Technical Information

TEGO® Pep 4–17

The ECM boosting peptide

Intended use

Active for skin care

Benefits at a glance

- TEGO® Pep 4–17 is derived from a bioinformatic approach of prediction of biologically active ECM breakdown products
- TEGO® Pep 4–17 is a peptide sequence based on skin's own structure
- TEGO® Pep 4–17 is a preservative-free peptide solution
- TEGO® Pep 4–17 shows superior collagen, hyaluronic acid and fibronectin boosting activity
- TEGO® Pep 4–17 minimizes the appearance of all kinds of wrinkles and reduces skin roughness
- Usage concentration: 0.5 – 5.0%

INCI (PCPC name)

Tetrapeptide-21; Glycerin; Butylene Glycol; Aqua

Chemical and physical properties (not part of specifications)

Form	viscous liquid
Active matter	2000 ppm

Properties

The extracellular matrix (ECM) is the structural backbone of many tissues, especially of the skin and, therefore, represents a main target for cosmetic applications. Alongside, ECM proteins have been described recently to play a pivotal role in cellular migration, proliferation and gene regulation during wound healing. The question of how these versatile activities are evolved has been answered by the fact that fragments from ECM constituents are capable of stimulating ECM biosynthesis to compensate for tissue destruction. This mechanism has been implicated in wound healing, skin aging and responses to UV irradiation.

Based on this concept we used bioinformatic (*in silico*) methods to identify highly repetitive amino acid motifs in several human ECM proteins to identify sequences with inherent anti-aging activities. Using the amino acid sequences of collagen I, II, III, IV, V, elastin, and proelastin, tetrapeptide sequences were identified by their frequency of occurrence.
We found several dozen tetrapeptides to be highly abundant in ECM proteins. These peptides have been tested primarily for induction of collagen protein production.
Finally, the most active tetrapeptide with the sequence GEKG (Glycine – Glutamic Acid – Lysine – Glycine) was evaluated *in vivo* for its potential anti-aging effects.

- ***In vitro* evaluation of the induction of collagen protein synthesis by Tetrapeptide-21**

Collagen is one of the main components of the extracellular matrix that is responsible for skin

strength and elasticity and its degradation leads to wrinkles that accompany aging.

Method: To examine the induction of collagen protein synthesis Tetrapeptide-21, the active ingredient in TEGO® Pep 4-17, was applied at concentrations of 1 or 10 ppm for 24 hours to human dermal fibroblast cell cultures. Afterwards, the concentration of collagen in the cell culture supernatant was quantified by Sircol™ Collagen Assay.

Results: Tetrapeptide-21 increased the amount of secreted collagen protein in the supernatant of human dermal fibroblast cultures at a concentration of 1 ppm by about 2.5-fold versus the control. This effect is even strongly increased at a concentration of 10 ppm. Compared to the market standard, Palmitoyl Pentapeptide-3, the collagen production in the presence of Tetrapeptide-21 is almost 2-fold higher. All results for the peptides were statistically significant ($p<0.05$).

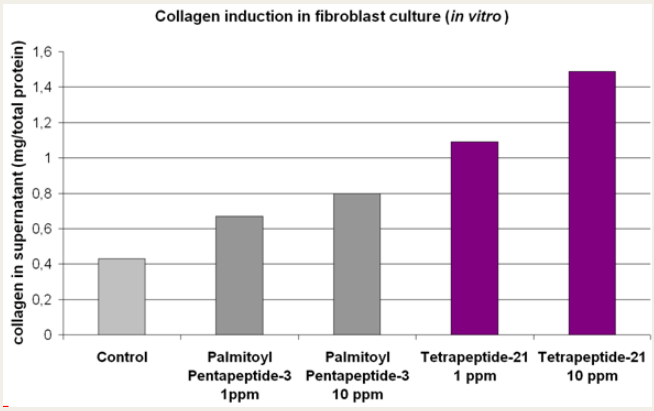

Figure 1: Effects of Tetrapeptide-21 on secreted collagen levels

- ***In vitro* evaluation of the induction of other dermal skin components by Tetrapeptide-21**

Besides collagen, there are a number of other important ECM constituents such as hyaluronic acid and fibronectin that play a crucial role in skin-aging. Hyaluronic acid is able to bind enormous amounts of water, thus ensuring the elasticity of the skin. Fibronectin is a glycoprotein that helps to create a cross-linked network within the extracellular matrix by having binding sites for other ECM components such as hyaluronic acid and collagen.

