DERMA-LUCIA’S NEUROPEPTIDES FOR ANTI-AGING

Derma-Lucia Skinceuticals LLC
BIO-FD&C Co., Ltd

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The Technology of Designing and Synthesis for Functional Peptides

Designed advanced peptides for anti-aging based on structural biology
Neuropeptides, Phytochemical fused peptides, Self assembled peptides, Nanoparticle binding peptides, and TDP

Peptides are short amino acid sequences that are components of proteins, such as collagen and growth factor. Peptides are functional molecules to perform biological actions in our body.

Advantages of Peptide as Cosmetic Active Ingredients

- High stability compare with protein, due to the short polymer chains.
- Economical synthesis than recombinant proteins.
- Prolonged activity in the cosmetic formula.
- Very low biological toxicity.
- Can be enhanced the efficacy by its sequence repertoire for anti-aging.

Solid-phase peptide synthesis (SPPS) is the most common technique for peptide synthesis. Usually peptides are synthesized from the carbonyl group side (C-terminus) to amino group side (N-terminus) of the amino acid chain by their sequence repertoire in this SPPS method.
**Peptide Manufacturing Process**

**Procedure of Peptide Synthesis**

1. Resin swelling
2. Amino acid activation
3. Amino acid conjugation
4. Completion of amide reaction by its sequence repertoire
5. Cleavage from peptide-conjugated resin
6. Concentration/Crystallization

**Purification & Lyophilization**

1. Purification by Prep HPLC
2. Freeze drying
3. Screening in biological tools

**Aging**

**Active skin dynamics**
- High synthesis rate of collagen and elastin
- Keeping of ample water-holding proteins

**For anti-aging**
- Exogenous phytochemicals, peptides, and growth factors

**Aged Skin**
- Decreased expression level of growth factors
- Decreased epidermal thickness
- Reduced skin elasticity and plasticity through fragmentation of elastic fiber

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**Wound healing process in human skin**

**Exogenous peptides and growth factors for anti-aging**
- Peptides: Neuropeptide, Self-assembled peptide, Oligopeptides
- Growth factors: EGF, bFGF, IGF-1, PDGF, VEGF, KGF

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**Young Skin**

**Active skin dynamics**
- High synthesis rate of collagen and elastin
- Keeping of ample water-holding proteins

**Young Vs Aged Skin**

**Fat**
- Platelet plug
- Blood vessel
- Fibrin clot
- Macrophage
- Neutrophil
- Neutrophil
- Fibroblast
- Fibroblast
- FGF-2
- IGF
- KGF
- PDGF
- PDGF BB
- VEGF
- PTGF-β1
- TGF-β1
- TGF-α
- TGF-α
- TGF-β1

**Dermis**
- Epidermis
- Platelet plug
- Blood vessel
- Fibrin clot
- Macrophage
- Neutrophil
- Neutrophil
- Fibroblast
- Fibroblast
- FGF-2
- IGF
- KGF
- PDGF
- PDGF BB
- VEGF
- PTGF-β1
- TGF-β1
- TGF-α
- TGF-α
- TGF-β1

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Neuropeptides

Mediators and sensitization pattern of nociceptive and pruriceptive neurons in the skin.

Sensitizing and activating mediators in the skin target receptors on primary afferent nerve fibers involved in itch and pain processing. During inflammation, mechanoinsensitive "sleeping" nociceptors and itch histamine-sensitive mechanoinsensitive preceptors and probably mechanosensitive preceptors transmit the response to the spinal cord. In the spinal cord noxious input can induce central sensitization for pain, and perceptive input can provoke central sensitization for itch. Via the contra lateral tractus spinothalamicus, the stimuli from primary afferent sensory nerves will be transmitted to specific areas in the CNS.

Neuropeptides

Leu-Enkephalin → Delta Opioid Receptor (DOR)
Met-Enkephalin

α-Neoeendorphin → Kappa Opioid Receptor (KOR)
β-Neoeendorphin
Dynorphin A

α-Endorphin → Mu Opioid Receptor (MOR)
β-Endorphin
γ-Endorphin

The opioid receptors are G-protein coupled receptors (GPCRs) that inhibit neurotransmitter release by reducing $\text{Ca}^{2+}$ ion currents and increasing $\text{K}^+$ ion conductance.

