

Analysis of the efficacy of the main ingredient complex of “S Clinic Biological Solutions Stem Cell Extract Ampoule”

* Hereinafter referred to as “S.clinic main ingredient complex”.

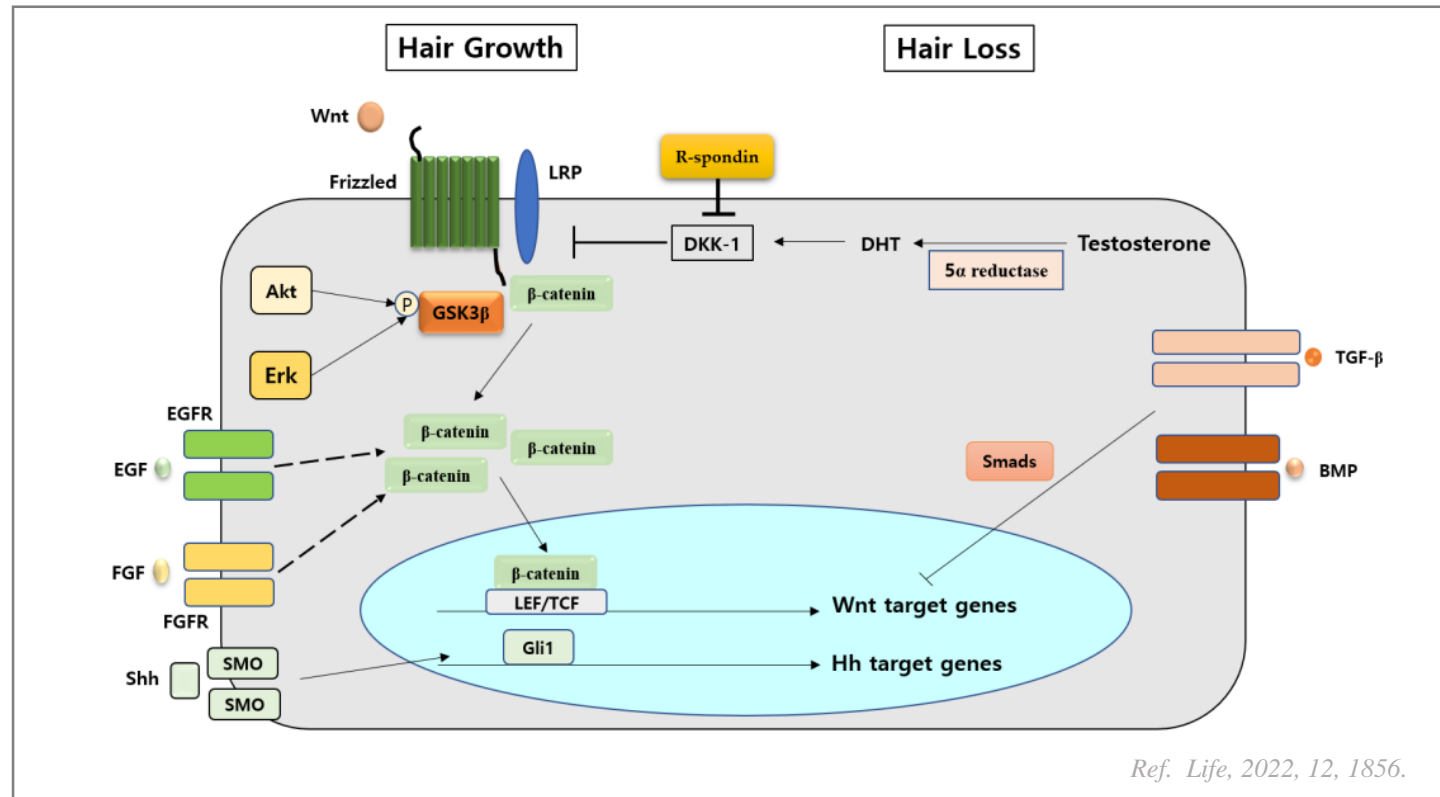


Medicinal Nanomaterial Institute

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1-1. Introduction

Alopecia, commonly known as hair loss, is a dermatological condition that can cause emotional distress and impact quality of life. Various factors interact with the Wnt/ β -catenin signaling pathway in relation to both hair growth and hair loss. The Wnt/ β -catenin pathway plays a key role in influencing hair follicles, the hair cycle, and the regeneration of hair cells. By regulating cell signaling pathways, Dickkopf-1 (DKK-1), which blocks Wnt signaling, can affect the hair growth cycles. Bone morphogenetic protein-4 (BMP-4) plays a role in regulating cell differentiation as well as the regeneration and cycling of hair follicles. The androgen receptor (AR) plays a crucial role in controlling hair growth and follicles, especially in reaction to hormones. Each factor contributes to the complex regulation of hair follicle function and growth. This study assessed the impacts of “S’Clinic main ingredient complex” on hair follicle dermal papilla cell (HFDPC) proliferation and mRNA expression levels of DKK-1, BMP-4, and AR.



1-2 Preparation

- HFDPC (Promo Cell, lot. 485Z014.1) maintained in DMEM medium (Dulbecco's Modified Eagle's Medium) containing 10% FBS (fetal bovine serum), penicillin (100 IU/ml), and streptomycin (100 µg/ml) at 37°C in a humidified, 5% CO₂ atmosphere.
- "S'Clinic main ingredient complex" was prepared in FBS-free DMEM medium immediately before use.
- The stock solution of 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Invitrogen, cat. M6494) was prepared with 5 mg/mL in phosphate buffered saline (PBS) and filtered.

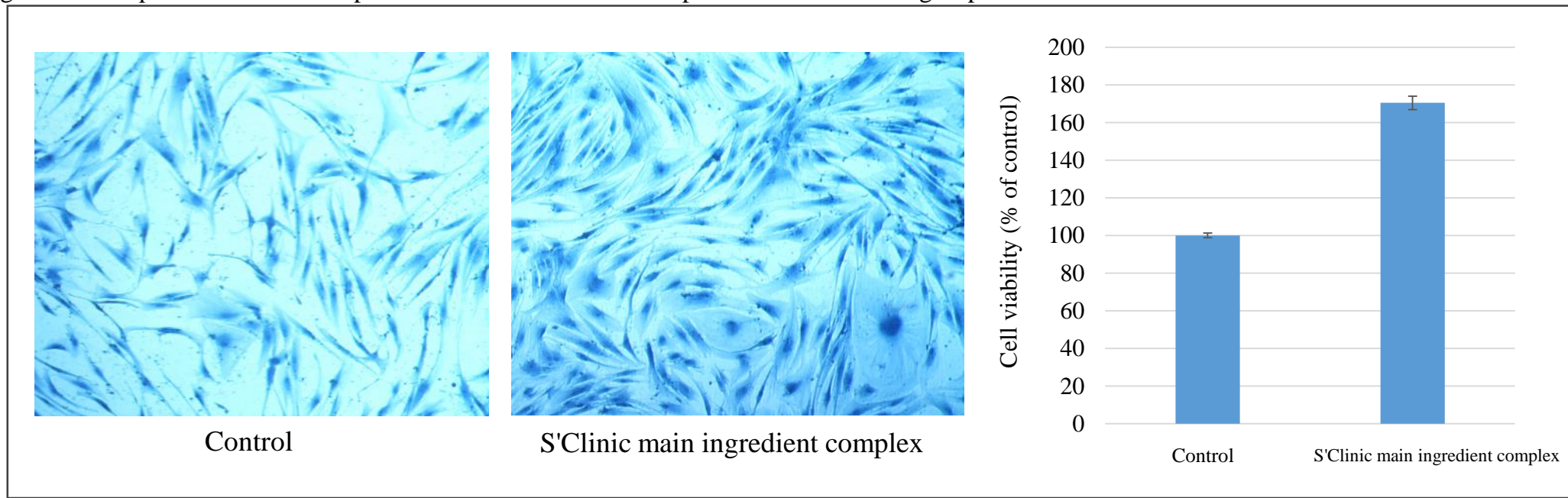
- Effect of “S’Clinic main ingredient complex” on HFDPC proliferation.

