1. Introduction

Skin dryness is a multifactorial disorder characterized by impaired epidermal barrier function, increased trans-epidermal water loss (TEWL), and persistent pruritus, often progressing to chronic inflammation or severe forms such as atopic dermatitis [1,2]. As a significant public health and cosmetic concern, skin dryness is exacerbated by both intrinsic aging and extrinsic factors collectively termed the exposome, which includes ultraviolet (UV) radiation, air pollution, low humidity, psychological stress, and hygiene practices [3,4]. These external stressors destabilize the stratum corneum (SC), leading to abnormal keratinization, dehydration, and immune dysregulation [5]. Filaggrin plays a critical role in maintaining epidermal structure by promoting the aggregation of intermediate filaments and the formation of the corneocyte matrix [6,7]. Synthesized initially as profilaggrin in the granular layer, filaggrin undergoes dephosphorylation and proteolytic cleavage to generate functional filaggrin monomers, which are subsequently degraded into natural moisturizing factors (NMFs) such as pyrrolidone carboxylic acid (PCA) and trans-urocanic acid (UCA). These NMFs are essential for water retention and maintenance of the skin's acidic pH. Genetic mutations in filaggrin or cytokine-induced downregulation compromise barrier function, leading to xerosis, increased TEWL, and weakened antimicrobial defense. Given the critical role of filaggrin in skin barrier integrity, recent studies have explored filaggrin-derived peptides as potential therapeutic and functional cosmetic agents [8]. These peptides, designed to mimic endogenous filaggrin sequences, aim to enhance epidermal hydration and promote biochemical and physical barrier reconstruction. In this study, we developed a human filaggrin-derived peptide (sh-polypeptide-170) as a novel bioactive strategy to mitigate skin dryness and restore barrier function. We designed a series of experiments to elucidate the mechanism of sh-polypeptide-170 action, focusing on their involvement in processing, epidermal differentiation, and cytoskeletal reorganization. To model pathological conditions of dryness, an IL-17A-induced cytokine model and an SDS-compromised reconstructed skin model were employed [9, 10]. Particular emphasis was placed on assessing whether sh-polypeptide-170 could facilitate the sequential conversion of profilaggrin into functional filaggrin and NMFs, interact with keratin filaments during terminal differentiation, and enhance tight junction protein expression and antimicrobial barrier functions [11]. Through these investigations, we aimed to determine whether sh-polypeptide-170 not only replenishes NMFs components but also actively participates in structural and immunological reorganization of the epidermis, offering a mechanistically grounded approach for treating filaggrin-deficient dry skin under exposomal stress.

2. Materials and Methods

2.1. Preparation of sh-polypeptide-170

Bioactive peptides derived from human filaggrin were generated by segmenting the full-length filaggrin gene into ten domains (S1–S10) based on genome-based 3D structural predictions. Codon-optimized DNA sequences corresponding to each domain were synthesized for recombinant expression. The genes were cloned into the pET-28a(+) vector using BamHI and XhoI restriction sites and transformed into *E. coli* BL21 (DE3) cells. Expression was induced with 0.5 mM IPTG at 25 °C for 16 hr. Recombinant peptides were purified from the supernatants using Ni-NTA affinity chromatography (GE Healthcare, USA). Purified peptides were eluted

and confirmed as single bands by Western blot analysis using a 6xHis-tag antibody (Invitrogen, USA).

2.2. Cell culture and treatments

Normal human epidermal keratinocytes (NHEKs) were purchased from Cascade Biologics, Inc. The cells were cultured in Epilife medium (Cascade Biologics, USA) supplemented with human keratinocyte growth supplement. Once the culture reached 80% confluence, the cells were treated with sh-polypeptide-170 and incubated at 37 °C in 5% CO_2.

2.3. Real-time PCR (RT-PCR)

Total RNA was extracted from the cells using QIAzol™ lysis reagent (QIAGEN, Netherlands). The RNA extract was used as a template for subsequent cDNA synthesis using the Applied Biosystem™ High-capacity RNA-to-cDNA kit (Applied Biosystems, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was performed using the 7500 Fast Real-time PCR system (Applied Biosystems, USA) with SYBR-Green PCR Master mix (Applied Biosystems, USA). The following primers were used for amplification : Profilaggrin, Caspase-14, Filaggrin, TGase-1, NHE-1, TARC, HBD-2.

