Technical Report

Product

TELANGYN™

Date

June 2012

Revision

1

Pol. Ind. Camí Ral C/ Isaac Peral, 17
08850 Gavà Barcelona (Spain)
Tel. +34 93 638 80 00
www.lipotec.com
commercial@lipotec.com

Lipotec Group

telangyn™

Aimtec™

Contents

Skin redness and extra reactivity

Millions of people suffer from inflammatory overreactions linked to skin redness, which cause general discomfort and influence personal wellness and social life. Facial inflammation of capillaries and pilosebaceous units can be found in these skin disorders, which at the end lead to **flushing skin, temporary or even persistent erythema (mainly in the nose and cheek), telangiectasia (dilated capillaries), pustules, papules and itching, concentrated on the central third of the face** [1, 2]. These extra redness disarrangements are basically facial conditions, but they can also occur in other areas like the neck, ears, scalp and the upper portion of the chest [2].

Rosacea is a chronic skin disorder that typically manifests in people with pale skin and northern-western European descent ("curse of the Celts"), being **women over 30s the most affected population** [1, 2]. Although less frequently, men and other ethnic groups can also suffer from this common inflammation presenting the same distressing consequences [1, 2]. The stress that this alteration causes to the skin is commonly translated into a dull appearance, among other effects.

Although **its etiology is already uncertain**, there are many studies which connect facial erythema to strong emotions (anger, stress or embarrassment), sport practise, UV radiation, certain foods (spicy) or drinks (alcohol), environmental conditions (strong wind, overheating and cold weather) and certain topical treatments [1, 2].

What it is certainly demonstrated is that there is an **increase of skin inflammatory metabolites and reactions** in people affected by facial redness and spider veins, suggesting that inflammation and its metabolites play a central role on these manifestations and disorders.

In the natural response of the immune system to potential harmful agents and conditions, **Kallikreins (KLK) and antimicrobial compounds like cathelicidines have an important role. They induce the release of Interleukines (IL)**, which are among the molecules with increased levels in facial skin redness and vascular alterations [1-3]. Their increment certainly generates capillary dilation, papules, redness, inflammation and Post-inflammatory Hyperpigmentation (PIH) at times. PIH normally occurs after cutaneous injury or local inflammation, being linked to tyrosinase and melanocyte activity increase and melanin raise [4].

Acting on inflammation and its metabolites becomes highly useful to diminish skin redness and spider veins.

Cathelicidins role in skin inflammatory disorders

The skin has an essential role protecting from environmental pathogens as it can mount an innate immune response, which involves the **production of antimicrobial peptides** [5]. The innate immune protecting function of the skin is highly enhanced by an antimicrobial peptide barrier, activated when physical obstacles fail to avoid pathogen entry. These antimicrobial peptides act as natural antibiotics (against bacteria, fungi and/or viruses), providing fast broad-spectrum protection. Besides, they can also **modify the local inflammatory reaction and activate mechanisms of cellular and adaptive immunity** [5].

Cathelicidins are a relevant family of **antimicrobial peptides** expressed in human leukocytes and some epithelial cells, where they participate in the innate immune response [1, 3]. They show potent **endogenous antibiotic activity**, promote the expression of Extracellular Matrix (ECM) components, leukocyte chemotaxis, angiogenesis and act as signalling molecules, coordinating local vascular function and wound healing [1, 6, 7].

Human cathelicidins are secreted as an inactive pro-protein named 18kDa Cationic Antimicrobial Protein (**CAP18**), which needs proteolytic processing to become **biologically active as** 37-amino-acid peptide **(LL-37)** [1, 3, 6]. **Kallikrein-5** (KLK5), also known as Stratum Corneum Tryptic Enzyme (SCTE), is the key serine protease from the kallikrein family that cleaves CAP18 to LL-37 in human epidermis [3, 6-7].

Once LL-37 is locally activated, a cascade of pro-inflammatory reactions leads to an **increase of pro-inflammatory metabolites like cytokines, IL-6, IL-8** and Tumor Necrosis Factor-α (TNF-α), together with macrophages, neutrophils and mast cells, which end up causing the visible effects of inflammation [1, 6, 8]. Besides, LL-37 has chemotactic and angiogenic activity [1].

Both CAP18 and KLK5 are naturally found in the skin, being KLK5 one of the major trypsin-like KLKs in the stratum corneum [9-10]. KLK5 also degrades corneosome proteins, a vital event prior to desquamation, which can lead to dry skin and scaling [9-12].

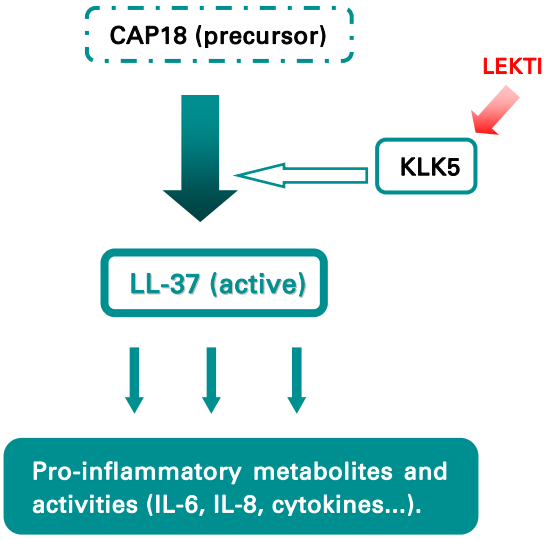

Fig. 1. Inflammation cascade in the skin.

As a KLK5 regulating factor, the endogenous skin serine protease inhibitor Lymphoepithelial Kazal-Type-related Inhibitor (**LEKTI**) specifically inhibits KLK5, indirectly **down-regulating its pro-inflammatory effects, cathelicidin activation and release of ILs** [6, 12].

