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A NOVEL AND UNIQUE ACTIVE(*) INTEGRATING DERMAL AND EPIDERMAL ACTIVITIES

(*) Integrine®-60 (Vevy Europe, Genova, Italy)

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A novel and unique active(*) integrating dermal and epidermal activities

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ABSTRACT

Several research projects are aimed at developing bioactive materials to enhance keratinocytes and/or fibroblasts activities. One promising methodology, widely adopted to identify active substances, is the screening of natural extracts and the isolation of the targeted active compounds from them. Rice (*Oryza sativa L.*) is a worldwide basic food and many studies highlighted the benefits associated to rice proteins but there is still much to find out about their functional properties. After a long focused and systematic research, Vevy Europe isolated a novel compound named "Oligopeptide 43785" (**). This specific low molecular weight (avg <1kDa) oligopeptide of rice pure protein was obtained by controlled enzymatic hydrolysis through proteases from botanical source. It was demonstrated that Oligopeptide 43785 stimulates the synthesis of Fibronectin, Elastin, Collagen type I, IV, VII and XIII in cultured human keratinocytes and fibroblasts, potentially leading to an improvement of the Extra Cellular Matrix (ECM) and thus to dermis eutrophism. In addition to this, it stimulates Filaggrin production and this could lead to support the homeostasis of the upper layers of the epidermis and a substantial increase of the Natural Moisturizing Factor (NMF), which means a greater moisturization of the epidermis.

(**) "Oligopeptide 43785" is the active component of Integrine®-60 (Vevy codex 18.5500)

INTRODUCTION

Epidermis

Epidermis is a stratified squamous epithelium consisting of several cell types. The most abundant cell type of epithelial layer of the epidermis is the keratinocyte, constituting 90% of the cells found in the outermost layer[1].

The primary function of keratinocytes is the formation of a barrier against environmental damage from pathogen bacteria, fungi, parasites, viruses, heat, UV radiation and water loss. If pathogens start to invade the upper layers of the epidermis, keratinocytes can also react by producing pro-inflammatory mediators, particularly chemokines to recruit immunocompetent cells.

Keratinocytes are interconnected each other with cellular junctions, among which are desmosomes, in a constant state of transition from the deeper layers to the superficial ones.

The epidermis varies in thickness based on the tissue of origin. The four layers of the epidermis are stratum basale (basal or germinativum cell layer), stratum spinosum (spinous or prickle cell layer), stratum granulosum (granular cell layer) and stratum corneum. These layers are formed by the differing stages of keratinocyte maturation[2].

Epidermal skin layers

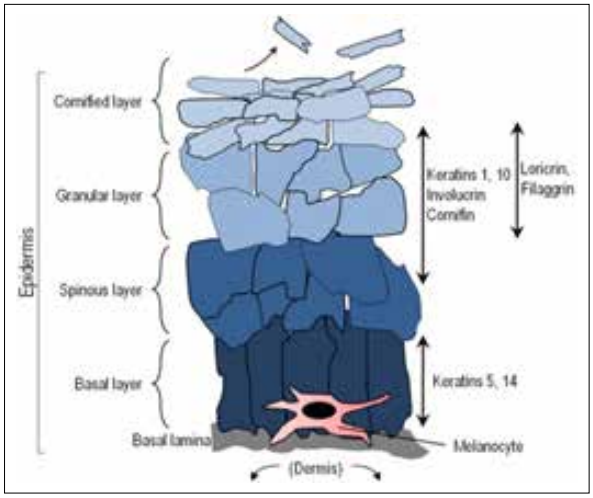


Image Courtesy of D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T - Int J Mol Sci (2013)

The anchorage of epidermis to dermis is mediated by the Lamina lucida and the Lamina densa in the Basal membrane by a set of anchoring fibrils (Collagen VII and Collagen IV included) with a fast turnover.

In fact, Collagen VII and Collagen IV have a turnover of a few weeks while Collagen I has a turnover of 15-30 years.

The anchoring fibrils are the most subject to wear and tear since the epidermis is subjected to continuous mechanical stress (due to its continuous movement on the joints, on the face in smiles and grimaces, etc.) and oxidative stress (when a keratinocyte is damaged the consequent micro-inflammation and the subsequent arrival of macrophages and polynuclear cells cause an oxidative burst that damages the proteins between the keratinocyte and the dermis, in particular anchoring fibrils, Collagen VII and Collagen IV).

Keratinocytes produce a number of structural proteins (e.g. Filaggrin, Keratin) contained in the keratohyalin granules, enzymes (proteases), lipids and antimicrobial peptides (defensins) which contribute to maintain the fundamental barrier function of the skin. Keratinization is part of the physical barrier formation (cornification), in which keratinocytes produce more and more keratin and undergo the terminal differentiation. The fully cornified keratinocytes forming the outermost layer are constantly shed off and replaced by new cells[3].

The main proteinaceous component of the keratohyalin granules is the huge, repetitive polyprotein Profilaggrin (pro-FILament AGGRegating proteIN, approx. 500KDa). When granular layer cells commit to terminal differentiation to form the flattened squamae of the stratum corneum, Profilaggrin is rapidly cleaved into multiple copies of the about 35kDa Filaggrin monomer, which binds to and condenses the keratin cytoskeleton, thereby facilitating cellular compression. Within the stratum corneum, Filaggrin is broken down to form Natural Moisturizing Factor, a pool of amino acids and derivatives exerting multiple effects. Filaggrin is therefore essential for the normal stratum corneum biogenesis and physiology[3].

For these reasons, Filaggrin plays a crucial role in preventing dryness and non-physiological peeling of skin. Indeed, it is able to aggregate keratin filaments in order to form horny lamellae and it organizes their alignment in a regular distribution, avoiding differences of level. This alignment is responsible for the homogeneous, compact and smooth aspect of skin.

Therefore, a lack of Filaggrin in stratum corneum is responsible for dryness, exfoliation and more serious skin damages such as atopic dermatitis[12].

Moreover, several studies demonstrated the presence and localization of Collagen type XIII in normal human skin and cultured keratinocytes, closely associated with adherent type junctions suggesting a role of this protein in maintaining the compactness of skin[4], contributing to the elasticity of the skin in a non-negligible way.

In this context, primary cell cultures of keratinocytes, *in vitro*, provide a system to study the effect of new dermal compounds on epithelial cell growth and differentiation as well as on neo-synthesis of compounds such as Filaggrin and Collagen XIII, regulating the healthy skin status.