2.4. Immunoblotting analysis

NHEKs were incubated with lysis buffer. An equal amount of protein (30 μg) was dissolved in Bolt NuPAGE LDS Sample buffer (Invitrogen, USA). The lysates were boiled at 70 °C for 10 min and loaded and run on 4-12% Bolt NuPAGE Bis-Tris gels (Invitrogen, USA). The proteins were transferred onto polyvinylidone fluoride membranes (Invitrogen, USA) and blocked in 5% skim milk. The membranes were probed with anti-Caspase-14 (Santa Cruz, USA), β-actin (Santa Cruz, USA) antibodies overnight at 4 °C, followed by incubation with a secondary antibody, anti-mouse horseradish peroxidase-conjugated IgG antibody. The protein bands were detected using ChemiDoc (ATTO, Japan). Densitometric analysis of the protein bands was performed using LuminoGraph II image analysis software (CS Analyzer, ATTO).

2.5. Lucifer yellow assay

A Lucifer yellow (LY, Sigma-Aldrich) solution of 1 mM in ddH_2O was applied topically to the skin models. After 2 hr of incubation in the CO_2 incubator, superficial LY was removed by washing with PBS. Pre-treatment with 0.25% SDS 24 hr prior to LY staining served as positive control. Formalin-fixed paraffin sections were placed onto slides and deparaffined. For combined nuclear staining and mounting, the DAPI histology mounding medium was used. Data were derived from n=2 experiments. For quantification, three pictures per samples were taken and measured via Image J.

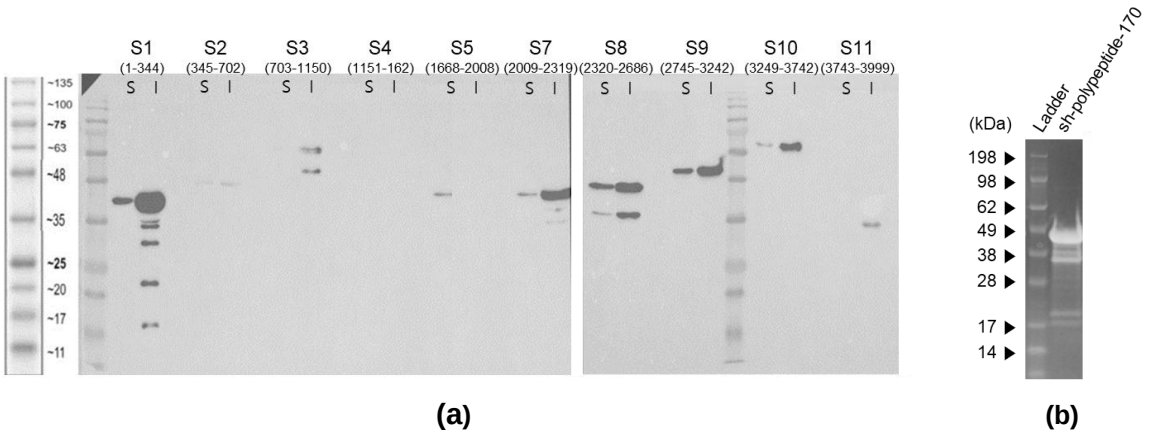
2.6. Immunocytochemistry

NHEKs were incubated with fresh serum-free medium with or without samples at 37 °C in 5% CO_2. NHEKs in culture were fixed 4% paraformaldehyde and perforated with 0.1% Triton in phosphate buffered saline (PBS) for 10 min. Cells were blocked for 1 hr with 1% bovine serum albumin in PBS. Cells proved for the expression of cytokeratin 10 (Invitrogen, USA), Filaggrin (Invitrogen, USA) with specific antibodies directed against anti-cytokeratin10, anti-Filaggrin overnight at 4 °C, followed by a 1 hr incubation with a fluorescent Alexa 488® anti-mouse and anti-rabbit secondary antibody (Invitrogen, USA). Cells were also stained for the nucleus with DAPI (4’6-diamidino-2-phenylindole) and preserved by the addition of VectaShield Mounting Medium (Vector Laboratories, USA). The protein intensity were detected using Confocal Laser Scanning Microscope (Leica, Germany).