Method: To determine the effect of Tetrapeptide-21 on the gene expression of marker genes for collagen I, hyaluronic acid-synthase and fibronectin, human dermal fibroblasts were treated with 1 ppm of the peptide for 24 hours and RNA was extracted in order to perform quantitative real-time PCR.

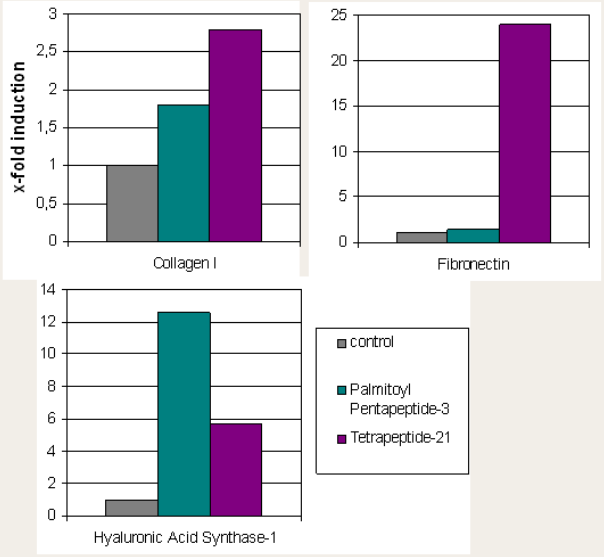

Figure 2: Effects of Tetrapeptide- 21 on gene expression of different ECM marker genes

Results: The expression of all three tested ECM marker genes was induced by Tetrapeptide-21 compared to the control. In case of collagen I and fibronectin gene expression, Tetrapeptide-21 outperformed the market standard Palmitoyl Pentapeptide-3.

Overall, the *in vitro* studies clearly show that Tetrapeptide-21 increases the amount of secreted collagen protein and furthermore enhances the gene expression of important ECM proteins like collagen I and fibronectin and stimulates hyaluronic acid production by inducing hyaluronic acid-synthase.

- ***In vivo* efficacy study: Skin biopsy study on induction of ECM proteins**

In support of the *in vitro* data, we conducted an *in vivo* skin biopsy study to demonstrate that the efficacy of TEGO® Pep 4-17 is not limited to *in vitro* models, but also applies to real human skin on genetic and protein level.

Method: The study was performed at the Institute for Molecular Preventive Medicine (IUF) at the Heinrich Heine University in Duesseldorf, Germany. Ten healthy volunteers older than 40 years were recruited for the study. They applied a vehicle formulation and a test formulation containing 50 ppm Tetrapeptide-21. Every panelist applied the formulations on the buttock and on the arm once a day. After 8 weeks 4mm punch biopsies were taken from the treated and untreated areas of the upper gluteal region. Collagen I is encoded by the COL1A1 gene whose expression was measured by RT-PCR. In addition, induction of collagen and fibronectin protein and hyaluronic acid was determined by immunohistostaining of the taken punch biopsies after 8 weeks.

Results: The *in vivo* biopsy study demonstrated that the topical application of Tetrapeptide-21 leads to a a statistically significant increase in the expression of Collagen I.