Epidermal LEKTI deficiency results in KLK5 hyperactivity, accelerated shedding and loss of **skin barrier function**, whose **disruption** alone was demonstrated to stimulate cytokine and chemokine production, **causing inflammation** [12].

In skin facial disorders with redness and dilated capillaries, like rosacea, **LL-37 expression is found to be abnormally high as well as IL-6 and IL-8** levels [1-10, 13]. In fact, it has been observed that LL-37 expression is upregulated in common inflammatory skin lesions like psoriasis and after skin wound healing, but also in certain skin disorders presenting erythema in newborns [5, 13]. Comparing skin samples from normal skin and skin with redness, cathelicidins were found to be much more abundant in the altered skin [6]. The *in vivo* effects of LL-37 were also studied and it was found that its presence induced erythema and vascular dilation in skin after 48 h [6]. These results were in accordance with LL-37 *in vitro* effects, where IL-8 release was induced by this compound in human keratinocytes [6].

In order to know more about this process, KLK5 was evaluated *in vivo*, observing that this protease also induced erythema and inflammatory cell infiltration, but also the formation of active cathelicidins [6].

Adding that the skin response to inflammation and experimental sunburn lesions resulted in the release and increase of several pro-inflammatory cytokines and chemokines, including IL-6 and IL-8, it can be said that **inflammation, LL-37 and skin disorders with erythema are linked** [8].

All these compounds are thought to be the **responsible agents** for the uncomfortable manifestations accompanying this exacerbated innate immune response, affecting skin appearance [6]. When the elevated levels of LL-37 are not temporary, the effects of **skin inflammation, including facial redness and visible capillaries, can become chronic and highly difficult to minimise** [1].

Due to an increase of LL-37, IL-6 and IL-8 are abnormally raised in skin redness disorders so decreasing their cutaneous levels would help to reduce the appearance of erythema, telangiectasia and other undesired manifestations.

Inflammation and tissue degradation

The facial consequences of an exacerbated immune system response involve erythema and spider veins, which are derived from the local vascular system dilation. When capillaries are fragile and permeable, **they can easily dilate and blood can arrive to the surrounding tissue, becoming more visible as red zones**. When local inflammation appears, **skin firmness and elasticity are also affected.** These properties are basically influenced by the two most important fibrous components of the ECM: collagen and elastin.

Collagen is the most abundant protein in the skin (70-80% dry weight) providing mechanical and structural integrity [14]. **Type I collagen** represents the 80% of the dermal collagen and it assembles into collagen fibrils (<300 nm diameter), which in turn aggregate into larger cable-like collagen fibres (fibrillogenesis) [15]. The resulting collagen bundles are responsible for **skin strength and resistance** [14, 16]. **Type III collagen** constitutes most of the other collagen in adult human dermis [15].

Elastin is a connective tissue protein which is the major component of the elastic fibres. These fibres are insoluble structures with a central core of hydrophobic cross-linked elastin surrounded by fibrillar structures (10-12 nm). Although being less abundant than collagen, elastin is certainly crucial for **skin elasticity, resiliency and recoil** [14].

The dermal connective tissue may be damaged as a result of an inflammatory response. Harsh weather conditions and UV exposure stimulate reactive species production, boosting the skin inflammatory process and activating the proteolytic degradation of the ECM.

Phagocytes like neutrophils infiltrate into the skin from capillaries and secrete cytokines, which raise the recruitment of more inflammatory cells. The recruited **neutrophils release proteases** that cause more inflammation and **activate Matrix Metalloproteinases (MMP)** [17-18].

MMPs are zinc-dependent endopeptidases that **degrade ECM components**, and act in dermal turnover and remodelling. In normal skin, MMPs are kept inactivated and poorly expressed but inflammation activates them, rising ECM degradation. MMP-1 starts the cleavage of type I and III collagen, which is further degraded by MMP-2 and -9 [17]. Released by macrophages, MMP-12 is the most active MMP against elastin, degrading type IV collagen too [19]. The result of a **raise in collagen and elastin degradation due to MMPs is a flaccid and soft skin.**

LL-37 proved to clearly inhibit type I and III collagen mRNA expression in human dermal fibroblasts and kallikreins increase is linked to **ECM degradation, MMP activation, blood pressure changes and type I and III collagen alteration** [15, 20].

A skin decrease in LL-37 and MMPs would help to improve skin firmness and elasticity, properties that local disorders with inflammation and redness tend to reduce.

TELANGYN™, the solution to facial redness

TELANGYN™ is an innovative tetrapeptide specifically designed to decrease the appearance of facial redness and telangiectasia caused by an exaggerated inflammatory response.

As facial inflammation and redness are related to an increase of IL-6 and IL-8 levels, **TELANGYN™ was tested *in vitro* in human keratinocytes cultures, where the release of these both IL, induced by LL-37, was reduced in a dose-dependent manner.** It also showed to improve skin firmness by highly decreasing *in vitro* collagen degradation, which happens to be a usual result of an exacerbated immune response and normally occurs in skin with redness.

Moreover, **it provided high cellular photoprotection**, which helps to lower cell damage and inflammation negative effects. Its additional **anti-tyrosinase activity and melanin content reducing capacity** facilitate to increase the evenness of the skin tone, diminish the hyperpigmentation that sometimes is linked to inflammatory processes and improve the usually found dull appearance [4].

TELANGYN™ demonstrated beneficial effects also *in vivo*, providing a visible reduction of facial erythema, skin redness and extent just after 7 days, ameliorating skin roughness too. After 4 weeks, it diminished these parameters even more, together with the **number of red spots and their size and intensity.**

TELANGYN™ is an excellent active ingredient to reduce the appearance of local erythema, increase photoprotection and improve general skin properties like firmness and tone homogeneity.