3. Results

3.1. Design and selection of sh-polypeptide-170 for skin dryness

Filaggrin monomers play a critical role in epidermal barrier formation by aggregating keratin intermediate filaments during terminal differentiation, thereby contributing to the mechanical integrity of the SC [12]. To mimic the structural and functional properties of native filaggrin monomers, the full-length filaggrin protein was segmented into ten discrete peptide regions (S1–S10) based on genome-derived 3D domain predictions. Each segment was selected to reflect distinct structural motifs potentially involved in keratin interaction or barrier modulation. The peptides were expressed in *E. coli* BL21 (DE3) cells, and their molecular weights were analyzed by Western blot using a His-tag antibody (Figure 1a). Among the ten designed constructs, five peptides (S1, S7, S8, S9, and S10) were successfully expressed. The S1 domain, containing a known S100-like domain, was excluded from further analysis due to its extensive prior characterization. Notably, the S8 domain (amino acid residues 2320–2686) exhibited the highest number of functional motifs, including one linker domain (SFLYQVST) and three caspase-14 cleavage sites (HSED, FSQD). These features suggested strong potential for application in mitigating skin dryness [13]. Based on its production yield and functional relevance, the S8 domain was selected as the sh-polypeptide-170 for further investigation (Figure 1b).



(a) **(b)**

Figure 1. Western blot of sh-polypeptide-170 recognized by an 6xHis-tag mAb.
(a) Expression profiles of filaggrin peptide candidates corresponding to each domain were analyzed. Following lysis, samples were fractionated into soluble (S) and insoluble (I) parts. (b) The sh-polypeptide-170 (S8) was overexpressed, and its molecular weight was confirmed to be approximately 45 kDa.

3.2. Activation of NMFs processing pathways by sh-polypeptide-170

In human skin, profilaggrin undergoes proteolytic processing to generate functional filaggrin repeat units, which play a critical role in epidermal barrier formation by binding to the keratin cytoskeleton and facilitating transglutaminase 1 (TGase-1) mediated crosslinking [14]. Degradation of monomeric filaggrin produces free amino acids, contributing to NMFs essential for epidermal hydration and maintenance of the acidic pH of the SC. Elevated pH of SC has been reported in filaggrin-deficient atopic dermatitis patients, with sodium/hydrogen exchanger 1 (NHE-1) playing a key role in regulating acidification [15]. To investigate the effects of sh-polypeptide-170 on filaggrin processing, we treated human keratinocytes with sh-polypeptide-170 and analyzed mRNA expression of profilaggrin, filaggrin, caspase-14, TGase-1, and NHE-1 over a 3-day period (Figure 2). Based on the established role of caspase-14 in filaggrin deg-

radation and NMFs production, we hypothesized that sh-polypeptide-170 treatment would induce early upregulation of caspase-14 and TGase-1, followed by downstream modulation of skin barrier–associated genes. Following sh-polypeptide-170 treatment, caspase-14 and TGase-1 expression increased markedly from early phase, preceding changes in other genes. During the early phase (Days 1–2), profilaggrin and filaggrin expression slightly decreased, consistent with caspase-14–mediated proteolytic processing. By Day 3, expression of both profilaggrin and filaggrin recovered and exceeded initial levels, suggesting a compensatory feedback response. In parallel, NHE-1 expression progressively increased and remained elevated by approximately 24% even after 5 days of treatment, indicating sustained promotion of epidermal acidification. Mechanistically, sh-polypeptide-170 was internalized into keratinocytes, where its linker domains were proteolytically cleaved. sh-polypeptide-170-induced filaggrin monomers functionally interacted with TGase-1, promoting keratin–intermediate filament (KIF) crosslink formation and leading to degradation into amino acids, both processes contributing to barrier stabilization. These findings suggest that sh-polypeptide-170 reinforces the epidermal barrier through a multi-step mechanism involving enhanced NMFs production, cytoskeletal reorganization, and maintenance of SC acidity.