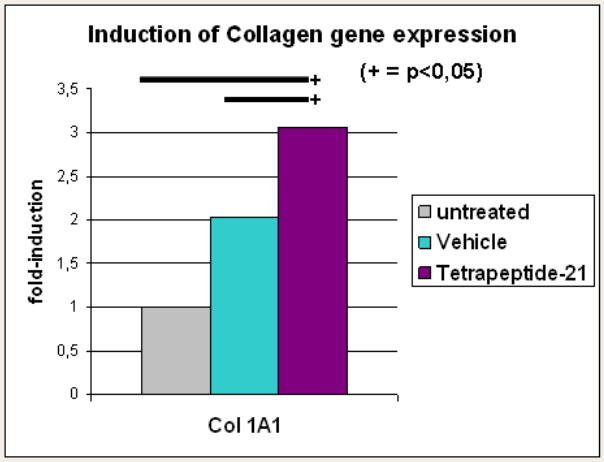

Figure 3: Induction of Collagen gene expression in human skin after 8 weeks of application

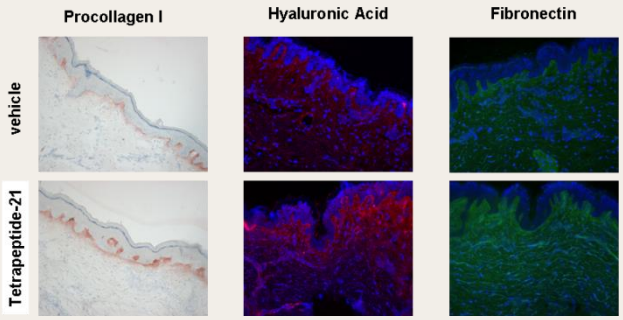

Figure 4: Immunohistostainings of punch biopsies after 8 weeks of application

Compared to the vehicle formulation, treatment with Tetrapeptide-21 led to a considerable induction of protein production of the ECM components collagen, fibronectin and hyaluronic acid (Procollagen I = red, hyaluronic acid = red, fibronectin = green, figure 4).

The potent ECM boosting activity of Tetrapeptide-21 – already demonstrated in various *in vitro* studies – was further substantiated with this *in vivo* skin biopsy study. A significant increase of collagen gene expression has been shown. This result was verified on protein level by immunostaining for different important ECM proteins. The well balanced induction of all important ECM constituents by Tetrapeptide-21 on gene and protein level further supports a strong anti-aging effect of TEGO® Pep 4-17.

Within this study the skin elasticity on the arm has been evaluated as well. Figure 5 shows that different important elasticity parameters were improved after 8 weeks by Tetrapeptide-21 compared to the vehicle. That shows an overall increase in skin elasticity by the peptide.

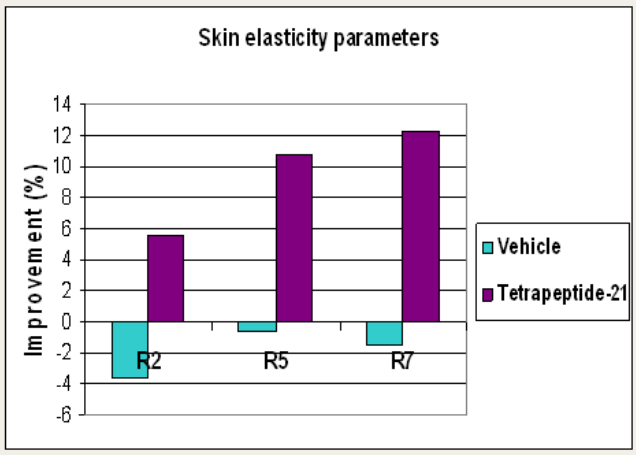

Figure 5: Skin elasticity after 8 weeks of application compared to start value

- ***In vivo* efficacy study: Long-term evaluation of skin volume and roughness**

Method: In order to demonstrate the cosmetic anti-aging effect *in vivo*, 60 volunteers (15 volunteers/formulation) applied a formulation containing 10 ppm or 100 ppm Tetrapeptide-21 topically over a period of eight weeks. The study was placebo controlled; 10 ppm Palmitoyl Pentapeptide-3 served as positive reference. Every panellist got one formulation and applied it on the inner forearm twice a day. Before and after eight weeks of application, skin elasticity, skin volume as well as skin roughness were analyzed using the Cutometer and Visioscan VC 98 device (Courage & Khazaka electronic GmbH, Germany).
The parameter "volume" calculates the theoretical amount of a liquid necessary to fill the wrinkles and generate a plain surface. A reduction of this parameter shows an overall improvement of skin structure resulting from reduction of skin wrinkles in number and depth.
Results: Figure 6 demonstrates that application of 10 ppm Tetrapeptide-21 reduced the skin volume by 8% and even inverted to a 10% improvement at 100 ppm Tetrapeptide-21.

