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# Synergistic peptide action revitalises skin and hair


## ABSTRACT

Dermatopoietin-Plus is a mixture of two peptides which act synergistically to renew the dermal skin structure and reduce hair loss. One component is recombinant interleukin-1 alpha, an epidermal cytokine, which is expressed by keratinocytes on a constitutive basis. This 159-amino acid protein is responsible for the homeostasis of normal skin. It acts as a paracrine messenger of epidermal cells and triggers fibroblasts to rebuild the fibrous network of the dermis. It also affects papilla cells in hair bulbs to express several factors needed for hair growth and preventing hair loss. The other component of the mixture is a novel hexapeptide with unique physiological properties: it enhances capillary microcirculation and controls the release cycle of interleukin-1 alpha. The mixture represents a new cosmeceutical with proven efficacy against skin ageing, cellulite skin and hair loss.


Dermatopoietin-Plus is a synergistic mixture of two peptides: Dermatopoietin and Hexadeltine. Dermatopoietin (INCI name: rh-Polypeptide-17) is the proprietary trade name for human interleukin-1 alpha (IL-1 alpha), a protein of 159 amino acids (produced by recombinant biotechnology in *Escherichia coli*). Hexadeltine is the trade name for a novel artificial hexapeptide with unique properties. Based on the role of interleukin-1 alpha in skin physiology and the efficacy profile of Hexadeltine, the mixture shows great promise for use in dermatology and cosmetics. Its full potential has not been explored yet, much needs still to be done, however the present review summarises the current state of knowledge about the mechanisms of the two peptides and their use as active ingredients in cosmetic applications with focus on anti-ageing, anti-cellulite and anti-hair loss products.

## Scientific background of Dermatopoietin

### Pleiotropic factor: The many functions of interleukin-1 alpha

Human IL-1 alpha is a protein of 159 amino acids and belongs to the large cytokine superfamily. A cytokine is a factor that, being secreted a one cell, acts on a neighbouring cell to produce an effect, usually differentiation or proliferation. Cytokines are involved in cell-to-cell communication, and thus, vital for organogenesis. To understand the physiology of IL-1 alpha we must know the cell types producing and releasing the factor, the target cells expressing receptors for IL-1 alpha and the effects elicited upon binding IL-1 alpha.

IL-1 alpha is best known for being released by macrophages and monocytes and activating an immune response by the body against infection. Most biologists therefore associate inducible IL-1 alpha with pro-inflammatory and pyrogen effects. Indeed, intravenous injection of nanomolar concentrations of IL-1 alpha has immunomodulatory effects and causes fever. But as a typical pleiotropic factor, IL-1 alpha has a much broader efficacy profile.

Pleiotropy occurs when a single factor has multiple effects due to different cell

**Figure 1:** *Three-dimensional structure of Dermatopoietin, a 159-amino acid polypeptide chain. It acts as master regulator, or architect, of skin renewal.*

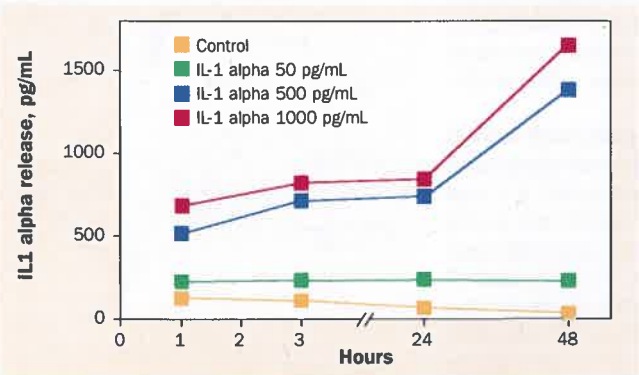


**Figure 2:** *Release of IL-1 alpha from cultured human keratinocytes pre-treated for one hour with various concentrations of IL-1 alpha.*

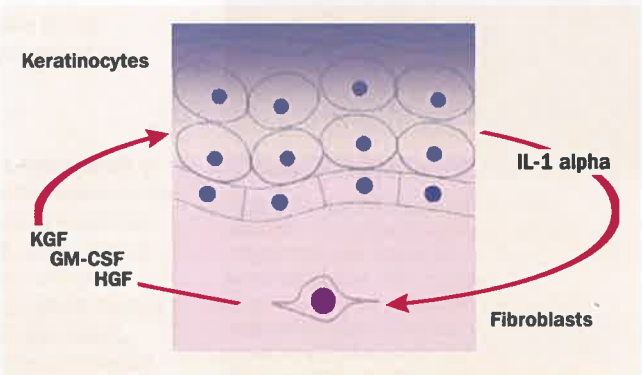


**Figure 3:** *Scheme of the paracrine regulatory mechanism of epidermal renewal. HGF: hepatocyte growth factor, GM-CSF: granulocyte-macrophage colony-stimulating factor, KGF: keratinocyte growth factor.*

types producing the factor and different effector systems in different tissues. Dermatological data accumulated since the 1980s identified IL-1 alpha as a major constitutive epidermal cytokine involved in skin renewal and in the regulation of hair growth. The effects of IL-1 alpha in skin are completely unrelated to those in the immune system: they are local and mediated by autocrine and paracrine mechanisms. IL-1 alpha is the key conductor that orchestrates skin renewal upon injuries or environmental impacts on the epidermis. Also emerging is the dominant role of IL-1 alpha in the regulation of the cycle of hair follicles and of hair loss. In the following, the current knowledge about the dermatological functions of IL-1 alpha is summarised.