In vitro efficacy

EVALUATION OF THE INHIBITION OF LL-37-INDUCED IL RELEASE

The aim of this study was to evaluate the **efficacy of TELANGYN™ on the inhibition of the activity of the antimicrobial peptide LL-37** by means of measuring the key pro-inflammatory **cytokines IL-6 and IL-8** in culture supernatants from **human primary keratinocytes** by ELISA.

Keratinocytes were seeded on well plates 48 h before the assay and 0.05 mg/mL of LL-37 was pre-incubated alone or with TELANGYN™ (at 0.025, 0.01 or 0.5 mg/mL) for 1 h at room temperature in a microtube rotator. After pre-incubation, **cells were treated with** 50 µL of **LL-37 alone or with TELANGYN™** and incubated for 1 h at 37 ºC. Afterwards, medium was added and cells were incubated 5 h more in order to allow the release of cytokines.

Supernatants were subsequently harvested for cytokine quantification by **ELISA**, adding anti-human IL-6 or IL-8 specific capture antibody in the medium and measuring absorbance in a microtiter plate reader at 450 nm. **Crystal Violet assay** permitted to determine the number of cells per well, by measuring the optical density at 630 nm.

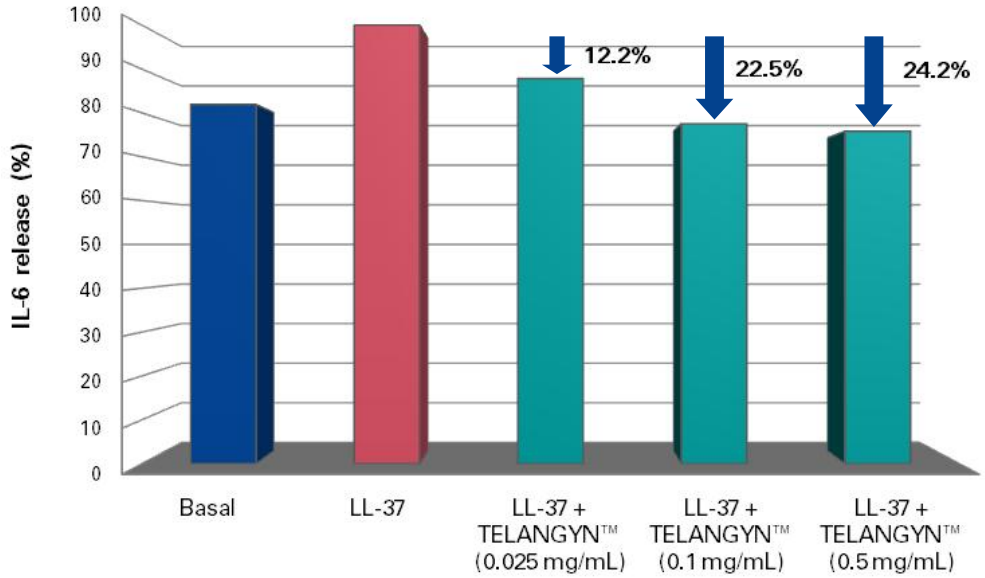

Fig. 2. Release of IL-6 after treatment with LL-37 plus TELANGYN™, showing the inhibiting effect of TELANGYN™ on its LL-37-induced release.

Keratinocytes treated with TELANGYN™ released lower levels of IL-6, diminishing its LL-37 induction by 12.2%, 22.5% and 24.2% at the tested concentrations.

TELANGYN™ proved to have a statistically significant effect in decreasing the LL-37-induced levels of IL-6 in human keratinocytes.

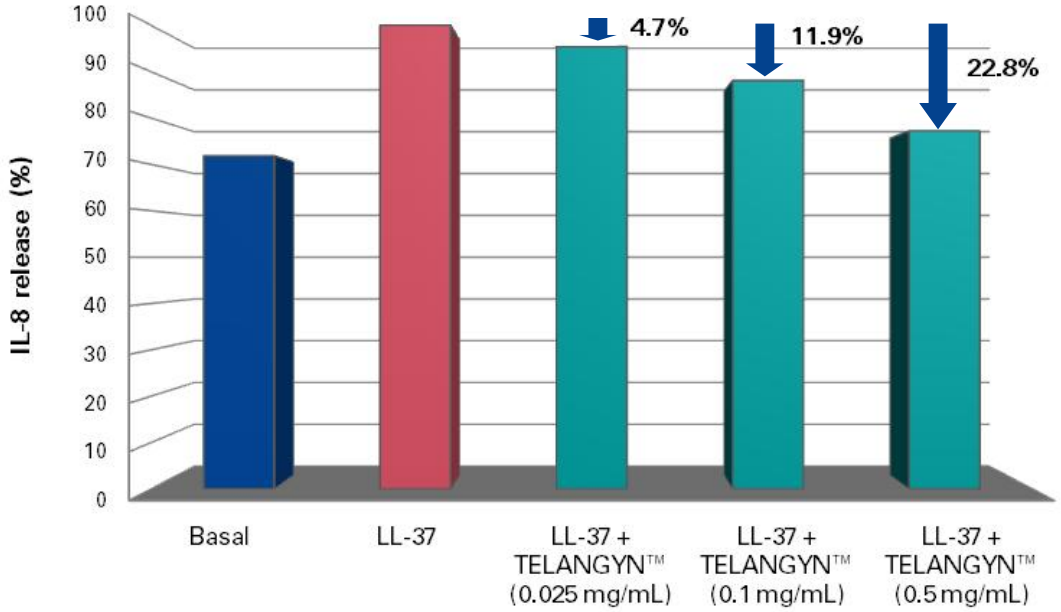

Fig. 3. IL-8 levels after treatment with LL-37 and TELANGYN™, showing the inhibiting effect of TELANGYN™ on LL-37 induced release.