Human Follicle Dermal Papilla Cells (HFDPCs) are specialized mesenchymal cells located at the base of hair follicles. These cells play a pivotal role in regulating hair follicle growth, cycling, and regeneration. Enhancing the proliferation of HFDPCs in the scalp has profound implications for hair health and scalp homeostasis, as these cells are critical for maintaining a healthy hair growth cycle and mitigating hair loss.

Stimulating the proliferation of HFDPCs has significant implications for scalp health and hair growth. By enhancing the functionality of these cells, it is possible to create a more favorable environment for hair follicle regeneration and to address scalp disorders that lead to hair thinning or loss.

HFDPCs influence the behavior of hair matrix cells, the rapidly dividing cells at the base of the hair follicle that form the hair shaft. By promoting HFDPC proliferation, the downstream effects include improved matrix cell activity, resulting in healthier and more structurally sound hair strands.

The effects of an “S’Clinic main ingredient complex” on the growth of human follicle dermal papilla cells were evaluated. The treatment group of “S’Clinic main ingredient complex” showed a cell proliferation rate of 171% compared to the untreated group.

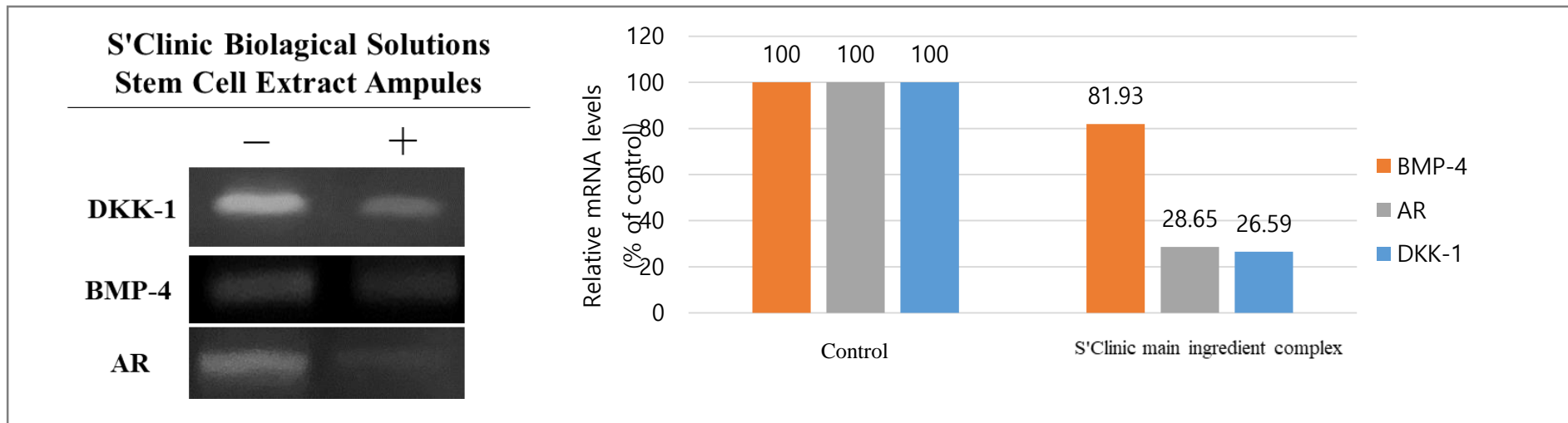


1-4-2. Results

- Effect of “S’Clinic main ingredient complex” on inhibiting hair loss and stimulating hair growth.

DKK-1, BMP-4, and AR are central to the regulation of hair follicle biology and scalp homeostasis, with significant implications for hair growth and loss. DKK-1 mediates Wnt inhibition, driving follicle miniaturization, while BMP-4 modulates stem cell activity and follicle cycling, and AR amplifies androgen sensitivity, contributing to hair loss in androgenetic alopecia. Targeting these pathways offers promising therapeutic avenues for managing hair loss and improving scalp health.

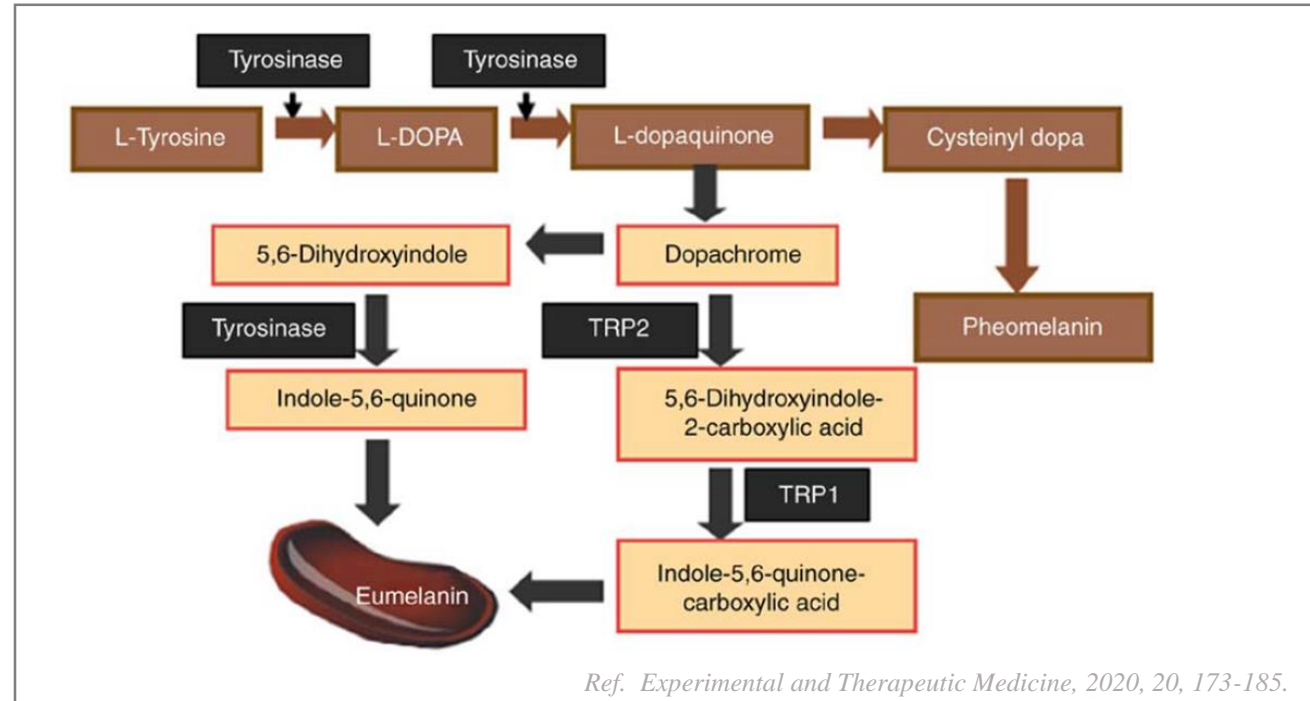
The effects of the “S’Clinic main ingredient complex” on the mRNA levels of factors that regulate the hair follicle were assessed. The treatment group showed a reduction in DKK-1, BMP-4, and AR mRNA levels compared to the control group. These findings indicate that the use of “S’Clinic main ingredient complex” may have the potential to inhibit hair loss and promote hair growth.



