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Natural matrikine-like peptides for skin rejuvenation

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Abstract

A person's perceived age is determined by physical traits on face and neck. These traits mostly reflect the integrity of the extracellular matrix, which provides structure, homogeneity and elasticity to skin.

Matrikines are dermal messenger peptides that instruct skin cells to reorganize and build up the extracellular matrix. So far, synthetic matrikines have been used in cosmetics. Now, for the first time, Lipoid-Kosmetik AG investigated and isolated natural matrikines-like peptides from cedar nuts and presents them in a novel anti-aging concentrate: PhytoCodine®.

Here we show that plant-derived, matrikine-like peptides mimic the activity of matrikines that naturally occur in our skin. Plant-derived, matrikine-like peptides activate the formation of extracellular matrix components, optimize skin structure, and improve age-related properties of mature skin, such as elasticity, density, wrinkles and sagging.

Altogether, PhytoCodine® is the first concentrate of plant-derived, natural matrikine-like peptides. It reprograms fibroblasts to build up extracellular matrix components characteristic of younger looking skin and thereby directly contributes to a person's perceived age.

Our skin is an integrated network of cells that communicate with each other using biological messengers - small molecules that carry information. Cellular signaling and cell-to-cell communication control e.g. inflammation, skin repair, melanogenesis or aging. Examples of signaling messenger molecules in skin are cytokines, neurotransmitters, growth factors, or matrikines, which freely diffuse between cells and transmit their messages, for example by binding to cell surface receptors.[1] With age, the activity of signaling and communication declines. This strongly affects the extracellular matrix impacting skin texture and topography.[2]

Synthetic matrikines and their use in cosmetics

Matrikines are one type of dermal messengers that naturally occur in our skin. Matrikines are short peptides with defined amino acid sequences. Depending on their sequence, matrikines activate intracellular signaling pathways that lead to cellular events, such as cell proliferation, protein synthesis, or matrix formation.[3,4]

Matrikines promote ECM synthesis. The extracellular matrix (ECM) consists of a network of interwoven macromolecules that give structure and elasticity to skin. Dermal fibroblasts are the factories of extracellular matrix proteins, producing collagen, elastin, or fibronectin. Matrikines regulate fibroblast activity by binding to specific receptors on their cell surface,

Figure 1: Lipoid Kosmetik's technology produces natural matrikine-like peptides and makes them available for cosmetic applications. Proteases generate unique sets of short peptides from cedar nut proteins with an ideal average amino-acid chain length of about 4 amino acids, which were analyzed by mass spectrometry, de novo peptide sequencing. Using homology searches - matching original matrikine peptide sequences of our skin with cedar nut peptide sequences – we revealed several matrikine homologs (peptides with very similar sequences). Peptide sequences in the illustration are read from left to right using the amino acid 1-letter code.[10]