TELANGYN™ demonstrated to diminish the LL-37-induced release of IL-8 in human keratinocytes by 4.7%, 11.9% and 22.8% at the different tested concentrations.

TELANGYN™ showed to highly decrease the LL-37-induced levels of the pro-inflammatory cytokine IL-8.

ANTI-COLLAGENASE ASSAY

In order to evaluate the anti-collagenase effect of TELANGYN™, a high sensitivity method for **measuring collagenase activity** and for screening inhibitors in a high-throughput format was used (**Molecular Probes EnzChek Gelatinase/Collagenase Assay kit**).

Initially, 80 µL of reaction buffer alone, **metalloproteinase inhibitor** (1,10-Phenantroline solution) **or samples with TELANGYN™** (at 2, 3 or 10 mg/mL) was incorporated into the wells of a microplate. Then, 20 µL of the fluorescein conjugate (gelatine) was added as well as 100 µL of **collagenase solution** or reaction buffer alone (used as negative control). The metalloproteinase inhibitor was used as a positive control.

Finally, the plate was incubated during 2 h, **measuring fluorescence** at 515 nm (excitation at 495 nm) in a microtiter plate reader. Fluorescence intensity was proportional to proteolytic activity: **low fluorescence implied higher collagenase inhibition**.

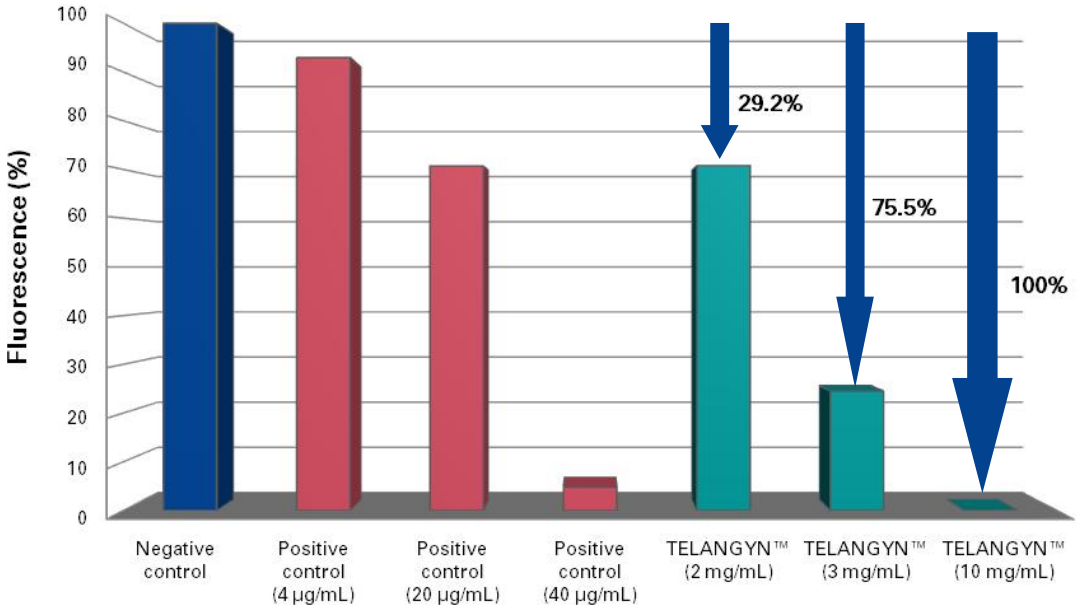

Fig. 4. Fluorescence and anti-collagenase activity provided by TELANGYN™ versus negative control.

Results showed that TELANGYN™ extremely inhibited collagenase activity by 29.2% (2 mg/mL), 75.5% (3 mg/mL) and 100% (10 mg/mL) versus negative control.

TELANGYN™ demonstrated a statistically significant inhibitory effect on collagenase activity, reducing collagen degradation.

ANTI-TYROSINASE ASSAY

The *in vitro* mushroom tyrosinase inhibition assay was performed in order to measure the **inhibitory effect of TELANGYN™ on tyrosinase activity**, which is a key enzyme for skin pigmentation.

TELANGYN™ and kojic acid were pre-incubated at 37 ºC in a microplate with **L-Dopa** (tyrosinase substrate at 5mM) during 30 min. Afterwards, **tyrosinase** (5 U/µL) was added and samples were incubated 10 min more at 37 ºC. Distilled water was used as the negative control.

Finally, the reaction was quenched by cooling samples at -20 ºC during 5 min. Read at 490 nm in a microtiter plate reader, the **absorbance values** were proportional to tyrosinase activity. **Low absorbance signified high tyrosinase inhibition**.

