Innovative Peptide Technologies for Even, Young and Healthy Looking Skin


Introduction

With age, skin performance is decreasing and as a consequence, overall skin appearance is dramatically changed. This article describes two cosmetic peptides which were tailor-made to successfully combat two of the most visible signs of the aging process, the appearance of wrinkles and age spots.

Tetrapeptide-21 (GEKG: Glycine – Glutamic Acid – Lysine – Glycine) is a tetrapeptide-motif that is present in several human extracellular matrix (ECM) proteins. Its topical application induces restoration of the skin’s most important structural dermal components. It is available as a preservative-free, clear, water-soluble solution of 2000 ppm active matter as TEGO® Pep 4-17 (INCI: Tetrapeptide-21, Glycerin, Butylene Glycol, Aqua).

Besides the desire to counteract the depletion of different ECM components, a safe and skin-friendly way of inhibiting the pigmentation process is of major cosmetic concern. Tetrapeptide-30 (PKEK: Proline – Lysine – Glutamic Acid – Lysine) is a skin-derived tetrapeptide which diminishes post-inflammatory hyperpigmentation. Several in vivo studies show that PKEK is a powerful active ingredient which is able to balance the skin tone in multiple ways. It is made available to the market as a preservative- and glycol-free, water-soluble solution of 1600 ppm active matter as TEGO® Pep 4-Even (INCI: Tetrapeptide-30, Glycerin).

Tetrapeptide-21 –
The ECM Boosting Peptide

The ECM is the structural backbone of many tissues, especially of the skin. During regular turnover, ECM is degraded

Abstract

Many peptides have been developed and commercialized for the dermatological and skin care markets. The present review summarizes the development and bioactivity of two such peptides, Tetrapeptide-21 and Tetrapeptide-30. The efficacy of the peptides was shown in several in vitro, ex vivo and in vivo studies.

The results show that Tetrapeptide-21 is highly efficacious in boosting the major extracellular matrix (ECM) components in a well balanced way. As a consequence, skin elasticity and volume are improved and skin roughness and scaliness are reduced. The ingredient is effective in reducing all kinds of wrinkles and thus has a high anti-aging potential.

Tetrapeptide-30 has strong skin tone modulating and skin brightening effects. It acts by a smart mechanism via reducing keratinocyte-induced activation of melanocytes. It is active on all kinds of skin types (Caucasian, Asian and African) where it is able to reduce acne lesions, alleviate melasma and visibly diminish hyperchromatic spots. It acts in a safe and skin-friendly way and as a result inhibits the skin pigmentation process, which is of major cosmetic concern.
and it is well-known that several of its breakdown products are able to stimulate ECM resynthesis, in order to compensate for tissue destruction. An *in silico* (computational modeling) approach was used to develop peptides by identifying highly repetitive amino acid motifs in several ECM proteins (collagen I, II, III, IV, V, elastin, and proelastin) (1, 2). Approximately 30 tetrapeptide motifs, particularly abundant in different ECM proteins, have been identified. These peptides have been synthesized and tested for induction of collagen protein production in human dermal fibroblasts on genetic and protein level. The most active peptide had the sequence GEKG and was subsequently assessed for its effects on the formation of ECM components *in vitro* and *in vivo*.

**Tetrapeptide-21 induces ECM Gene Expression and Protein Production**

Collagen protein expression was investigated in cell culture supernatant of human dermal fibroblasts after stimulation for 24 hours with 1 and 10 ppm Tetrapeptide-21 or Palmitoyl Pentapeptide-4 (pal-KTTKS) as a reference standard. Tetrapeptide-21 significantly (*p*<0.05) increased the amount of secreted collagen protein dose-dependently compared to control. When comparing to Palmitoyl Pentapeptide-4, the collagen production was almost doubled at either concentration (Fig. 1).

On mRNA level, the tested ECM marker genes (COL1A1, Fibronectin and HAS1) were clearly and significantly (*p*<0.05) induced by treatment with Tetrapeptide-21 at 1 ppm (data reported in 3). The well balanced induction of ECM constituents by Tetrapeptide-21 suggests a strong anti-aging effect of this molecule. This assumption was further tested in an *in vivo* biopsy study enrolling 10 volunteers aged above 40 (average 48.2 years). An O/W vehicle with and without 50 ppm Tetrapeptide-21 was applied once daily on different areas of the upper gluteal region and on the inner forearm for 8 weeks. Thereafter, punch biopsies were taken from the treated sites and an untreated site. Quantitative real-time PCR (qRT-PCR) was used to quantify increases in COL1A1, the gene that encodes the major component of type I collagen. It was demonstrated that topical application of Tetrapeptide-21 led to a statistically significant increase in expression of COL1A1 (*p*<0.05). For immunohistochemical investigations, 4 representative subjects were selected and their tissue samples were analyzed for procollagen I, fibronectin and hyaluronic acid (Fig. 2). Compared to vehicle, a considerable induction of the ECM components collagen, fibronectin and hyaluronic acid was observed (3).