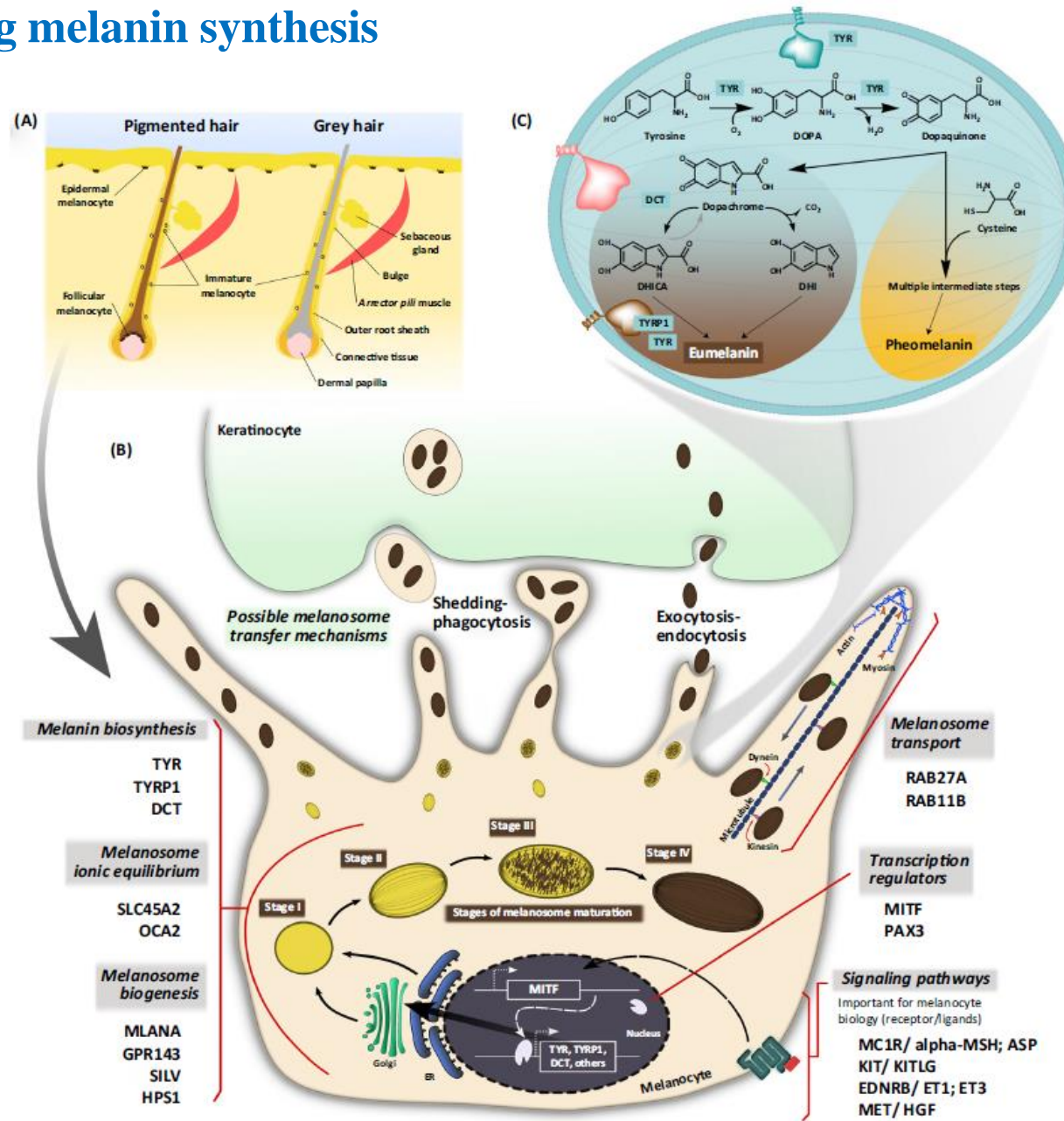
2-1. Introduction

Melanin is a natural pigment responsible for determining the coloration of hair, skin, and eyes. Melanin is generated by specialized cells known as melanocytes situated in the basal layer of the epidermis and within the hair follicles. In addition to its involvement in pigmentation, melanin serves a protective purpose by absorbing and dispersing ultraviolet rays from the sun, thereby shielding cells from harm. As the production of melanin decreases with age, the hair undergoes a transition from its original color to grey and ultimately white, resulting in a change in its visual presentation.

Melanogenesis is a multifaceted process influenced by transcription factors including tyrosinase, TRP1 (tyrosinase-related protein-1), and DCT (dopachrome tautomerase; TRP2; tyrosinase-related protein-2). As the expression level of these factors increases, melanin synthesis is stimulated. This study evaluated the impact of “S’Clinic main ingredient complex” on melanogenesis and the mRNA expression level of tyrosinase in melanocytes.



Stimulating melanin synthesis



Melanocyte Function in HF (hair follicle) Pigmentation.

(A) Schematic drawing of a pigmented and a grey HF, where some structures and cells are highlighted.

(B) Differentiated melanocyte showing its typical cellular processes and some of the most frequently associated genes.

(C) Biochemical synthesis of both types of melanin, which occurs inside specialized organelles called melanosomes.

2-2. Preparation

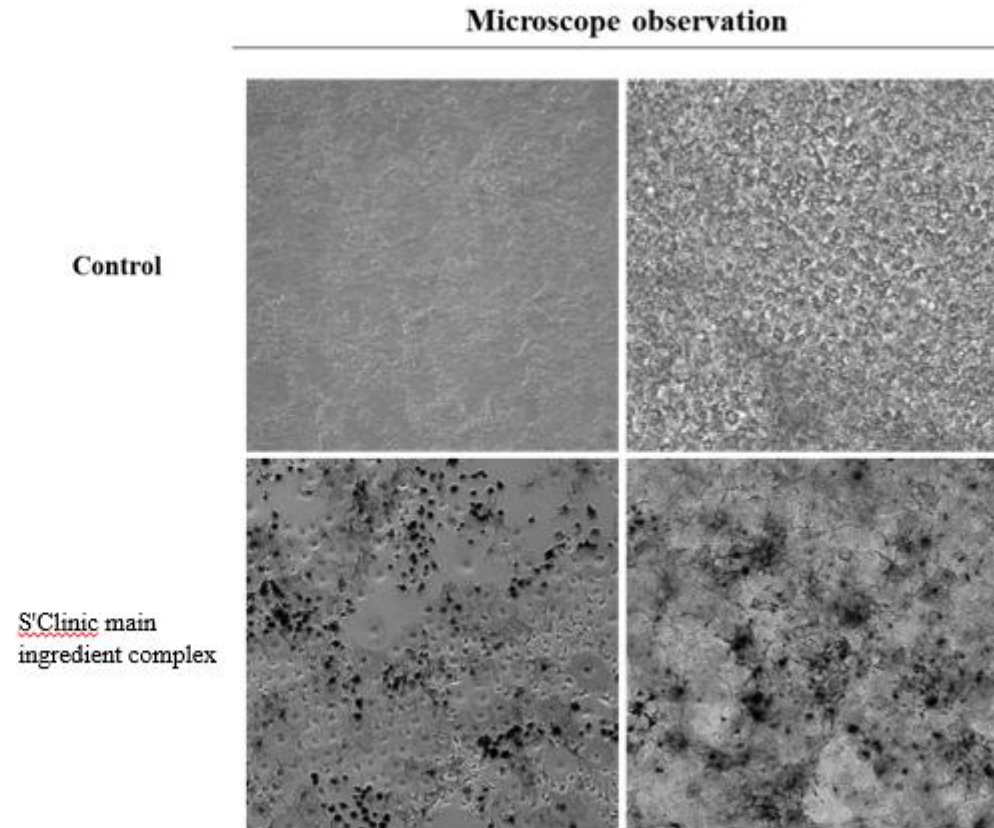
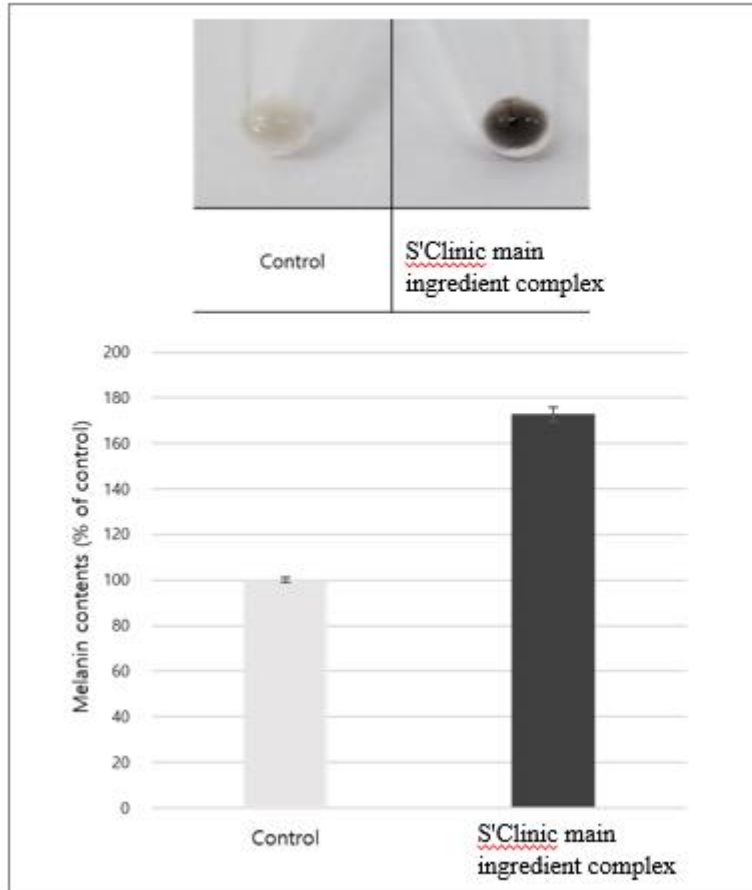
- Murine melanoma cell line, B16F10 cells (ATCC, no. CRL-6475) maintained in DMEM medium (Dulbecco's Modified Eagle's Medium) containing 10% FBS (fetal bovine serum), penicillin (100 IU/ml), and streptomycin (100 µg/ml) at 37°C in a humidified, 5% CO₂ atmosphere.
- “S'Clinic main ingredient complex” was prepared in DMEM medium immediately before use.