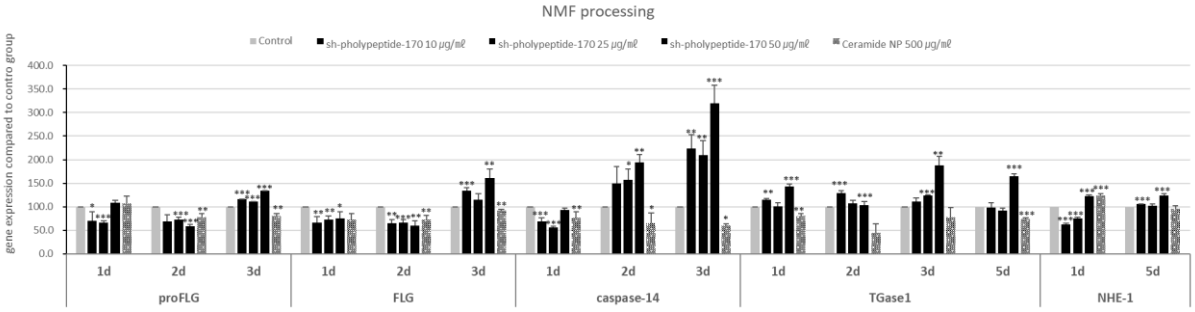


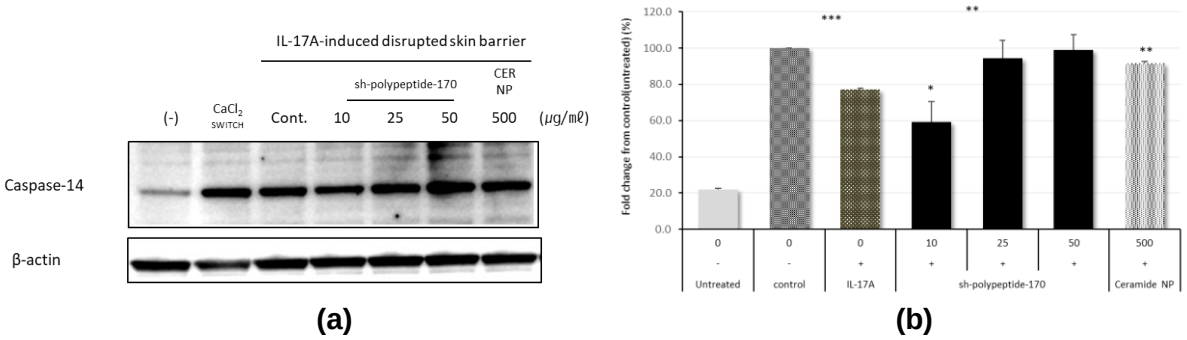
Figure 2. The mRNA expressions of profilaggrin, filaggrin, caspase-14, TGase-1, NHE-1 were increased by sh-polypeptide-170 treatment of cultured keratinocyte *in vitro*.
After 1, 2, 3 days or 5 days of treatment with sh-polypeptide-170, the gene expressions were determined by real-time PCR. Ceramide NP was used as a positive control for differentiation and barrier formation. The data are expressed as the mean ± STDEV from three independent experiments performed in triplicate (n=3). (*p <0.05, ** p <0.01, *** p <0.001 vs. the respective control group).

3.3. Strengthen the skin barrier in disrupted skin models

To investigate the therapeutic potential of sh-polypeptide-170 under compromised skin conditions (dry skin and atopic dermatitis–like models), we designed a series of experiments using various skin model systems. We analyzed modulation of caspase-14 expression in a dry skin model established by IL-17A–induced downregulation (Figure 3). Treatment with sh-polypeptide-170, which contains multiple caspase-14 cleavage sites, was found to promote the expression of caspase-14. Caspase-14 expression, which was reduced to approximately 75% of the normal skin level by IL-17A treatment, was restored to normal levels following sh-polypeptide-170 treatment at concentrations of 25 μg/mL and 50 μg/mL.