Dermal skin layer

The extracellular matrix (ECM) is the largest component of the dermal skin layer and it is mainly responsible for skin structure, in terms of providing tensile strength, elasticity and resiliency. The ECM is composed of ground substance (proteoglycans, glycoproteins, and other complex polysaccharides) and protein fibers (Collagen, reticular, elastic)[5]. In the dermis, most of the ECM proteins are synthesized by fibroblasts[6]. The importance of ECM in maintaining the skin texture is evident in the collapse of dermal connective tissues and reduction of ECM concurrent with skin aging signs, such as wrinkle formation and reduction of elasticity[7]. Therefore, it has long been believed that the enhancement of fibroblast activity, in the context of ECM production, may be a key feature to maintain healthy skin textures.

AIM OF THE STUDY

Many researchers tried to develop bioactive materials to enhance both keratinocyte and fibroblast activities. One promising methodology, still being widely adopted to identify active substances, is the screening of natural extracts and the isolation of the targeted active compounds from them.

Rice (*Oryza sativa L.*) is a worldwide basic food and many studies highlighted the benefits associated to rice proteins but there is still much to find out about their functional properties.

Efficacy improvements due to protein hydrolysis are known and hydrolyzed rice proteins may potentially be used in a variety of cosmetic and food systems due to their improved functionality.

In the course of a screening, aimed to the identification of natural bioactive compounds involved in the stimulation of keratinocytes and fibroblasts activities, Vevy Europe isolated a novel compound named "Oligopeptide 43785". This specific low molecular weight (avg <1kDa) oligopeptide of rice pure protein was obtained by controlled enzymatic hydrolysis through proteases from botanical source.

Since Vevy researchers surmise that this compound could stimulate keratinocytes and fibroblasts activity either in survival and cell proliferation or Filaggrin as well as ECM protein neo-synthesis, the aim of this study was to evaluate the effect of Oligopeptide 43785 compound on:

1. Keratinocytes and fibroblasts viability/ cytotoxicity;
2. Keratinocytes and fibroblasts cell proliferation;
3. Filaggrin and Collagen XIII expression in keratinocytes;
4. Fibronectin, Collagen type-I, -IV, -VII and elastin expression in fibroblasts.

For this purpose, it has been used two cell models well representing epidermis and dermal skin layer: normal adult human primary epidermal keratinocytes and normal adult human dermal fibroblasts.

Finally, it was demonstrated that this specific phytoderivative, integrating both dermal and epidermal activities, is able to modulate fibroblast and keratinocyte activity in terms of eutrophism and Extra Cellular Matrix (ECM) selective protein secretion.

MATERIALS AND METHODS

Experimental Compounds

1. Oligopeptide 43785 (20.9 mg/ml) was diluted in culture medium to obtain the experimental conditions.
2. Recombinant human IGF-I (Sigma-Aldrich, St. Louis, MO) was suspended in PBS and diluted in culture medium and used as positive control for cell proliferation[8] and Collagen type-I production[9,10,11].

Cell cultures

Normal adult human dermal fibroblasts and normal adult human primary epidermal keratinocytes (American Type Culture Collection-ATCC, VA) were grown at 37°C in a humidified CO_2 incubator in monolayer (Figure 1A and 1B). Culture media were Dulbecco's MEM (Biochrom, Berlin, Germany), supplemented with 10% FBS, 1% non-essential amino acids, 20mg/dl gentamycin, 200mM glutamine for fibroblasts and Dermal Cell Basal Medium (ATCC® PCS-200-030) added with Penicillin-Streptomycin Solution, Phenol Red and one Keratinocyte Growth Kit (ATCC® PCS-200-040) and the following growth supplements: Bovine Pituitary Extract (BPE), rh TGFα, L-glutamine, hydrocortisone hemisuccinate, insulin, epinephrine and apotransferrin for keratinocytes. Confluent cells were harvested with trypsin-EDTA (Euroclone, UK) and were seeded in 60mm/tissue culture dishes (IWAKI, J) or in 96-well plates (Costar, NY, USA) depending on the experiments.

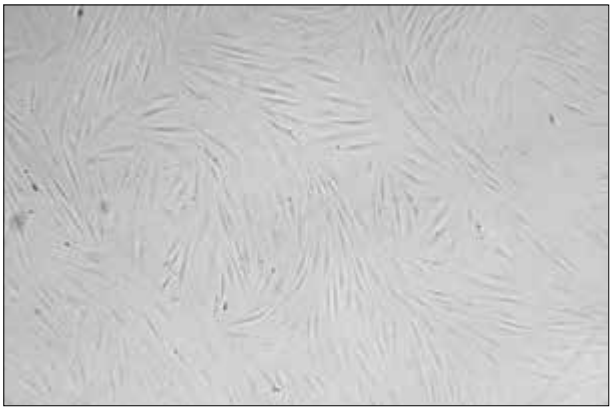


Figure 1A: *Normal adult human dermal fibroblasts.*

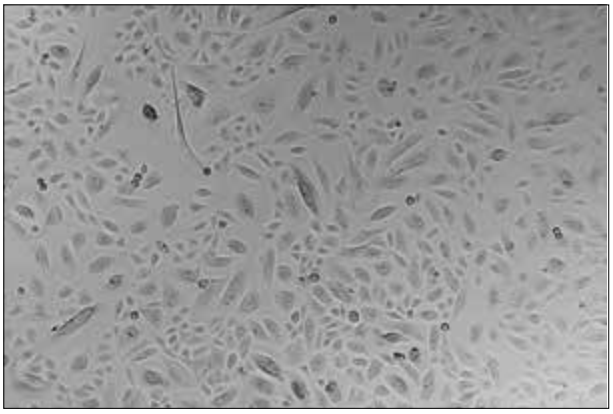


Figure 1B: *Normal adult human primary epidermal keratinocytes.*

Cytotoxicity studies

To estimate the effect of Oligopeptide 43785 on cell cytotoxicity, 3.000-5.000 keratinocytes and fibroblasts were seeded in 96-well plates in Dermal Cell Basal Medium and D-MEM 10% FBS, respectively, in a final volume of 200µl per well for 24h.

The culture medium of fibroblasts was replaced by D-MEM/0.1% BSA to rest cells in G0 phase of cell cycle. After 24h the culture medium was replaced

by adding 200µl of experimental medium (Dermal Cell Basal Medium or D-MEM/0.1% BSA) containing Oligopeptide 43785 (range: 1ng/ml-1mg/ml) and cells were incubated for further 48h.

After the incubation period, cells were centrifuged at 250rcf for 10min and 100µl per well of conditioned medium were transferred in an optical clear 96-well plate and 100µl Reaction Mixture (LDH-Cytotoxicity Assay Kit, BioVision Incorporated, Milpitas, USA) was added.

The absorbance at 492 nm was measured in Tecan I-Control system after 30 min. Results were obtained by determining the mean value of 4 replicates.

Conditioned medium obtained by cells treated with 1% Triton X-100 was used as positive control, conditioned medium obtained by untreated cells was used as negative control and medium alone was used to calculate the background.

