Anti-aging, Skin rejuvenation

**BIO-Placenta** *Pseudo-Placenta with synergic effects and safety*

Contains five different growth factors (EGF, IGF-1, acidic FGF, basic FGF and VEGF), amino acid and vitamin. These growth factors are safely synthesized.
Commonly known effects of dermatological efficacy of the placenta are **Anti-aging & Skin rejuvenation**

**Fetus**
Fetus obtains all the nutrients and GF made by placenta. GF will stimulate the expansion of cells in the body system.

**0~3 years old**
Infants produce nutritional values and GF on their own.

**10~13 years old**
GF production decreases in body and growth rate starts to slow down.

**More than 18 years old**
GF production and the growth process decreases rapidly. Minimal amount is produced for metabolism.

**Aging is caused by loss of Growth Factors (GF)**

GF decrease and absent of cell replacement leads to aging.

Reference: Placental growth factors, B.V.Rama Sastry, Placental pharmacology, p126~150
BIO-Placenta VS Human Placenta
Components of BIO-Placenta are similar to Human Placenta which is based on ‘Placental Pharmacology’

Synthesized Growth factors
Components are safely synthesized

Proven to contain Growth Factors
The inclusion of growth factors can be found by HPLC analysis

Synergic Effect
Combination of 5 growth factors and essential factors make synergic effect on skin
## BIO-Placenta VS Human Placenta

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Human Placenta</th>
<th>Effect</th>
<th>BIO-Placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>Skin rejuvenation</td>
<td></td>
<td>EGF</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Anti-wrinkle</td>
<td></td>
<td>IGF-1</td>
</tr>
<tr>
<td>acidic FGF</td>
<td>Anti-wrinkle</td>
<td></td>
<td>acidic FGF</td>
</tr>
<tr>
<td>basic FGF</td>
<td>Elasticity enhance</td>
<td></td>
<td>basic FGF</td>
</tr>
<tr>
<td>VEGF</td>
<td>Nutritional supplement</td>
<td></td>
<td>VEGF</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Anti-wrinkle</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>The minority parts</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Essential Factor</td>
<td>Vitamin</td>
<td>Cell vitalization</td>
<td>Vitamin : Vitamin B₉</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Energy supplement</td>
<td></td>
<td>Amino acids : Acetyl Glutamine</td>
</tr>
</tbody>
</table>

Reference: Placental growth factors, B.V.Rama Sastry, Placental pharmacology, p126~150

Components of BIO-Placenta are similar to human placenta
Manufacturing Process of Growth factors

EGF DNA sequence

<table>
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<tr>
<th>Component</th>
<th>DNA Sequence</th>
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<tbody>
<tr>
<td>EGF</td>
<td>5'-GTT ACC AGC GAC TCC GAA TGC CCG CTG AGC CAT GAC GGC-3'</td>
</tr>
<tr>
<td></td>
<td>3'-CAA TTG TCG CTG AGG CTG CGG CGG GAC TCG GTA CCG-5'</td>
</tr>
</tbody>
</table>

Advantages

- Recombinant E.coli
- Fermentation

Injection

Components are safely originated from microbial fermentation
Different from commercial human placenta products, the inclusion of growth factors can be found by HPLC analysis.
Synergic Effect

Advantages

Combination of 5 growth factors and essential factors make synergic effect on skin

Fig. Effect of growth factor singling on wound epithelialization.

Ref; Journal of Cell Science 116, 3227-3248
The migration ability of Keratinocyte is improved by using BIO-Placenta
Stimulation of Cell Growth

MTT assay

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Human Derma Fibroblast neonatal (HDFn, LONZA)</th>
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<tr>
<td>Detection method</td>
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BIO-Placenta is more effective than commercial human placenta product in stimulation of cell growth
Biosynthesis of Collagen

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<tr>
<td>Detection method</td>
<td>Western blot</td>
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In vitro assay