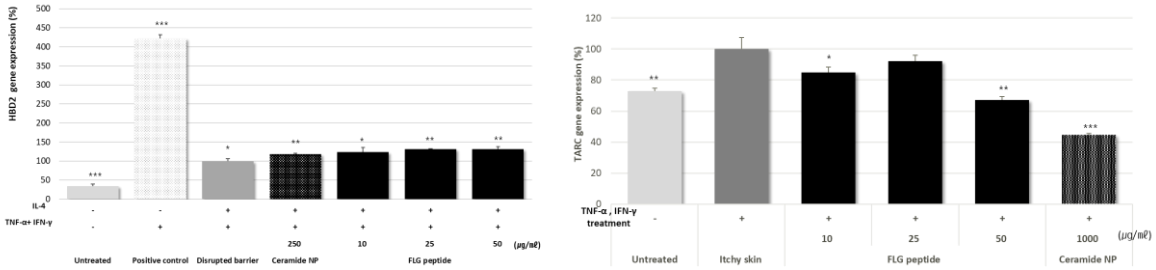
Skin dryness is known to induce both a weakened antimicrobial barrier and itch-related inflammation. We designed a microbial-sensitive model and an itch-associated inflammatory model. In skin with abnormally reduced antimicrobial peptide secretion, sh-polypeptide-170 treatment led to an increase of up to 31.7% in HBD-2 expression (Figure 4a). Building on previous findings, three days after sh-polypeptide-170 treatment, the increase in NMFs pro-

duction driven by caspase-14 activation promoted epidermal acidification, which in turn contributed to the strengthening of the antimicrobial barrier. In the itch-associated inflammatory cytokine model, TARC expression, one of the chemokines frequently detected in patients with atopic dermatitis and individuals with sensitive skin, was reduced by 33.7% following sh-polypeptide-170 treatment (Figure 4b).



(a) (b)

Figure 3. sh-polypeptide-170 promoted caspase-14 expression in human keratinocyte. (a) Normal human keratinocytes were treated with sh-polypeptide-170, and the protein expression levels of caspase-14 were evaluated by western blotting. β-actin was used as a loading control. (b) The data are presented as the mean ± STDEV of three independent experiments performed in triplicate (n=3). Calcium chloride and ceramide NP (CER NP) were used as positive controls for keratinocyte differentiation. Statistical comparisons were performed using Student's t-test (**p < 0.01, ***p < 0.001 vs. the respective control group).

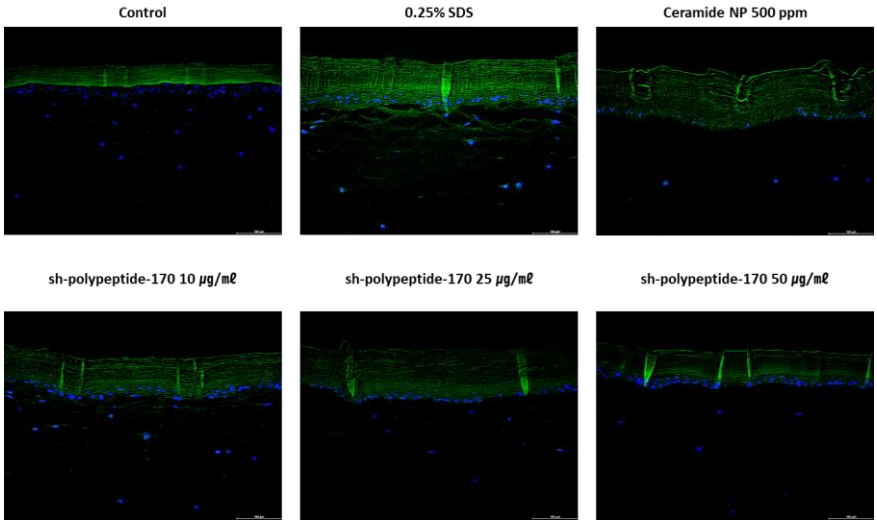


(a) (b)