**Produced and released by keratinocytes**

The skin is the richest source of IL-1 alpha across the whole body. Keratinocytes in the epidermis seem to produce the cytokine on a constitutive basis. It accumulates in the *stratum corneum* when cells move to the surface, keratinise and die. In normal epidermis, 50% of IL-1 alpha present was recovered from the *stratum corneum* and 50% from living cells. In quantitative terms, *stratum corneum* contains about $6x10^5$ IU IL-1 alpha/g tissue (lymphocyte activation test) which corresponds to about 6 µg/g of WHO standard grade IL-1 alpha with a specific activity of $1x10^8$ IU/mg. As reference, the plasma concentration of IL-1 alpha is about 2 pg/ml. The skin is thus by far the most important production site for IL-1 alpha in the human body. Furthermore, keratinocytes from newborns produce significantly more IL-1 alpha than keratinocytes from adult or even elderly individuals, suggesting a role for IL-1 alpha in skin ageing.

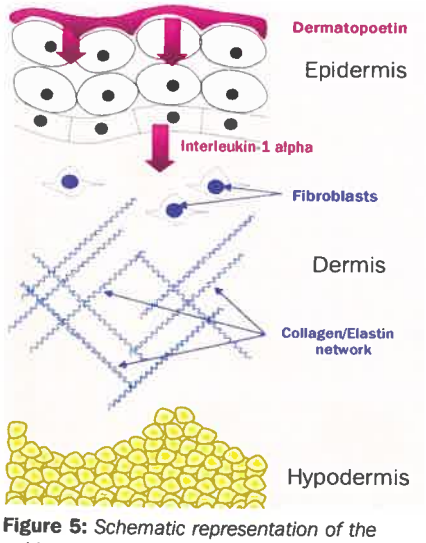


**Figure 5:** *Schematic representation of the epidermal-mesenchymal interaction in skin.*

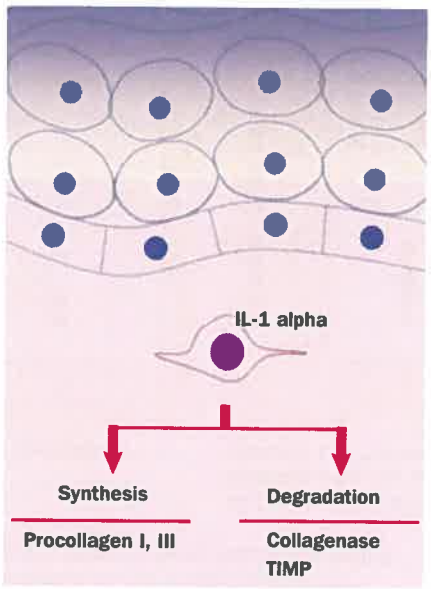


**Figure 4:** *Keratinocyte-derived IL-1 alpha regulates collagen turnover in the dermis by tight control of both collagen synthesis and degradation pathways.*

**Stimulating release via positive (self-reinforcing) feedback loop**

Keratinocytes, besides producing and releasing IL-1 alpha, also carry IL-1 alpha receptors which, upon binding with IL-1 alpha, activate the production and release of additional IL-1 alpha, i.e. of its own ligand. This mechanism is the basis of an autocrine and paracrine signalling cascade: the release of IL-1 alpha by cells of the *stratum corneum*, e.g. by injury, results in a spatial propagation of the IL-1 alpha message across the epidermis by repetitive cycles of activating IL-1 receptors on neighbouring cells and eliciting the release of IL-1 alpha. This effect of keratinocytes releasing IL-1 alpha upon treatment with IL-1 alpha is shown in Figure 2. The figure shows that IL-1 alpha induces a dose- and time-dependent secretion of IL-1 alpha. Cells were incubated for one hour with the concentration of IL-1 alpha indicated, washed, re-suspended and the concentration of IL-1 alpha in the incubation medium measured at the indicated time points.

**Dermal fibroblasts**

Keratinocyte-derived IL-1 alpha binds to type 1 IL-1 receptors on fibroblasts in the dermis and initiates multiple responses. Upon stimulation by IL-1 alpha, dermal fibroblasts, in turn, affect epidermal cells by releasing a set of growth factors (KGF, GM-CSF, and HGF) that result, among other things, in the proliferation and differentiation of keratinocytes (Fig. 3). It is noteworthy that IL-1 alpha does not directly stimulate proliferation of keratinocytes, but indirectly through the release of growth factors from fibroblasts.