Stimulating melanin synthesis

2-3-1. Results

- Effect of “S’Clinic main ingredient complex” on melanin synthesis

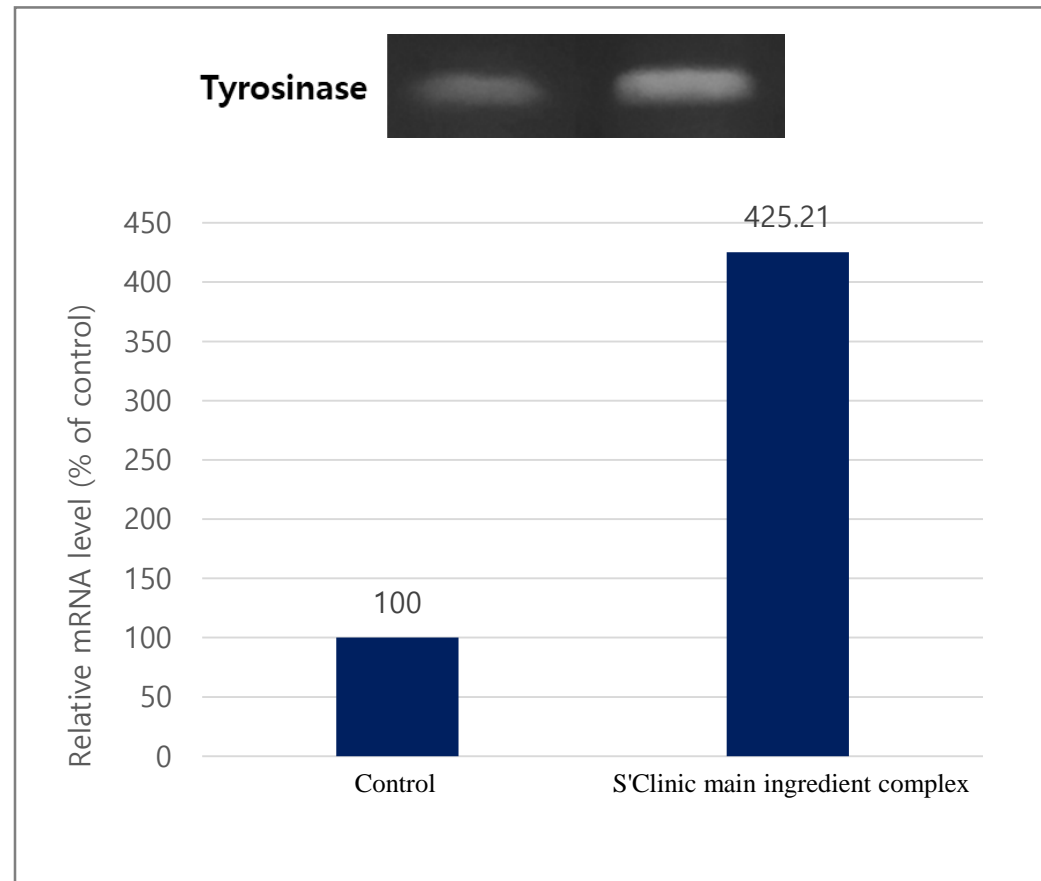
The results showed that “S’Clinic main ingredient complex” significantly increases melanin biosynthesis in B16F10 cells compared to the control without causing cytotoxicity.



2-3-2. Results

- Effect of “S’Clinic main ingredient complex” on the mRNA level of melanin synthesis factor

The result indicated a notable elevation in the mRNA expression level of tyrosinase following treatment with the “S’Clinic main ingredient complex”. Therefore, “S’Clinic main ingredient complex” has an effect as a pigmenting agent.



3-1. Introduction

Collagen, the most abundant structural protein in the human body, plays a critical role in maintaining the structural integrity and functionality of various tissues, including the scalp. In the scalp, collagen is predominantly found in the dermis, where it provides mechanical strength, elasticity, and support to hair follicles and surrounding structures. Collagen types I and III are the most common forms present in the scalp and are crucial for maintaining dermal health and hair growth.

Collagen surrounds the hair follicles and contributes to their structural stability. It supports the dermal papilla, a key structure that regulates hair follicle cycling and growth. The degradation of collagen in the scalp has been linked to hair thinning and reduced hair density, which are often observed in aging and scalp disorders. In the event of injury or inflammation in the scalp, collagen plays a key role in wound healing by serving as a substrate for fibroblast migration and tissue repair. Collagen synthesis is upregulated during the repair process to restore tissue integrity, making it vital for maintaining scalp health in the presence of conditions such as seborrheic dermatitis or psoriasis. Collagen contributes to the retention of moisture in the dermis by interacting with glycosaminoglycans (e.g., hyaluronic acid), which help maintain hydration. A well-hydrated scalp supports a robust barrier function, reducing trans-epidermal water loss (TEWL) and preventing microbial invasion. Age-related declines in collagen synthesis and quality lead to thinning of the scalp dermis, reduced elasticity, and weakened hair follicles. Studies has shown that promoting collagen synthesis or supplementation with collagen peptides can improve scalp dermal thickness and hair follicle vitality.

This study evaluated the effects of “S'Clinic main ingredient complex” on the proliferation of HFDPC, collagen biosynthesis in fibroblasts.

