32nd IFSCC Congress
London 19-22 September 2022

华熙生物
BLOOMAGE BIOTECH

STUDY OF THE SOOTHING AND REPAIR EFFECTS OF AN INNOVATIVE ACTIVE INGREDIENT COMPLEX

Poster ID:320

Kan, Hongling; Liu, Xiaoyun; Dong, Jianjun; Shen, Fengtong; Qu,Wenjie; Li,Feng
Research and Development Centre，BLOOMAGE BIOTECHNOLOGY CORPORATION LIMITED., Jinan, China

Introduction:

The skin serves as a barrier between the human body and the surrounding environment, minimizing environmental aggression[1,2]. Functionally, the skin barrier can be separated into microbiome, chemical, physical, and immune layers. Although each layer has a particular function, they are highly interdependent and they all contribute to the overall integrity of the skin barrier[3,4].

Proper skin hydration helps to recover and maintain the skin protection barrier, and nearly 90% of the skin barrier function exists in the stratum corneum(SC). The SC's lipid-filled extracellular compartment and humectant/water-filled intracellular compartment, are critical for the skin's barrier function and water-holding properties[5]. Normally, the skin barrier protects epidermis from exposure to environmental chemicals and symbiotic bacteria when the barrier function is complete, however, when barrier function is disordered, bacteria, fungi, and viruses gain access to the epidermis and then taken up by Langerhans cells which present antigens to the immune system, activating it and inducing an inflammatory response[6]. As many papers indicated, skin barrier dysfunction is frequently linked to a variety of skin and sebaceous gland disorders，which are typically accompanied with some skin lesions.

Here, the moisturizing, soothing and repairing effects of the complex was evaluated from different perspectives (in vitro reconstructed epidermis, cell culture, clinical trials, etc.). Specifically, the complex's moisturizing effect was assessed by measuring retained water and transepidermal water loss on the reconstructed epidermis. The soothing effect research was carried out by detecting IL-6, IL-1alpha, and TNF-alpha through ELISA test. Likewise, the repairing effect was obtained by investigating the expression of Ki-67 on the epidermis through immunohistochemical staining as well as the formation of glycosaminoglycan and collagen in human fibroblasts. Moreover, in the clinical test, clinical scoring and pH measurement was used to record the changes at day0 and day28.

Materials & Methods:

1-Preparation of active substances

The complex is comprised of hydrolyzed hyaluronic acid, hydrolyzed sodium hyaluronate, sodium acetylated hyaluronate, adenium obesum leaf cell extract, vitis vinifera (Grape) flower cell extract, and vegetable glycerin. The cell extracts are acquired from dedifferentiated plant cells.

2-Moisturizing assessment on recombinant epidermis SKINETHIC®

This study consisted in using the products under consideration and tritiated water (1μCi) to treat the reconstituted epidermis SKINETHIC® previously dried at the level of the stratum corneum. In the study of "Reservoir effect – bound water", product contact time with epidermis is 15 and 30 minutes, after that, the epidermis was washed quickly on the surface, peeled from the filter and sonicated, and then the radioactivity was counted in a Packard counter. In the study of "Dynamic effect – Free Water", product contact time with epidermis is 15, 30 and 60 minutes, after that, 20 μl of medium were collected and the radioactivity of the medium was counted in a counter.

3-Ki-67 assessment

This reaction was carried out with antibody MIB1 (Immunotech), recombining peptide of the nuclear antigen by Ki-67 pretreatment. The revelation was made by the method peroxydase-antiperoxydase after antigenic unmasking by pretreatment with heat. The staining by DAB chromogene reveals in brown the Ki-67 nuclear sites of the cells fraction in growth expressed in phases. The epidermises were frozen at -180°C. After inclusion in blocks of paraffin, these epidermises were cut then treated by immunohistochemistry.

4-Anti-inflammatory assessment

After the treatment of reconstituted epidermises;the culture mediums were taken, and the assessment of inflammatory mediators was performed according to the protocols described in the IL1-α kit、IL-6 kit and TNFα kit.

5-Assessment of the glycosaminoglycans content

The fibroblasts were distributed in multiwell plates (6 wells) and the radioactive precursor ([3H]-Glucosamine) was added to the cultures 18 hours, and then the cells were treated for 24 h at 37°C. After having eliminated the medium by aspiration, the cells were washed 2 times with medium without serum, then the cells were collected. This base were realized the proteoglycans assay by FPLC (Fast protein liquid Chromatography). An aliquot of 50μl was taken for the counting of the radioactivity incorporated in the total glycosaminoglycans.