Furthermore, in response to IL-1 alpha, fibroblasts express and release tropoelastin (precursor of elastin), type I and III pro-collagen, precursors of matrix collagen, and PGE2, an inhibitor of the conversion to collagen. In addition, IL-1 alpha stimulates the production of collagenase as well as its inhibitor TIMP (Fig. 4). Although little detail is known about the biochemical regulation of the fibrous networks turnover in the dermis, these factors comprise a complete set of tools needed to degrade and rebuild the dermal matrix consisting of collagen and elastin fibres. Figure 5 summarises the mechanism of epidermal and dermal renewal initiated by topical application of IL-1 alpha onto the skin surface. In this example, the product stimulates the uppermost layer of living keratinocytes in the epidermis to produce and release IL-1 alpha, which initiates and propagates a cascade of endogenous IL-1 alpha release throughout the epidermis. This "IL-1 alpha wave" eventually reaches the fibroblasts and triggers them to restructure the dermal network of collagen and elastin.

Besides that, IL-1 alpha up-regulates the expression of its receptor on fibroblasts through the release of endogenous prostaglandin E2 (PGE2), thus representing another positive feedback loop.

**No skin penetration**

As a 159-amino acid polypeptide, IL-1 alpha is not expected to penetrate the skin. Indeed, measurement on artificial human skin patches exposed during

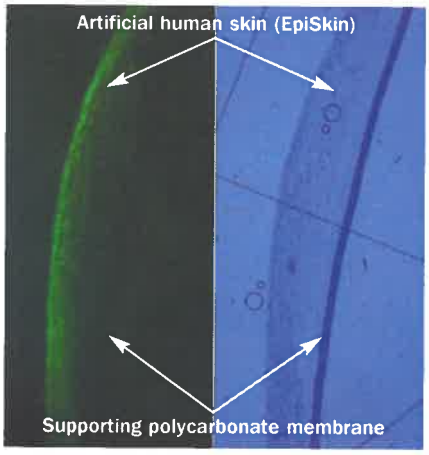


**Figure 6:** *Artificial epidermis (EpiSkin), mounted on a supporting polycarbonate membrane, was exposed to a high concentration of FITC-labelled Dermatopoietin. Left panel: fluorescence microscopy. Green fluorescence indicates the presence of labelled Dermatopoietin in the* stratum corneum *only. Right panel: light microscopy of the same field as shown in the left figure. Magnification: 100x. Small circle: 10 µm, large circle: 25 µm.*

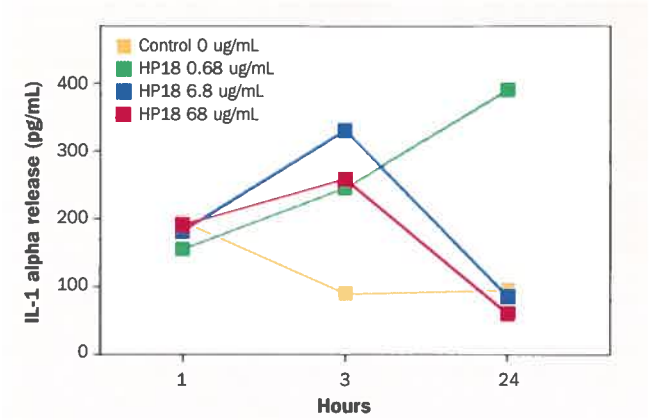


**Figure 7:** *Release of IL-1 alpha by cultured human keratinocytes after pre-incubation with Hexadeltine (HP-18).*

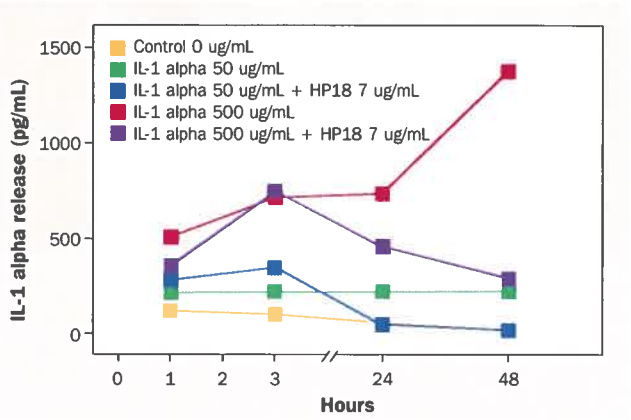


**Figure 8:** *After 24 and 48 hours, but not at shorter time intervals, Hexadeltine (HP-18) inhibits the release of IL-1 alpha stimulated by IL-1 alpha in human keratinocytes.*

24 hours to high concentrations of fluorescence-labelled product (about 480,000 higher than the in-use concentration) showed that the first peptide is absorbed by the *stratum corneum* in a reversible manner without penetrating deeper layers (Fig. 6). The detection limit of this method was estimated to be 20 pg/cm².

In summary, these data indicate that IL-1 alpha is a master cytokine that originates in the epidermis and activates a whole programme of skin renewal in the dermis.

## Scientific background of Hexadeltine

### Enhancing microcirculation and controlling IL-1 alpha release

Hexadeltine (now referred to as "the hexapeptide") is the trade name for the novel artificial hexapeptide Tyr-D-Ala-Gly-Phe-Leu-Asp. Its INCI name is Hexapeptide-18.

A crucial finding for the use of this peptide in cosmetics was its ability to control the release of IL-1 alpha from keratinocytes. Figure 7 shows that its effect is time- and dose-dependent. At three hours after pre-incubation, the release of IL-1 alpha by keratinocytes was slightly increased for the entire range of the hexapeptide concentrations studied. Interestingly, after an interval of 24 hours, only the lower concentration of 68 ng/ml induced the release of IL-1 alpha while the higher concentrations were ineffective.