Cell proliferation studies

To estimate the effect of Oligopeptide 43785 on cell proliferation of sub-confluent cells, $1.5x10^3$ keratinocytes and $2.5x10^3$ fibroblasts were seeded in 96-well plates in Dermal Cell Basal Medium and D-MEM 10% FBS, respectively, in a final volume of 200µl per well for 24h. The culture medium of fibroblasts was replaced by D-MEM/0.1% BSA to rest cells in G0 phase of cell cycle. After 24h the culture medium was replaced by adding 200µl of experimental medium (Dermal Cell Basal Medium or D-MEM/0.1% BSA) containing Oligopeptide 43785 (range: 1ng/ml-2mg/ml) and control cells were incubated for further 48h.

200µl of medium containing recombinant human IGF-I (50ng/ml) was used as positive control for cell proliferation. 20µl of 10X 5' -bromo-2' deoxyuridine solution (BrdU) were added to the cultures 24h before the end of the incubation time.

Cell proliferation was measured by BrdU cell proliferation assay kit (Cell Signaling, Danvers, MA) according to the manufacturers. The absorbance at 450nm was measured in a Tecan I-Control system and results were obtained by determining the mean value of 3 replicates.

Regarding the effect of Oligopeptide 43785 on confluent fibroblasts, thus mimicking the physiological state of fibroblasts *in vivo*, $5x10^3$ fibroblasts were seeded in 96-well plates and the same setting of experiments as previously described were performed.

Fibronectin and Collagen type-I expression in fibroblasts studies

To evaluate the effect of Oligopeptide 43785 on Fibronectin and Collagen type-I secretion, $2x10^5$ fibroblasts were seeded in petri dishes for 48h.

The culture medium was replaced by D-MEM/0.1% BSA to rest cells in G0 phase of cell cycle. After 24h the culture medium was replaced by adding 5 ml of experimental medium (D-MEM/0.1% BSA) containing Oligopeptide 43785 (range:1ng/ml-2mg/ml).

After a 72h incubation, conditioned media from cell lines were collected and Fibronectin and Collagen type-I secreted into conditioned media were measured by using specific commercially available assay kit.

In detail, Fibronectin and Collagen type-I were measured by using human Fibronectin DuoSet ELISA kit and human Pro-Collagen type-I alpha 1 (COLIA1) DuoSet ELISA kit (R&D Systems, Minneapolis, USA), respectively.

Elastin, Collagen type-IV and Collagen type-VII expression in fibroblast lysates by western blot analysis

After collection of conditioned medium for secretion studies, adherent fibroblasts were directly washed in each well with 1X PBS supplemented with protease inhibitors (200µM Na_2VO_4, 200µM PMSF, 10µg/ml leupeptin, 10µg/ml aprotinin), solubilized in RIPA buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 1% Triton X-100, 0.10% SDS, 200µM Na_2VO_4, 200µM PMSF, 10µg/ml leupeptin, 10µg/ml aprotinin), incubated 60 min on ice and subsequently centrifuged at 14.000rcf for 15 min at 4°C.

The supernatants were collected and the protein content was measured by use of BCA protein assay (T-Pro BCA Protein Assay Kit, T-Pro Biotechnology, Taiwan R.O.C.).

50µg of total proteins were denatured and fractionated on 10% SDS-PAGE and then transferred electrophoretically to Hybond C-extra nitrocellulose membrane (GE Healthcare, Chalfont, St Giles, UK).

After transfer, non-specific binding sites were blocked by treating membranes with Tris-buffered saline-Tween (TBS-T) containing 10% BSA for 90 minutes at room temperature on a rotating shaker.

After blocking and three washes with TBS-T, to evaluate the presence of Elastin, Collagen type-IV and type-VII in total proteins, membranes were incubated for 16h at 4°C with a 1:200 dilution Elastin monoclonal antibody, a 1:1000 dilution Collagen type-

IV polyclonal antibody and a 1:200 dilution Collagen type-VII monoclonal antibody (Novus Biologicals Europe, Abingdon, United Kingdom).

Membranes were washed with TBS-T and then incubated for 1 h at 22°C with a 1:5000 dilution of horseradish peroxidase (HRP)-linked anti-rabbit/ mouse IgG.

For β-actin evaluation (used to normalize protein expression), membranes were incubated for 1h, at room temperature, with a HRP-conjugated β-actin primary antibody (dilution 1:10.000).

Membranes were washed in TBS-T, immersed for 5 min in a detection solution and analyzed using a dedicated chemiluminescence imaging system (UVITEC Alliance, UVITEC, Cambridge, UK).

Filaggrin and Collagen type-XIII expression in keratinocyte lysates

To evaluate the effect of Oligopeptide 43785 on Filaggrin expression, $2x10^5$ keratinocytes were seeded in petri dishes. After 48h the culture medium was replaced by adding 5 ml of experimental medium (Dermal Cell Basal Medium) containing Oligopeptide 43785 (range: 1ng/ml-2mg/ml).

After a 48h incubation, cells were collected, lysed by freeze-thaw cycles and subsequently centrifuged at 1.500rcf for 10min at 4°C. The supernatants were collected and the protein content was measured by use of BCA protein assay (T-Pro BCA Protein Assay Kit, T-Pro Biotechnology, Taiwan R.O.C.).

Filaggrin production in keratinocyte lysates was measured by using human Filaggrin ELISA kit (LSBio, LifeSpan BioSciences, Inc., Seattle, WA, USA). Collagen type-XIII expression was detected by western blot analysis using a 1:1000 dilution Collagen type-XIII polyclonal antibody (LSBio, LifeSpan BioSciences, Inc., Seattle, WA, USA) as previously described in "*Elastin, Collagen type-IV and Collagen type-VII expression in fibroblast lysates*" paragraph. β-actin was used to normalize Collagen type-XIII expression.

Collagen type-XIII expression by immuno-fluorescence

To support Collagen type-XIII expression on keratinocytes, cells were seeded in 8-well chamber slides in a final volume of 250µl per well and incubated at 37°C in a humidified 5% CO_2 atmosphere for 72h. Cells were fixed in 4% paraformaldehyde in PBS for 15 min at room temperature (RT).

Unreacted aldehydes after fixation were blocked with 100mM glycine in PBS for 10 min. Non-specific binding sites were blocked with PBS-10%BSA for 1h at RT and then incubated for 1h with the following antibodies: Collagen type-XIII polyclonal antibody (LSBio, LifeSpan BioSciences, Inc., Seattle, WA, USA), diluted 1:100 in PBS containing 1% BSA.

