BIOTIDE, a vitamin-peptide hybrid, a new approach for dermal niche control

Incopharm Corporation
Growth Hormone

- A 191-amino acid, single-chain polypeptide
- Peptide hormone that stimulates growth, cell reproduction and regeneration in humans and animals
- Synthesized, stored, and secreted by the somatotroph cells within the lateral wings of the anterior pituitary gland
- Growth Hormone activities include:
  - Increases protein synthesis
  - Plays a role in homeostasis
  - Through JAK-STAT signaling pathway, the production of IGF-1
  - Stimulates the immune system
Hexapeptide-2 (GHRP-6)

- His-dTrp-Ala-Trp-dPhe-Lys-amide
- One of several synthetic met-enkephalin analogs
- Potent stimulator of GH release
- Distinct from growth hormone releasing hormone (GHRH) in that they share no sequence relation and derive their function through action at a completely different receptor, called the GH secretagogue receptor.
- Benefits of increased Growth Hormone levels through GHRP-6 include:
  - an increase in strength
  - muscle mass and body fat loss
  - increase of fat accumulation in adipocytes
  - rejuvenation and strengthening of joints
  - connective tissue and bone mass
- Stability issues
Your Ingredients for Cosmetic and Pharmaceutical Applications

Biotin – vitamin B7

- 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid
- Water-soluble B-complex vitamin (vitamin B7)
- Coenzyme in the metabolism of fatty acids and leucine
- Necessary for cell growth, production of fatty acids, and metabolism of fats and amino acids
- Biotin is often recommended for strengthening hair and nails.
- Deficiency in biotin results in the development of:
  - Hair loss (alopecia)
  - Conjunctivitis
  - Dermatitis
  - Breaking, chipping, or flaking nails
  - Eczema and seborrheic dermatitis (children)
A Conjugate of Biotin and Hexapeptide-2

⇒ Trade Name: “Biotide”
Patent #: 2666780 (EU), 9,180,082 (US)
INCI Name: Biotinoyl Hexapeptide-2 Amide
Your Ingredients for Cosmetic and Pharmaceutical Applications

- **Activity by hexapeptide-2 and growth hormone**
  - increase in cellular total collagen synthesis
  - increase in muscle mass
  - increase of fat accumulation in adipocytes by stimulating growth hormone secretagogue receptor (GHSR)
  - rejuvenation of connective tissue

- **Activity by biotin**
  - Skin rejuvenation (anti-aging)
  - relief from dermatitis
  - relief from alopecia

⇒ Use as skin anti-ageing agent
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Cell viability was measured by collagen type-1 assay using neonatal HDF cells. Results indicate that only Biotide showed significant collagen synthesis stimulating activity whereas hexapeptide-2, biotin, hexapeptide-2+biotin mixture did not have collagen synthesis stimulating activity.
Cells: HDFn, passage 10, 3000 cells/well/96 well plate
Method: Cells were treated with product A or Biotide 36 hours after seeding, and supernatant were collected for assay 1, 2, 4, 8 and 24 hours after treatment.

Results: Biotide showed higher collagen synthesis activity than product A.

Additional studies: Earlier passage cells will be also used for assays.
Cell viability was measured by collagen type-1 assay using neonatal HDF cells. Results indicate that Aquestide 2 showed significant collagen synthesis stimulating activity up to 100 mM concentrations.
Biotide

3-D Artificial Skin Test

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Collagen synthesis tested in *in vitro* 3-D human epithelial TISSUE equivalents

Methods: The EpiDermFT™ Skin Model (MatTek, USA) was used to test product M and biotide

Results:
1. Biotide actually can penetrate into the epidermis and dermis.
2. Biotide showed significantly higher collagen producing activity compared to product M.
3D culture : Epidermal/Dermal 3D culture system
Condition : 3D culture system were treated w/ or w/o biotide and cultured for 7 days, and tissue were fixed and stained using Masson Trichrome method (red : keratin, blue : collagen, black : nuclei)
Results : **Biotide treated skin tissue produced 50% higher of collagen production level**
Collagen producing dermis (fibroblast) layer is significantly healthier, indicating reduction of wrinkles
Keratinocyte-Conditioned Media Regulate Collagen Expression in Dermal Fibroblasts

Abdi Ghaffari1,2, Ruhangiz T. Kilani1,2 and Aziz Ghahtay1

Excessive extracellular matrix (ECM) production during dermal wound healing often leads to fibrotic conditions such as keloids and hypertrophic scarring (HSc). Type I collagen is the predominant form of collagen in the human skin and is produced mainly by dermal fibroblasts. It has been suggested that abnormalities in epidermal-dermal interaction can lead to excessive production of collagen by fibroblasts. To identify and further characterize any possible keratinocyte-derived collagen-inhibitory factors (KD-CIFs), we investigated the expression of pro-α1(I) collagen at the level of mRNA and protein in human fibroblasts that had been either co-cultured with keratinocytes or treated with keratinocyte-conditioned medium (KCM). Fibroblasts in both groups demonstrated a significant reduction in the steady-state levels of collagen mRNA and protein. Further characterization of KD-CIFs revealed a high-molecular-weight factor (>30 kDa) that showed stable activity at high temperature (56°C) and acidic pH (pH 2). Keratinocyte differentiation did not alter the release of KD-CIFs into KCM. These results provide further evidence that type I collagen expression and synthesis in fibroblasts are regulated by a keratinocyte-releasable factor(s) with an apparent molecular weight between 30 and 50 kDa.