This biphasic time course at higher concentrations of the hexapeptide is, in fact, an initial stimulation that turns into an inhibition of IL-1 alpha release as shown by incubating keratinocytes with mixtures of both peptides. Indeed, as shown in Figure 8, the hexapeptide also inhibited at long time intervals alpha-stimulated release of IL-1 alpha. This antagonism is the main reason for combining both peptides to control the release of IL-1 alpha by allowing IL-1 alpha to be released for a few hours but blocking the late "exponential" increase due to high concentrations of IL-1 alpha. Cosmetic preparations containing both peptides can therefore be applied daily without risking hyperkeratosis or exfoliation of skin. The mixture has clear efficacy and safety benefits over the single compounds.

Besides that, the hexapeptide exhibits a significant effect on skin microcirculation (Fig. 9) which underlines its proven efficacy for the cosmetic treatment of dark circles and undereye bags (data not shown).

### No skin penetration

The absorption and penetration of the hexapeptide was measured by exposing artificial human skin patches during 24 hours to a high concentration of fluorescence-labelled product (about 880 times higher than the in-use concentration). Fluorescence microscopy indicated that the hexapeptide was absorbed by the *stratum corneum* only without penetrating deeper layers (Fig. 10). The detection limit of this method was estimated to be 20 pg/cm².

Similar to IL-1 alpha, the hexapeptide must also initiate on the skin surface a signalling cascade that reaches progressively deeper structures to exert its effects, which include i) stimulation of cutaneous microcirculation, ii) inhibiting skin pigmentation in response to exogenous stimuli (not shown) and iii) preventing IL-1 alpha release from "overshooting".

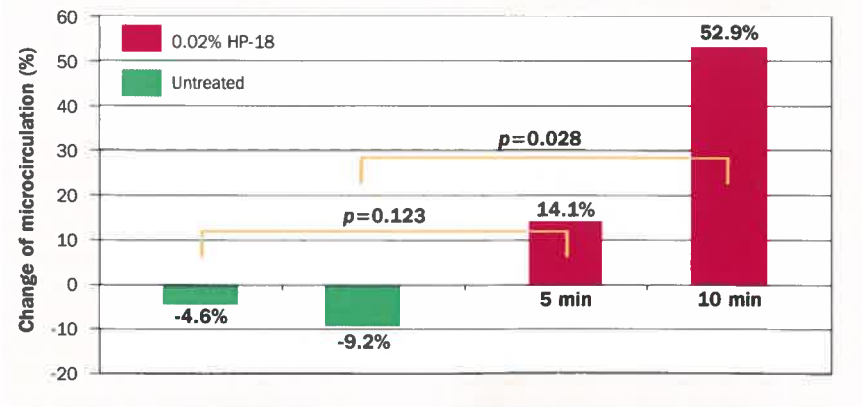


**Figure 9:** *Measurement of cutaneous microcirculation by laser Doppler flowmetry in 15 volunteers.*

## Role in hair life cycle and hair loss prevention

### Background

The effects of IL-1 alpha on hair follicle cycling are complex (Fig. 11). Dermal papilla cells are fibroblast-like cells within the hair bulb. Upon stimulation by interleukin-1 alpha they produce a set of growth factors which are essential for hair growth, and PN-1, an inhibitor of the protease involved in hair loss. Cell division in the hair matrix is responsible for the cells that form the major structures of the hair fibres. Similar to skin, there is also in hair follicles an epidermal-mesenchymal interaction involving IL-1 alpha stimulating dermal papilla cells to release growth factors for the epithelial matrix.

*In vitro* data show that IL-1 alpha and IL-1 beta directly inhibit the proliferation

of matrix keratinocytes. This effect seems to be mediated by cAMP. IL-1 alpha does not inhibit hair fibre growth. At the same concentration at which IL-1 alpha inhibits proliferation of matrix keratinocytes, it also strongly stimulates follicular papilla cells to express growth factors (KGF, HGF, GM-CSF, VEGF) required for keratinocyte proliferation, hair follicle development and differentiation which lead to hair growth, hair elongation, and proper vasculature around the hair follicle during the anagen growth phase.

IL-1 alpha further stimulates the expression of PN-1, the main marker of anagen follicles. Protease nexin-1 (PN-1) is a cell-secreted protein that inhibits certain proteases, particularly thrombin. Thrombin receptors are present in hair follicles only during the catagen phase. Activated by thrombin, they reduce the hair growth-supporting activity of follicle papilla cells (switch to catagen). PN-1 co-localises with thrombin. As inhibitor of thrombin, PN-1 is thus believed to prevent hair follicles from progressing to catagen or, expressed positively, to keep them in anagen phase.

If indeed, as suggested, exogen phase (hair shedding) is an active process mediated by a protease cleaving the hair shaft, one might hypothesise that the protease involved is thrombin and the induction of PN-1 by IL-1 alpha is responsible for reducing hair shedding.