After washing with PBS, cells were incubated with a goat anti rabbit IgG AlexaFluor 488 (Invitrogen, California, USA), diluted 1:400 in 1% BSA-PBS, for 30 min at RT. Negative controls were performed by omitting the primary antibody.

Cell nuclei were stained with DAPI (2 µg/ml). Slides were cover slipped and examined at a Leica TCS SP5 confocal fluorescence microscope (Mannheim, Germany).

RESULTS

Citotoxicity

Oligopeptide 43785 did not show any cytotoxic effect, measured as LDH release.

At all tested concentrations cytotoxicity was close to the baseline (within random background). Results are expressed as percentage of cell cytotoxicity calculated as (Test Sample - Low Control)/(High Control - Low Control)x100 (Figure 2).

Cell Proliferation

Oligopeptide 43785 was able to stimulate fibroblast proliferation at 5 out of 10 concentrations. In detail, Oligopeptide 43785 increased cell proliferation at 10ng/ml (20.7% vs. untreated cells) and showed a dose-response trend in the dose-range 100µg/ml – 2mg/ml (20.9% - 264.8% increase of cell proliferation vs. untreated cells). As expected, IGF-I induced a 208.9% increase of cell proliferation vs. untreated cells at 50ng/ml. No effect on fibroblast proliferation was demonstrated on confluent cells (data not shown).

Regarding keratinocyte proliferation, Oligopeptide 43785 was unable to increase cell proliferation, while Oligopeptide 43785 showed a dose-response trend in the dose range 500µg/ml – 2mg/ml (-31.6% – -49.8% reduction of cell proliferation vs. untreated cells). IGF-I was unable to stimulate cell proliferation at 50ng/ml (Figure 3).

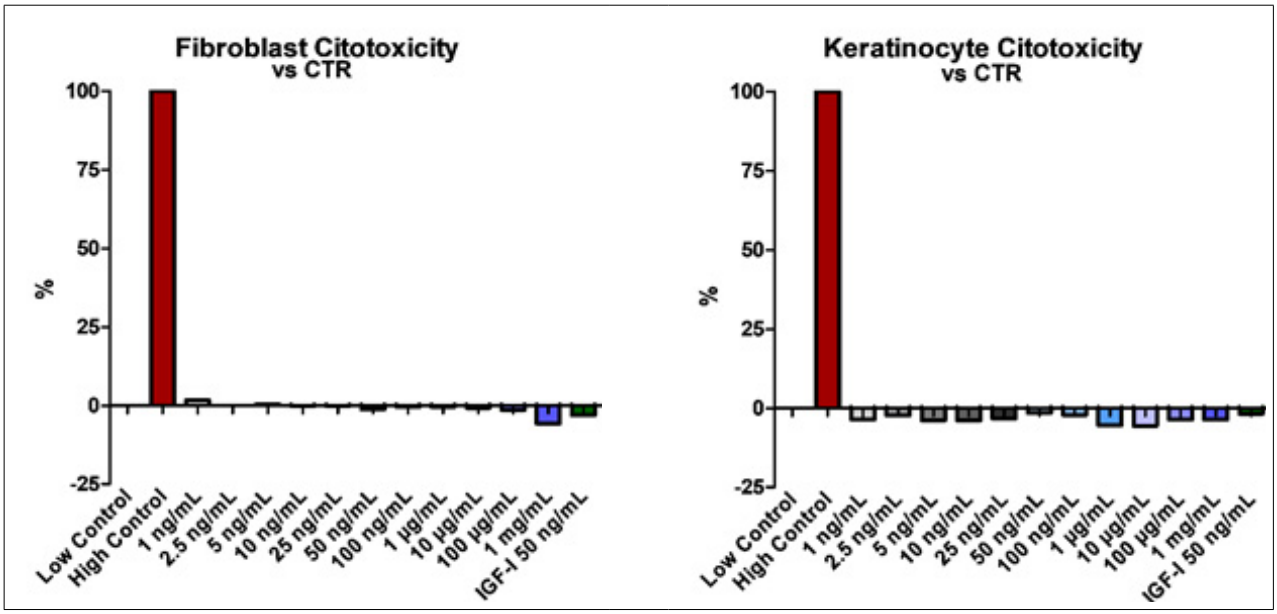


Figure 2: *Data are expressed as % variation vs untreated cells. Low Control: assay medium obtained by untreated cells; H igh Control: assay medium containing 1% Triton X-100. [CTR, untreated cells].*

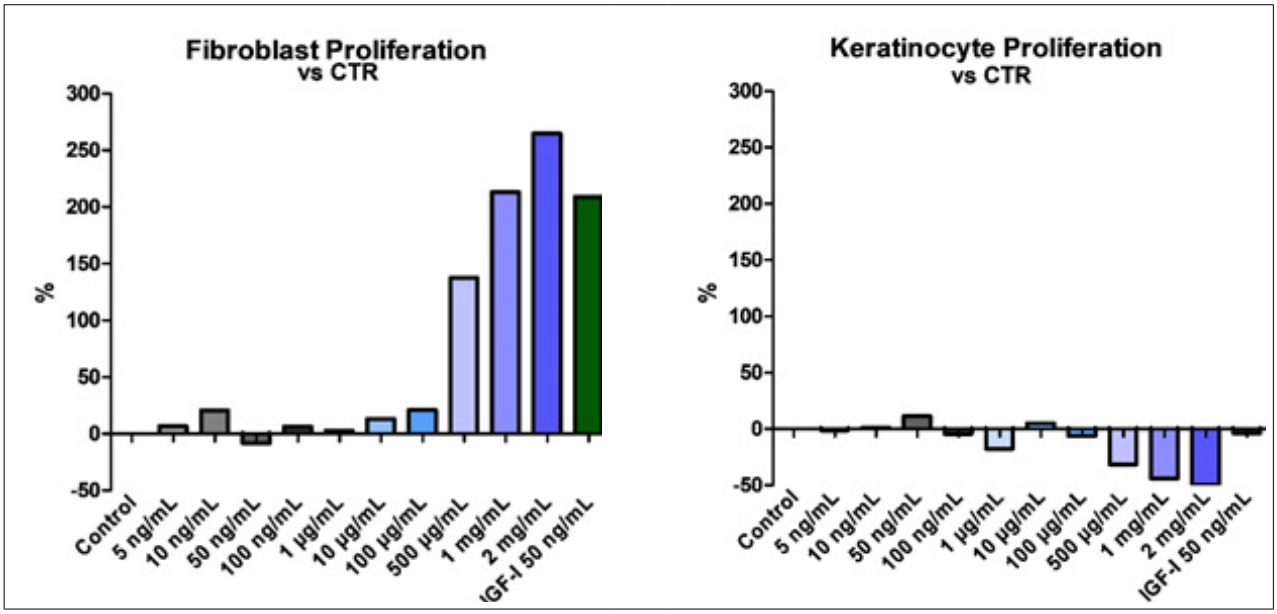


Figure 3: *Data are expressed as % variation vs untreated cells. [CTR, untreated cells].*

Fibronectin and Collagen type-I expression in fibroblasts

Oligopeptide 43785 was able to stimulate Fibronectin and Collagen type-I synthesis by fibroblasts in the low range of concentrations (1ng/ml-10µg/ml) compared to untreated cells (Figure 4, 5).

