A novel transdermal EGF for skincare

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Transdermal epidermal growth factor (T-EGF) is a fusion protein that can be absorbed through the skin. In T-EGF, a transdermal short peptide (TD1) and epidermal growth factor (hEGF) are fused using a Linker (Figure 1).

The function of TD1 is to improve the transdermal transport efficacy of the EGF. This article focuses on the evolution and advantages of T-EGF for its application in novel skin care products.

Epidermal growth factor (EGF)

In 1962, American scientist Stanley Cohen discovered an active ingredient that could induce newborn mice to open their eyelids and grow teeth.[2] By adding this active ingredient to cultured skin he found that it can promote the growth of human epidermal cells.

This active ingredient was since named epidermal growth factor (EGF). Dr. Cohen won the 1986 Nobel Prize in Physiology and Medicine jointly with Dr. Levi-Montalcini for this discovery.

EGF is a hydrophilic protein comprised of 53 amino acids. It plays a significant role in promoting various cellular processes including cell division, collagen, and glycoprotein secretion. EGF is known to enhance cell plumpness, ensure tight arrangement of muscle fibres, restore skin elasticity and vitality, and effectively combat the formation of wrinkles.[3-5]

Modern medical research places a strong emphasis on EGF's ability to stimulate cell proliferation and differentiation, making EGF a promising candidate for applications in skin damage repair solutions.[6] Despite its promising skincare functionality, EGF's capacity to penetrate the intact skin barrier is limited.[7]

As a result, current conventional EGF treatments are primarily applicable to treat skin conditions with open wounds and eye-related ailments, while it has minimal efficacy on normal condition skin with an intact barrier. This poses challenges to apply EGF in the broader field of the skincare and cosmetics industry.

Delivery of macromolecules through the skin barrier

The skin is primarily composed of the epidermis, dermis, and subcutaneous tissue (Figure 2). Functioning as both a defence and excretion organ, the skin fends off the intrusion of foreign substances and safeguard against the depletion of water and essential nutrients within the body.

Throughout the course of extended evolutionary development, skin tissue has developed a barrier function, with the pivotal element being the stratum corneum situated in the outermost layer of the epidermal tissue. While comprising primarily of deceased keratinocytes rich in protein and lipid components, this impervious network system poses a significant challenge for the transdermal delivery of particularly larger biomacromolecules.[8]

Over the years, extensive efforts have been dedicated to finding the most effective and safe method to deliver drugs across the skin barrier. The traditional approaches can be broadly categorized into two main groups: chemical penetration enhancers and physical assistance techniques.[9-11]

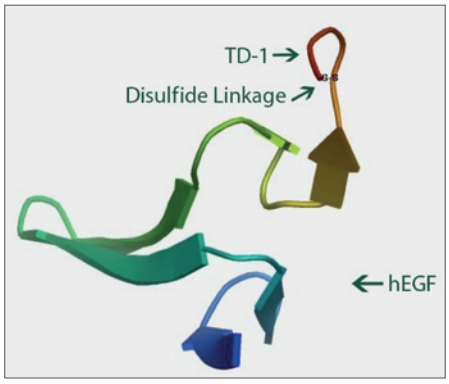

Figure 1: Schematic diagram of T -EGF structure[1]