Furthermore, IL-1 alpha has been shown to suppress androgen receptor expression by follicular papilla cells, a clear anti-androgenetic alopecia effect. These data suggest that the effects of IL-1 alpha are mediated by follicular papilla cells and that these effects may be related to the anagen phase of hair follicles.

*In vivo* data showed that IL-1 alpha protected animals and humans at least partially from hair loss in several models of alopecia, as well as against chemotherapy-induced hair loss. These data and the observation that hair growth is accompanied by simultaneous expression of IL-1 alpha strongly suggest an important role of IL-1 alpha in the regulation of the hair cycle. This role includes the inhibition of hair loss and the promotion of anagen mechanisms in hair follicles.

## Reducing signs of ageing and cellulite

Dermatopoietin-Plus (now referred to as "the novel mixture of IL-1 alpha and a hexapeptide") was tested in a double-blind and placebo-controlled study on 22 female volunteers for its anti-ageing effects. The volunteers all showed visual signs of skin ageing. They were randomly assigned to receive topical application of the test preparation on one forearm and of the placebo on the other forearm for eight weeks twice daily. Test parameters were:

- Mechanical properties of skin as measured with a Cutometer.
- Dermal thickness and structure as measured by ultrasonography.

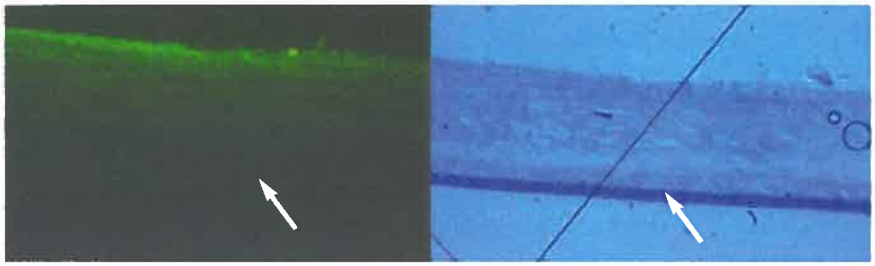

**Figure 10:** *Artificial epidermis (EpiSkin), mounted on a supporting polycarbonate membrane (arrows), was exposed to a high concentration of FITC-labelled Hexadeltine. Left panel: fluorescence microscopy. Green fluorescence indicates labelled Hexadeltine absorbed by* stratum corneum. *Right panel: light microscopy of the same field. Magnification: 100x. Small circle: 10 µm, large circle: 25 µm.*

The results are summarised in Table 1 and Figure 12. The placebo was a rich formulation containing special oils, vitamins and plant extracts. The test preparation in addition contained 50 µg/l of IL-1 alpha and 200 mg/l of the hexapeptide. Interestingly, both formulations resulted in a strong reduction of dark pixels in the dermis (water and fat inclusions) and in an increase of protein, as shown by ultrasonography. They also led to the same increase of "epidermal and dermal thickness" (not shown). But only the test formulation improved the mechanical properties of skin, shown as an increase of elasticity and decrease of viscosity of skin. In other words, the active ingredients were needed to turn the deposited collagen and elastin into elastic networks.

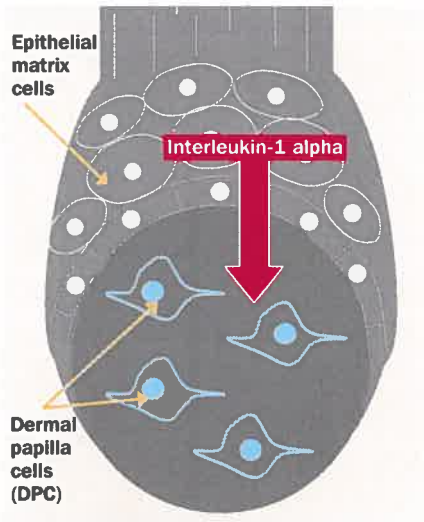


**Figure 11:** *Schematic representation of a hair follicle. Epithelial matrix cells produce IL-1 alpha which triggers dermal papilla cells to release several growth factors and PN-1, a protease inhibitor. These factors are vital for hair growth and preventing hair loss.*

In a second trial, the novel mixture was evaluated in a double-blind and placebo-controlled study with 20 female volunteers showing clear signs of cellulite. One of their thighs was randomly assigned to receive placebo application while the other thigh was treated with the test preparation. The application was twice daily and lasted eight weeks. Test parameters were the number of dark pixels (fat inclusion in the dermis) and the hypodermal – dermal junction distance as measured by ultrasonography. As shown in Table 2 and Figure 13, treatment during four and eight weeks with a cosmetic preparation containing the mixture resulted in a clear reduction of dermal fat inclusions in cellulite skin. This was accompanied by clear improvement of the visual appearance on the skin surface. Upon termination of treatment, the effect seems to increase for some time. No reduction of thigh girths was observed.

In general, efficacy parameters for aged skin seemed to reach a plateau after about four weeks of application and slowly declined over longer periods. For this reason, and to obtain optimal effects, cosmetic products containing the novel mixture of IL-1 alpha and a hexapeptide are recommended to be used in turn with a product devoid of actives. The cyclic application regimen in skin care consists of a four week treatment with the novel mixture followed by a four week maintenance phase with a "placebo", after which a new cycle can be started.

