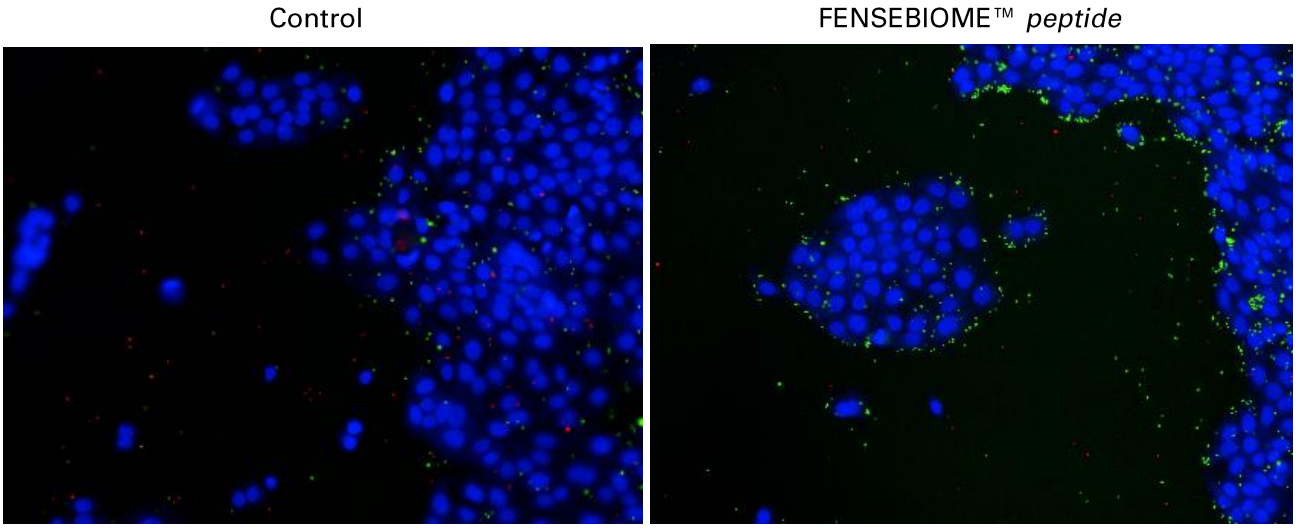


Fig. 11. Keratinocytes nuclei (blue) pre-incubated with only the medium or with the active treatment, showing adhesion of *S. epidermidis* (green) and of *S. aureus* (red).

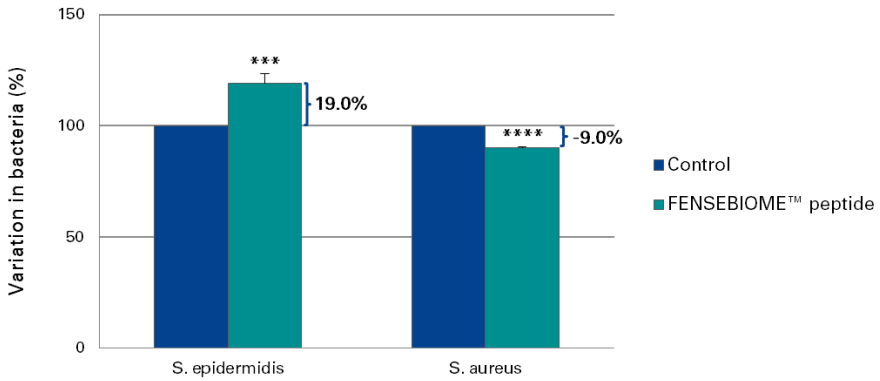


Fig. 12. Relative content of specific bacteria under different treatments (***p<0.001; ****p<0.0001).

FENSEBIOME™ *peptide* **favors the adhesion of *S. epidermidis*** with respect to the potentially pathogenic *S. aureus* in keratinocytes.

Promoting a shift in the skin microbiota that favors the presence of the beneficial bacteria.

Modulation of the immune response

The expression profile of a set of genes related to the activation of the immune response was obtained by means of a transcriptomics study.

Human keratinocytes were treated with 50 µg/mL FENSEBIOME™ *peptide*, while cells treated with culture medium were used as a control, for 24 hours.

RNA was purified from the cells and processed through RT-PCR arrays to determine gene expression levels.

Table 1. Genes related to the activation of the immune response.