*Figure 3. Crude determination of KD-CIF molecular weight.* (a) KCM was passed through a 50 kDa cutoff centrifugal filter, and corresponding filtrate and retentate were used to treat dermal fibroblasts. Total KCM and non-conditioned medium (NCM) were also used as positive and negative controls, respectively. COL1A1 expression level was determined by northern blot analysis. Total KCM (lane 1), retentate (lane 2, >50 kDa), and filtrate (lane 3, <50 kDa) of 50 kDa cutoff filter, as well as the same fractions for NCM, are shown here. (b) The same experimental protocol as above but with a 30 kDa cutoff filter. Total KCM (lane 1), retentate (lane 2, >30 kDa), and filtrate (lane 3, <30 kDa) of 30 kDa cutoff filter and corresponding fractions of NCM are presented. Expression of 18S ribosomal RNA was used as a control for loading. (c) Graph represents the mean ratio of COL1A1/18S RNA (percentage of control, which is NCM-treated cells) ± SD of four separate experiments. *P<0.01 and **P<0.001 between control and KCM or KCM fraction of 30-50 kDa treated cells, respectively.

**Dermal Niche Control by Biotide**

- Biotide treated (Biotide) and non-treated (blank) keratinocyte cell cultured media (KCM) were used to culture fibroblast cells. Fibroblast cell proliferation and collagen synthesis were measured.
- Collagen synthesis in fibroblast cells were suppressed by KCM by 50-60%, whereas collagen synthesis was recovered and increased by Biotide treated KCM.
- This proves Biotide’s role of inhibition of keratinocyte’s control of collagen synthesis in fibroblasts, thus dermal niche control.

**Fibroblast proliferation**

- Serum starvation vs no serum starvation

**Collagen synthesis**

- Serum starvation vs no serum starvation
Biotide stimulates not only collagen gene expression in fibroblast cells, but also keratinocyte cells to secret paracrine factors (FGF2, FGF5 and Interleukins) which activate dermal fibroblast cells to synthesize collagen actively.
• **Biotide increases cell-cell interaction protein expression**
  - 100 uM Biotide was treated to keratinocytes, and tight junction proteins as well as signal transduction protein expression was measured. Collagen IV, Collagen XVII, Laminin-5 and Integrin-α6 were increased.
100 ppm (5%) of Biotide 2000 was applied daily for 9 weeks

**Fine Wrinkle Reduction**

Before

4 weeks

9 weeks
• 100 ppm (5%) of Biotide 2000 was applied daily for 5 weeks

**Before**

**5 weeks**
Your Ingredients for Cosmetic and Pharmaceutical Applications

PRODUCT: Biotide (INCI Name: Biotinoyl Hexapeptide-2 Amide)
LOT NUMBER: ICP.AVB-0714-1
SEQUENCE: Biotin-HwAWfK-NH2
MOLECULAR FORMULA: C56H70N14O8S1

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<th>TEST</th>
<th>SPECIFICATION</th>
<th>RESULT</th>
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<tr>
<td>Peptide Identification by HPLC</td>
<td>&gt; 90% at 230nm</td>
<td>&gt; 93.60%</td>
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<tr>
<td>Appearance</td>
<td>White amorphous powder</td>
<td>Conform</td>
</tr>
<tr>
<td>Odor</td>
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<td>pH at 0.1mg/ml in water (22°C)</td>
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PRESERVATIVE INFORMATION: Contains no preservatives.
STORAGE CONDITIONS: Store at room temperature (15 to 25°C), away from direct sunlight. Use the whole package after opening. Otherwise, store at -20°C. Microbiological control recommended before reusing.
SHELF LIFE: 30 months under normal storage conditions, in original unopened containers.
**Biotide 2000**

Specifications - Solution

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**PCPC/INCI Name**

- Biotinoyl hexapeptide-2 amide

**Application**

- Anti-ageing
- Anti-wrinkle

**Recommended Dosage**

- 2~5% for cream, cream, lotion and essence formulation
- 0.1~1% for masksheets

**Composition**

- 0.2% Biotide
- 2.0% 1,2-Hexanediol
- 97.8% Water