## Reduction of hair loss

Pilot studies with the novel mixture in volunteers suffering from excessive hair shedding have demonstrated a consistent reduction of hair loss to normal levels. Consumer reports confirmed that the product containg Dermatopoietin-Plus led to a reduction of abnormal hair shedding in the majority of cases. Systematic clinical studies have been initiated to explore the mechanism of action and to better define the responder and non-responder groups. The following is a summary of the still-

limited and preliminary knowledge of the effects of the novel mixture on the hair cycle.

- A short treatment during only a few days, typically four, is sufficient to reduce excessive hair shedding to normal levels (Fig. 14).
- The optimal regime consists of a four day treatment with the novel mixture followed by a ten day maintenance period during which a nourishing gel is applied. A full treatment cycle lasts 14 days.
- Best effects are achieved by two consecutive treatment cycles.
- Effect remains for at least 60 days.
- Responder groups include subjects with stress-, trauma- and age-related hair loss. To what extent early stages of androgenic alopecia and disease-related hair loss respond to this novel mixture is currently being investigated.
- Even subjects with normal hair growth seem to benefit from repeated treatments by gaining stronger and denser hair.

In view of the dominant role IL-1 alpha seems to play in regulating the cycle of hair follicles as inducer of "hair growth factors" and anagen markers, one should not be surprised if the novel mixture did not only inhibit hair loss but also increase hair growth by prolonging the anagen phase at the expense of the telogen phase.

In summary, these data strongly indicate that subjects with hair problems may benefit from cosmetic products containing the novel mixture.

**Table 1: Results of a double-blind anti-ageing study in 22 female volunteers.**

Elasticity was measured by cutometry; dark pixels refer to low echogenic material (water and fat inclusions) in ultrasonographic recordings.

| Test parameter | | Placebo | Test article | Significance level test *vs.* placebo |
|---|---|---|---|---|
| Change of gross elasticity | 28 days | –6.1% | +20.7% | $p=0.03$ |
| | 56 days | 0% | +15.2% | $p=0.12$ |
| Change of viscoelasticity | 28 days | +16.7% | –4.7% | $p=0.0008$ |
| | 56 days | +9.4% | +1.1% | $p=0.12$ |
| Number of dark pixels | 28 days | –54.4% | –53.0% | ns |
| | 56 days | –46.2% | –49.2% | ns |

ns: not significant.

## Conclusion and outlook

Skin epidermis, especially *stratum corneum*, is the richest endogenous source for interleukin-1 alpha. By applying a cosmetic product containing IL-1 alpha, one adds only some 10%-20% of what is already present in the skin. Interleukin-1 alpha has been called the master regulator of skin renewal. It is released by epidermal cells upon injury, chemical stress or UVB and initiates a signal cascade that eventually leads to the renewal and strengthening of epidermal and dermal structures and functions. Hexadeltine improves capillary microcirculation in skin and controls the release cycle of IL-1 alpha, thereby improving efficacy and tolerability of Dermatopoietin. With the novel mixture a product is available that exploits the endogenous skin renewal mechanism in a safe way for cosmetic and dermatological applications. Dermatopoietin-Plus has already proven effective for rejuvenating skin and hair. The future will without doubt reveal many other applications for this