In detail, Oligopeptide 43785 increased Fibronectin secretion by fibroblasts as follows: 0.27ng/µg – 0.29ng/µg tot prot. vs. 0.21ng/µg tot prot. untreated cells, 27.9% – 41.9% vs. untreated cells (Figure 4); and it increased Collagen type-I secretion as follows: 1.07ng/µg – 1.14ng/µg tot prot. vs. 0.86ng/µg tot prot. untreated cells, 22.3% – 32.3% vs. untreated cells (Figure 5).

Elastin, Collagen type-IV and Collagen type-VII expression in fibroblasts by immunoblot analysis

Oligopeptide 43785 showed a good increase of Elastin and Collagen-VII protein expression, while Oligopeptide 43785 was able to increase Collagen-IV expression at almost all tested concentrations compared to untreated cells. In detail, Oligopeptide 43785 increased Elastin expression at 50ng/ml and 1µg/ml (16.2% and 14.7% vs. untreated cells), Collagen type-IV expression in 6 out of 7 concentrations (5ng/ml: 25.4%; 50ng/ml: 17.4%; 1µg/ml: 29.1%; 500µg/ml: 25.4%; 1mg/ml: 21.8%; 2mg/ml: 21.0% vs. untreated cells) and Collagen type-VII expression with a sinusoidal curve, with the highest stimulatory effect at 1µg/ml (9.8% vs. untreated cells) (Figure 6, 7, 8).

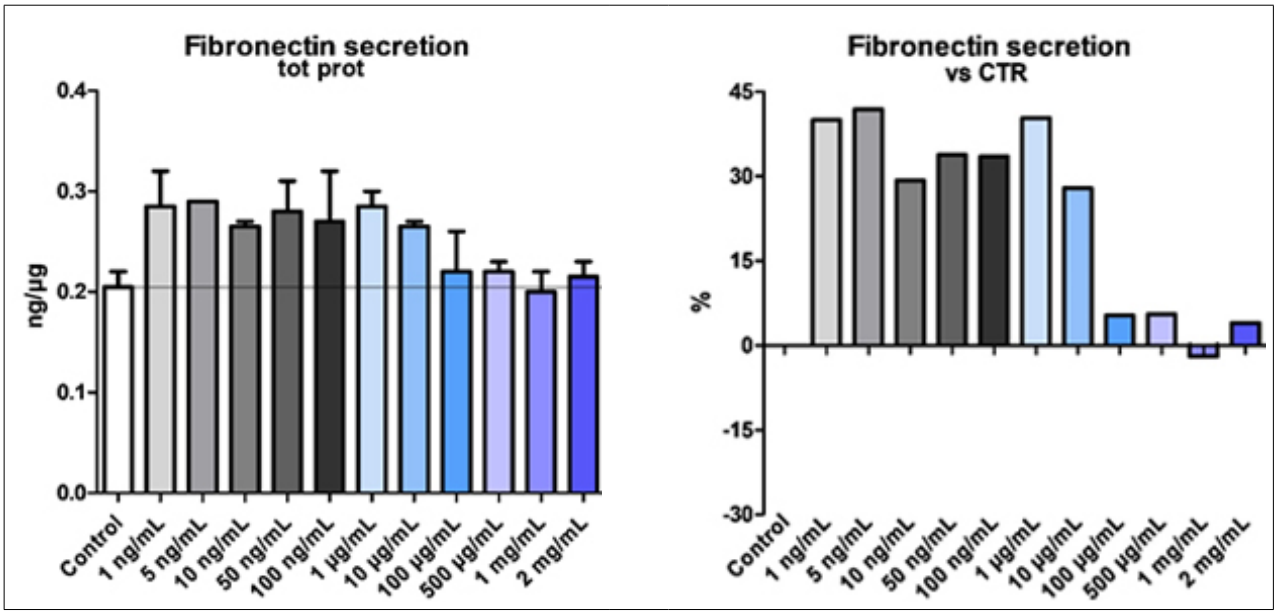


Figure 4: *Data are expressed as % variation vs untreated cells. Low Control: assay medium obtained by untreated cells; H igh Control: assay medium containing 1% Triton X-100. [CTR, untreated cells].*

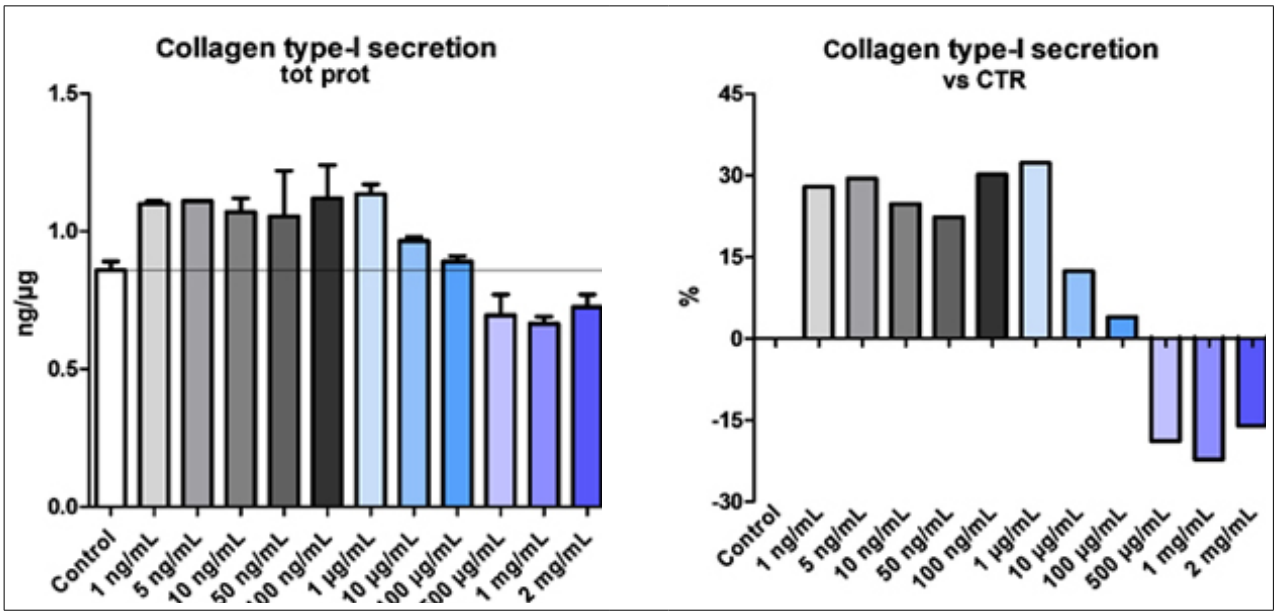


Figure 5: *Data are expressed as % variation vs untreated cells. [CTR, untreated cells].*

