Introduction of Cell Transducible Growth Factor for skin renewal

SP1-EGF & SP1-FGF1

ADBIOTECH & Bioceltran
Aim of the Study

Development of Cell Transducible Growth Factors

1) Skin Permearble Epidermal Growth Factor (PTD-EGF)
2) Skin Permearble Fibroblast Growth Factor (PTD-FGF)
3) Skin Permearble Superoxide Dismutase (PTD-SOD)
4) DDS technology of skin permeable therapeutic humanized antibodies

We aim to develop cosmeceuticals which cover from skin care, skin regeneration to burn injury through the development of cell transducible growth factors with PTD technology.
The Superiority and Creativity of PTD Technology
- Increase skin regeneration by improving skin permeability

◆ Comparison with the third party product

Cosmetics containing even very small amounts of EGF, FGF is quite expensive in the current market. Notwithstanding the high price, protein itself is very difficult to permeate through the skin so liposome formulated one is widely used. However, liposomal formulation also has its limitation. Thus we improved those limitation with Protein Transduction Domain (PTD) Technology.

As shown in the figure, PTD conjugated product (protein) showed better permeability compared to existing liposome. (Green fluorescence represent protein permeated deeply into the skin)
Introduction of PTD Technology

ADBIOTECH & Bioceltran
The Structure of Human Skin

1. **Epidermis**
   is the outermost layer of the skin and protective wrap over the body's surface

2. **Dermis**
   is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain.

3. **Subcutis**
   Due to the protective role of dermis and epidermis, absorption of skin care cosmetics is not easy
**EGF (Epidermal growth factor)**

*Fount at 1986 by Stanley Cohen; Nobel Prize*

1. **Structural Characteristics**
   - Consists of 53 amino acids
   - Molecular Weight 6.2kDa
   - 3 S-S bond (Disulfide bond)
     (6-20, 14-31, 33-42)

2. **Function**
   - Angi-aging & Improve pre-made winkles by skin regeneration
   - Improve skin tone
   - Soft Skin, Brightening and Rejuvenation
   - Remove skin scar by forming new epidermis

**FGF (Fibroblast growth factor)**

1. **Structural Characteristics**
   - Consist of 155 amino acids
   - Molecular Weight 17.3kDa

2. **Function**
   - Prevent winkles, aging, dry skin by synthesizing Collagen, Elastin and Glucosaminoglucan under the skin.
   - Promote skin elasticity by acting on the cells in the dermis.
Macromolecules such as Protein, DNA and Peptide are very difficult to transfer into the cells. However, PTD (Protein Transduction Domain) technology enables effective transfer of these macromolecules possible. Adbiotech developed 1:1 conjugated PTD and Growth factors such as PTD-EGF and PTD-FGF.

Adbiotech’s PTD technology is superior in terms of using native protein and superior permeability.
Core Technology

1. PTD+Growth Factor (EGF, FGF1)

2. Penetration

3. Efficient Delivery

4. Skin Renewal

Core technology of this product is PTD technology enabled macromolecules transfer through the skin into the dermis which results in the increase of skin elasticity and alleviation of skin wrinkles (SP1-EGF & SP1-FGF1), and maximize antioxidant effect (SP1-SOD)
The Fusion Technology of PTD-Growth Factor

We have

The world 1st fused PTD- Growth Factor Expression Technology
Recombinant fusion protein purification Technology
Refolding Technology while keeping protein activity
Advanced assay system for physiological activity
Expression of SP1-EGF, SP1-FGF1

This data shows that recombinant E-coli expressed right EFG and FGF by western blotting.
Activity of EGF & FGF1

(A) PEP-1-EGF

![Image of phosphorylation status of EGF receptor](image)

Phospho EGF receptor (Tyr1068)

(B) FGF1-PEP-1

![Image of phosphorylation status of FRS2-alpha](image)

Phospho FRS2-alpha (Tyr196)

The data above shows the dose dependent activity of PEP-1-EGF, FGF1-PEP-1 by showing phosphorylation status of EGF receptor as low as 10ng/ml concentration.
Effects of SP1-EGF & SP1-FGF1 in cell proliferation

A: Control
B: SP1-EGF
C: SP1-PEP
D: SP1-EGF + SP1-FGF1 Mixture

The data above shows the proliferative activity of SP1-EGF, SP1-FGF1 in cells. Compared to control A, all the combination of growth factors showed proliferative effects.
The effects of SP1-EGF & SP1-FGF1 in Cell Proliferation

The figure above is representative bar graph of results 4. There is dose dependent proliferative effects from the concentration of 0.01ng/ml.
The effects of SP1- EGF & SP1-FGF1 in Collagen Synthesis