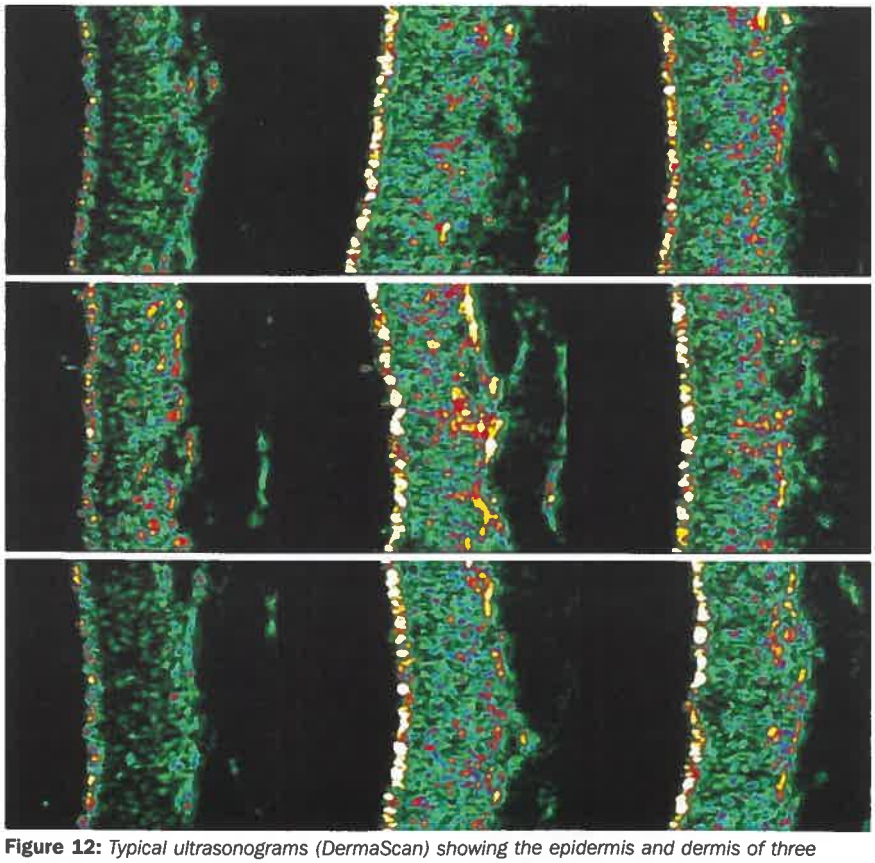

**Figure 12:** *Typical ultrasonograms (DermaScan) showing the epidermis and dermis of three volunteers on day 0 (baseline) and after 28 and 56 days, respectively. Both formulations were equally effective concerning a strong reduction of SLEB (sub-epidermal low echogenic band) as well as an increase of dense material in the epidermis and dermis.*

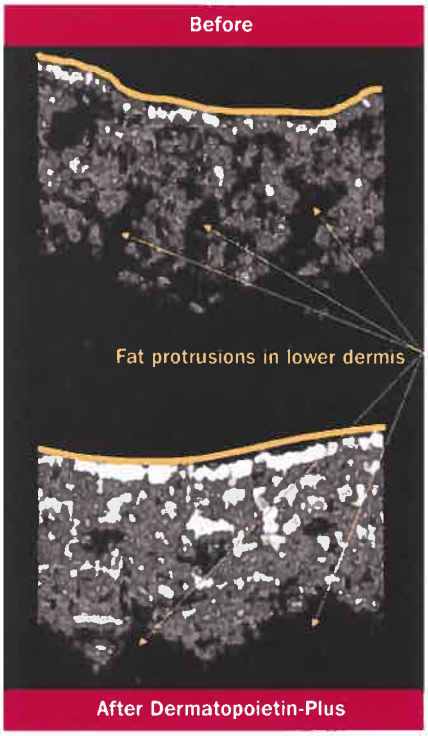


**Figure 13:** *Ultrasonographic pictures of cellulite-affected skin before and after treatment for four weeks with a preparation containing 100 µg/l Dermatopoietin and 200 mg/l Hexadeltine. The treatment resulted in a strong reduction of dark pixels (fat inclusions) and visible smoothing of skin surface.*

innovative concept not only in cosmetics but also in medicine. Potential examples are wound healing, including burns, treatment of scars, laser injuries, diabetic foot, and others. PC

## References


**Review**

1 Pomytkin I. Interleukin-1 alpha, an epidermal cytokine critical for skin renewal. *SOFW J*, 135, 2-6 (2009).

**Interleukin-1 alpha: release from keratinocytes and interaction with fibroblasts**

*For interleukin-1 alpha and its role in skin physiology see the above review and the references cited therein.*

**Interleukin-1 alpha and the hair cycle**

2 Hussein A.M., Jimenez J.J., McCall C.A., Yunis A.A. Protection from chemotherapy-induced alopecia in a rat model. *Science*. 249, 1564-1566 (1990).

3 Hussein A.M. Interleukin-1 protects against 1-beta-D-arabinofuranosylcytosine-induced alopecia in the newborn rat animal model. *Cancer Res*, 51, 3329-3330 (1991).

4 Jimenez J.J., Wong G.H., Yunis A.A. Interleukin-1 protects from cytosine arabinoside-induced alopecia in the rat model. *FASEB J*, 5, 2456-2458 (1991).

5 Groves P.W., Williams I.R., Sakar S., Nakamura K., Kupper T.S. Analysis of epidermal IL-1 family members in vivo using transgenic mouse models. *J Invest Dermatol*, 102, 556 (1994).

6 Danilenko D.M., Ring B.D., Yanagihara D., Benson W., Wiemann B., Starnes C.O., Pierce G.F. Keratinocyte growth factor is an important endogenous mediator of hair follicle growth, development, and differentiation. Normalization of the nu/nu follicular differentiation defect and amelioration of chemotherapy-induced alopecia. *Am J Pathol*, 147, 145-154 (1995).

7 Shimaoka S., Tsuboi R., Jindo T., Imai R., Takamori K., Rubin J.S., Ogawa H. Hepatocyte growth factor/scatter factor expressed in follicular papilla cells stimulates human hair growth *in vitro*. *J Cell Physiol*, 165, 333-338 (1995).

8 Yu D.-W, Yang T., Sonoda T., Gaffney K., Jensen P.J., Dooley T., Ledbetter S., Freedberg I.M., Lavker R. Sun T.-T. Message of nexin-1, a serine protease inhibitor, is accumulated in the follicular papilla during anagen of the hair cycle. *J Cell Sci*, 108, 3867-3874 (1995).

9 Hoffmann R., Eicheler W., Huth A., Wenzel E., Happle R. Cytokines and growth factors influence hair growth *in vitro*. Possible implications for the pathogenesis and treatment of alopecia areata. *Arch Dermatol Res*, 288, 153-156 (1996).

10 Sredni B., Xu R.H., Albeck M., Gafter U., Gal R, Shani A., Tichler T., Shapira J., Bruderman I., Catane R., Kaufman B., Whisnant J.K., Mettinger K.L., Kalechman Y. The protective role of the immunomodulator AS101 against chemotherapy-induced alopecia studies on human and animal models. *Int J Cancer*, 65, 97-103 (1996).