Reference Studies

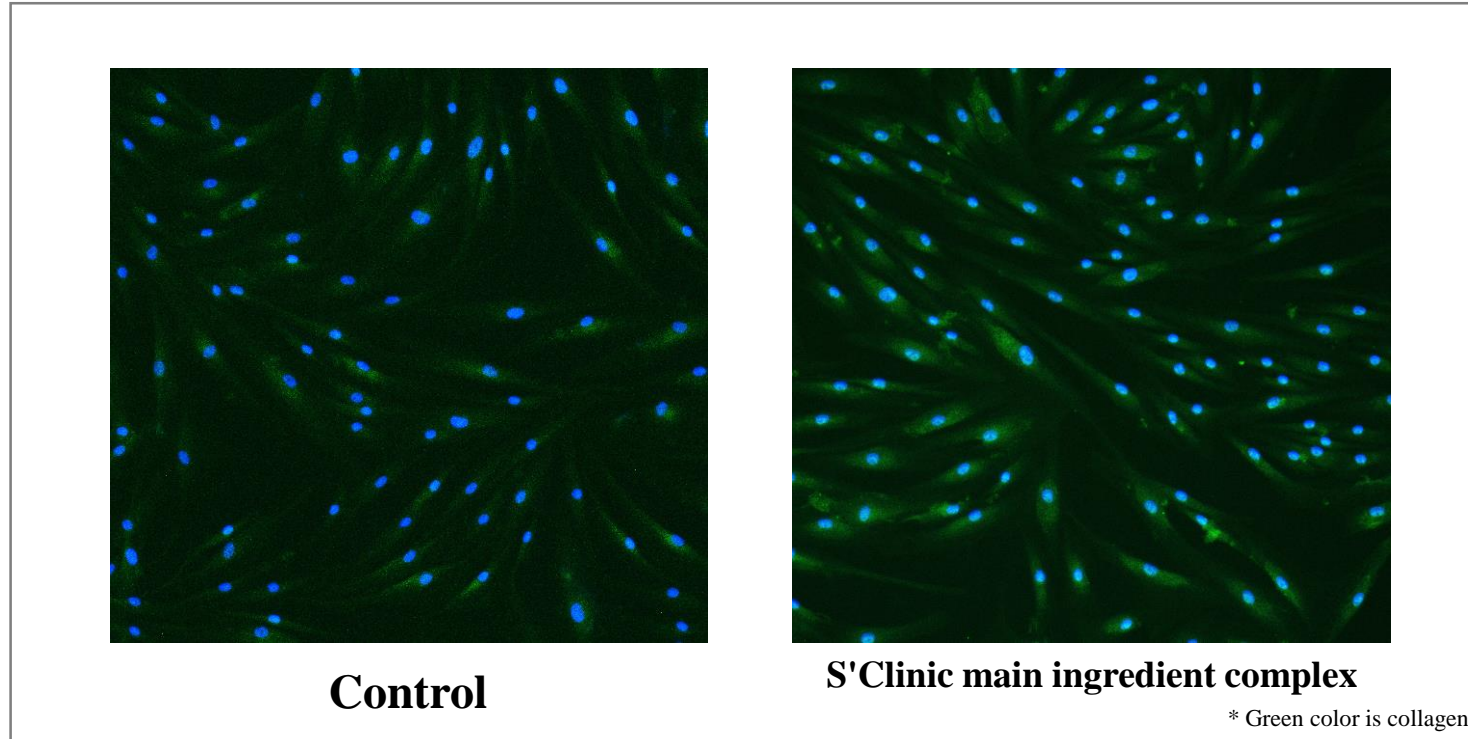
1. Choi, S. H., et al. (2018). The Role of Collagen in Skin and Hair Health. *Journal of Cosmetic Dermatology*, 17(6), 1002-1008.
2. Shin, H., et al. (2020). Collagen Supplementation and Its Effects on Hair Growth and Dermal Health. *Dermatological Research*, 312(4), 545-553.
3. Zhang, Z., et al. (2016). Collagen Degradation in Skin Aging: Implications for Scalp and Hair Health. *International Journal of Dermatology*, 55(2), 127-135.

3-2. Preparation

- HaCaT (Human Epithelial Keratinocyte, American Type Cell Culture, USA), CCD-986sk (Human Skin Fibroblast, Korean Cell Line Bank, Korea), and HFDPC (Human Follicle Dermal Papilla Cell, Promo Cell, Germany) maintained in DMEM medium (Dulbecco's Modified Eagle's Medium) containing 10% Fetal Bovine Serum (FBS), penicillin (100 IU/ml), and streptomycin (100 µg/ml) at 37°C in a humidified, 5% CO₂ atmosphere.
- **“S'Clinic main ingredient complex”** was prepared in FBS-free DMEM medium immediately before use.
- Anti-collagen I antibody (Abcam, UK) was used for immunofluorescent staining techniques to validate collagen synthesis in fibroblasts.

3-3. Results

- Effect of “S'Clinic main ingredient complex” on Collagen Biosynthesis



The effect of “S'Clinic main ingredient complex” on collagen biosynthesis was evaluated in human skin fibroblasts. The total amount of collagen type I (green) increased to 145.8% compared to the negative control, indicating its potential to enhance scalp elasticity, strengthen the extracellular matrix, and promote a healthier scalp environment.

4-1. Introduction

The scalp, as a specialized part of the skin, plays a critical role in maintaining the health of hair follicles and providing a barrier against environmental and microbial insults.

The scalp barrier is a complex system that ensures hydration, protects against environmental stressors, and supports hair follicle function. The integrity of this barrier relies on various structural proteins, including filaggrin and occludin, which are crucial for epidermal differentiation and tight junction stability, respectively. Understanding their roles is essential for developing therapeutic strategies for scalp-related disorders such as seborrheic dermatitis, atopic dermatitis, and hair loss conditions.

Filaggrin, synthesized as its precursor profilaggrin in the granular layer of keratinocytes, plays a central role in epidermal barrier formation. In the scalp, filaggrin contributes to keratinocyte differentiation by promoting the aggregation of keratin filaments, which enhances the mechanical resilience of the epidermis. Additionally, the enzymatic breakdown of filaggrin produces natural moisturizing factors (NMF), including free amino acids and their metabolites. These NMF components maintain scalp hydration, regulate the pH, and provide antimicrobial defense, which are critical in the context of the sebaceous-rich scalp environment.

Filaggrin deficiency or dysfunction can disrupt the scalp barrier, leading to increased transepidermal water loss (TEWL), heightened susceptibility to allergens and microbial invasion, and an inflammatory response. Such disruptions are associated with scalp conditions like atopic dermatitis and chronic itching, which can further affect hair follicle health.

Occludin is a transmembrane protein that is a key component of tight junctions, which are vital for the scalp's barrier function. Tight junctions are located in the upper layers of the epidermis and help regulate paracellular permeability, preventing the penetration of harmful substances while retaining essential nutrients and moisture. Occludin ensures the structural integrity of tight junctions and modulates signaling pathways involved in inflammation and barrier repair.

In the scalp, occludin is particularly important in areas with high environmental exposure and physical stress from grooming practices. A compromised occludin function has been implicated in conditions characterized by barrier disruption, such as seborrheic dermatitis and psoriasis, where tight junction integrity is compromised, leading to increased permeability and inflammation.

Filaggrin and occludin function in complementary ways to maintain the scalp's barrier homeostasis. While filaggrin enhances the physical and biochemical resilience of the epidermis, occludin strengthens the paracellular barrier through tight junction stabilization. Together, these proteins protect the scalp from environmental insults, maintain hydration, and support a healthy microenvironment for hair follicles.

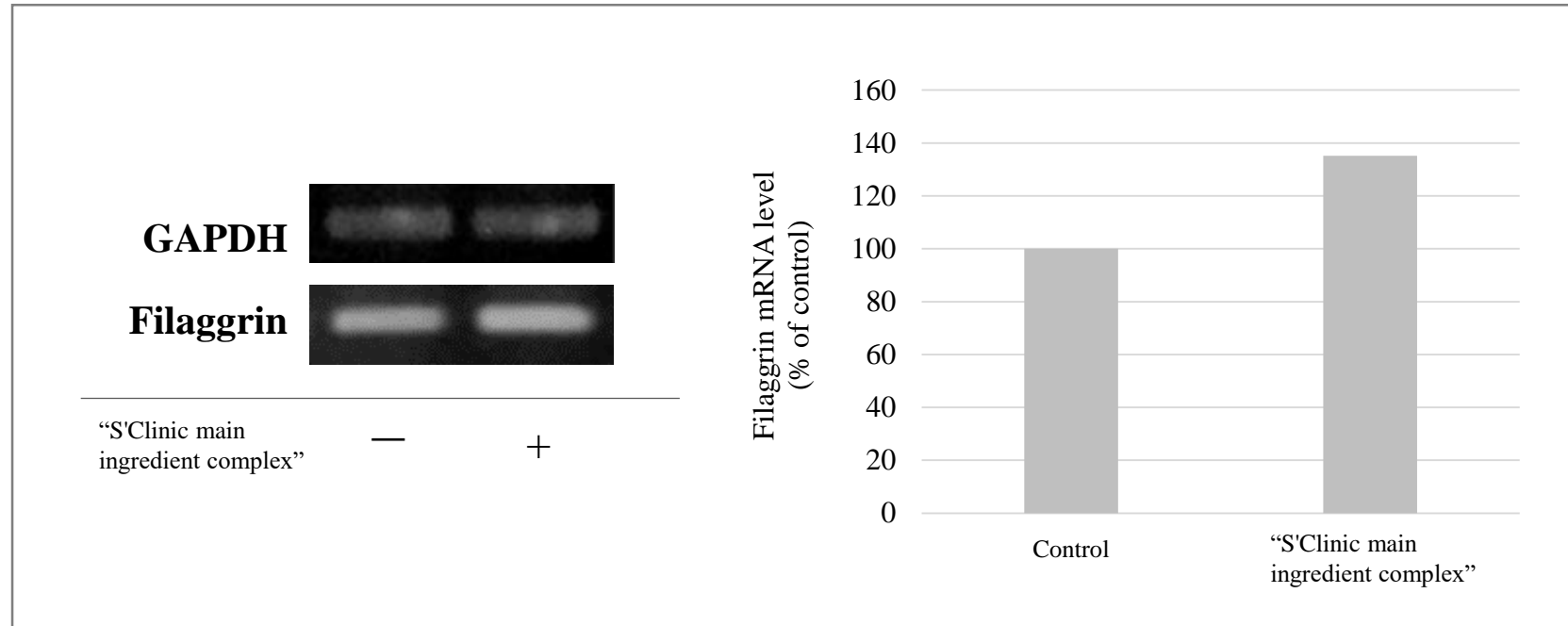
Thus, we conducted an experiment to determine whether it increases the mRNA expression levels of essential proteins for improving the skin barrier, filaggrin and occludin.