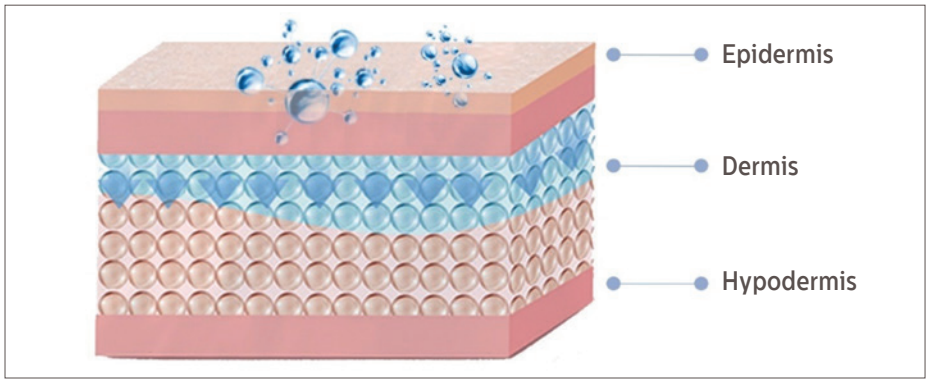

Figure 2: Schematic diagram of skin structure

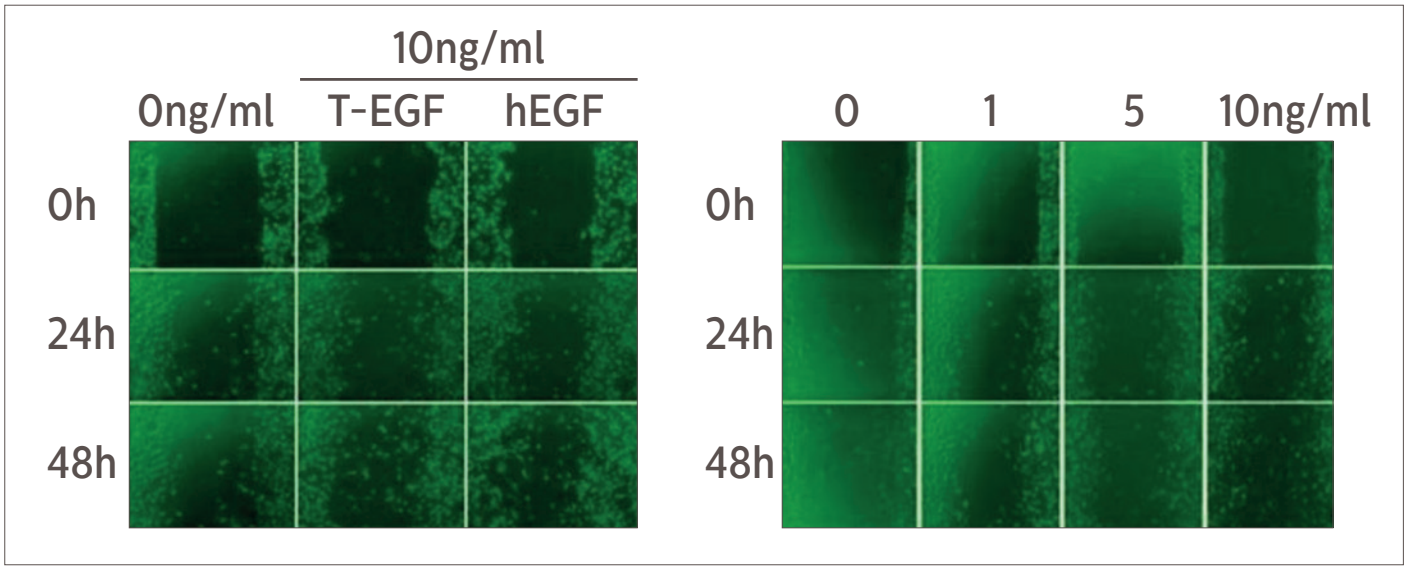

Figure 3: (*Left*) Cells were treated with TD1-hEGF (10 ng/ml) and hEGF (10 ng/ml) after scratch was made for zero hours, 24 hours and 48 hours. Lane 0 ng/ml: untreated cell sample was negative control. Lane hEGF are native hEGF for standard. Microscopic images were captured after 24 hours and 48 hours. (*Right*) Dosage dependent scratch assay from adding 0 to 10 ng/ml TEGF. Microscopic images were captured at T0 and after 24 hours and 48 hours for each concentration. Figure is taken from published doctoral thesis

Chemical penetration

Chemical penetration enhancers include using chemicals (e.g. surfactants) that alter the arrangement of lipids within the skin's outermost layer. Their action induces swelling and softening of the stratum corneum, in turn, leads to enlargement of sweat gland and hair follicle openings. Through this mechanism, the enhancers facilitate the passage of molecules through the skin barrier, fostering transdermal absorption.

Though these substances exhibit a definite capability to enhance skin permeability, there are two drawbacks: (a) tendency to cause skin allergies and (b) inability to significantly facilitate the transdermal delivery of hydrophilic macromolecules e.g. proteins.[12]

Physical assistance

Physical assistance includes iontophoresis, ultrasonic, microneedle, electroporation, and laser treatment. These methods are effective to deliver a diverse array of substances. However, they are not well-suited for wide home usage due to the necessity of specialized equipment and limited flexibility in forms of dosage, all while inducing various degrees of pain and discomfort.