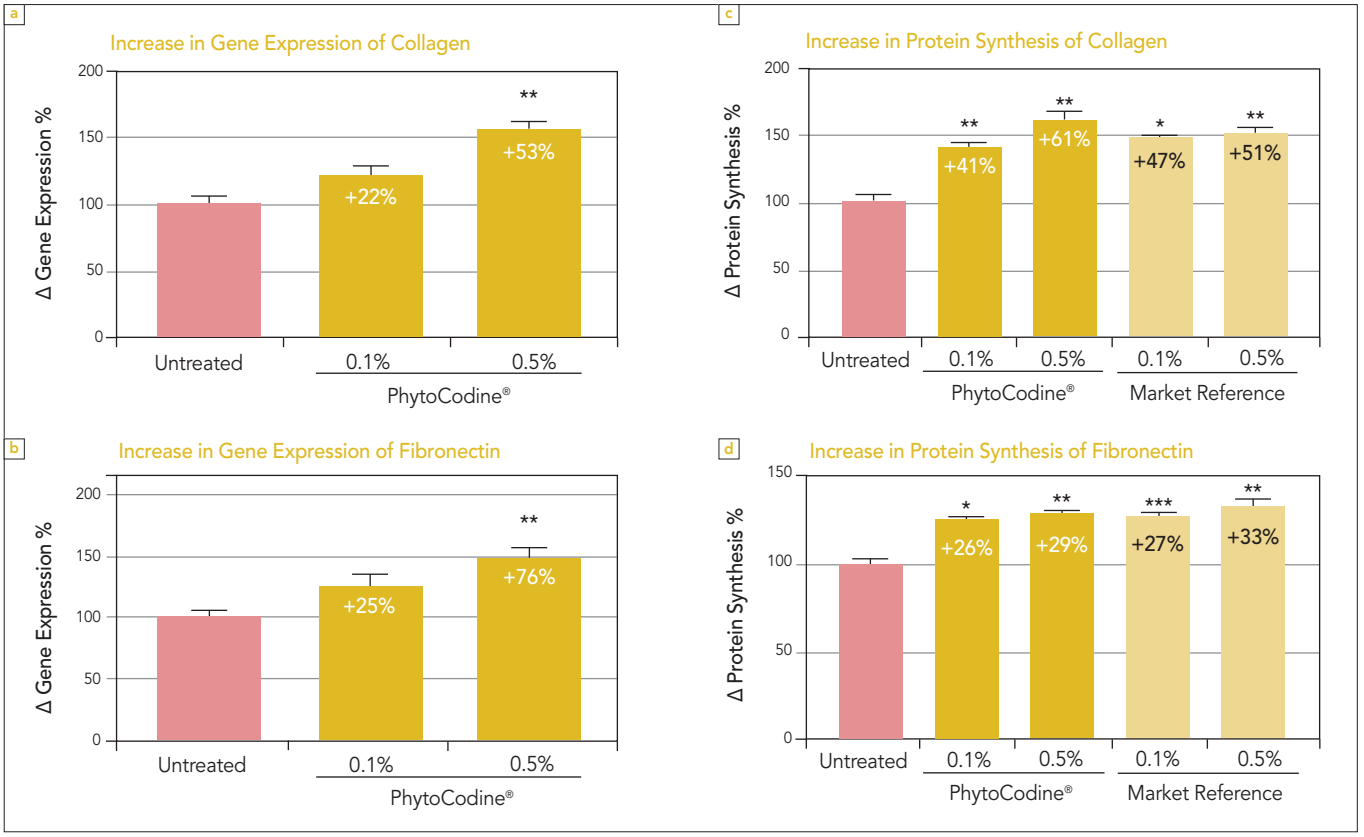

Figure 2: Plant-derived, matrikine-like peptides activate ECM genes and protein synthesis in a dose dependent manner. In a cell culture assay, primary human dermal fibroblasts were treated for 48h with the active or a commercially available matrikine market reference. (A, B) 0.5 % of the active ingredient activated collagen gene expression by > 50 % and fibronectin gene expression by > 75 % versus untreated. (C, D) Similarly, 0.5 % of the active ingredient increased the production of the extracellular matrix proteins pro-collagen and fibronectin by 61 % and 29 %, respectively. As expected, plant-derived, matrikine-like peptides performed as good as the market reference (a commercially available, synthetic matrikine). N = 3; Mean + SEM; Student's t-test vs. baseline; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

initiating a series of intracellular processes that finally lead to enhanced ECM production. This way, matrikines directly contribute to skin elasticity, strength and resilience.

Synthetic matrikines are a success in cosmetics. Three specific matrikines have been shown to stimulate the formation of ECM components when used in cosmetic preparations, including collagen, I, III, and VII, elastin, or fibronectin - all of which deplete considerably when skin ages.[2] Not surprisingly, they have been used in anti-aging preparations.[1,5]

A novel technology to produce natural matrikines

Lipoid-Kosmetik AG developed a unique technology to make natural matrikine-like peptides available for cosmetics. We systematically searched and identified matrikine-like peptide sequences from cedar nuts.

In a first step, we pre-screened diverse plant resources for criteria based on proteomics, amino acid profiles, appropriate protein amount, as well as scientific literature, and selected cedar nut as the best natural source for matrikine-like peptides. In a second step, we tested a variety of proteases (enzymes that cleave proteins at defined positions) and generated several unique sets of peptides. Finally, we sequenced each set and fed thousands of peptides into a database. Using a homologous search (BLAST - Basic Local Alignment Search), we identified one set containing several cedar nut peptides with strong homology to naturally occurring matrikines of our skin (Fig.1).