4-2. Preparation

- HaCaT (Human Epithelial Keratinocyte, American Type Cell Culture, USA), CCD-986sk (Human Skin Fibroblast, Korean Cell Line Bank, Korea), and HFDPC (Human Follicle Dermal Papilla Cell, Promo Cell, Germany) maintained in DMEM medium (Dulbecco's Modified Eagle's Medium) containing 10% Fetal Bovine Serum (FBS), penicillin (100 IU/ml), and streptomycin (100 µg/ml) at 37°C in a humidified, 5% CO₂ atmosphere.
- “S'Clinic main ingredient complex” was prepared in FBS-free DMEM medium immediately before use.

4-3-1. Result

- Effect of mRNA Regulation of Filaggrin on Skin Barrier Function

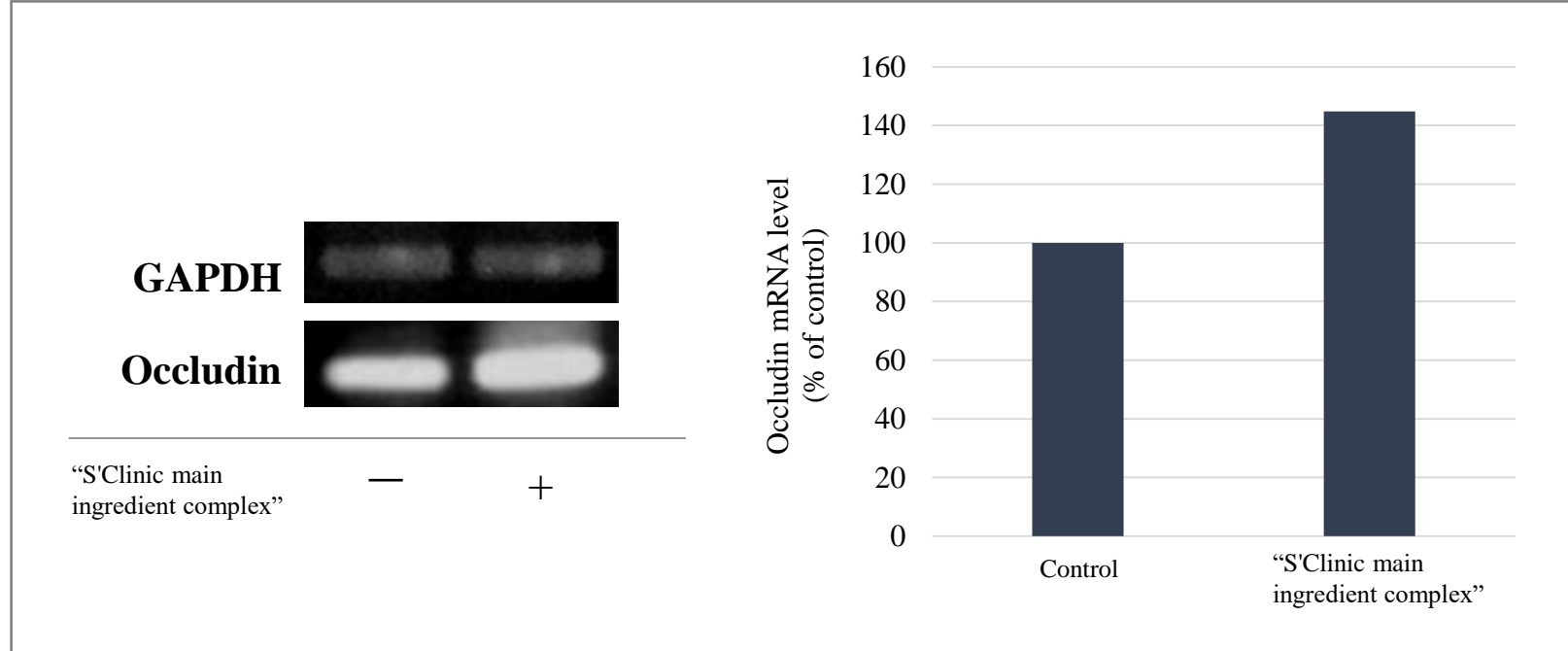


Thus, filaggrin is indispensable for the mechanical and biochemical properties of the skin barrier, underscoring its significance in dermatological health and disease.

The effect of “S'Clinic main ingredient complex” on skin barrier function was evaluated by assessing the expression level of filaggrin. The results showed that filaggrin mRNA expression increased to 135.16% compared to the negative control. This elevated expression of filaggrin, a crucial protein for maintaining the skin barrier, signifies an enhancement in scalp barrier function, which aids in improving moisture retention and reducing transepidermal water loss. These findings highlight the potential of “S'Clinic main ingredient complex” in promoting a healthier and more resilient scalp environment.

4-3-2. Result

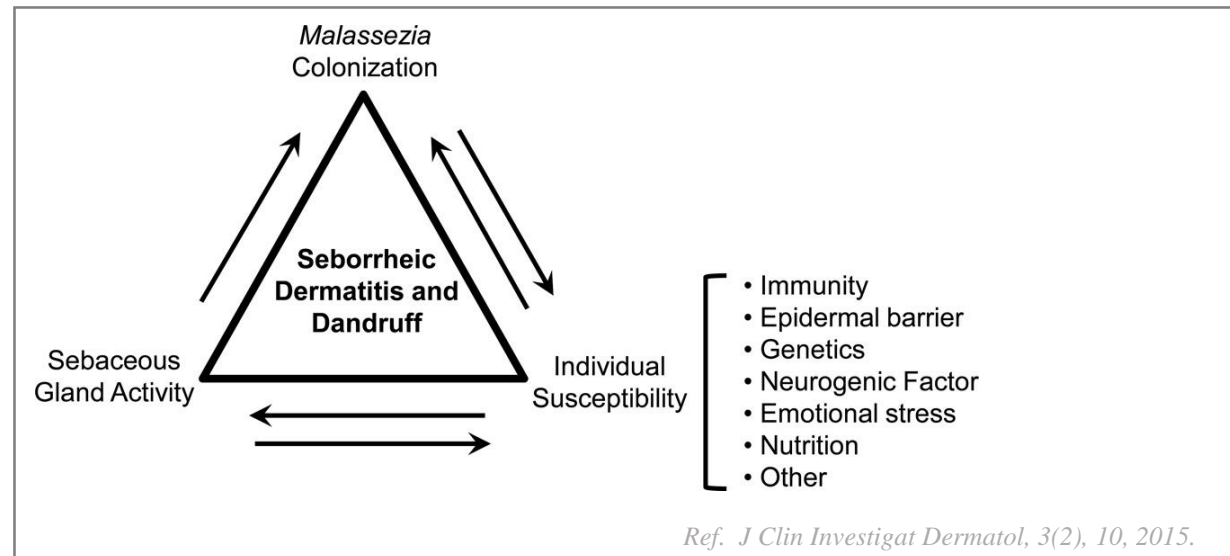
- Effect of mRNA Regulation of Occludin on Skin Barrier Function



The effect of “S’Clinic main ingredient complex” on skin barrier function was evaluated by measuring the expression level of occludin. Occludin mRNA expression of “S’Clinic main ingredient complex” was increased to 144.82% when compared to the negative control. The increased expression of occludin, a key tight junction protein, indicates improved cellular cohesion and a strengthened scalp barrier, contributing to enhanced protection against environmental aggressors and better overall scalp integrity.