	Symbol	Gene name	Description
Receptors for pathogen recognition	TLR5	toll-like receptor 5	Membrane receptor
	CD14	CD14 molecule	Co-receptor of TLRs, required for their activation
	NOD1	nucleotide-binding oligomerization domain containing 1	Intracellular receptor
	NOD2	nucleotide-binding oligomerization domain containing 2	Intracellular receptor
Adaptors	MYD88	myeloid differentiation primary response 88	Transducer of signals from TLRs
Transcription factors	STAT6	signal transducer and activator of transcription 6	Activator of gene expression
	IRF3	interferon regulatory factor 3	Activator of gene expression

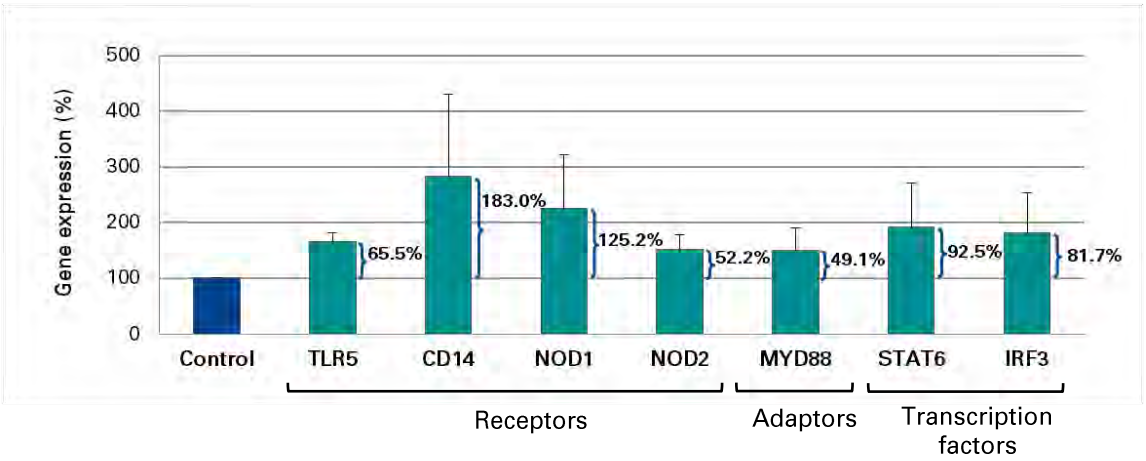


Fig. 13. Expression of genes from TLRs and NLRs pathways.

Induction of different genes related to TLR and the NLR signaling pathways that could help prepare the skin cells to **efficiently respond** against a wide spectrum of harmful microbes in case of need.

Upregulation of genes associated with an improved immunological barrier.

Reinforcing the key epidermal barrier compartments

1) Increased expression of intercellular junctions

The aim of this test was to study the ability of the peptide to increase gene expression linked to an improved functionality of the epidermal barrier.

Human keratinocytes were treated with 50 µg/mL FENSEBIOME™ *peptide* for 24 hours or were treated with culture medium as a control.

Then, RNA was purified from the cells and analysed by transcriptomics (RT-PCR arrays) to assess expression levels of genes related to tight junctions.

Table 2. Genes related to tight junctions.

	Symbol	Gene name
Membrane proteins	OCLN	occludin
	CLDN1	claudin 1
	JAM3	junctional adhesion molecule 3
Membrane-associated proteins	TJP1	tight junction protein 1
	TJP2	tight junction protein 2
	TJP3	tight junction protein 3
Cytoskeletal actin	ACTA1	alpha-actin

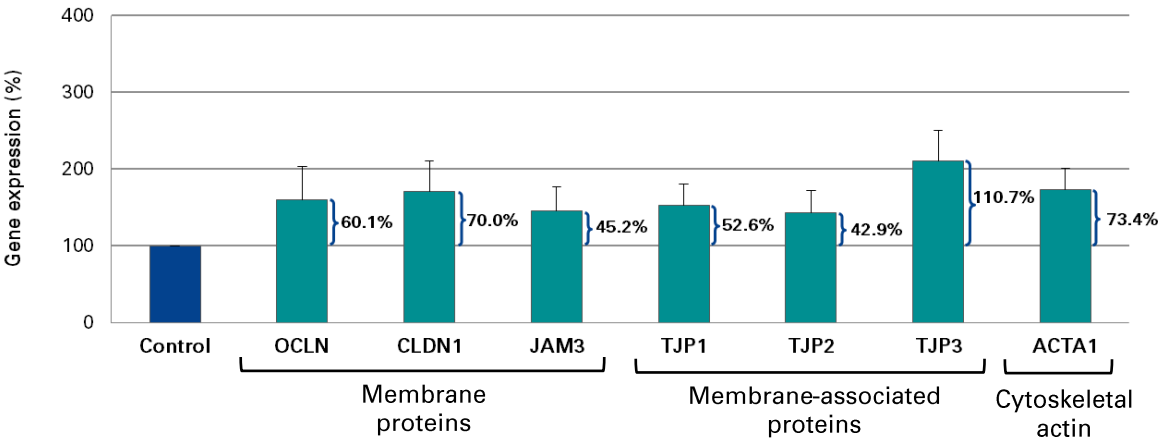


Fig. 14. Expression of genes related to tight junctions and hence, to the physical skin barrier.