Panel A ***Panel B***

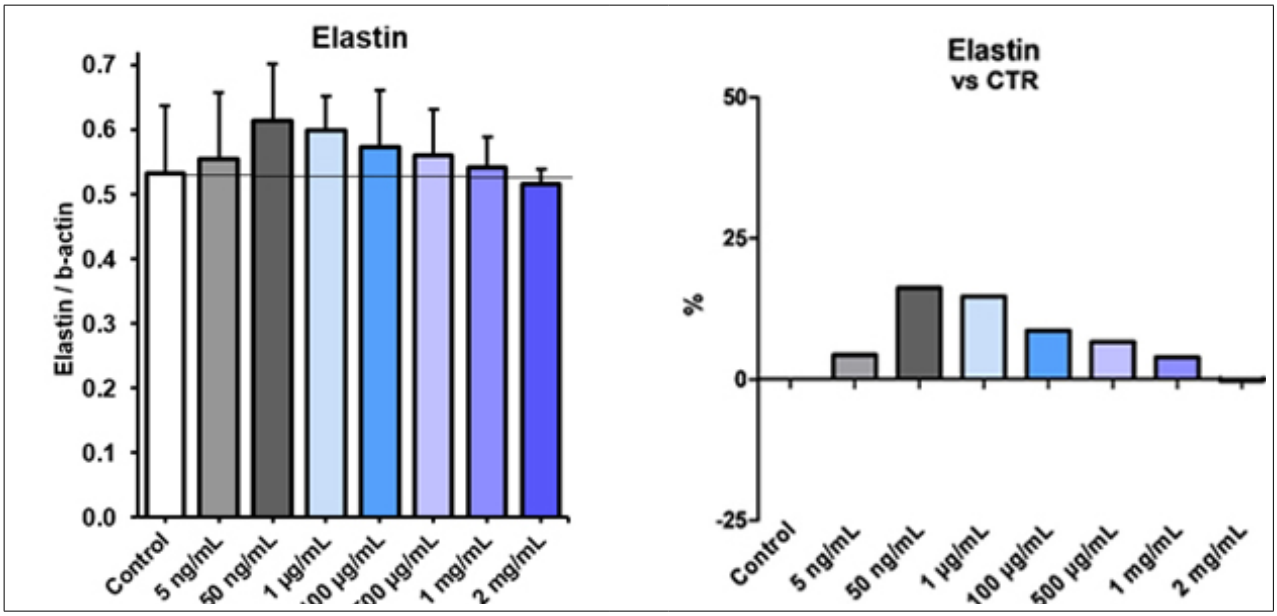


Figure 6: *Data are expressed as mean ± st error of two experiments (Panel A) and % variation vs untreated cells (Panel B). Elastin values were normalized according to β-actin expression/conditions. [CTR, untreated cells].*

Panel A ***Panel B***

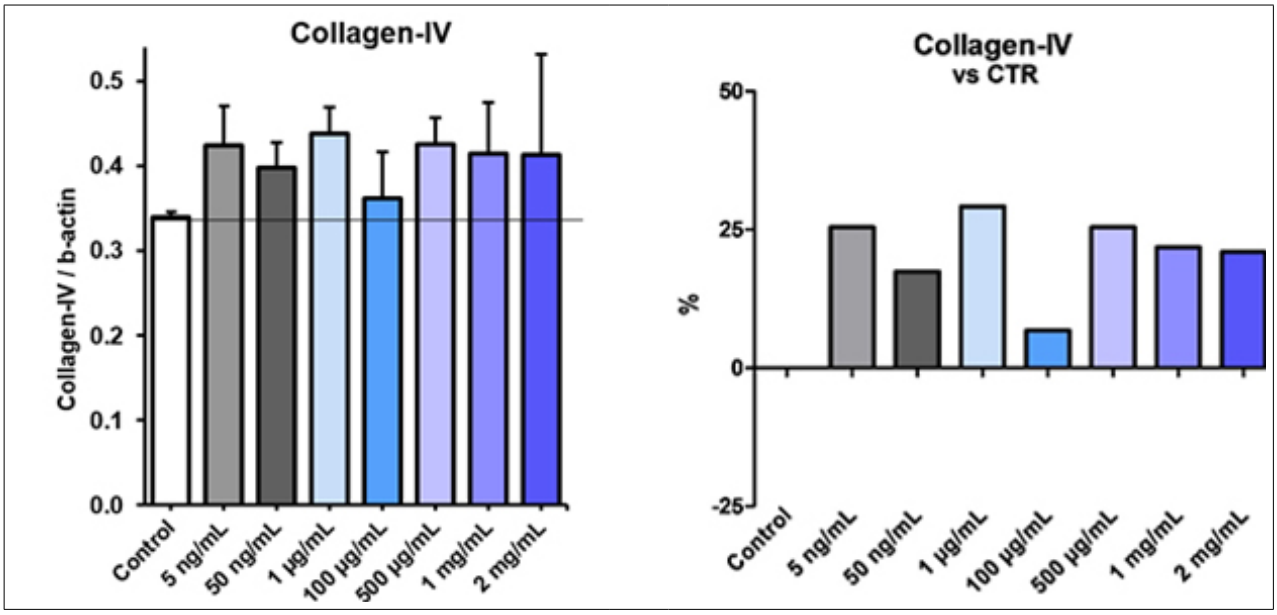


Figure 7: *Data are expressed as mean ± st error of two experiments (Panel A) and % variation vs untreated cells (Panel B). Collagen type-IV values were normalized according to β-actin expression/conditions. [CTR, untreated cells].*