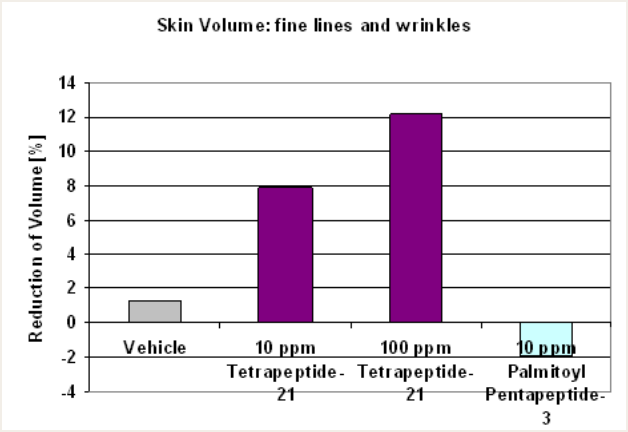

Figure 6: Skin volume after 8 weeks of application compared to start value

Figure 7 shows an increased reduction of skin roughness with increasing concentrations of Tetrapeptide-21. The parameter roughness describes the depth of fine and coarse wrinkles.
10 ppm Tetrapeptide-21 showed a 15% improvement whereas 100 ppm Tetrapeptide-21 showed nearly 25% improvement.

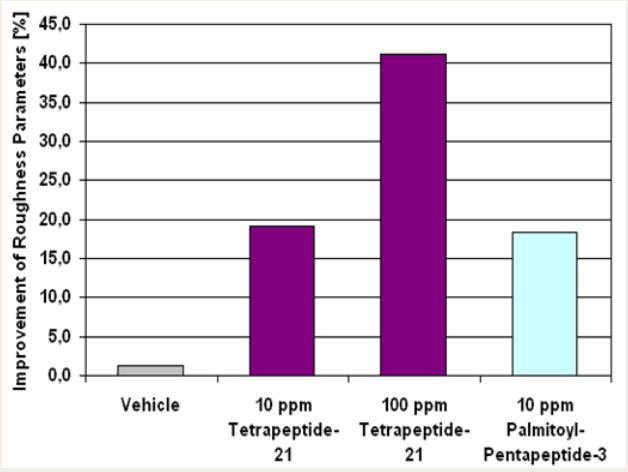

Figure 7: Skin roughness after 8 weeks of application

The skin elasticity is described by the parameter R1 which is the remaining deformation after stretching. Skin with a strong elasticity shows a very small remaining deformation.
Figure 8 clearly shows that the application of Tetrapeptide-21 leads to a strong reduction of the remaining skin deformation after stretching. The parameter R1 is reduced by approx. 41% already with

10 ppm Tetrapeptide-21. A further increase of the peptide concentration is not required.