Increased expression of components of the cell **tight junctions** that provide impermeability to the barrier, preventing loss of water and solutes and reinforcing the protection against the invasion of pathogens.

Induction of TJ elements for a better epidermal barrier integrity.

2) Improved stratum corneum lipids profile

Differences regarding the presence of ceramides between control and treated skin were studied through a lipidomics study, an omics platform that assists in the mapping of the present lipids.

Reconstructed human epidermis models were treated with 50 µg/mL FENSEBIOME™ *peptide* for 24 hours or were treated with culture medium as a control.

Then, lipids were extracted from the models and the endogenous lipidomic profiles were obtained by means of ultra performance liquid chromatography coupled to mass spectrometry.

Ceramides levels in treated reconstructed human epidermis were calculated with respect to those of control epidermis models. Depending on their molecular composition, there are several classes of ceramides, arising from the combination between the different sphingolipids (dihydrosphingosine (dS), sphingosine (S) and phytosphingosine (P)), and the fatty acid chains, such as the non-hydroxy fatty acid (N). N-type ceramides represent the most abundant in the stratum corneum.

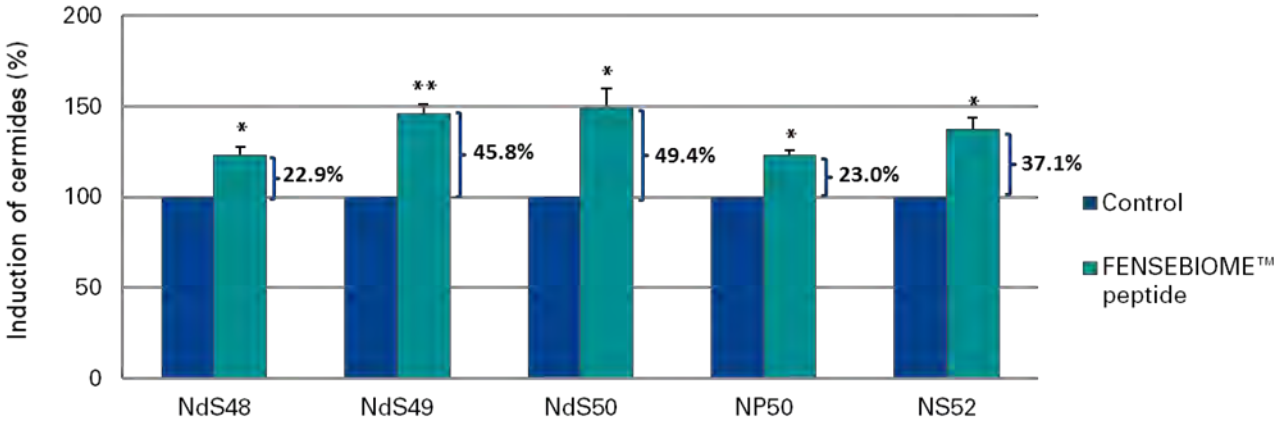


Fig. 15. Changes in the level of N-type ceramides detected in the epidermis at different conditions (*p<0.05; **p<0.01).

Increased amount of different **ceramides with long chains**, key for the formation of tightly packed impermeable lipid lamellae for a **proper barrier function**.

Boost of long-chain ceramides, essential for the structure and impermeability of the barrier function.

Improved epidermal barrier functionality

The ability of the active ingredient to reinforce the epidermal barrier and protect from aggressors was evaluated on reconstructed human epidermis models. For this purpose, two different dye penetration assays, which can be used as an *in vitro* indicator of transepidermal water loss (TEWL), were performed.

1) Stratum granulosum integrity

Performance of the TJs in the granular layer of the epidermis was tested using biotin as a tracer molecule. Biotin can pass through intercellular spaces but not through properly functioning TJs. Therefore, its diffusion through the stratum granulosum indicates a disrupted barrier. The ability of FENSEBIOME™ *peptide* to enhance barrier function by inhibiting tracer diffusion through the different layers in the presence of sodium dodecyl sulfate (SDS), which causes the functional deterioration of TJs, was studied.

