IFF | LUCASMEYER COSMETICS

ACTIVE INGREDIENTS

TECHNICAL FILE

PROGELINE™

MODULATES PROGERIN, A MARKER OF SENESCENCE

Biomimetic peptide derived from Elafin

-

Improves the appearance of wrinkles and sagging skin for a complete remodelling effect

PRESERVATIVE FREE · ECO FRIENDLY · CHINA COMPLIANT · in-cosmetics Innovation Zone Best Ingredient Award 2012 GOLD

SUMMARY

INCI NAME **CAS** **EINECS**	Glycerin (1^{o}) (and) Water (2^{o}) (and) Dextran (3^{o}) (and) trifluoroacetyl Tripeptide-2 (4^{o}) (1^{o}) 56-81-5, (2°) 7732-18-5, (3^{o}) 9004-54-0, (4) 64577-63-5 (1^{o}) 200-289-5, (2°) 231-791-2, (3^{o}) 232-677-5
ORIGIN	3 amino acids peptide biomimetic of elafin, an enzyme inhibitor involved in extracellular matrix integrity
COSMETIC PROPERTIES	• Reduction of progerin synthesis • Inhibition of dermis elastase activity • Inhibition of MMP-1, MMP-3, MMP-9 activity • Contraction of collagen matrices • Increase of syndecan synthesis
SKIN BENEFITS / POTENTIAL CLAIMS	• Reduces the signs of aging: sagging skin and wrinkles • Has a remodelling effect, improves the jawline • The skin is firmer and appears lifted and denser
APPLICATIONS	• Anti-aging • Firming care • Remodelling skin care • Alternative to surgery • Skin care for mature skin • Men's care • Body care • Post-pregnancy care
RECOMMENDED DOSAGE	• Intensive treatment: 2% • Body care: 1% • Preventive care: 0.5–2%
USAGE PH RANGE	pH: 4–6
INCORPORATION	At the end of the formulation (< 40˚C)
INCOMPATIBILITIES	Not known

INTRODUCTION

Aging changes the appearance in usually unflattering ways: the skin sags, and wrinkles are of major concers. Where once only women past the age of 40 were trying to reverse the clock, now aging concerns affect those in their 20s as well as men.

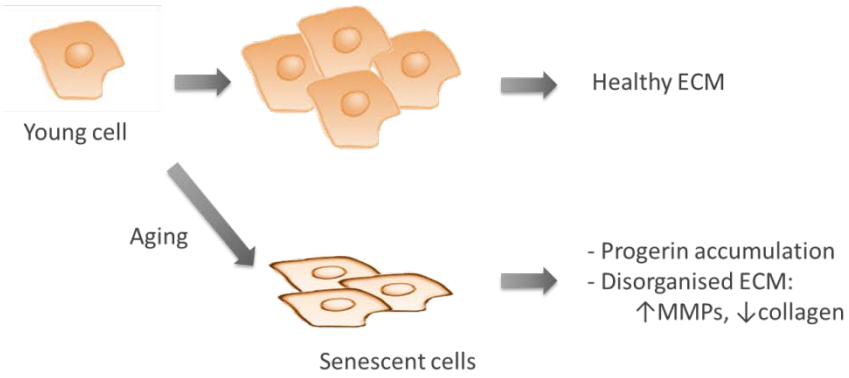
The number of 35–44 year olds is falling, and in contrast, the number of 55–64 year olds will grow by 31% in the U.S., while the 65–74 age group will increase by 31%. A similar situation will emerge in Western Europe by 2013, with demographic projections in France showing 2% more 45–54 year olds, 8.5% more 55–64 year olds and a 9.3% increase in those over 65; results in Italy showing 12.1% more 45–54 year olds, 4% more 55–64 year olds and a 6% increase in the over-65 age group; or results in the U.K. showing that the number of 45–54 year olds will rise by 10%, the number of 55–64 year olds will fall by 0.8% and the over-65 age group will grow by 9.8%.

With population growth and more information available on how unhealthy lifestyles increase aging, products that can help consumers look younger are more popular than ever.

In the global anti-aging market, the **appearance** segment is expected to generate $105 B in 2013, after an annual growth rate of 10.4% in the past few years.

Appearance and skin vitality is a subject of primary importance in daily life and for social well-being.
With the progress of scientific knowledge, in 2012 the fight is no longer only about signs of aging, but also about delaying senescence.

Senescence or biological aging is the change in the biology of an organism as it ages. Such changes range from those affecting cells and their function to those affecting the whole organism. The word "senescence" is derived from the Latin word *senex*, meaning old man, old age, or advanced in age. Cells are the fundamental structure composing our bodies, thus cellular decline contributes to the aging process. Cellular senescence, or senescent cells, is the phenomenon by which normal cells lose the ability to divide but remain active. Senescent cells acquire a large and flat cellular appearance, decrease contact with other cells, and increase adhesion to the extracellular matrix. Senescent cells also display increased metalloproteinase activity which degrades the extracellular matrix and contributes to the appearance of aging.



The constant need for innovative anti-aging ingredients and advances in skin biochemistry have inspired us to develop a sophisticated anti-aging skin care peptide acting on progerin a new target in skin senescence.