6-Collagen content assessment

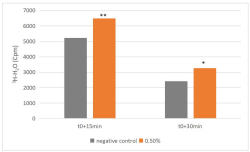
After incubation time, fibroblasts were recovered by centrifugation. The pellets were digested by collagenases. After centrifugation at 10000g, collagens were precipitated in NaCl at 1 M, the precipitate being suspended and dialyzed. Primary amino acids were derivated by ophtaldehyde acid (OPA), eliminating their interference. Hydroxyproline and proline were the derivated by NBD-Cl by coupling with NBD-Cl amino groups. The NDB-Hyp was separated and identified by HPLC in reverse phase. Hydroxyproline was dosed by fluorescence measure after separation of HPLC in reverse phase.

7-Clinical test

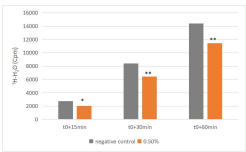
20 subjects with oily skin or oily combination skin with acneic tendencies to the face, with at least 10 retentional lesions, 5 inflammatory lesions and brown areas (scars), the range of 18 to 35 years old. 20 volunteers used emulsion with 0.5% complex on the face twice a day for 28 days.Assessment of the anti-redness, imperfection, pigmentary spot effect, pH measurements and Self-assessment.

Results & Discussion:

1-The effect of the innovative active ingredient on skin barrier function

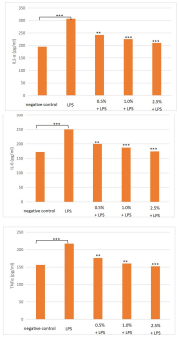


The levels of radioactivity in epidermis demonstrated an increase in water retention in the epidermis of 24% and 35% respectively.



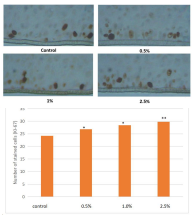
A decrease in the trans-epidermal passage of tritiated water of 27%, 24% and 21% respectively.

2-Evaluation of the innovative active ingredient complex's effect on the immune system of the skin



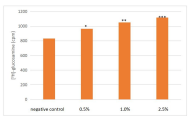
The concentration of IL1-α ,IL-6, TNF-α detected by Elisa significantly decreased.

3-Evaluation of the innovative active ingredient complex's effect on the epidermis stimulation and regeneration

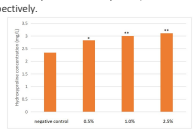


KI-67 expression on reconstructed epidermis respectively by 11%, 17% and 23% respectively.

4-Effect of the innovative active ingredient complex on the extracelluar matrix in dermis

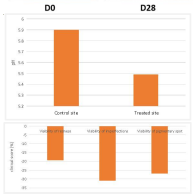


The glycosaminoglycans rate in fibroblast significantly increases by 16%; 27% and 35% respectively.



The Collagen content significantly increases by 21%, 28% and 33% respectively.

5-Clinical effect of the innovative active ingredient complex on face area



The redness, imperfection and pigmentary spot on the face area of the volunteers' significant decreased, compared to D0/T0.And a statistically significant improvement of the pH measurements on D28.

N°	Items	Subject satisfaction percentage
		D28
1	My skin is soothed. I feel less sensations of irritation	60%
2	My skin is more radiant	79%
3	My skin is more hydrated	74%
4	I feel a sensation of well-being and confort	84%
5	My imperfections are less apparent	79%

Conclusions:

The innovative active ingredient complex resulted in an increase in water retention and a decrease in the trans-epidermal passage of tritiated water. In the meanwhile, the complex promoted cellular proliferation and accelerated the production of glycosaminoglycans and collagen by human fibroblasts, which stimulated the regeneration of the epidermis and dermis. Moreover,when it comes to the effect on immune system, monocytes were cultured and the anti-inflammation effect were tested. It is clarified that the innovative active ingredient complex could significantly reduce the release of IL1-α, IL6 and TNFα induced by LPS.

Clinical trials revealed that the complex caused a statistically significant reduction in facial redness, and 58% of the subjects declared that their skin was soothed and they felt less irritated. Additionally, 79% of the participants said that their imperfections were less apparent in their Self-assessments. The pH values from D0 to D28 likewise showed a statistically significant improvement.

In general, the innovative active complex helps the water retention and decreases the TWEL, stimulating epidermis and dermis regeneration. At the same time, it provides a soothing effect by reducing skin inflammation. Thanks to these associated processes, the complex contributes to the development of stronger, more uniform, and healthier-looking skin.

References:

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