5-1. Introduction

Seborrheic Dermatitis and dandruff are common dermatological problems that affect the seborrheic areas of the body. Various intrinsic and environmental factors, such as sebaceous secretions, skin surface fungal colonization, individual susceptibility, and interactions between these factors, all contribute to the pathogenesis. Seborrheic dermatitis on the scalp, is caused by increased male hormones, development of sebaceous glands, and environmental changes such as temperature and humidity that promote the proliferation of *Malassezia furfur*. The entry of *Malassezia* and its metabolites is facilitated, stimulating the epidermis and triggering an immune response, which results in inflammation. This inflammatory response of the scalp causes hair loss. In this study, we evaluated whether “S’Clinic main ingredient complex” could improve seborrheic skin of the scalp by inhibiting the proliferation of *Malassezia furfur*. Additionally, we tested whether the mRNA expression level of IL-6, an inflammatory cytokine, was suppressed.

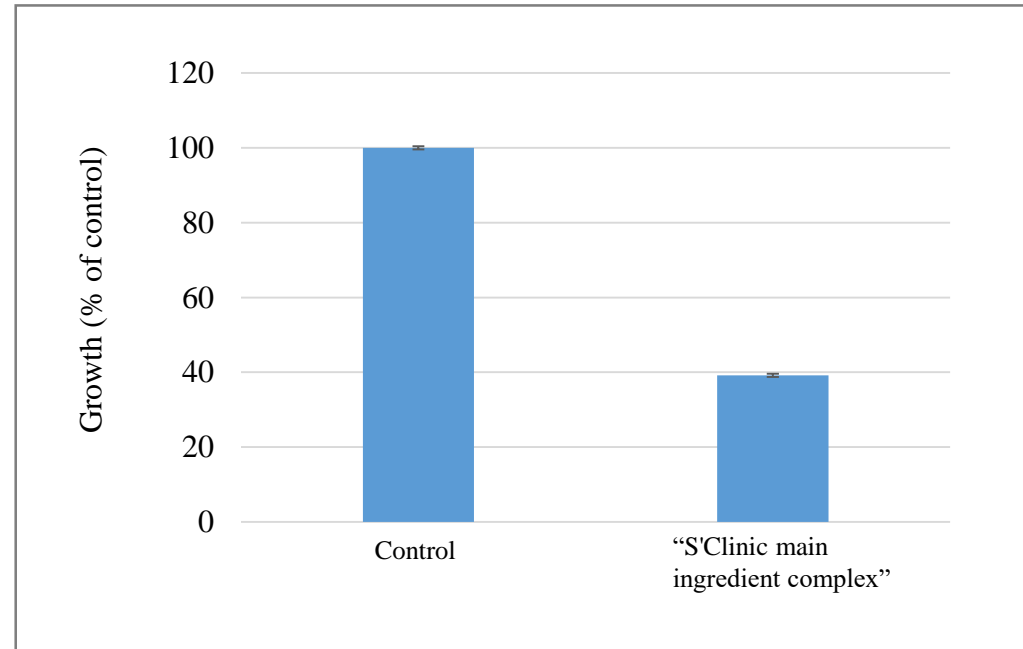


5-2. Preparation

- *Malassezia furfur* (Korean Collection for Type Culture, Korea) maintained in modified Leeming & Notman Broth (mLMB, MB Cell, Korea) containing tween 40 and glycerol.
- “S’Clinic main ingredient complex” was prepared in mLMB liquid medium immediately before use.

5-3-1. Result

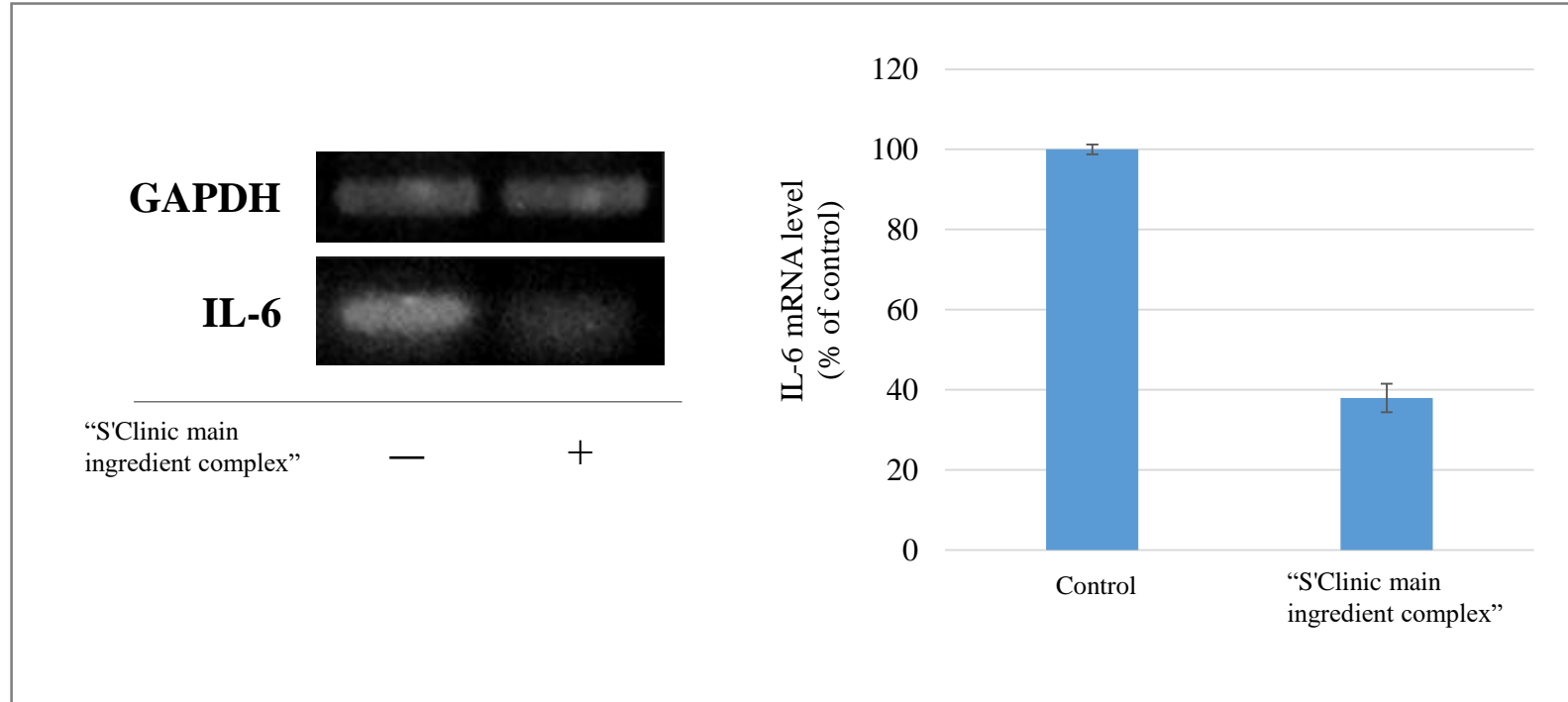
- Effect of “S’Clinic main ingredient complex” on Antifungal activity



The antifungal efficacy of “S’Clinic main ingredient complex” against *M. furfur* was evaluated, and the results demonstrated that “S’Clinic main ingredient complex” significantly inhibited the growth of *M. furfur*, achieving an inhibition rate of approximately 60% compared to the negative control. This indicates its potential effectiveness in managing scalp conditions associated with fungal overgrowth.