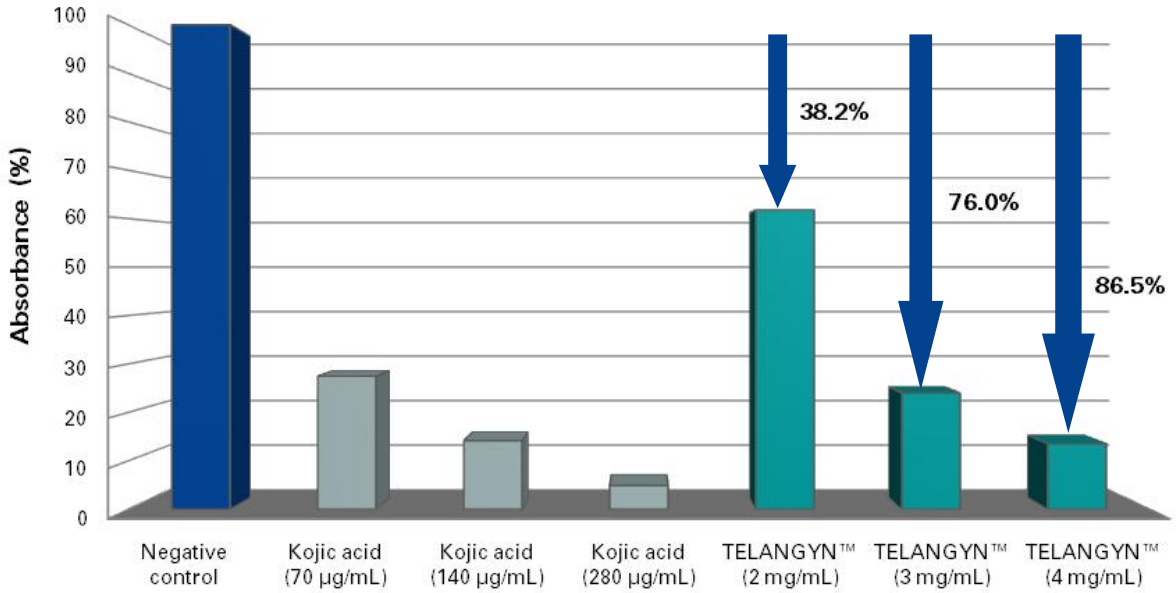

Fig. 5. Absorbance values and tyrosinase inhibition due to TELANGYN™, compared to control and kojic acid.

The absorbance values demonstrated that TELANGYN™ had a **statistically significant inhibitory effect** on tyrosinase, decreasing its activity. **TELANGYN™ inhibited tyrosinase activity by 38.2%, 76.0% and 86.5%** at 2, 3 and 4 mg/mL respectively, versus negative control.

TELANGYN™ proved to clearly minimise tyrosinase activity, being able to reduce it up to 86.5%.

MELANOGENESIS INHIBITION ON HUMAN EPIDERMAL MELANOCYTES

Primary human melanocytes cell cultures were used to study the **efficacy of TELANGYN™ inhibiting melanogenesis** at two different concentrations (0.0625 mg/mL and 0.125 mg/mL).

Melanocytes were allowed to grow in the seeding medium during 72 h and subsequently, medium was changed to culture medium until confluence (2 weeks). After overnight incubation at 37 ºC, cells received the first treatment: medium was aspirated and **fresh medium with TELANGYN™ was added**. Wells with medium alone were used as plate negative controls. The same treatment was repeated on days 3, 6, 8 and 10.

After the 13 days treatment period, cells were lysed and centrifuged. Melanin concentration was quantified by **measuring the absorbance** at 450 nm in a plate reader and normalising with respect to the number of cells per well.

Melanin concentration was determined from a standard curve plotted with synthetic melanin at known concentrations.

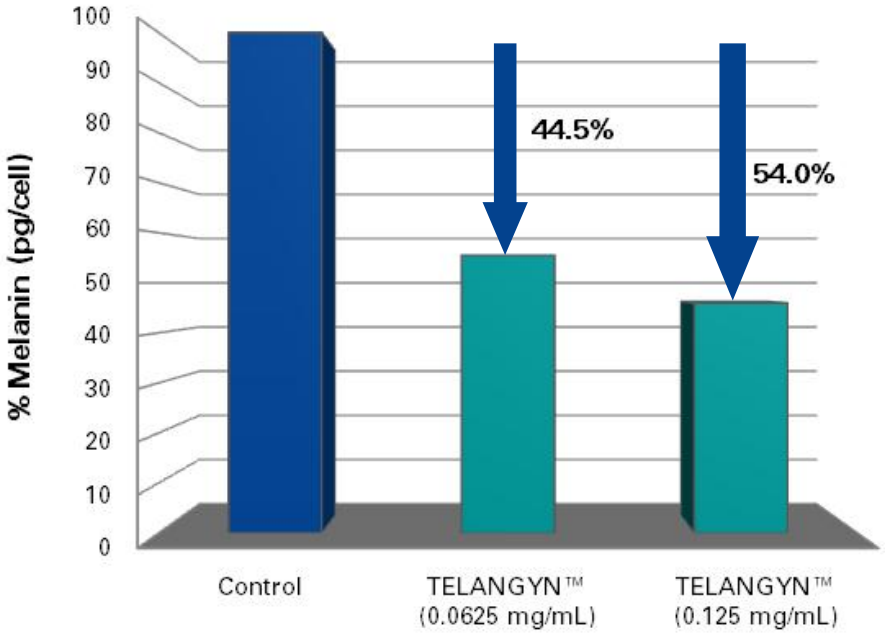

Fig. 6. Melanin content after treatment with TELANGYN™ with respect to control.

Results showed that the percentage of **melanin was reduced by 44.5% and 54.0%** versus control **due to TELANGYN™ application** at 0.0625 mg/mL and 0.125 mg/mL respectively.

TELANGYN™ provided a statistically significant inhibitory effect on melanogenesis in human epidermal melanocytes.

PHOTOPROTECTION TEST ON HUMAN DERMAL FIBROBLASTS

This test was performed to determine the **protective effect of TELANGYN™** application when human dermal fibroblasts (HDF) were **exposed to a cytotoxic dose of UV radiation**.

After maintaining HDF in culture plates during 24 h, they were **incubated with TELANGYN™** (1 or 10 mg/mL) or the medium alone (non-treated cells) during 1 h at 37 ºC. Thereafter, cells were **exposed to UV irradiation** (about 36 J/cm^2) **during 150 min**. A plate was kept in the dark during the same period, acting as the control for non-irradiated cells.

Finally, the treatment medium was replaced by the culture medium and cells were incubated during 24 h more. **Neutral Red Uptake (NRU)** was the method used to quantify the optical density of the samples at 540 nm in a spectrophotometer and **determine cell viability**. Cells can internally accumulate NR, decreasing its levels when cell surface alterations are induced.