Opioid receptors modulate a range of brain functions, including instinctive behavior and emotions and skin functions including wound healing and senescence.
Opioid Receptor

Opioids bind to specific receptor molecule that mediates its effects. Several opioid specific receptors have been cloned: Mu (μ), Kappa (κ), and Delta (δ) receptors. These receptors belong to G protein-coupled seven transmembrane receptor family. The amino acid sequences are approximately 65% identical among these receptors, but they have little homology with other G protein-coupled receptors.

1. μ, δ, and κ are functionally coupled to pertussis toxin sensitive heterotrimeric G proteins (Gi) to inhibit adenylyl cyclase activity.
2. Activates receptor-activated K⁺ currents which increase K⁺ efflux (hyperpolarization) reduces voltage-gated Ca⁺⁺ entry.
3. Hyperpolarization of membrane potential by K⁺ currents and inhibition of the Ca⁺⁺ influx prevents neurotransmitter release and pain transmission in varying neuronal pathways.

Mechanism of Opioid Receptor Function
Neuropeptides

What's different between our Neuropeptide and Botox like peptide?

Acetyl Hexapeptide

Muscles are contracted when they receive neurotransmitter released from inside a vesicle. The SNARE (SNAP REceptor) complex is essential for this neurotransmitter release at the synaps.

It is a ternary complex formed by the proteins VAMP, Syntaxin and SNAP-25 (SyNaptosomal Associated Protein). This complex is like a cellular hook which captures vesicles and fuses them with the membrane for the release of neurotransmitter.

Acetyl hexapeptide is a mimic of the N-terminal end of SNAP-25 which competes with SNAP-25 for a position in the SNARE complex, thereby modulating its formation. If the SNARE complex is slightly destabilized, the vesicle can not release neurotransmitters such as acetylcholine efficiently and therefore muscle contraction is attenuated, preventing the formation of lines and wrinkles (see right figure).

Neuropeptides

- Derma Lucia Peptides
- Acetyl Hexapeptide

- Active site
- Deeper active site than Neuropeptide's
- More difficult in skin penetration
Neuropeptide

Irritation Data of Peptides

Skin Irritation Test

1. Distilled Water (DW) as negative control
2. 10% Sodium Lauryl Sulfate (SLS) as positive control
3. Neuropeptide-1
4. Neuropeptide-2
5. α-Endorphin
6. γ-Endorphin
7. Neoendorphin
8. Met-Enkephalin
9. Caffeoyl Neuropeptide-1
10. Gold-binding Peptide-1

(Treated Samples to Volunteers after 48hrs)
Neuropeptides

Skin Cell Images on Culture for MTT Assay of Neuropeptides

Control  Met-Enkephalin  Neoendorphin  Neuropeptide-1

Neuropeptide-2  α-Endorphin  CA-Neoendorphin
The INCI name of the Decapeptide-9 is changed to the sh-Decapeptide-9 by PCPC.
Neoendorphin is a sort of neuropeptide which are found in neural tissue. Neuropeptides are enkephalin, neoendorphin, \(\alpha\)-endorphin, \(\beta\)-endorphin, dynorphin, neurotensin, neuromedin U, and somatostatin. These peptides are all released centrally and act on other neurons at specific receptors. Peptide signals play a role in information processing that is different to that of conventional neurotransmitters, and many appear to be particularly associated with specific behaviors. For example, neoendorphin has striking and specific effects on skin retexturizing in signal communication between skin and neural system. Neuropeptide such as neoendorphin acts a driving force to repair scars and wrinkles, to increase production of collagen, to accelerate wound healing effect.
MTT Assay of Neoendorphin

Proliferation Effect of Neoendorphin on Human Skin Cell (Keratinocytes, HaCaT)

- **Control**
- 1 nM
- 100 nM
- 1 μM
- 5 μM
- 10 μM
- 1 nM EGF

24 hrs Treatment

48 hrs Treatment
Total cellular RNA was isolated from CCD986sk at 24 hour after treating Neoendorphin (20 ppm) cDNA was synthesized by reverse transcription and quantitation real-time PCR was performed to determine PCOLCE RNA levels by using Rotor-Gene Q Series software (QIAGEN, 5-Plex).