Panel A ***Panel B***

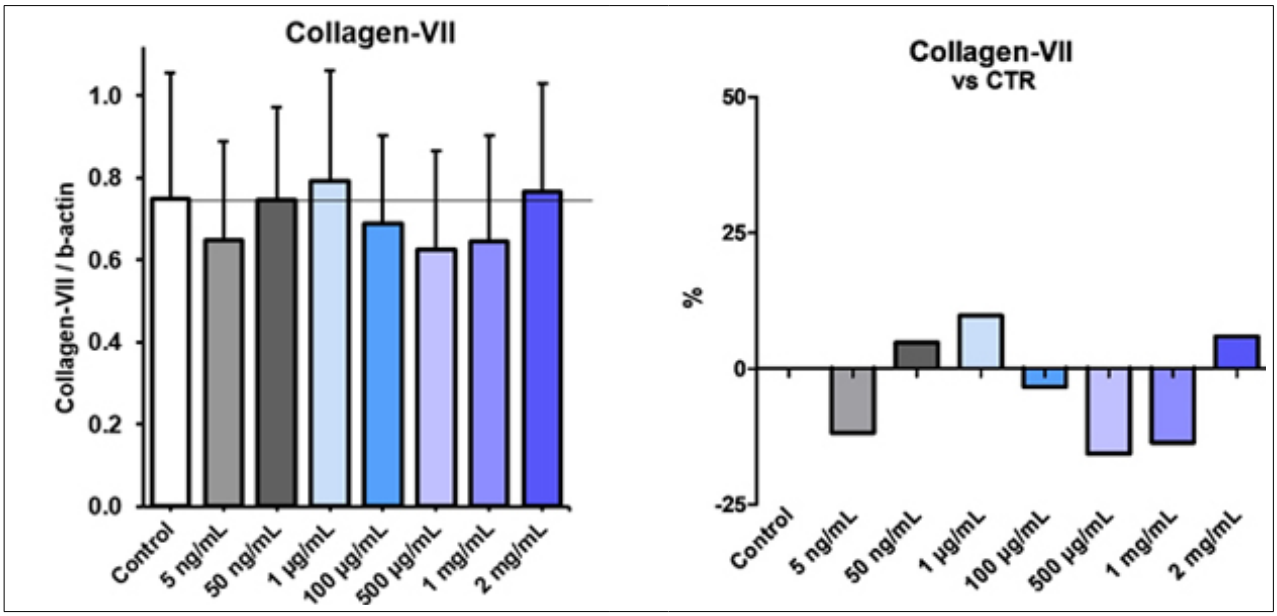


Figure 8: *Data are expressed as mean ± st error of 2 experiments (Panel A) and % variation vs untreated cells (Panel B). Collagen type-VII values were normalized according to β-actin expression/conditions. [CTR, untreated cells].*

Filaggrin and Collagen type-XIII expression in keratinocytes

Enzyme immunoassay (ELISA) on keratinocyte lysates demonstrated that Oligopeptide 43785 was able to increase Filaggrin expression in keratinocytes with a sinusoidal curve without a statistical significance besides 1µg/ml and 10µg/ml concentrations, which increased Filaggrin concentration by 20.7% and 50.7%, respectively (Fig. 9).

Conversely, immunoblot analysis of the same lysates demonstrated that Oligopeptide 43785 was able to stimulate Collagen type-XIII production at all tested concentrations with the maximum effect at 1-10µg/ml (33-37%, respectively) (Fig.10).

Panel A ***Panel B***

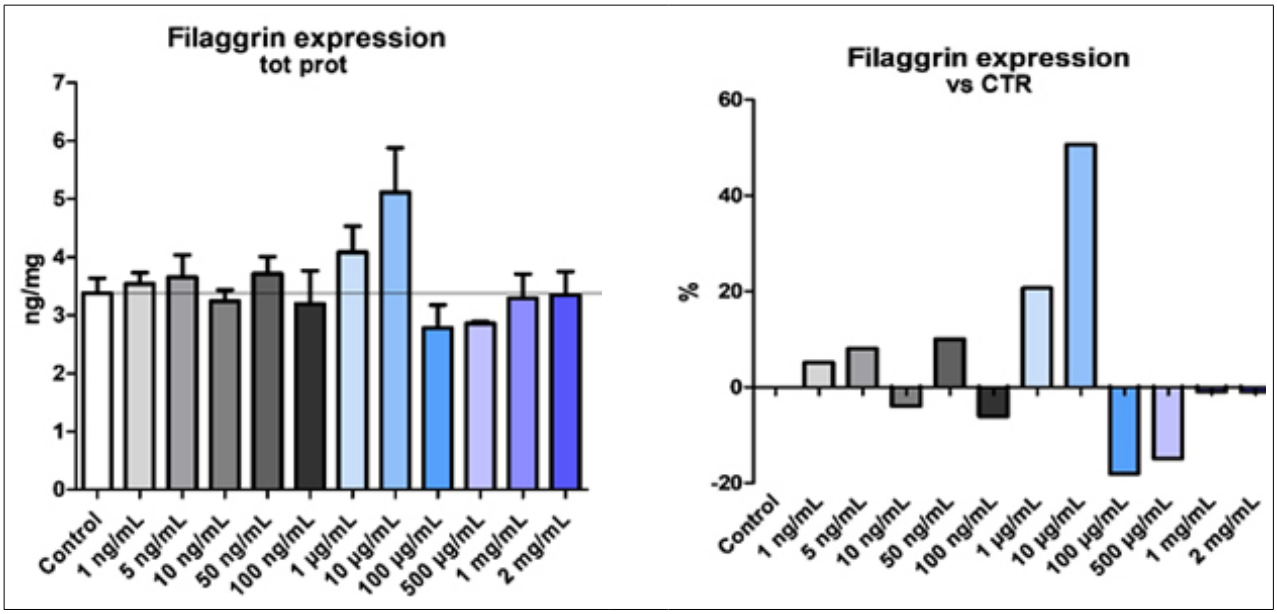


Figure 9: *Data are expressed as mean ± st error of 2 experiments (Panel A) and % variation vs untreated cells (Panel B). Filaggrin values were normalized according to mg of total protein/conditions. [CTR, untreated cells].*