Matrikines are small molecules that must penetrate skin layers to reach dermal fibroblasts - their point of action. Therefore, PhytoCodine® (now referred to as 'the natural matrikine-like peptide') comes with a liposomal carrier system for hydrophilic active ingredients that enhances penetration. In fact, *in vivo* studies using Raman spectroscopy demonstrate that penetration of model actives increased by 50% after topical application.

Matrikine peptides are sensitive to proteolytic degradation. Protease activity in the extracellular space quickly cleaves peptides, extinguishing their biological activity. Likewise, collagen and elastin are sensitive to proteolytic degradation. Therefore, the natural matrikine-like peptide contains natural eleutherosides, potent protease inhibitors from Siberian ginseng (*Eleutherococcus senticosus*).

In vitro activity: Activation of extracellular matrix protein synthesis

We analyzed the effect of the active on gene activation and protein synthesis of two major extracellular matrix (ECM) proteins, collagen and fibronectin. Gene expression was tested by quantifying RNA transcripts using qPCR. Protein production was tested by quantifying protein release using ELISA. As a result, the active mimics the function of matrikines that naturally occur in our skin. It activates extracellular matrix gene expression and protein synthesis (Fig 2) confirming that plant-derived, matrikine-like peptides are active and functional.

In vitro activity: plant-derived, matrikine-like peptides optimize skin structure

We studied the effect of plant-derived, matrikine-like peptides on skin texture by analyzing the morphology and structural integrity of skin, together with the organization and distribution of extracellular matrix proteins like elastin and collagen. As a result, the skin surface appears smoother after treatment with plant-derived, matrikine-like peptides, and the distribution of elastin, the skin's major component of elastic fibers, is more

regular. Further, collagen I and III fibers appear denser and finer textured than in the untreated control (not shown).

In vivo activity: plant-derived, matrikine-like peptides improve age-related properties of facial skin

We studied the rejuvenating effects of plant-derived, matrikine-like peptides on mature facial skin with two approaches: First, in a placebo-controlled, instrumental assessment we focused on age-related, biomechanical parameters (Fig 3). And in a second approach, we analyzed skin topography (Fig 4).

Summary: Action of plant-derived, matrikine-like peptides

In chronological aging, the capacity of fibroblasts to secret extracellular matrix protein declines. At the same time, elastin and collagen loose organization. This compromises skin elasticity and resilience and finally contributes to the appearance of wrinkles.

Plant-derived, matrikine-like peptides improve the synthesis, organization and distribution of elastic fibers in the extracellular matrix (ECM) and contribute to a more youthful appearance of the skin. PC

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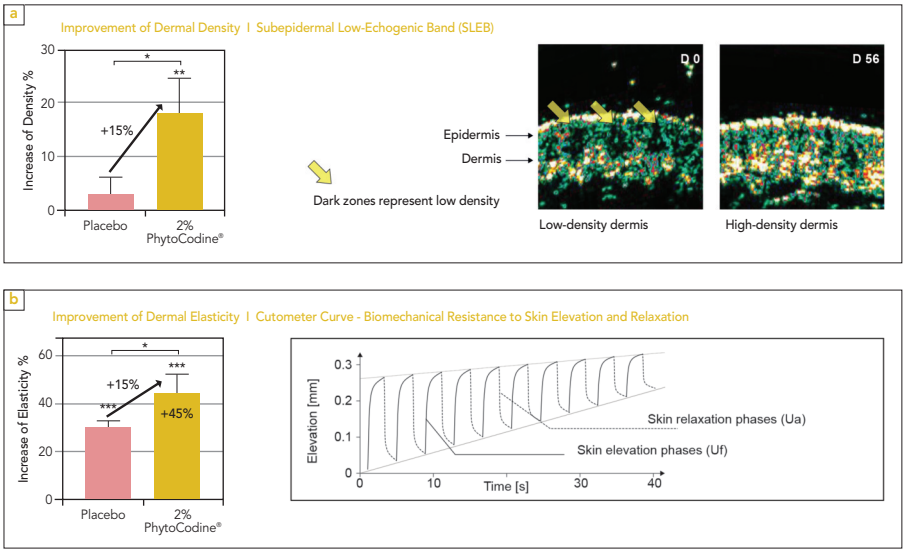