**Tetrapeptide-21 improves Skin Elasticity**

Within the initial *in vivo* biopsy study, skin elasticity had been evaluated on the inner forearm with a cutometer. The improvement of several important elasticity parameters was indicated by treatment with Tetrapeptide-21, but no significance was reached due to the small panel. Therefore, in a second *in vivo* study 60 volunteers were recruited and each 15 volunteers received the test formulation containing 10 ppm or 100 ppm Tetrapeptide-21, 10 ppm Palmitoyl Pen-
tapeptide-4 or the O/W vehicle formulation. Test formulations were applied twice daily over a period of 8 weeks on the left inner forearm. A Visioscan VC 98 was used to evaluate skin volume, skin roughness and skin scaliness. Skin elasticity was assessed with a Cutometer. Treatment with 10 ppm Tetrapeptide-21 significantly (p<0.10) reduced skin scaliness and improved skin volume compared to vehicle. Further improvements (p<0.01) were detected when 100 ppm Tetrapeptide-21 were employed. Increasing concentrations of Tetrapeptide-21 also decreased skin roughness. Compared to Palmitoyl Pentapeptide-4, 10 ppm Tetrapeptide-21 showed comparable activity whereas 100 ppm doubled the effect (results are reported in (4)). R1, the remaining deformation after the first stretching cycle, was significantly improved after treatment. The improvement was maximal already at the lowest concentration applied. The efficacy of 10 ppm Tetrapeptide-21 was superior to 10 ppm Palmitoyl Pentapeptide-4 (Fig. 3).

Tetrapeptide-21 – Facial Anti-Wrinkle Study

To further manifest the beneficial effects of the ECM boosting tetrapeptide in a cosmetically relevant context, an *in vivo* facial anti-wrinkle study was conducted on 60 volunteers. One half of the panel received an O/W formulation containing 80 ppm Tetrapeptide-21, the other half received vehicle only. Test formulations were applied twice daily in the face for 8 weeks. Cutaneous roughness was assessed with a Primos Pico, and digital images of the periorbital region were recorded before treatment and after 4 and 8 weeks of application, respectively. The parameters Sa (arithmetic average of surface roughness) and Sz (average of the 5 highest peaks and 5 deepest valleys form the entire measuring field) were significantly reduced after 4 and 8 weeks of application, while the vehicle was not effective in reducing wrinkles (Fig. 4). The following images indicate the efficacy of the tetrapeptide in reducing wrinkles (Fig. 5). It is clearly visible that the product is able to reduce fine lines as well as medium or deep wrinkles notably. First effects were visible after 4 weeks and were further manifested with increasing application time. Taken the results of the various *in vitro* and *in vivo* studies together, Tetrapep-
tide-21 shows superior collagen, hyaluronic acid and fibronectin boosting activity and thus improves skin elasticity and reduces skin roughness. The skin is strongly smoothed and firm, and the appearance of all kinds of wrinkles is minimized. The high anti-aging potential of Tetrapeptide-21 turned out to be particularly suitable for anti-wrinkle and anti-aging eye care applications.

- **Tetrapeptide-30 – Balancing Skin Tone**

  The skin pigment melanin is synthesized in melanocytes in the basal layer of the epidermis, by a process which is controlled by a plethora of mechanisms and reactions (melanogenesis). One of these processes depends on the interaction between keratinocytes and melanocytes, which can be regulated by the pigmentation-inducing soluble factor melanocyte-stimulating hormone (α-MSH). It is generated by cleavage of the precursor peptide proopiomelanocortin (POMC) and secreted by the keratinocytes. Additionally, pro-inflammatory cytokines secreted from keratinocytes modulate melanocyte activation and proliferation. Skin brightening and the pigmentation process can be influenced in many different ways. A commonly used approach is inhibition of the main responsible enzyme for melanin synthesis (tyrosinase). Inhibition of melanocyte activation and blocking of melanin transfer into keratinocytes are alternative and equally effective approaches.