Panel A *Panel B*

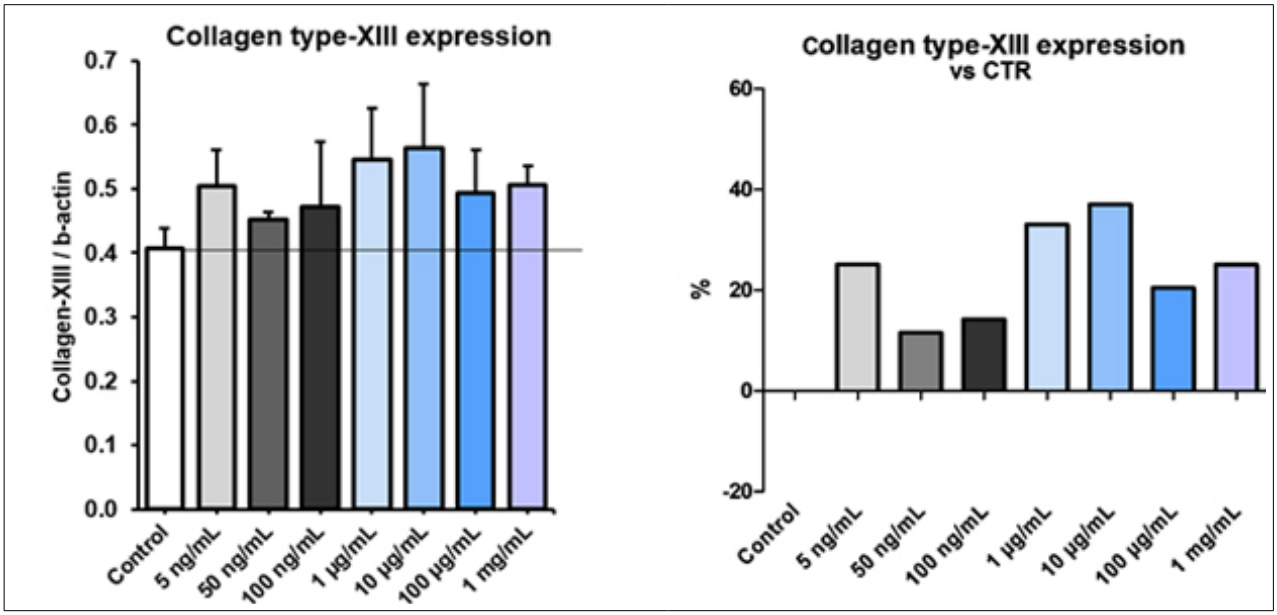


Figure 10: *Data are expressed as mean ± st error of two experiments (Panel A) and % variation vs untreated cells (Panel B). Collagen type-XIII values were normalized according to β-actin expression/conditions. [CTR, untreated cells].*

Collagen type-XIII expression in keratinocytes by immunofluorescence

Immunofluorescence analysis confirmed the presence of Collagen type-XIII in keratinocytes both in basal and stimulated conditions (Fig.11).

CONCLUSIONS

Cytotoxicity

Cytotoxicity assay demonstrated that Oligopeptide 43785 does not have any cytotoxic effect and all tested concentrations show percentages of cytotoxicity below or close to the baseline.

Proliferation

Proliferation studies performed on sub-confluent fibroblasts showed that Oligopeptide 43785 induces a dose-dependent response on cell proliferation with a maximum effect at the highest concentrations.

As expected, IGF-I (positive control), increased fibroblasts proliferation at all tested concentrations.

The same setting of experiments, performed on confluent cells mimicking the physiological state of fibroblasts *in vivo*, demonstrated that the Oligopeptide 43785 does not interfere with fibroblasts proliferation highlighting the safety of this compound in physiological condition of the skin.

Proliferation studies on keratinocytes demonstrated that neither Oligopeptide 43785 nor IGF-I do have any stimulating effects on keratinocyte proliferation at all tested concentrations.

Collagen type-I and Fibronectin expression in fibroblasts

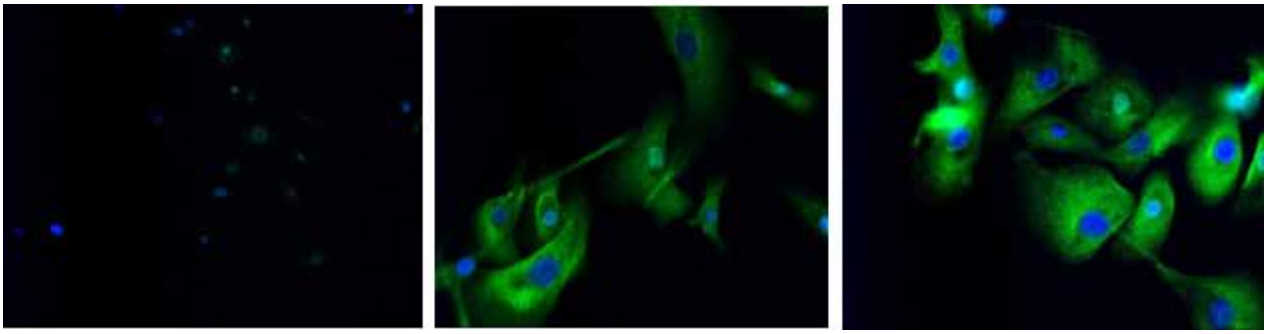
Oligopeptide 43785 stimulated Fibronectin secretion by fibroblasts with an increase in 7 cultures out of 11 within concentrations from 1ng/ml to 10µg/ml, while Oligopeptide 43785 showed a stimulating effect on Collagen type-I secretion by fibroblasts at concentrations among 1ng/ml and 1µg/ml. These data suggest a role of Oligopeptide 43785 in the regulation of the Fibronectin and Collagen type-I amount in the extracellular matrix in a not dose-dependent way.

Elastin, Collagen type-IV and Collagen type-VII expression in fibroblasts

Data obtained by immunoblotting demonstrated that Oligopeptide 43785 shows a good increase of Collagen type-IV expression and it is able to stimulate Elastin and Collagen type-VII protein expression too.

Filaggrin and Collagen type-XIII expression in keratinocytes

Oligopeptide 43785 modulates Filaggrin and Collagen type-XIII expression with an increase at 1µg/ml and 10µg/ml concentrations suggesting a role of this compound in the regulation of the both protein expression in keratinocytes.



CTR neg **CTR** **Oligopeptide 43785 10µg/ml**

Figure 11: *Collagen type-XIII immunofluorescence. [CTR, untreated cells] (400X).*

DISCUSSION

Oligopeptide 43785 is a safe peptide as demonstrated by the lack of cytotoxic effect at all tested doses on both dermal and epidermal cells as well as by its inability to affect cell proliferation in physiological condition of the skin.

Regarding the effect of Oligopeptide 43785 on extracellular matrix (ECM) compounds, it is able to stimulate the synthesis of Collagen type VII, IV and XIII, thus contributing to the maintenance and restoration of the basal conditions of the skin. Therefore, an increase in the elasticity and resiliency of the basal membrane and a natural consequent improvement of smoothness, luminosity, wrinkling, moisturization, elasticity and appearance of epidermis, can be expected by the use of this novel compound.

Since Oligopeptide 43785 is a peptide capable to stimulate the synthesis of anchoring fibrils and Collagen type-XIII, it deserves full attention, not only for its positive properties on ECM, but also because of the wide range of concentrations in which its activity is expressed, without changing the normal rhythm of fibroblast and keratinocyte growth. In addition, the stimulation of Filaggrin production should lead to an increase in moisturization of the skin.

Therefore, this specific phytoderivative, integrating both dermal and epidermal activities, is able to modulate fibroblast and keratinocyte activity in terms of eutrophism and Extra Cellular Matrix (ECM) selective protein secretion.

It can be concluded that a very innovative product of remarkable properties has been developed.

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