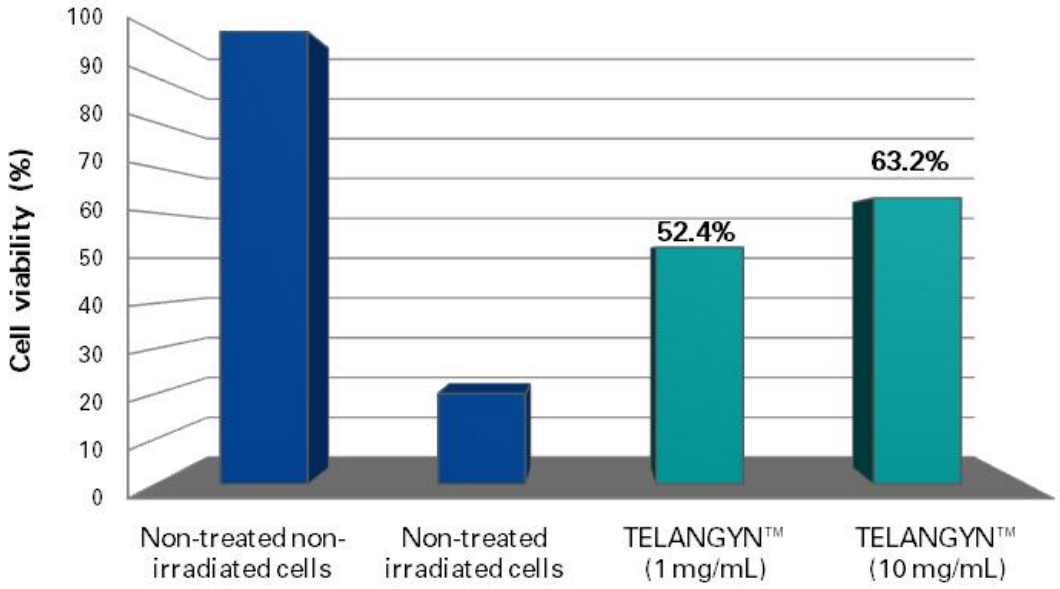

Fig. 7. Cell viability after irradiation in cultures previously treated with TELANGYN™.

Fibroblasts treated with TELANGYN™ and irradiated showed an increased cell viability respect to non-treated irradiated cells. Applied at 1 and 10 mg/mL, **TELANGYN™ raised cell viability up to 52.4% and 63.2% respectively.**

TELANGYN™ offered a statistically significant photoprotective effect on human dermal fibroblasts.

In vivo efficacy

DERMATOLOGICAL EVALUATION

In order to determine the *in vivo* efficacy of TELANGYN™ in reducing the appearance of facial erythema, a panel of **20 volunteers** (25-65 years old) with **mild rosacea** was evaluated. They were asked to apply a cream with **2% TELANGYN™ SOLUTION on the face, twice a day** for **28 days**. Its effect on skin redness, erythema and extent intensity was analysed as well as skin roughness, feature which can be abnormally increased due to the inflammation processes. These parameters were determined by a trained specialist after 1 and 4 weeks of treatment via the *In-vivo* Touching Evaluation method, using an analogous scale going from "no intensity" to "maximum intensity".

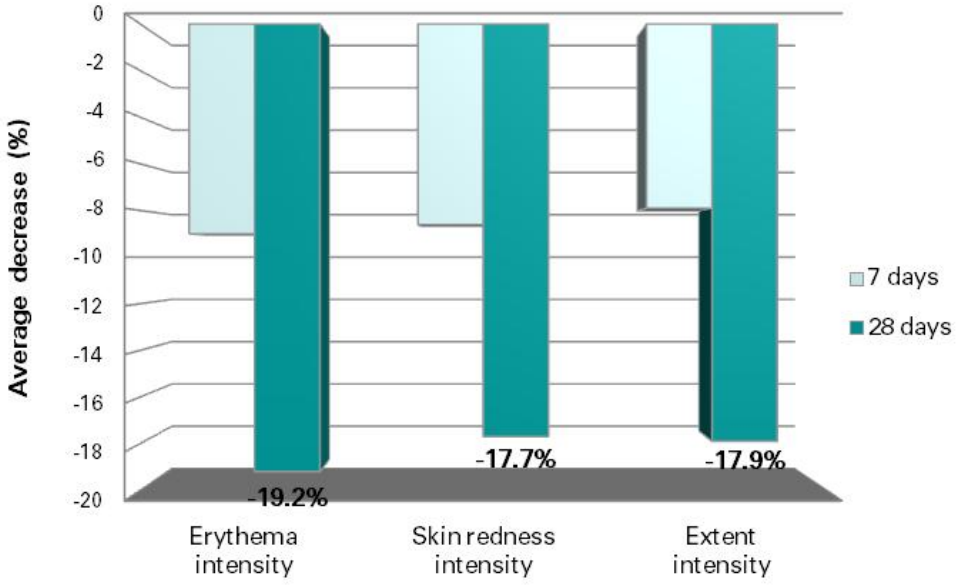

Fig. 8. Erythema, skin redness and extent intensity decrease after a treatment with a cream containing TELANGYN™.

After 7 days, **TELANGYN™ already had a visible effect on facial erythema, skin redness and extent, diminishing its intensity by 9.0%, 8.6% and 7.9%** respectively.

The induced improvement after 4 weeks was clearly higher, averagely decreasing the intensity by 19.2%, 17.7% and 17.9% according to erythema, skin redness and extent original values respectively.

Skin roughness values were also averagely reduced by 5.4% and 7.5% after 1 and 4 weeks of treatment with TELANGYN™ respectively.

TELANGYN™ minimised the appearance of skin redness, erythema, extent and skin roughness just after one week, providing excellent improvements after 4 weeks.