  Tetrapeptide-30 acts by a smart mechanism via reducing keratinocyte-induced activation of melanocytes. The tetrapeptide contains the KEK motif, which is present in the antimicrobial polypeptide cathelicidin LL-37 (1, 2). In vitro and in vivo studies indicate a strong efficacy of Tetrapeptide-30 in balancing skin tone.

- **Reduction of Keratinocyte-Secreted Factors of Melanogenesis**

  The effect of Tetrapeptide-30 on keratinocyte-secreted factors of melanogenesis was investigated in vitro on UVB-irradiated primary normal human epidermal keratinocytes (NHEKs) from neonatal foreskin. NHEKs were cultivated in medium containing 0.7 ppm or 7 ppm Tetrapeptide-30 or vehicle for 24 hours prior to exposure to a dose of 160 J/m² UVB radiation. Afterwards, cells were cultivated for another 6 hours and 24 hours, respectively. Gene expression was assessed by qRT-PCR applying total RNA. It was found that irradiation of NHEKs with UVB led to up-regulation of interleukin-6 (IL-6) and interleukin-8 (IL-8) gene expression 6 hours post irradiation, an effect that was significantly (p<0.05) reduced by the action of Tetrapeptide-30. Interleukin increase was followed by an induction of the α-MSH precursor POMC gene expression after 24 hours, an effect that was completely and significantly (p<0.05) blocked with either 0.7 or 7 ppm Tetrapeptide-30 (Fig. 6). Thus, the induction of POMC gene expression remained at basal level (5). The results of this study show that Tetrapeptide-30 efficiently reduces UVB-induced melanogenesis in cultured keratinocytes by reducing POMC/α-MSH as well as interleukin gene expression. The data suggests that the activation of melanocytes and thus production of the skin pigment melanin is efficiently reduced by a mechanism involving keratinocyte to melanocyte communication.

- **Reduction of Melanogenesis Activation in Human Skin**

  The in vivo relevance of these findings was explored in the context of a biopsy study enrolling 10 volunteers aged 25-
69. The volunteers were treated once daily over a period of 4 weeks with vehicle or formulation containing 40 ppm Tetrapeptide-30 on the upper medial quarter of the buttock. Thirty minutes after the final application, skin color was measured with a colorimeter and volunteers were irradiated with a dose of 1.5 MED (minimal erythema dose) of broadband UVB. One day after irradiation, the change in skin color was recorded and punch biopsies were taken from a UVB-
treated and -untreated area from each a vehicle and Tetrapeptide-30 treated region. It was found that expression of key inflammation marker genes IL-6 and IL-8, TNF-α and COX-2, as well as expression of genes for tyrosinase and POMC was induced in UVB-irradiated, vehicle-treated skin. This effect was significantly reduced by treatment with Tetrapeptide-30 (Fig. 7) (5).

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UVB-irradiation led to a significant decrease in ITA° values, which represents an increase in skin melanin content (tanning). Treatment with Tetrapeptide-30 led to a moderate change of ITA°, thus preventing UV-induced melanin formation by trend (data reported in (5)).

Lightening the Skin Tone on the Hand

The previous results confirmed the relevance of the in vitro findings in an in vivo context. In a next step, a broader investigation of in vivo effects was started and 38 panelists aged 35-59 were enrolled in a skin lightening study. A hand cream containing 1.5% sodium ascorbyl phosphate (SAP; A commonly accepted lightening active ingredient and known inhibitor of tyrosinase activity) or a combination of 1.5% SAP plus 40 ppm Tetrapeptide-30 was applied twice daily for 8 weeks. Before and after 4 and 8 weeks of application, skin color was determined on the back of the hand with a colorimeter, and skin redness and erythema were assessed with a Mexameter. The combination of Tetrapeptide-30 and SAP led to a strong and significant (p<0.01) reduction of skin redness (a*), yellow skin color (b*) and erythema, while SAP alone did not show significant effects. Also a significant (p<0.10) increase in ITA° (skin lightening) was detected for the combination of active ingredients but not for SAP alone (5) (Fig. 8).

In summary, it could be clearly demonstrated that a combination of 1.5% SAP plus 40 ppm Tetrapeptide-30 is effective in visibly reducing hyperchromatic age spots on the hands and in lightening the hand skin tone.