Figure 3: Plant-derived, matrikine-like peptides improve age-related biomechanical parameters of facial skin. 21 women applied a cream with 2 % of the active ingredient to one hemiface and a placebo cream to the other for 56 days. (A) The active ingredient increases sub-epidermal density. Images show changes in subepidermal density (light areas = high skin density, dark areas low density), measured with a skin ultrasonography ultrasound system Dermascan C. (B) The active ingredient increases elasticity and firmness. Elasticity (ratio between the ability of returning to the original position (Ua) and the maximal amplitude of elevation (Uf)) was measured with a Cutometer® dual MPA 580. N = 21; Mean + SEM. Student's t-test vs. baseline and between treatments; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

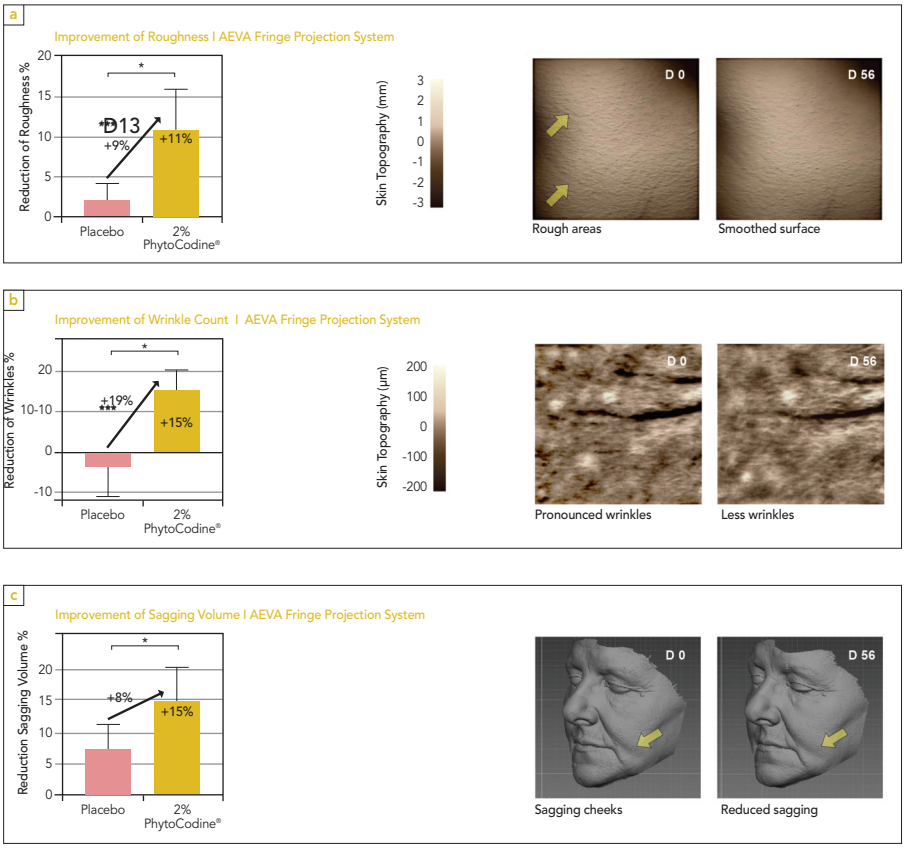

Figure 4: Plant-derived, matrikine-like peptides improve the topography of facial skin. In a placebo-controlled consumer test, 21 women with mixed to dry skin applied a cream containing 2 % of the active ingredient to one hemiface and a placebo cream to the other hemiface for 56 days. (A) The active ingredient reduces skin roughness. Representative images where obtained by Stereo-Fringe projection system AEVA®. (B) The active ingredient reduces the quantity of wrinkles. Representative images obtained by Stereo-Fringe projection system AEVA®. (C) The active ingredient has a lifting effect on facial skin. Representative images obtained by Stereo-Fringe projection system AEVA®. N = 21; Mean + SEM. Student's t-test vs. baseline and between treatments; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.