A novel biological approach – TD1 transdermal peptide

In 2006, Professor Wen Longping and his team of the University of Science and Technology of China applied '*in vivo* phage display technology' to the field of transdermal drug delivery, and successfully discovered a short transdermal peptide composed of 11 amino acids, which can transport a variety of molecules, including large protein polymers, through the skin effectively.[13]

The transdermal peptide acts on the hair follicle to temporarily open the skin barrier, assists macromolecular substances to pass through and reach the subcutaneous tissue.[13]

Professor Mark R. Prausnitz, a respected international expert, published commentary citing this work: "*Chen et al* have taken a biological approach to increase skin permeability, in contrast to the chemical and physical approaches previously investigated... protein delivery from a patch with equal efficacy and similar cost would almost certainly make hypodermic injection of many proteins obsolete. This prospect is what makes finding a peptide chaperone for transdermal delivery such an exciting advance."[14]

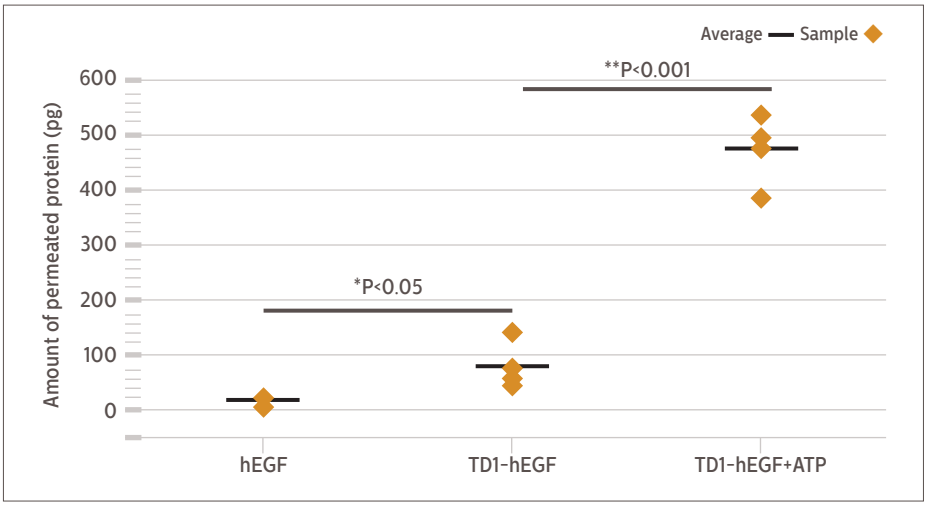

Figure 4: T-EGF Transdermal in human skin. TD1-hEGF+ATP,TD1-hEGF and hEGF (50µg/mL) were administered to human skin separately for 16 hours. hEGF levels were measured by ELISA kit. Mean±s.e.m. (n=4) *P < 0.05. **P < 0.0011

The introduction of a transdermal EGF

Following the discovery of TD1, Longsheng Biotech created a fusion of TD1 and EGF. TD1-EGF (T-EGF) not only retains the same biological efficacy in promoting skin cell growth as conventional EGF but also boasts a transdermal efficiency that surpasses that of traditional EGF by over ten times.[15]

The beneficial efficacy to cell activity, repairing and moisture effects demonstrates, among others, how this remarkable enhancement in transdermal efficiency significantly widens the scope of EGF application within the cosmetics industry.

Cell activity and transdermal efficiency

The ability of T-EGF to promote the migration of balb/c 3T3 cells was demonstrated through cell scratch experiment.[15] The experimental results showed that, in the presence of 10 ng/mL T-EGF or human recombinant EGF (hEGF), after 24 hours of cell culture, cells migrated towards the centre of the scratch.

In comparison to the control group, following 48 hours of continuous culture, a significant number of cells migrated to the scratch's centre when treated with 10ng/mL T-EGF, resembling the response seen with hEGF. The promotion of cell migration by T-EGF exhibited a concentration-dependent manner, with a higher concentration of T-EGF corresponding to a stronger ability to induce cell migration.

The results unequivocally demonstrated that the activity of promoting cell migration is similar

between T-EGF and hEGF (Figure 3).

The transdermal efficacy of T-EGF in human skin was also verified. The skin tissue was obtained from routine plastic surgeries performed at Hefei No.1 People's Hospital. The patients signed informed consents to have their removed skin tissue collected for this research purpose.

Following the careful removal of subcutaneous fat, the skin was placed in a Franz diffusion tank. The transdermal ability of T-EGF (50μg/mL) was monitored for 16 hours.