BIO-Placenta stimulates collagen biosynthesis of fibroblasts

Pro-collagen type 1
## SUMMARY OF THE CLINICAL STUDY

<table>
<thead>
<tr>
<th>Subjects of experiment</th>
<th>12 female aged 43 to 50 (average age : 45.75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place of experiment</td>
<td>Constant temperature and humidity room (22±1°C, 45±5%)</td>
</tr>
<tr>
<td>Experimental period</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Test area</td>
<td>Facial region including crow’s feet</td>
</tr>
<tr>
<td>Test sample</td>
<td>BIO-Placenta Cream (BIO-Placenta : 3%)</td>
</tr>
<tr>
<td>Test material application</td>
<td>Spread suitable quantity on facial region including crow’s feet</td>
</tr>
</tbody>
</table>
| Evaluation method               | 1. Evaluating wrinkle improvement through PRIMOS Lite and Robo Skin Analyzer  
                                  | 2. Evaluating skin elasticity improvement through DermaLab elasticity probe and Robo Skin Analyzer  
                                  | 3. Evaluating moisturizing improvement through DermaLab moisture probe  
                                  | 4. Visual evaluating according to global photodamage  
                                  | 5. Evaluation of abnormal response  
                                  | 6. Survey                        |
| Statistic analysis methods      | SPSS 17.0 for Windows                       |
| Test institute                  | Korea Institute for Skin and Clinical Sciences |
Anti-wrinkle

<table>
<thead>
<tr>
<th>Test area</th>
<th>Crow’s feet</th>
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<tr>
<td>Detection method</td>
<td>PRIMOS Lite 3D Face and Skin ScannerAnalyzing System, GFMesstechnik</td>
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Reduces wrinkles by 13% in 2 weeks and 16% in 4 weeks on crow’s feet.
In vivo assay

Anti-wrinkle

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Ra : Average of all heights and depths to the reference plane

Reduces wrinkles by 13% in 2 weeks and 16% in 4 weeks on crow’s feet
Anti-wrinkle

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Reduces wrinkles by 38% in 2 weeks and 46% in 4 weeks on below eyes
In vivo assay

Anti-wrinkle

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Reduces wrinkles by 38% in 2 weeks and 46% in 4 weeks on below eyes
Anti-wrinkle

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Before

Total length of wrinkle: 47mm

After

Total length of wrinkle: 21mm

Reduces wrinkles by 10% in 2 weeks and 21% in 4 weeks on crow’s feet
In vivo assay

Anti-wrinkle

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Reduces wrinkles by 10% in 2 weeks and 21% in 4 weeks on crow’s feet
Skin elasticity has been improved by 32% in 2 weeks and 47% in 4 weeks
Skin Moisturizing has been improved by 83% just after applying, 50% in 2 weeks and 64% in 4 weeks.
Mitogenic effect of cells

Wound healing effect by epithelialization

Growth, differentiation and migration of cells

Wrinkle care

Activation of hyaluronan synthase 2 in human epidermal keratinocyte

IGF-1
Insulin-like Growth Factor-1

- Effect of IGF-1 restoration on phospholipid profile in skin
- Angiogenic effect
- Induction of synergic effect of EGF on wound healing and growth stimulation of fibroblast cell
- IGF-1 increases the synthesis of hyaluronan and chondroitin sulfate proteoglycan by tissue fibroblast


Fig. Effect of growth factor signalling on wound epithelialization.

Ref: Journal of Cell Science 116, 3227-3248
FGF
Fibroblast Growth Factor

- Growth and mitogenesis stimulation of fibroblast cells
- Stimulation of skin re-generation
- Stimulation of Collagen and Elastin synthesis in fibroblast cells
- Wound healing effect
- basic FGF (FGF-2) activates hyaluronan synthesis with IGF-1 in human epidermal fibroblast

**VEGF**

Vascular Endothelial Growth Factor

- Management of capillary vessels
- Induction of nutrient supplements to fibroblast cells
- Growth and migration of cells
- Wound healing effect

The formation of B₉-Vitapol is made through structural changes of folic acid into the natural form of polyglutamate.

It is produced to improve the negative characteristics (pH sensitivity and low solubility) of folic acid through Korean traditional fermentation technology.
Amino acid
Hydracell Q

- Acetylated Glutamine, which is reformulated to improve the stability.

Glutamine → Improve Stability → Hydracell Q

The amines in glutamine is very unstable.