Reconstructed human epidermis models were incubated for 24 hours with 50 µg/mL FENSEBIOME™ *peptide* and 0.02 mg/mL SDS. Skin models treated with medium were used as a control and reconstructed human epidermis models incubated with 0.02 mg/mL SDS and medium were used as a positive control for barrier disruption.

Then, a biotin tracer was added at the bottom of the epidermis models for 30 minutes to allow its inside-out diffusion. Presence of biotin tracer in the different layers was revealed by incubating sections of the reconstructed human epidermis with fluorescently-labelled streptavidin during 1 hour and by quantifying microscopy images.

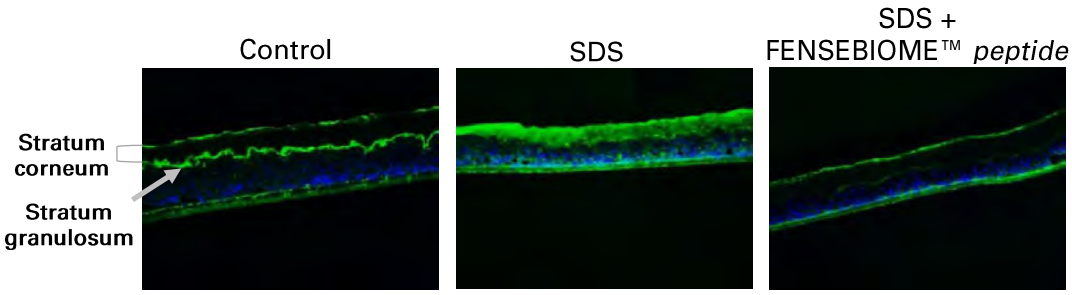


Fig. 16. Inside-out diffusion through reconstructed human epidermis models. Presence of tracer is observed in green (cell nuclei are stained in blue). Due to nonspecific binding of fluorescent streptavidin to lipids, top surface of stratum corneum was also stained in green.

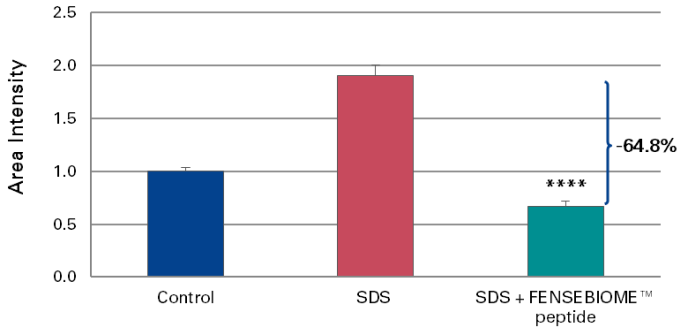


Fig. 17. Quantification of dye diffusion into the stratum granulosum and stratum corneum under each treatment (****p<0.0001 vs SDS).

Biotin **penetration** through the stratum granulosum and **into the stratum corneum** was **inhibited** by 64.8% compared to the positive control, suggesting a reinforced barrier that **can resist damage** induced by SDS.

Protection and reinforcement of the permeability barrier to water loss.

2) Stratum corneum integrity

The intercellular lipids play a key role in the integrity of the stratum corneum, which can be evaluated by measuring the outside-in diffusion of the toluidine blue dye from the epidermal surface. The ability of the peptide to enhance the barrier functionality was measured.

Reconstructed human epidermis models were treated with 0.02 mg/mL SDS, in order to induce a disruption of the stratum corneum, in the presence or absence of 50 µg/mL FENSEBIOME™ *peptide*. Models treated with medium were used as a control.

After 24 hours, the toluidine blue dye was added on top of the epidermis models for 10 minutes and its diffusion across the epidermis was evaluated by microscopy imaging.

Blue staining in each condition was visually scored according to the following scale: slight (1), moderate (2), quite strong (3), strong (4).

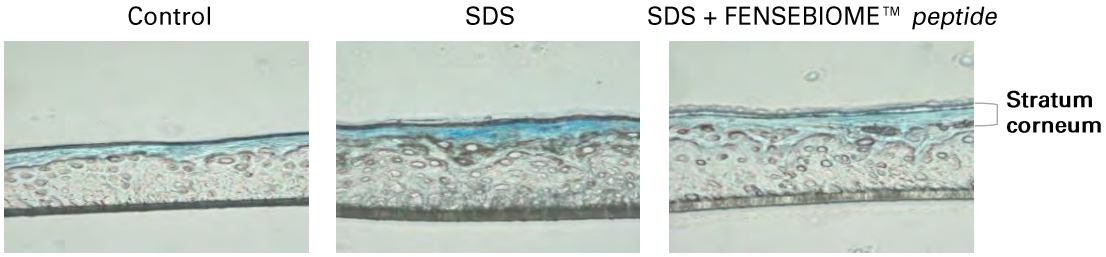


Fig. 18. Outside-in diffusion of the toluidine blue dye into the stratum corneum under different conditions.