The results indicated that, in human skin, the transdermal efficiency of T-EGF is significantly higher than that of the control group (hEGF). Furthermore, it was observed that the skin permeation process of T-EGF is energy dependent. Upon addition of ATP, the transported T-EGF through skin barrier exhibited a substantial increase compared to the experimental group without ATP addition (Figure 4).

The repairing and moisturizing effect

To substantiate the efficacy of T-EGF in terms of skin repair and moisturization, a comprehensive evaluation was conducted through a third-party commissioned institution (Report No. B136-20230407-01-R1-V1, EviSkin).

A cohort of 30 Asian individuals, exhibiting a baseline facial trans epidermal water loss (TEWL) value equal to or exceeding 16 g/m–h, were selected to participate in this investigation. T-EGF formulation at a concentration of 10μg/ml was used in this test.

A significant reduction ($p<0.05$) in TEWL value post-application compared to pre-application indicated that the product possessed skin repair function.

Additionally, data on the subjective improvements of facial skin condition as well as the general skincare suitability was investigated through a survey. An effectiveness rate equal to or surpassing 50% was regarded as indicative of a favourable outcome within the scope of the assessment parameters.

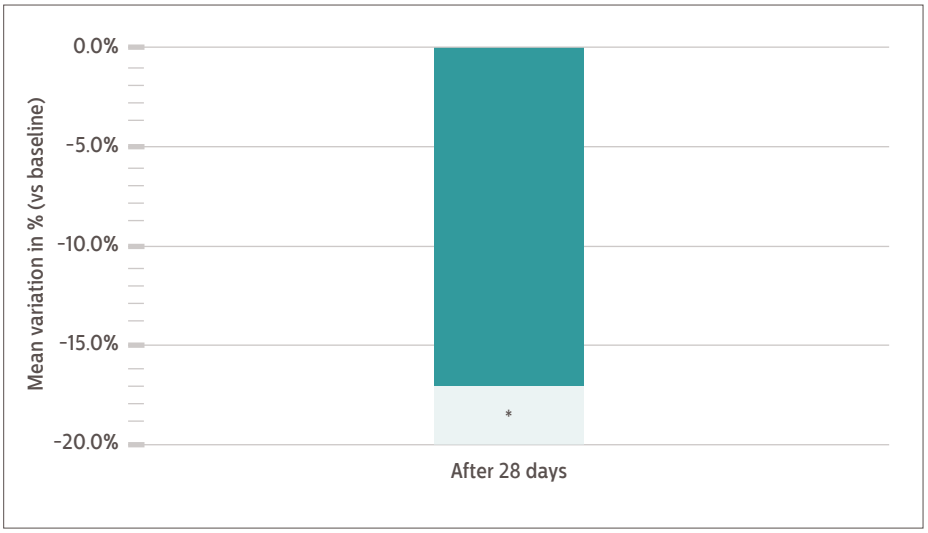

Figure 5: Variations (versus baseline) in percentage on the mean of TEWL values. * indicates that the difference between the test value and the baseline value at this time point is statistically significant

TABLE 1: RESULT FOR *IN VITRO* SKIN IRRITATION BY RECONSTRUCTED HUMAN EPIDERMIS (%). TEST GROUP RESULT IS SIGNIFICANT COMPARED TO THE POSITIVE CONTROL. SDS = SODIUM DODECYL SULFATE

Group	Positive control (5 mg/ml SDS)	Negative control (Ultra-pure grade water)	Test group (T-EGF) 100μg/ml
Mean OD ± SD	0.431 ± 0.009	1.059 ± 0.013	1.036 ± 0.057
Viability ± SD (%)	41.596 ± 0.988	100	97.890 ± 5.376
IL1$^{\alpha}$ (pg/ml)	37.03 ± 4.51	--	8.646 ± 4.34

The findings (Figure 5) revealed a statistically significant reduction in the mean TEWL value following 28 days of employing the T-EGF samples in contrast to baseline value ($P < 0.05$).

Following a 28-day application of T-EGF sample, all 30 participants reported notable improvements. One hundred percent of the subjects noted a reduction in facial skin dryness, with an equal percentage attesting to heightened facial skin hydration.