The images of the volunteers at the end of the treatment demonstrated the elevated beneficial effects of TELANGYN™ in reducing the extension and intensity of facial erythema and spider veins after its application for 28 days.

Fig. 9. Real images of a volunteer before (left) and after (right) applying TELANGYN™ for 28 days.

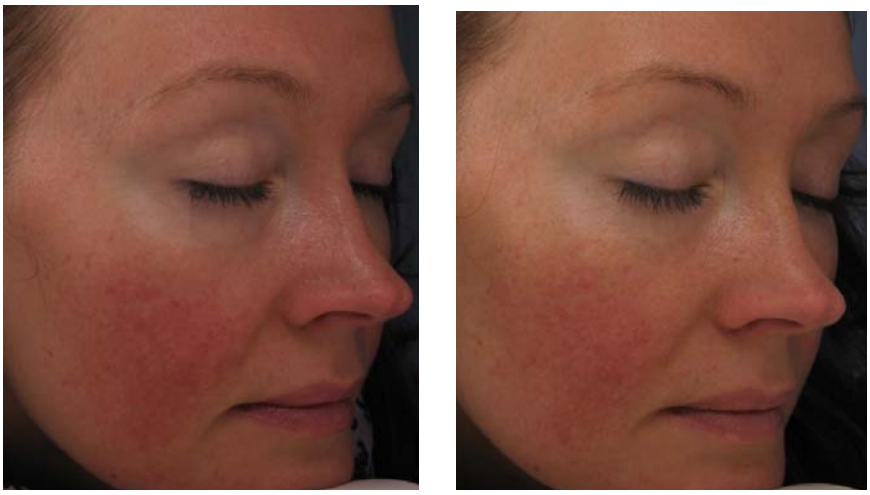

Fig. 10. Pictures of a volunteer at the beginning (left) and at the end of the treatment (right) with TELANGYN™.

TELANGYN™ proved to manifestly diminish the appearance of facial skin redness as well as its intensity after 28 days.

DIGITAL ANALYSIS OF SKIN REDNESS WITH THE VISIA™ SYSTEM

The **VISIA™ system** allows the comparison of skin characteristics and conditions at different times, **objectively displaying any parameter**. Pictures from the volunteers are taken using a camera unit, which results in a diagram of the volunteer complexion after software analysis.

Five volunteers from the *In-vivo* Touching Evaluation panel with **mild rosacea**, that applied a **cream with 2% TELANGYN™ SOLUTION twice a day for 28 days**, were chosen for this test. In this study, the VISIA™ analysis was performed to examine the skin redness parameter before and after the treatment (4 weeks). Feature counts were considered as the number of red areas and Absolute scores implied the size and intensity of these red areas.

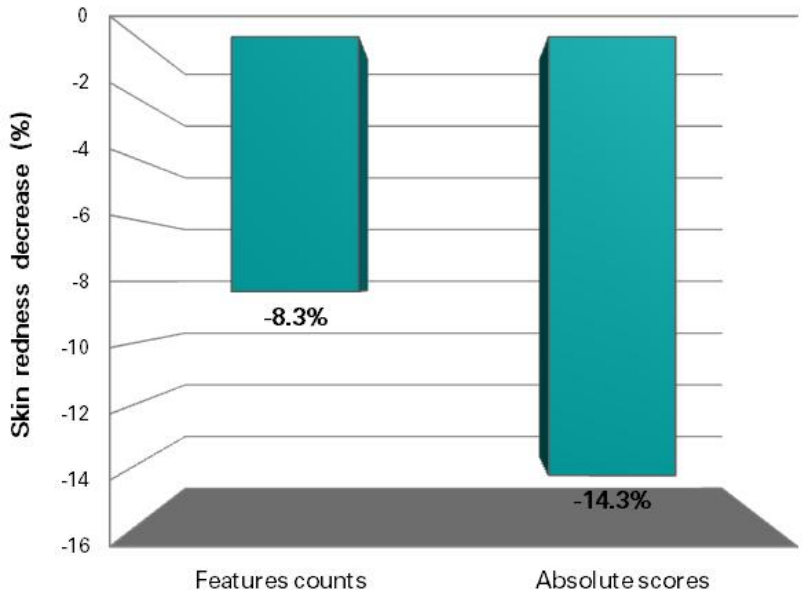

Fig. 11. Skin redness improvements obtained via VISIA analysis in volunteers applying TELANGYN™ for 28 days.

After 4 weeks, **the number of red areas was averagely reduced by 8.3% due to TELANGYN™ and also their sizes and intensities, that averagely diminished by 14.3%.**

The images from the volunteers clearly showed these effects and the overall **excellent improvement of the skin facial redness, which was visibly minimised after 28 days.**

TELANGYN™ decreased the main parameters of facial redness after 28 days, reducing the average number of red areas as well as the size and intensity.

Cosmetic properties

TELANGYN™:

- **is a new tetrapeptide to diminish the appearance of skin redness and visible capillaries, related to local inflammation.**
- proved to have a **statistically significant effect reducing** the levels of LL-37-induced cytokines **IL-6 and IL-8,** getting to inhibit them **by 24.2% and 22.8%** respectively (at 0.5 mg/mL) in human keratinocytes.
- extremely **inhibited *in vitro* collagenase activity** (linked to collagen degradation) **by 29.2%, 75.5% and 100%**. Its effects were statistically significant.
- had a **statistically significant inhibitory effect on tyrosinase** (key enzyme for melanin synthesis), decreasing its *in vitro* activity by **38.2%, 76.0% and 86.5%**. It facilitated to ameliorate skin homogeneity and radiance.
- provided a **statistically significant reduction in melanin content: 44.5% and 54.0%** *in vitro* decrease in human dermal melanocytes. It can help to improve skin hyperpigmentation and dull appearance that may take place after inflammation.
- offered a **photoprotective effect increasing cell viability up to 52.4% and 63.2%,** in human fibroblasts cultures toxically exposed to UV radiation.
- notably **reduced the erythema (9.0%), redness (8.6%) and extent (7.9%) intensity,** as well as **skin roughness (5.4%)** just **after one week** in volunteers with mild rosacea.
- obtained *in vivo* excellent results **after 4 weeks diminishing** the intensity of the **erythema (19.2%), skin redness (17.7%), extent (17.9%) and skin roughness (7.5%).** It also averagely **decreased the number of red areas by 8.3% and the size and intensity** of these areas **by 14.3% at the end of the treatment.**