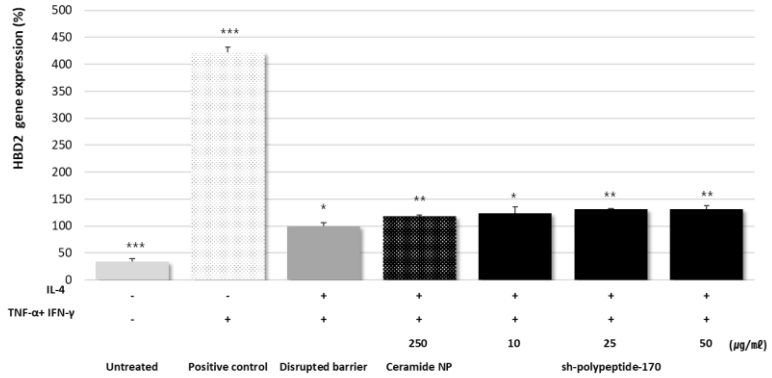
Figure 4. sh-polypeptide-170 suppressed HBD-2 and TARC expression in human keratinocytes. (a) The cells were stimulated with TNF-α/IFN-γ/IL-4 after sh-polypeptide-170 pre-treatment for 3 days. HBD-2 expression was evaluated by quatitative real-time PCR. Data are expressed as the mean fold-changes ± STDEV of independent experiments (N=3). Statistical comparisons were followed by Student's t-test. (b) The cells were stimulated with TNF-α/IFN-γ and treated with 10, 25, and 50 μg/mL of sh-polypeptide-170 or untreated control for 25 hr. The mRNA expression of TARC was measured by quatitative real-time PCR. The mRNA levels, relative to those in the TNF-α/IFN-γ-stimulated control groups, are presented as mean fold-changes ± STDEV of independent experiments (N=3). Statistical comparisons were followed by Student's t-test.

To evaluate skin barrier recovery, a reconstructed skin model with SDS-induced barrier disruption was treated with Lucifer Yellow, a hydrophilic fluorescent tracer (Figure 5). Following

sh-polypeptide-170 treatment, dye permeability decreased by up to 68.8%, indicating enhanced barrier resistance. These results suggest that sh-polypeptide-170 improves SC integrity, thereby strengthening skin barrier function at the tissue level.



(a)



(b)

Figure 5. Impaired cutaneous barrier function of SDS-disrupted skin models.
(a) Penetration of the Lucifer Yellow (LY) fluorescent dye (green) was used to visualize the permeability of skin equivalent stimulated with SDS compared to an unstimulated physiological negative control. Pretreatment with 0.25% SDS served as permeability control. DAPI (blue) was used for nuclear staining. (b) Quantification of LY fluorescence area (mean ± STDEV) of n=2 experiments using Image J software.

3.4. Visualization of Filaggrin-Keratin Aggregates by Immunocytochemistry
Keratin intermediate filaments (KIFs) protect the epidermis against mechanical force, support strong adhesion, help barrier formation. Monomeric filaggrin promotes aggregation and collapse of keratin intermediate filament resulting in the formation of barrier components by TGase-1 and strongly binds to bundle of the keratin cytoskeleton [16]. The keratin bound filaggrin is further degraded into amino acids. Human normal keratinocytes were treated with sh-polypeptide-170, and colocalization between filaggrin and keratin 10 was assessed (Figure 6a,

b). In untreated cells, only minimal colocalization of KRT10 with filaggrin was observed. In contrast, sh-polypeptide-170 treatment markedly enhanced the colocalization of KRT10 and filaggrin, indicating improved cytoskeletal organization. Line profile analyses clearly indicated colocalized peaks at the same pixel positions between filaggrin and KRT10 in sh-polypeptide-170 treatment group, supporting aggregation at these sites. Differences in absolute fluorescence intensity may reflect variations in protein expression or antibody labeling efficiency. These results highlighted an increased interaction between filaggrin and KRT10 upon sh-polypeptide-170 treatment.