Finally, the participants universally experienced enhanced facial skin texture, improved facial skin tightness, and alleviation of sensations like burning, tingling, and redness, all

TABLE 2: BCOP TEST RESULTS (MEAN±SD). OD VALUE REPRESENTS MEAN PERMEABILITY VALUE. IVS = *IN VITRO* SCORE. THE UN GHS GUIDELINES ARE FOLLOWED FOR THE CLASSIFICATION

Group	Mean corrected opacity value	Mean corrected OD value	IVS	UN GHS classification	Irritant classification
Negative control	0.427 ± 1.7154	0.0678 ± 0.0056	1.444 ± 1.7918	No category	Non-irritant
Positive control	32.9298 ± 0.450	1.2279 ± 0.4143	51.3488 ± 5.8910	No stand-alone prediction can be made	Impossible to predict
Test sample	-1.125 ± 0.2586	0.0013 ± 0.0023	-1.1064 ± 0.2926	No category	Non-irritant

of which contributed to an overall advancement in facial skin condition. Impressively, none of the participants encountered any discomfort throughout the entire 28-day testing period.

Skin irritation evaluation

A comprehensive skin irritation test (Report No. HK-R-20230609-02) was commissioned following the guidelines in 'In Vitro Skin Irritation: Reconstructed Human Epidermis Test OECD TG439'.

The assessment was executed using the laboratory method "Skin Model Irritation Test-MTT+IL-1α", which entails a comparative analysis against a positive control to ascertain the potential irritative impact of the test substance on the reconstituted epidermal model *in vitro*. The irritation induced by the test sample (T-EGF at 100 μg/ml) is determined by quantifying the percentage reduction in cellular viability.

The results indicate no irritating substance is present in the tested sample. The cell viability was determined to be 97.89% upon application of the sample, surpassing the threshold of 50%. The interleukin 1α (IL-1α) concentration was measured at 8.646 pg/mL (Table 1).

Ex vivo eye irritation test

The eye irritation test of T-EGF, according to the OECD TG437 bovine corneal turbidity and permeability (BCOP) guidelines, evaluated the potential irritation of T-EGF at a concentration of 100 μg/mL to the eyes.

Ultrapure water was used as negative control, and absolute ethanol was used as positive control. The results are shown in Table 2, concluding that T-EGF is classified as non-irritating.

Application of T-EGF in skincare formulations

The T-EGF raw material is a white solid substance obtained through freeze-drying. It can be dissolved into diverse aqueous solutions before adding to the production batch. Its versatility allows for incorporation into various cosmetic formulations, particularly suited for repair and sensitive skin.

T-EGF exhibits potency at very low concentrations. Guidelines regarding its optimal inclusion in cosmetic preparations are detailed in Table 3.

Best practices for T-EGF

Empirical studies revealed several factors that influence the stability of T-EGF formulations. Some key recommendations when applying T-EGF during production are listed below:

- Add T-EGF to the production batch when temperature is below 45°C
- Avoid using protein deactivating substances alongside T-EGF
- Optimal pH range for T-EGF is 4-5.5 or 6-8.5
- Combining with other peptides and moisturizing agents can enhance T-EGF activity
- Energy-generating substances can enhance T-EGF efficacy

TABLE 3: T-EGF BASIC PRODUCT INFORMATION AND DOSAGE RECOMMENDATIONS

Product name	T-EGF
INCI name (T-EGF)	sr-(Hexapeptide-40 Oligopeptide-232 sh-Oligopeptide-1
INCI name of the complete powder	Sodium Chloride Disodium phosphate sr-(Hexapeptide-40 Oligopeptide-232 sh-Oligopeptide-1) Sodium Phosphate
Storage conditions	The freeze-dried powder is protected and can be stored in room temperature (detailed updates expected in Q4 2023)
General production guidelines	Use a T-EGF solution of 100μg/ml at 0.2%-20% in the final product
Applications (final dosage)	Serum (5-10 μg/ml); Sheet masks, patches (0.2-2 μg/ml); Day and night cream (3-5 μg/ml); Lotion (1-2 μg/ml)

Conclusion

Overall, the development of TD1 presents a novel solution in the skincare industry for transdermal delivery of macromolecules. T-EGF stands as a testament to the remarkable possibilities that arise from deeply understanding and harnessing the intricacies of the human body's natural processes.

As research and technology continue to evolve, we expect significant momentum and interest from the industry and anticipate the emergence of more skincare formulations that benefit from inclusion of novel ingredient such as the transdermal epidermal growth factor. **PC**

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