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![Fig. 7 Effect of Tetrapeptide-30 on gene expression of POMC, Tyrosinase and inflammation markers COX-2 and TNF-α 24 hours post irradiation with UVB](image)

![Fig. 8 Visible fading of hyperchromatic spots and yellow skin color after application of 1.5% SAP plus 40 ppm Tetrapeptide-30 (from left to right: before, after 4 and 8 weeks)](image)
Reduction of Facial Age Spots

Subsequently, we investigated if the activity profile of Tetrapeptide-30 was also extendable to facial applications. A half-face study was performed on forty women aged 30-70 with hyperchromatic spots. They applied either vehicle, 40 ppm Tetrapeptide-30, 1.5% SAP or a combination of both twice daily for 6 weeks. Skin color of hyperchromatic spots and their surrounding area was determined and the severity of spots is represented by the contrast (difference in ITA°). The fading of age spots was described as the change in contrast between the spot and the surrounding area before and after treatment (Fig. 9).

A visible fading of hyperchromatic spots was detected, with similar efficacy, when Tetrapeptide-30 or SAP were applied. The fading was further increased when a combination of Tetrapeptide-30 and SAP was used (Fig. 10).
Improvement of Evenness of Skin Tone on Asian Skin

The proven skin lightening efficacy of Tetrapeptide-30 on caucasian skin indicated its potential also on asian skin, where skin brightening applications and applications targeting hyperchromatic spots and an uneven skin tone are a huge market need.

Thus, a mixed panel consisting of 27 volunteers aged 28-60 with Japanese background and an uneven skin tone were recruited. A test formulation containing 1.5% SAP as the vehicle compared to the same formulation containing 40 ppm Tetrapeptide-30 was applied twice daily for 8 weeks on one side of the face, while the other side remained untreated. A clinical assessment of skin conditions was performed, and skin color was recorded before and after 4 and 8 weeks of application.

Changes in ITA° values among the SAP (vehicle) group almost remained unchanged and skin tone was not affected, while Tetrapeptide-30 treatment significantly (p<0.01) lightened the skin compared to the vehicle (Fig. 11). No change in hyperpigmentation was observed among the SAP group by expert grading after 8 weeks, while rating values were reduced by 15% in the Tetrapeptide-30 group (5) (Fig. 12).

The study demonstrated that Tetrapeptide-30 is able to reduce some of the major concerns of asian skin. Skin tone is lightened, skin tone evenness is improved and hyperchromatic age spots are reduced. Therefore, it can be concluded that Tetrapeptide-30 is also highly recommended for cosmetic treatment of skin tone among asian population.

Improvement of Evenness of Skin Tone on African Skin

Finally, the high activity profile of Tetrapeptide-30 raised the question, if the active ingredient was also able to reduce melasma and/or post-inflammatory hyperpigmentation lesions among African ethnicities, who often suffer from an uneven skin tone. Melasma is a tan or dark skin discoloration that mainly affects women, and is especially pronounced in regions where the skin is exposed to intense sun irradiation. Post-inflammatory hyperpigmentation is the main cause of spot development after acne lesions.

Fifty women aged 18-50 with mild acne and/or melasma (Fitzpatrick type IV-V)
were recruited and panelists were asked to avoid excessive sunlight. Test formulations with either 40 ppm Tetrapeptide-30 or vehicle were applied twice daily over a period of 12 weeks on the full face. A clinical assessment of overall skin condition, evenness of skin tone, area of melasma (percentage values) and number of lesions (divided into three groups: <5, 5-10, >10 lesions) was performed on a five-level scale by experts before and after 2, 4, 8 and 12 weeks of application.

Skin overall appearance was significantly (p<0.05) improved after 12 weeks of treatment with Tetrapeptide-30 (Fig. 13), but not in case of vehicle-treated skin. Similar improvements were observed for evenness of skin tone after 8 and 12 weeks (p<0.10 and 0.01, respectively), and the number and appearance of post inflammatory hyperpigmentation/acne lesions already showed a significant decrease after 4 weeks of Tetrapeptide-30 treatment (p<0.05) (Fig. 14). Further improvements were registered after 8 and 12 weeks (p<0.01).

The study demonstrated that Tetrapeptide-30 is also able to reduce some of the major cosmetic concerns of African skin. There is much potential of the compound to significantly and visibly improve the skin’s evenness and overall appearance. In addition, the number of spots caused by acne lesions are reduced (6). Therefore, it can be concluded that Tetrapeptide-30 is highly recommended for treating skin tone problems on ethnic skin.

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References


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