Cosmetic applications

TELANGYN™ can be included in **products and formulations designed to reduce local facial redness and small visible capillaries, which are the result of an exaggerated local inflammatory response.**

Due to its additional firming, photoprotective and melanin decreasing activity, TELANGYN™ can also be incorporated in sun care, sensitive skin, firming and anti-aging facial treatments to complement their benefits, and in daily facial products with hydrating or nourishing effect.

Technical data

INCI NAME OF THE ACTIVE INGREDIENTS

Active ingredient	INCI name
TELANGYN™	Acetyl Tetrapeptide-33 (INCI proposed).

PRESENTATION AND PRESERVATIVE

Solution containing 0.05% of the peptide.

Code	Product presentation	Preservative
PD220	TELANGYN™ SOLUTION	Preservative free

Application data

PROCESSING

TELANGYN™ SOLUTION needs to be added in the aqueous phase. In case of emulsions, it should be added once the emulsion is formed and at temperatures below 40 ºC.

TELANGYN™ is stable at a pH range between 2.5 and 7.0.

INCOMPATIBILITIES

Not expected.

SOLUBILITY

Soluble in water.

DOSAGE

A dosage of 2% of TELANGYN™ SOLUTION is recommended in final cosmetic formulations.

References

1. Bevins CL, Liu F-T. Rosacea: skin innate immunity gone awry? *Nat Med*. 13 (8): 904-906, 2007.

2. Culp B, Scheinfeld N. Rosacea: a review. *Pharmacy and Therapeutics*. 34 (1): 38-45, 2009.

3. Murakami M, Ohtake T, *et al*. Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. *J Invest Dermatol*. 119 (5): 1090-5, 2002.

4. Chang MW. Disorders of hyperpigmentation. In: Bolognia JL, Jorizzo JL, Rapini RP, eds. *Dermatology*. 2nd ed. Elsevier Mosby, 2009.

5. Braff M, Bardan A, *et al*. Cutaneous defense mechanisms by antimicrobial peptides. *J Invest Dermatol.* 125 (1): 9-13, 2005.

6. Yamasaki K, Nardo A, *et al*. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med*. 13 (8): 975-80, 2007.

7. Yamasaki K, Scauber J, *et al*. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J.* 20 (12): 2068-80, 2006.

8. Angst MS, Clark JD, *et al*. Cytokine profile in human skin in response to experimental inflammation, noxious stimulation, and administration of a COX-inhibitor: a microdialysis study. *Pain.* 139 (1): 15-27, 2008.

9. Komatsu N, Saijoh K, *et al*. Quantification of human tissue kallikreins in the stratum corneum: dependence on age and gender. *J Invest Dermatol* 125: 1182-1189, 2005.

10. Voegeli R, Rawlings AV, *et al*. Increased basal transepidermal loss leads to elevation of some but not all stratum corneum serine proteases. *Int J Cosmet Sci*. 30 (6): 435-442, 2008.

11. Morizane S, Yamasaki K, *et al*. Kallikrein expression and cathelicidin processing are independently controlled in keratinocytes by calcium, vitamin D_3 and retinoic acid. *J Invest Dermatol.* 130 (5): 1297-306, 2010.

12. Briot A, Deraison C, *et al*. Kallikrein 5 induces atopic dermatitis-like lesions trough PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J Exp Med.* 206 (5): 1135-47, 2009.

13. Peric M, Koglin S, *et al*. IL-17A enhances vitamin D_3-induced expression of cathelicidin antimicrobial peptide in human keratinocytes. *J Immunol*. 181 (12): 8504-851, 2008.

14. Oikarinen A. Aging of the skin connective tissue: how to measure the biochemical and mechanical properties of aging dermis. Review. *Photodermatol Photoimmunol Photomed*. 10(2): 47-52, 1994.

15. Park HJ, Cho DH, *et al*. Collagen synthesis is suppressed in dermal fibroblasts by the human antimicrobial peptide LL-37. *J Invest Dermatol.* 129 (4): 843-50, 2009.

16. Pons Gimier L, Parra Juez JL. Ciencia Cosmética. Bases fisiológicas y criterios prácticos. *Consejo General de Colegios Oficiales de Farmacéuticos*. p. 5-15, 1995.

17. Pillai S, Oresajo C, *et al*. Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation - a review. *Int J Cosmet Sci*. 27 (1): 17-34, 2005.

18. Tsukahara K, Takema Y, *et al*. Selective inhibition of skin fibroblast elastase elicits a concentration-dependent prevention of ultraviolet B-induced wrinkle formation. *J Invest Dermatol.* 117 (3): 671-677, 2001. Erratum in: *J Invest Dermatol*. 118 (4): 742, 2002.

19. Saarialho-Kere U, Kerkelä E, *et al*. Accumulation of matrilysin (MMP-7) and macrophage metalloelastase (MMP-12) in actinic damage. *J Invest Dermatol*. 113 (4): 664-672, 1999.

20. Emami N, Diamandis EP. Human tissue kallikreins: a road under construction. *Clin Chim Acta.* 381 (1): 78-84, 2007.