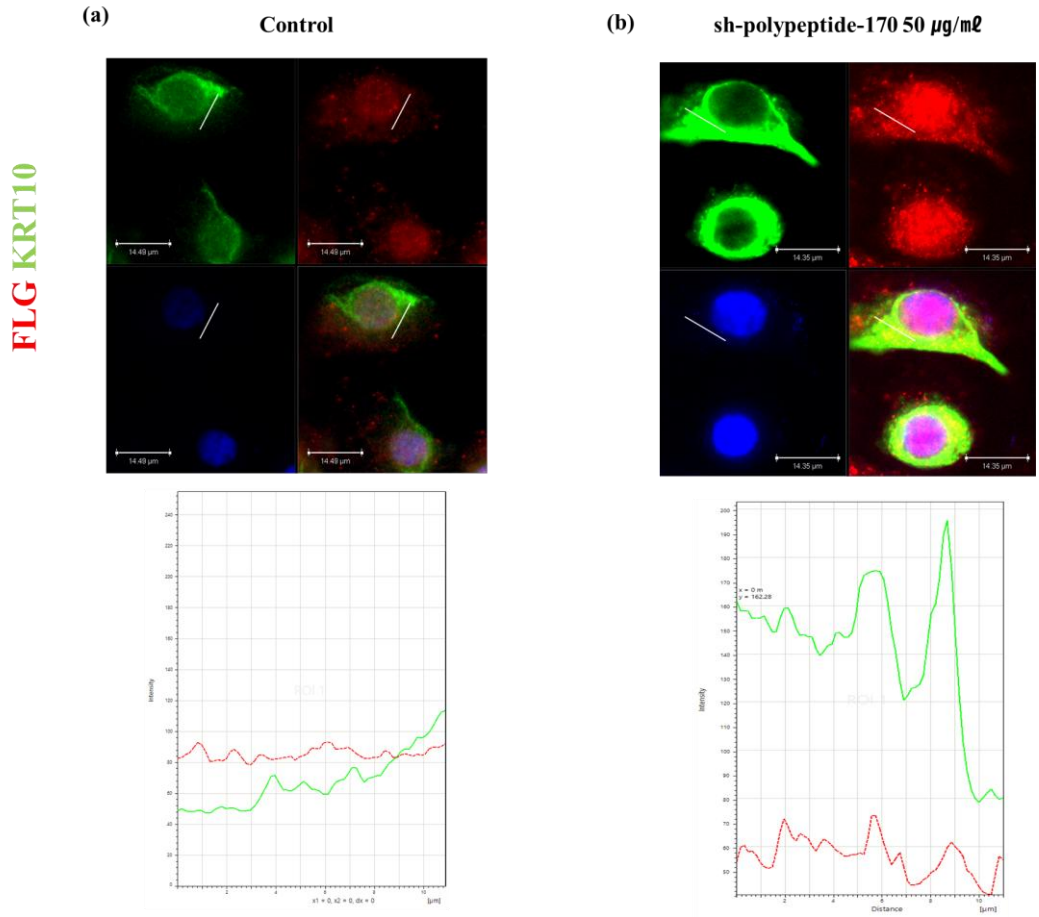


Figure 6. sh-polypeptide-170-dependent between KRT10 interaction with filaggrin and filaggrin processing.
Confocal microscopy of fixed human normal keratinocytes treated with sh-polypeptide-170 (a) or untreated (b). Cells were stained for nuclei (blue, DAPI), filaggrin (red, Alexa Fluor 594), and KRT10 (green, Alexa Fluor 488). The graphs in each figure show pixel intensity for red and green channels, respectively. Line profile analysis demonstrated simultaneous peaks in fluorescence intensity at identical pixel positions (X-axis) for both channels, indicating aggregation despite differences in absolute intensity values (Y-axis).

4. Discussion

The present study demonstrates the therapeutic potential of sh-polypeptide-170 in restoring the skin barrier under conditions of dryness and inflammatory stress. sh-polypeptide-170, mimic of key functional regions of native filaggrin, promoted filaggrin processing, reinforced structural barrier components, and contributed to antimicrobial defense. Mechanistically, sh-polypeptide-170 facilitated caspase-14 mediated degradation of filaggrin, leading to enhanced