5-3-2. Result

(2) Effect of mRNA Level Regulation for Anti-Inflammatory Effect



The effect of “S'Clinic main ingredient complex” on inflammation was evaluated by measuring the expression level of IL-6. IL-6 mRNA expression of “S'Clinic main ingredient complex” was reduced to 37.93% compared to the negative control. The decreased expression of IL-6, a pro-inflammatory cytokine, indicates a significant anti-inflammatory effect, suggesting that “S'Clinic main ingredient complex” can help soothe scalp irritation and reduce inflammation-related conditions, promoting a healthier scalp environment.

6-1. Introduction

The scalp, as a specialized skin region, is frequently exposed to physical, chemical, and environmental insults, which may lead to injuries ranging from minor abrasions to chronic wounds. The ability of the scalp to effectively regenerate and repair such injuries is crucial for maintaining scalp integrity and hair follicle function. Chronic wounds or delayed healing can perpetuate inflammation, which is a contributing factor to scalp disorders like seborrheic dermatitis and psoriasis. This not only disrupts scalp homeostasis but also compromises the surrounding follicular environment.

Therefore, the wound healing capacity of the scalp is critically important. In this study, we evaluated the ability of “S'Clinic main ingredient complex” to promote wound healing by assessing its effects on epithelial cells and fibroblasts.

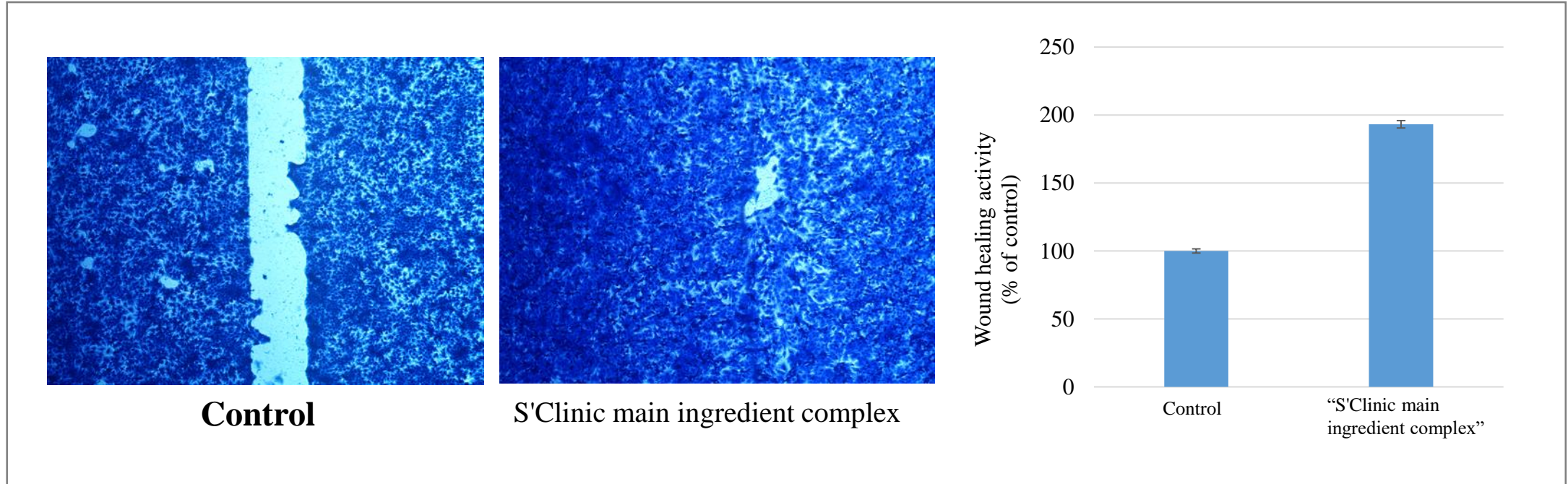
6-2. Preparation

- HaCaT (Human Epithelial Keratinocyte, American Type Cell Culture, USA), CCD-986sk (Human Skin Fibroblast, Korean Cell Line Bank, Korea), and HFDPC (Human Follicle Dermal Papilla Cell, Promo Cell, Germany) maintained in DMEM medium (Dulbecco's Modified Ealge's Medium) containing 10% Fetal Bovine Serum (FBS), penicillin (100 IU/ml), and streptomycin (100 µg/ml) at 37°C in a humidified, 5% CO₂ atmosphere.
- “S'Clinic main ingredient complex” was prepared in FBS-free DMEM medium immediately before use.

Wound Healing activity

6-3-1. Result

- Effect of “S'Clinic main ingredient complex” on Wound Healing in Human Keratinocytes

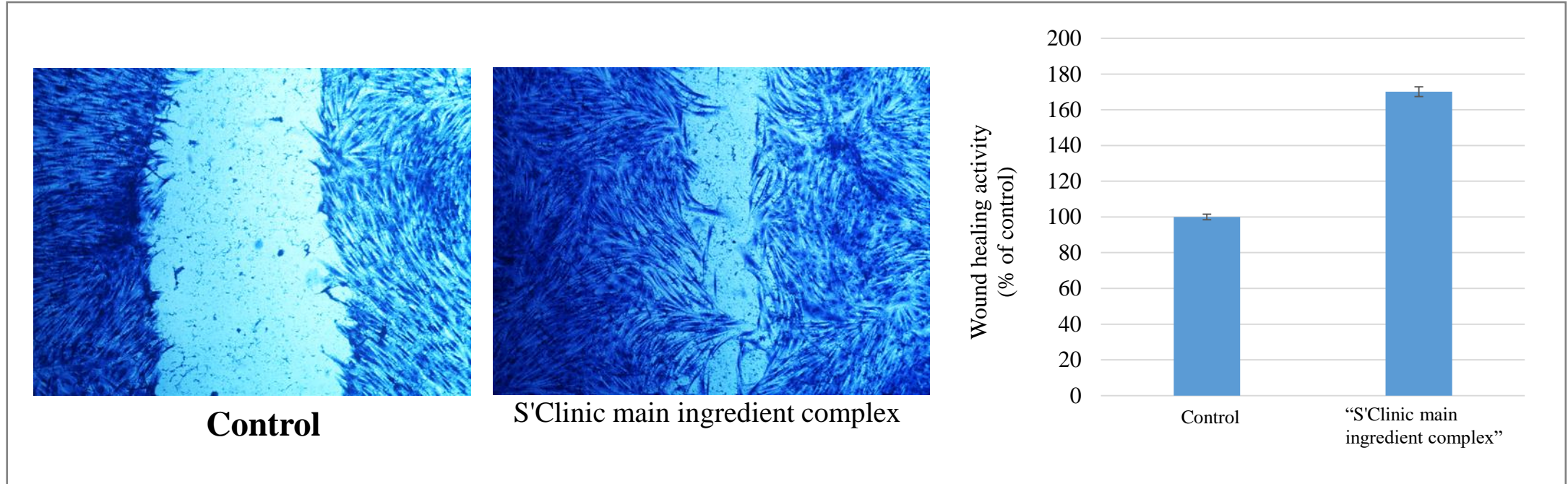


The effect of “S'Clinic main ingredient complex” on wound healing in human keratinocytes was evaluated. The treatment group showed a 193.15% increase in wound healing compared to the untreated control, indicating its potential to accelerate skin regeneration and repair.

Wound Healing activity

6-3-2. Result

- Effect of “S'Clinic main ingredient complex” on Wound Healing in Human Skin Fibroblast



The effect of “S'Clinic main ingredient complex” on wound healing in human skin fibroblasts was evaluated. The treatment group demonstrated a 170.14% increase in wound healing compared to the untreated control, highlighting its potential to enhance skin repair and regeneration.