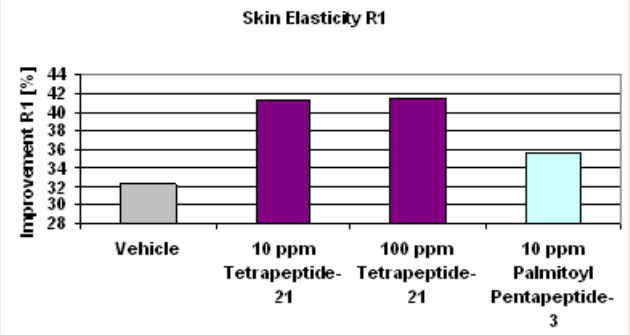

Figure 8: Improvement of skin elasticity after 8 weeks

- *In vivo* facial anti-wrinkle study

Method: For the study 60 volunteers were recruited. 30 volunteers got a test formulation containing 4% TEGO® Pep 4-17 (80 ppm Tetrapeptide-21), the others got a formulation which didn't contain an active ingredient (vehicle). They applied the formulation on their face twice daily for 8 weeks.
Before the application started as well as after 4 and 8 weeks of application cutaneous roughness was assessed using a Primos Pico (GFMesstechnik, Berlin, Germany) and digital images of the periorbital region were taken with a Fotofinder Dermoscope II.
The study was performed at an external institute (ISPE, Milan, Italy).

Results: The following graphs show the reduction of the parameters Sa and Sz after 4 and after 8 weeks application time assessed by Primos Pico. Sa is the arithmetic average of the surface roughness, Sz is the average of the 5 highest peaks and 5 deepest valleys form the entire measuring field.

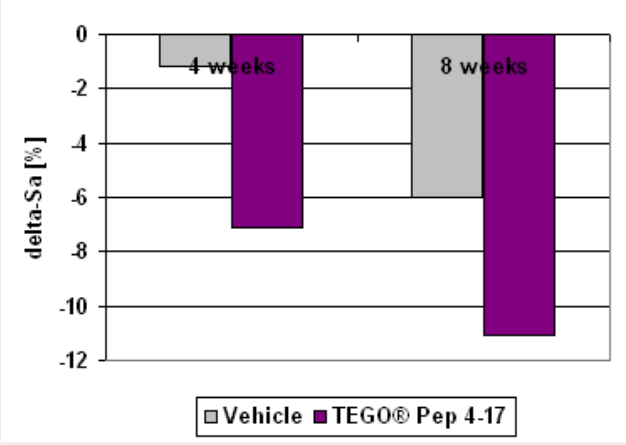

Figure 9: Reduction of the roughness parameter Sa after 4 and after 8 weeks

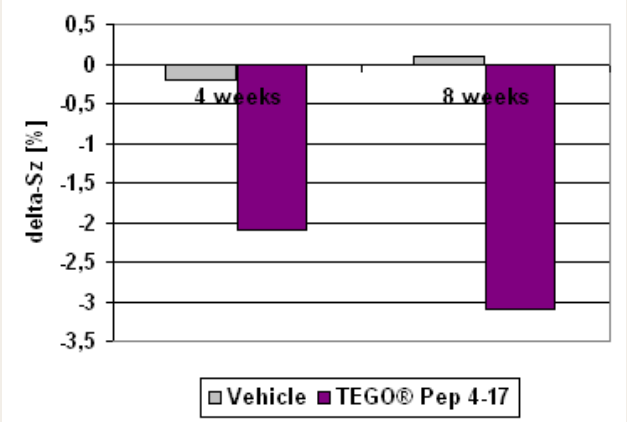

Figure 10: Reduction of the roughness parameter Sz after 4 and after 8 weeks

The vehicle formulation was not able to reduce wrinkles while the formulation containing TEGO® Pep 4-17 reduced fine lines as well as deep wrinkles significantly already after 4 weeks of application. This effect yet increased after 8 weeks. Also the following pictures taken before the application started (left side) and after 8 weeks (right side) prove the wrinkle reducing properties of TEGO® Pep 4-17.

Figure 11: Reduction of fine (row 1), medium (row 2) and deep (row 3) wrinkles by TEGO® Pep 4-17

In conclusion, TEGO® Pep 4-17 is an ideal active ingredient to prevent and minimize the appearance

of skin aging based on its ECM boosting activity, outperforming the chosen benchmark.

Preparation

TEGO® Pep 4-17 is added to the water phase and the emulsion is prepared as usual.