production of NMFs. This contributed to epidermal acidification, a key determinant of antimicrobial peptide function and skin homeostasis. The upregulation of NHE-1 and TGase-1 further supports the involvement of sh-polypeptide-170 in acidification regulation and barrier formation. In reconstructed skin models with SDS-induced barrier disruption, sh-polypeptide-170 significantly reduced Lucifer Yellow penetration, indicating enhanced SC integrity. Furthermore, due to early activation of caspase-14, the expression levels of profilaggrin and filaggrin initially declined. However, by Day 3, a feedback or compensatory response in keratinocytes led to a recovery and subsequent increase in both profilaggrin and filaggrin expression, exceeding baseline levels. In cytokine-induced models that mimic atopic and inflammatory skin conditions, sh-polypeptide-170 reduced TARC expression while enhancing HBD-2 levels, suggesting both anti-inflammatory and antimicrobial effects. The sh-polypeptide-170 does not just modulate filaggrin at the tissue level, but also alleviates symptoms of skin dryness at the cellular scale, demonstrating a synergistic effect on both structural restoration and immune regulation. This dual action highlights the peptide's synergistic role in both structural barrier reinforcement and immune modulation. Finally, through cytoskeletal fluorescence imaging, we observed the co-localization of filaggrin and keratin, confirming their coordinated distribution within differentiated keratinocytes. The localization of filaggrin and KRT10 confirmed the functional role of sh-polypeptide-170 in cytoskeletal reorganization, likely contributing to skin barrier network stability. Collectively, these findings support the notion that sh-polypeptide-170 can serve as a multifunctional agent to improve skin health by targeting both structural and immune-related components of the epidermal barrier.

5. Conclusion

This study identified and characterized a human sh-polypeptide-170 with therapeutic potential for dry and barrier-compromised skin. sh-polypeptide-170 enhanced skin barrier function by promoting filaggrin processing, NMFs production, and epidermal acidification. It also modulated the expression of key genes related to inflammation and antimicrobial defense. Through in vitro and reconstructed skin models, sh-polypeptide-170 demonstrated significant efficacy in restoring barrier integrity and reducing inflammatory chemokines. These results suggest that sh-polypeptide-170 may be a promising candidate for the development of novel cosmetic and dermatological products targeting dry, sensitive, or atopic skin.

References

[1] Loden M, Maibach HI. Dry skin and moisturizers: chemistry and function. *CRC Press* 2000.
[2] Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. *Exp Dermatol* 2008;17(12):1063–1072.
[3] Krutmann J, et al. The skin aging exposome. *J Dermatol Sci* 2017;85(3):152–161.
[4] Denda M, et al. Environmental humidity influences epidermal function. *J Invest Dermatol* 1998;111(5):858–863.
[5] Elias PM. Stratum corneum defensive functions: an integrated view. *J Invest Dermatol* 2005;125(2):183–200.
[6] Sandilands A, et al. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009;122(9):1285–1294.
[7] Candi E, et al. Epidermal differentiation: the role of keratinocyte cell death in skin homeostasis and regeneration. *Cell Death Differ* 2005;12(12):1494–1505.
[8] Egawa M, Tagami H. Functional analysis of filaggrin and its peptides as natural moisturizing factors in the stratum corneum. *J Dermatol Sci* 2008;51(2):113–117.
[9] Kim BE, Leung DYM. IL-17A downregulates filaggrin and affects keratinocyte expression of genes associated with cellular adhesion. J Invest Dermatol 2011;131(4):847–850.
[10] Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Otsuka A, Honda T, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. J Invest Dermatol 2015;135(3):757–764.
[11] De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in atopic dermatitis. J Allergy Clin Immunol 2011;127(3):773–786.e1–7.
[12] Candi E, Schmidt R, Melino G. Epidermal differentiation: the role of keratinocyte cell death in skin homeostasis and regeneration. *Cell Death Differ* 2005;12(12):1494–1504.
[13] Lippens S, et al. Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing. *Cell Death Differ* 2000;7(12):1218–1224.
[14] Denecker G, et al. Caspase-14: a novel protease involved in terminal differentiation of epidermis. *J Invest Dermatol* 2000;115(5):603–609.
[15] Kawasaki H, et al. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. *J Invest Dermatol* 2017;137(4):902–909.
[16] Steinert PM, Dale BA. The protein composition of cornified cell envelopes of epidermis and cultured epithelial cells. J Cell Biol 1981;90(3):541–546.