The graph above represents the ability of EGF and FGF in collagen synthesis. Collagen is distributed and acted in the dermis layer of the skin. SP1-FGF1 (FGF1-PEP-1) showed the best collagen synthesis when measured its messenger RNA levels.
The result above shows comparative results between PTD conjugated and non-conjugated growth factor. Green fluorescence in the left panel represents cell-tranduced growth factor inside cells.
SP1- EGF & SP1- FGF1: Degrees of Skin Permeability

The results above represent microscopically observed cross section of skin after fluorescent labelled SP1-EGF, SP1-FGF1 treatment to see the degree of tissue permeability. In contrast to other EGF and FGF, SP-EGF and SP-FGF showed superior permeability compared to others.
Regenerative effects of SP-EGF & SP-FGF1 against UV damage in the mouse skin

This figure shows the regenerative effects of SP1-EGF and SP1-FGF1. Although there was a minor recovery in the vehicle control group, regenerative effects of Adbiotech’s growth factor treated group showed much better efficacy in the damaged skin.
SP1- EGF & SP1-FGF1: Regenerative Effect against skin damage from UV

A : Sham  
B : UV  
C : EGF + FGF1  
D : SP1-EGF  
E : SP1-FGF1  
F : SP1-EGF + SP1-FGF1

This figure is microscopically investigated cross section of damaged skin in the area of epidermis and dermis after UV irradiation. Thick purple color area represent the degree of damage. SP1-EGF and SP1-FGF1 treated samples show the recovery of tissue damage in epidermis and dermis.
SP1- EGF & SP1-FGF1: Regenerative Effect against skin damage from UV

This graph representative bar graph of histological result. When compared to sham group (A), SP1-EGF+ SP1-FGF1 cotreatment showed synergic effect against damage from UV irradiation to the level of sham control.
This figure shows the microscopic comparison results of the EGF, FGF, SP1-EGF, SP1-FGF1 treatment in the re-synthesis of collagen after UV treatment.

Green color represents re-synthesized collagen after treatment of growth factors.

Treatment of SP1-EGF and SP1-FGF1 almost returned skin state before injury is made compared with controls.
Prototype of SP1- EGF containing cosmetics

This proves that SP1- EGF contains proper function Which can be used as a cosmetics.
SP1-EGF
(Skin Permeable - Epidermal Growth Factor)

Characteristics of SP1-EGF

PTD&EGF Fusin Protein

The PTD fusion protein with EGF makes skin permeability better than with protein itself so maximize its effects by deep penetration into the cells.

High Purity and High Activity

With purity more than 95%, suitable as a cosmetic Ingredient and more active than normal EGF.

Safety and Stability

Free from various microbial contamination and allergen. Suitable for the specification. Through the toxicity test, safety is assured. It is proved to be stable up to 6 month through long term stability study.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>SP1-EGF (INCI name: processing)</th>
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<tbody>
<tr>
<td>Source</td>
<td>E. coli</td>
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<tr>
<td>Purity</td>
<td>&gt;95% (HPLC)</td>
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<tr>
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<td>Activity unit</td>
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<td>Formulation</td>
<td>Lyophilized or Solution stock</td>
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<tr>
<td>Concentration</td>
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</tbody>
</table>
Purity of SP1-EGF

SDS-PAGE Western blot

HPLC Analysis

SP1-EGF 98.2%

Application of SP1-EGF

Stable up to 40 degrees in the manufacturing process
**Stability of SP1-EGF**

**SP1-EGF long term storage stability (8 weeks)**

**SP1-EGF Lyophilized Product**

1. EGF No treatment
2. Normal EGF
3. Store at -60°C
4. Store at 4°C
5. Store at 25°C (Room Temperature)

# Treat Conc.: 100ng/ml

**SP1-EGF Solution Product**

1. EGF No treatment
2. Normal EGF
3. Store at -60°C
4. Store at 4°C
5. Store at 25°C (Room Temperature)

# Treat Conc.: 100ng/ml

**Stability of SP1-FGF1**

**SP1-FGF1 long term storage stability (8 weeks)**

**SP1-FGF1 Lyophilized Product**

1. FGF1 No treatment
2. Commercial Sigma FGF1
3. Store at -60°C
4. Store at 4°C
5. Store at 25°C (Room Temperature)

# Treat Conc.: 100ng/ml

**SP1-FGF1 Solution Product**

1. FGF1 No treatment
2. Commercial Sigma FGF1
3. Store at -60°C
4. Store at 4°C
5. Store at 25°C (Room Temperature)

# Treat Conc.: 100ng/ml

Phospho-EGF receptor (Tyr1068)

PhosphoFRS2-alpha (Tyr196)
Clinical evidence of effect for skin renewal

Day 0

Day 28

Clinical picture
(Enlarged)

Replica Image

[A] : SP one Solution
Real Effective Cosmeceuticals

begin at

AD n BC