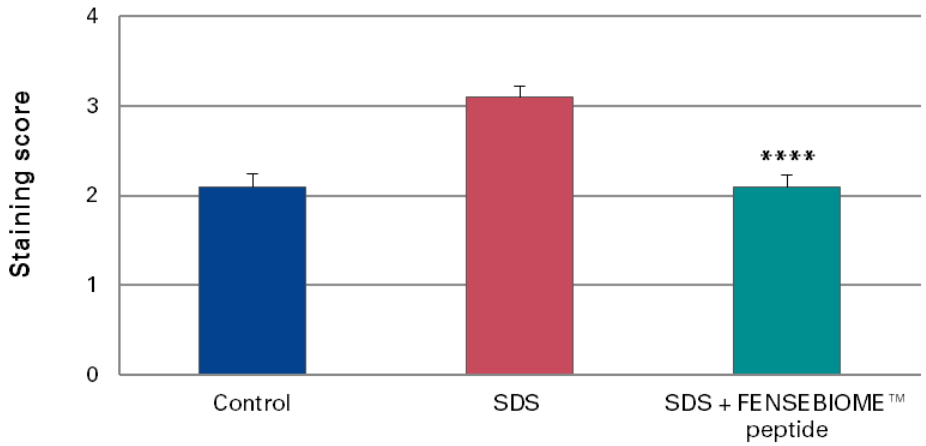


Fig. 19. Level of blue staining into the stratum corneum after the different treatments (****p<0.0001 vs SDS).

A **decreased penetration** of tracer dye through the stratum corneum was observed despite the presence of SDS.

Strengthening of the stratum corneum barrier that prevents alterations by chemical aggressors.

INGREDIENT PROPERTIES

FENSEBIOME™ *peptide*:

- heptapeptide that **reinforces** the skin's **microbial and physical barrier function**.
- applied *in vivo* as a 1% solution on subjects in an urban environment, it promoted a healthy microbiome similar to that of our ancestors in closer contact with nature. The ingredient favored the increase in **microbial diversity**, the **balance of the skin microbiota** and the **enhancement in beneficial bacteria on the skin.** It also helped **modulate metabolic pathways** that contribute to enrich the skin.
- decreased sloughed corneocytes by 14.7%, showing a **reinforced skin cell cohesion.**
- **reduced TEWL levels by 27.8%** after surfactant-induced damage, suggesting a **skin barrier protection** activity.
- *In vitro,* **favored the adhesion of beneficial bacteria** (*S. epidermidis*) over that of pathogenic bacteria (*S. aureus*) on keratinocytes.
- **induced genes** related to the **activation of the immune response** against pathogens in keratinocytes.
- **increased** the expression of **genes associated with tight junctions,** with a role in epidermal barrier integrity.
- modulated the profile of ceramides in reconstructed human epidermis models**, favoring an increase in the long-chain ceramides,** essential for a well-preserved barrier function.
- improved functionality of the epidermal barrier by **increasing its resistance to aggression by surfactants.**

COSMETIC APPLICATIONS

FENSEBIOME™ *peptide* can be incorporated into any formulation intended to strengthen the double barrier function of the skin and prevent dehydration. Moreover, it can be added into prebiotic and probiotic-inspired skin care products looking for a balance in the skin microbiota or an enhancement in beneficial bacteria that lead to a healthy skin. The ingredient is a good candidate to be introduced into formulations for skin exposed to urban conditions and sensitive skin types.

TECHNICAL DATA

INCI name of the active ingredient

Active ingredient	INCI name
FENSEBIOME™ *peptide*	Acetyl Heptapeptide-4

Presentation and preservative

Translucent solution containing 0.05% Acetyl Heptapeptide-4.

Code	Product presentation	Preservative
PD290	FENSEBIOME™ *peptide solution*	-

APPLICATION DATA

Processing

FENSEBIOME™ *peptide* can be formulated in the aqueous phase of formulations, in the final step of the manufacturing process. In case of preparing an emulsion, it is recommended to add it once the emulsion is formed and at a temperature below 40 ºC.

Recommended pH range between 4.0 and 8.0 for FENSEBIOME™ *peptide*.

Incompatibilities

Oxidants and electrophiles.

Solubility

Soluble in water. Insoluble in oils and silicones.

Dosage

A dosage of 1% of FENSEBIOME™ *peptide solution* is recommended in final cosmetic formulations.

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