Acetylated amino group
Biological Activity

Analysis of Growth factors

Cell strain: Human Derma Fibroblast neonatal (HDFn, LONZA)
Detection method: MTT assay (Sigma, US)

Comparison of Control, BIO-Placenta, EGF, IGF-1, a-FGF, bFGF, and VEGF.
**Biological Activity**

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**Analysis of Growth factors**

- **BIO-Placenta**
- **EGF**
- **IGF-1**
- **acidic FGF**
- **basic FGF**
- **VEGF**

**Graphs**

- Growth rate (%)
- BIO-Placenta (%)
- EGF (ng/ml)
- IGF-1 (ng/ml)
- aFGF (ng/ml)
- bFGF (ng/ml)
- VEGF (ng/ml)
rh-EGF
Recombinant Human Epidermal Growth Factor
RP- HPLC chromatogram & SDS-PAGE analysis

Lane M: Protein size marker
Lane 1: Bioprogen EGF
Lane 2: Authentic EGF
HPLC analysis
Spike assay

![Graph showing HPLC analysis of Bioprogen EGF, Authentic EGF, and Bioprogen EGF + Authentic EGF.](image)
MALDI-TOF analysis

MW of Bioprogen EGF : 6.2 kDa
Theoretical MW of authentic EGF : 6.2 kDa
rh-IGF-1
Recombinant Human Insulin-like Growth Factor-1

Analysis of Growth factors
SDS-PAGE analysis

Lane M: Protein size marker
Lane 1: Bioprogen IGF-1

Human recombinant IGF-1 (7.7 kDa)
Biological Activity of IGF-1

Condition

- Cell line: NIH3T3
- Inoculum: 1X10^4 cells/ well [96well plate]
- IGF incubation time: 24h
- BrdU incubation time: 5hrs

ED_{50} : 7 ng/mL

<table>
<thead>
<tr>
<th>IGF conc. [ng/mL]</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1397</td>
<td>0.6306</td>
</tr>
<tr>
<td>5.5731</td>
<td>0.6368</td>
</tr>
<tr>
<td>6.0430</td>
<td>0.6435</td>
</tr>
<tr>
<td>6.5525</td>
<td>0.6505</td>
</tr>
<tr>
<td>7.1050</td>
<td><strong>0.6579</strong></td>
</tr>
<tr>
<td>7.7040</td>
<td>0.6657</td>
</tr>
<tr>
<td>8.3536</td>
<td>0.6737</td>
</tr>
<tr>
<td>9.0580</td>
<td>0.6819</td>
</tr>
<tr>
<td>9.8217</td>
<td>0.6901</td>
</tr>
</tbody>
</table>
rh-acidic FGF / rh-basic FGF
Recombinant Human Fibroblast Growth Factor
SDS-PAGE analysis

Lane M : Protein size marker
Lane 1 : Bioprogen aFGF

Human recombinant acidic FGF (20 kDa)

Lane M : Protein size marker
Lane 1 : Bioprogen bFGF

Human recombinant basic FGF (17.3 kDa)

Analysis of Growth factors
rh-VEGF
Recombinant Human Vascular Endothelial Growth Factor
MALDI TOF & SDS-PAGE analysis

Human recombinant VEGF (16.3 kDa)

Lane M: Protein size marker
Lane 1: Bioprogen VEGF
Definition
Contains five different growth factors (EGF, IGF-1, acidic FGF, basic FGF and VEGF), amino acid and vitamin. These growth factors are safely synthesized.

INCI
Water, Lecithin, Acetyl glutamine, sh-Oligopeptide-1, sh-Oligopeptide-2, sh-Polypeptide-1, sh-Polypeptide-9, sh-Polypeptide-11, Bacillus/soybean/folic acid ferment extract, Sodium hyaluronate, Caprylyl glycol, Butylene glycol, 1,2-Hexanediol

Physical characteristics
Liquid form of liposome

Benefits & Uses
Anti-wrinkle
Skin rejuvenation
Elasticity enhancement
Moisturizing
Cell vitalization

Application
Skin, Body and Hair care

Recommended dosages
3.0%