Recommended usage concentration

0.5% - 5.0%

Applications

- Anti-wrinkle preparations
- Anti-aging eye care products

Packaging

1.0 kg package
5.0 kg package

Hazardous goods classification

Information concerning

- classification and labelling according to regulations for transport and for dangerous substances
- protective measures for storage and handling
- measures in case of accidents and fires
- toxicity and ecological effects

is given in our material safety data sheets.

Guideline formulations

Wrinkle Corrector MF 19/08	
Phase A	
TEGO® Care 450 (Polyglyceryl-3 Methylglucose Distearate)	3.0%
TEGIN® M Pellets (Glyceryl Stearate)	2.0%
TEGO® Alkanol 18 (Stearyl Alcohol)	1.0%
TEGOSOFT® TN (C12-15 Alkyl Benzoate)	9.5%
TEGOSOFT® CT (Caprylic/Capric Triglyceride)	9.5%
Phase B	
TEGO® Pep 4-17	3.0%
Glycerin	3.0%
Water	69.0%
Phase Z	
Preservative, Perfume	q.s.
Preparation: 1. Heat phase A and B separately to approx. 70-75° C. 2. Add phase A to phase B with stirring [1)]. 3. Homogenize. 4. Cool with gentle stirring. [1)] Important: If phase A has to be charged into the vessel first, phase B must be added without stirring.	

Deep wrinkle cream MAC 638/7/2	
Phase A	
TEGO® Care LTP (Sorbitan Laurate; Polyglyceryl-4 Laurate; Dilauryl Citrate)	2.0%
TEGIN® M Pellets (Glyceryl Stearate)	3.5%
TEGO® Alkanol 18 (Stearyl Alcohol)	3.5%
TEGOSOFT® OP (Ethylhexyl Palmitate)	8.0%
TEGOSOFT® CT (Caprylic/Capric Triglyceride)	8.0%
Phase B	
Skinmimics® (Ceteareth-25; Glycerin; Cetyl Alcohol; Behenic Acid; Cholesterol; Ceramide NP; Ceramide NS; Ceramide EOS; Ceramide AP; Carprooyl Phytosphingosine; Caprooyl Sphingosine)	5.0%
TEGO® Pep 4-17	1.0%
Glycerin	3.0%
Water	65.5%
Phase C	
TEGO® Carbomer 134 (Carbomer)	0.1%
TEGOSOFT® OP (Ethylhexyl Palmitate)	0.4%
Phase D	
Sodium Hydroxide (10% in water)	q.s.
Phase Z	
Preservative, Perfume	q.s.

Preparation:

1. Heat phase A and B separately to approx. 80° C.
2. Add phase A to phase B with stirring.[1)]
3. Homogenize.
4. Cool with gentle stirring to approx. 60°C and add phase C.
5. Homogenize again for a short time.
6. Cool with gentle stirring and add phase D below 40°C.

[1)] Important: If phase A has to be charged into the vessel first, phase B must be added without stirring.

Wrinkle smoothing cream MAC 638/3/1	
Phase A	
TEGO® Care 450 (Polyglyceryl-3 Methylglucose Distearate)	3.0%
ABIL® Care 85 (Bis-PEG/PPG-16/PPG16/16 Dimethicone; Caprylic/Capric Triglyceride)	1.0%
TEGIN® M Pellets (Glyceryl Stearate)	2.0%
TEGO® Alkanol 18 (Stearyl Alcohol)	1.0%
TEGOSOFT® DEC (Diethylhexyl Carbonate)	9.0%
TEGOSOFT® OS (Ethylhexyl Stearate)	9.0%
Phase B	
TEGO® Pep 4-17	2.0%
HyaCare® (Sodium Hyaluronate)	0.1%
Glycerin	3.0%
Water	69.9%
Phase Z	
Preservative, Perfume	q.s.

Preparation:

Heat phase A and B separately to approx. 70-75°C.
2. Add phase A to phase B with stirring.[1)]
3. Homogenize.
4. Cool with gentle stirring.

[1)] Important: If phase A has to be charged into the vessel first, phase B must be added without stirring.

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