11 Harmon C.S., Nevins T.D. Evidence that activation of protein kinase A inhibits human hair follicle growth and hair fibre production in organ culture and DNA synthesis in human and mouse hair follicle organ culture. *Br J Dermatol*, 136, 853-858 (1997).

12 Kozlowska U., Blume-Peytavi U., Kodelja V., Sommer C., Goerdt S., Majewski S., Jablonska S., Orfanos C.E. Expression of vascular endothelial growth factor (VEGF) in various compartments of the human hair follicle. *Arch Dermatol Res*, 290, 661-668 (1998).

13 Yano K., Brown L.F., Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. *J Clin Invest*, 107, 409-417 (2001).

14 Tang L., Cao L., Pelech S., Lui H., Shapiro J. Cytokines and signal transduction pathways mediated by anthralin in alopecia areata-affected Dundee experimental balding rats. *J Invest Dermatol Symp Proc*, 8, 87-90 (2003).

15 Anan T., Sonoda T., Asada Y., Kurata S., Takayasu S. Protease-activated receptor-1 (thrombin receptor) is expressed in mesenchymal portions of human hair follicle. *J Invest Dermatol*, 121, 669-673 (2003).

16 Alonso L., Fuchs E. The hair cycle. *J Cell Sci*, 119, 391-393 (2006).

17 Boivin W.A., Jiang H., Utting O.B., Hunt D.W.C. Influence of interleukin-1 alpha on androgen receptor expression and cytokine secretion by cultures human dermal papilla cells. *Exp Dermatol*, 15, 784-793 (2006).

18 Braun S., Krampert M., Bodó E., Kümin A., Born-Berclaz C., Paus R., Werner S. Keratinocyte growth factor protects epidermis and hair follicles from cell death induced by UV irradiation, chemotherapeutic or cytotoxic agents. *J Cell Sci*, 119, 4841-4849 (2006).

19 Feutz A.-C., Barrandon Y., Monard D. Control of thrombin signaling through PI3K is a mechanism underlying plasticity between hair follicle dermal sheath and papilla cells. *J Cell Sci*, 121, 1435-1443 (2008).


**Table 2: Change of structural parameters of cellulite skin.**

One thigh was treated with vehicle, the other with the test article containing 100 µg/l Dermatopoietin and 200 mg/l Hexadeltine during 56 days. From day 56 to day 70 no treatment took place. Test article resulted in a continuing improvement of skin structure, accompanied by smoothing of skin surface.

| Test parameter | | Placebo | Test article | Significance level test *vs.* placebo |
|---|---|---|---|---|
| Number of dark pixels | 14 days | +68.2% | +1.3% | ns |
| | 28 days | +41.0% | –12.9% | $p$=0.03 |
| | 56 days | +38.2% | –25.9% | $p$=0.006 |
| | 70 days | +14.3% | –36.8% | $p$=0001 |
| Hypodermal – dermal junction distance | 14 days | +35.6% | –6.6% | ns |
| | 28 days | + 38.4 % | –21.5% | $p$= 0.001 |
| | 56 days | +5.6% | –17.7% | $p$=0.001 |
| | 70 days | +18.6% | –21.1% | $p$=0.001 |

ns: not significant.

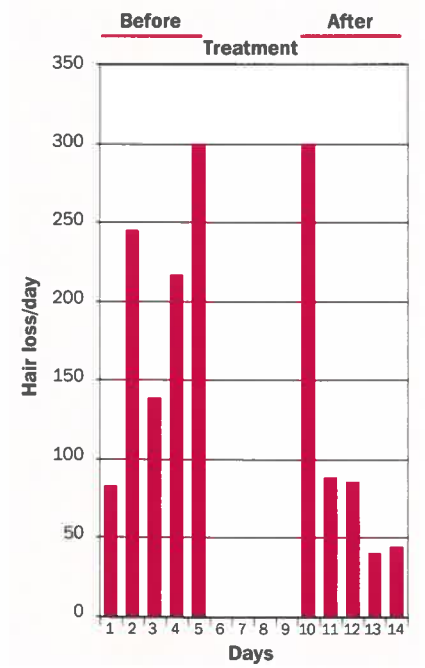


**Figure 14:** *Typical case of a volunteer with extensive hair shedding. Baseline hair loss was around 200 hairs per day. Treatment occurred over night during four consecutive days. On the first day after treatment hair loss was typically not or only marginally reduced. On the following days a major reduction of hair shedding was observed ranging from –50% to –73%. The effect remained for at least 60 days.*

Dermatopoietin-Plus is marketed by United Technologies UT AG in Schlieren/Zurich, Switzerland, which holds all the intellectual property rights for the use of IL-1 alpha in cosmetics and dermatology and Hexadeltine and similar peptides (for details consult www.unicos.ch). The authors would like to thank Alain Béguin, Skin Test Institute, Neuchatel, Switzerland, for the dermatological studies and Andreas Dreusch, MicroMol GmbH, Karlsruhe, Germany, for the EpiSkin measurements described in the paper.