Procollagen C-endopeptidase enhancer 1 is an enzyme that in humans is encoded by the PCOLCE gene. This gene encodes a glycoprotein which binds and drives the enzymatic cleavage of type I procollagen and heightens C-proteinase activity.
The levels of mRNAs encoding SIRT-6 was measured by real-time PCR

Total cellular RNA was isolated from CCD986sk at 24 hour after treating Neoendorphin (20 ppm) cDNA was synthesized by reverse transcription and quantitation real-time PCR was performed to determine SIRT-6 RNA levels by using Rotor-Gene Q Series software (QIAGEN, 5-Plex).

Sirtuin-6 (SIRT6) is a stress responsive protein deacetylase and mono-ADP ribosyltransferase enzyme encoded by the SIRT6 gene. SIRT6 functions in multiple molecular pathways related to aging, including DNA repair, telomere maintenance, glycolysis and inflammation.
Clinical Data of Neoendorphin

The clinical result of the neoendorphin contained cosmetic formula shown to reduced wrinkles dramatically after 8 weeks.

The clinical test was performed to volunteers organized by dermatologists in the hospital of Chungang University in S. Korea.
Clinical Data of Neoendorphin

Control

8 weeks later

Control

8 weeks later
### Product Information

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<th>Trade Name</th>
<th>Neoendorphin</th>
<th>Manufacturer</th>
<th>BIO-FD&amp;C Co., Ltd (<a href="http://www.biofdnc.com">www.biofdnc.com</a>)</th>
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<tr>
<td>Contact</td>
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<td>Sang Hyun Moh</td>
<td><a href="mailto:shmoh@biofdnc.com">shmoh@biofdnc.com</a></td>
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| Characteristics | ❖ Synthetic Peptide Materials (A sort of Neuropeptides)  
❖ Solubility: 3g / 100 ml (H₂O)  
❖ High Stability in Solution  
❖ M.W. 1228.4 Da |
| Function | ❖ Wound Healing Effect  
❖ Anti-Wrinkle Effect |
| IP | ❖ Patent Title  
Anti-inflammatory Cosmetic Composition for Wound healing and tissue Regeneration.  
❖ Registered Number  
10-2008-0095633  
❖ Country / Registered Date  
KR / 2008.09.30 |
2. “Activation of Delta Opioid Receptors Induces Receptor Insertion and Neuropeptide Secretion” Neuron  
Vol 37; 121-133 (2003) |
Alpha-endorphin

3D structure of alpha-endorphin

2D structure of alpha-endorphin

alpha-endorphin
Chemical Formula: C_{77}H_{120}N_{18}O_{26}S
Molecular Weight: 1745.95
Human skin fibroblast cells were cultured at a density of $1 \times 10^4$ cells/well into 24-well plates. The culture medium containing DMEM medium, 10% fetal bovine serum, 100 IU/ml penicillin and 100 $\mu$g/ml streptomycin. At 72 hours after plating, they were washed once with PBS (pH 7.4) and exposed to UVB radiation in a thin layer of PBS using three Philips TL 20W/01 lamps, emitting UVB peaking at 311 nm, which were placed 30 cm above the flasks. The emitted radiation was checked under the flask lid using a UVR radiometer with a UVB sensor. After irradiation, PBS was replaced by DMEM+10%FBS. The radiation stress was performed twice a day for 5 days. Control cells were kept in the same culture conditions without UVB exposure.

**Real time RT-PCR**

At 72 hours after the last stress, total cellular RNA was isolated from CCD986sk after treating $\alpha$-endorphin(20ppm). cDNA was synthesized by reverse transcription and quantitation real-time PCR was performed to determine **IL-1$\beta$, p21, JNK-2, PCNA**. RNA levels by using Rotor-Gene Q Series software (QIAGEN, 5-Plex).

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<th>Gene</th>
<th>Name</th>
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<td>Interleukin 1 beta</td>
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<td>p21</td>
<td>Cyclin dependent kinase inhibitor 1A</td>
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<td>JNK-2</td>
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<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
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Effects of α-endorphin in Human fibroblast to 10 exposures of UVB at 250 mJ/cm² per exposure on the mRNA level of senescence- and cell cycle-associated genes.

- Control
- Control-UVB
- α-endorphin(20ppm) -UVB

**Genes**
- IL-1β
- p21
- JNK-2
- PCNA
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“A neuropeptide courier for delta-opioid receptors?” by D. Julius, A. Basbaum in *Cell* (2005) Volume 122, Pages 496-498


“Activation of Delta Opioid Receptors Induces Receptor Insertion and Neuropeptide Secretion” by Z. Xu *et al.* in Neuron (2003) Volume 37, pages 121-133