THE CONCEPT OF PROGERIN

Many factors are involved in the aging process, and they result from chronological processes and are accelerated by environmental conditions such as UV radiation, stress, fatigue, pollution…. How may we understand each of those processes and the interactions between them? So many mechanisms are implicated in skin aging.
Of those factors, we question whether **pathological conditions** could explain a part of the "normal" process of aging?

A well-known but rare disease associated with accelerated aging is the Hutchinson-Gilford progeria syndrome (HGPS), or progeria. Progeria is a rare, fatal genetic condition characterized by the appearance of accelerated aging in children. Its name is derived from Greek and means "prematurely old." Although they are born looking healthy, children with progeria begin to display many characteristics of accelerated aging at around 18–24 months of age. Signs of progeria include growth failure, loss of body fat and hair, aged-looking skin, stiffness of joints, cardiovascular disease and stroke.

In popular culture, progeria is a disease that has inspired many movie characters including the famous *Benjamin Button.* The main character, Benjamin Button, is born as a seventy-year-old man and ages backwards.

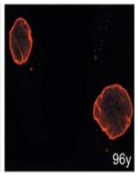
Pathologically, progeria is characterized by the buildup of a protein called progerin, a mutant form of the protein Lamin-A that is critical for the integrity of the nuclear structure and the organization of cells. Lamins play fundamental roles in the shape, integrity and function of the nucleus and in DNA replication. Mutations in *LMNA* are implicated in many disorders, and these disorders exhibit distinct clinical phenotypes associated with features such as myopathy, cardiomyopathy, lipodystrophy, neuropathy and premature aging.[1,2] The presence of progerin is concomitant with alterations of the dermis such as disorganized connective tissue.

Does progerin offer clues about the normal aging or senescence process?

Research published in the past few years has demonstrated that **progerin** is, in fact, present in the skin cells (fibroblasts and differentiated keratinocytes, mainly) of people without progeria. In normal individuals it accumulates at an estimated rate of 3% per year with higher levels later in life. With increasing age, the number of positive progerin cells increases in the upper dermis and progressively penetrates more deeply, creating a gradient of progerin-positive fibroblasts from the basement membrane to the reticular dermis. Skin derived from both sun-exposed areas and non-exposed areas has a similar distribution of progerin-positive fibroblasts.[3]

The accumulation of progerin in cells causes similar effects in HGPS patients. Cell nuclei in old individuals acquire defects similar to those in HGPS patients, including changes in the nuclear envelope and increased DNA damage.

Cell nucleus of a 9 year old



Cell nucleus of a 96 year old

In a study published in June 2011, the cause-and-effect relationship between normal aging and progerin production was established and associated with the mechanism implicated in cellular senescence. Progerin expression is now used as biomarker of normal cellular aging and is linked to terminal differentiation and senescence in elderly individuals.

Progeline™: Rethinking skin aging by modulation with progerin, the senescence marker

Recent developments have brought biomimetic synthetic molecules to the cosmetic market. These new molecules, composed of peptides, are designed to mimic natural products while increasing specificity and efficacy. Biomimetic peptides are inspired from the skin's natural peptides and are active on skin physiological processes with high specificity.

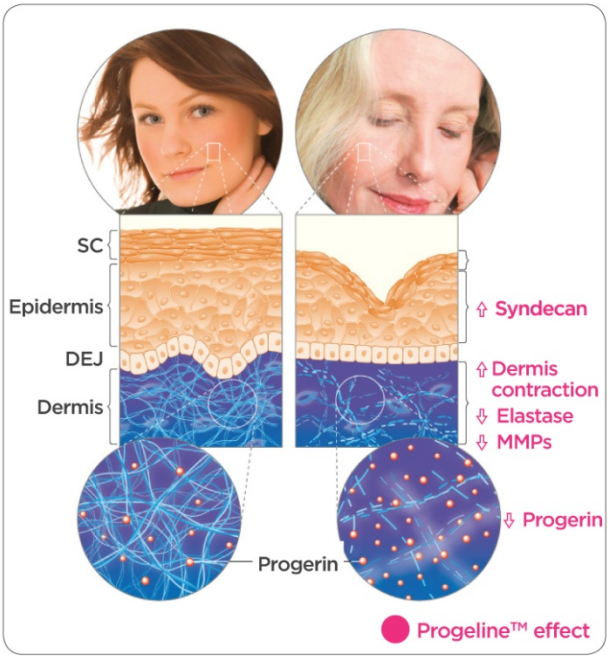
Progeline™ is a 3 amino acids peptide biomimetic of elafin, an elastase inhibitor produced by keratinocytes. Progeline™ decreases the synthesis of progerin, a new aging biomarker involved in skin cellular senescence. By acting directly on the senescence process, Progeline™ clinically improves the signs associated with skin maturation, such as wrinkles, sagging and slackness, for a complete remodelling effect.
Progeline™ is thus a sophisticated and high-tech product that fights the aging process and creates youthful and healthier-looking skin.

Progeline™ has also demonstrated its efficacy on syndecan-1, the most abundant syndecan in epithelial keratinocytes. The word "syndecan" comes from the root word "syndein," which means "to bind together." Located in the epidermis, it modulates cellular proliferation, migration, and adhesion and is involved in epidermal cohesion. Syndecan expression may be altered during the aging process, resulting in the loss of epidermal cohesion and consequently the loss of resistance and skin slackness.

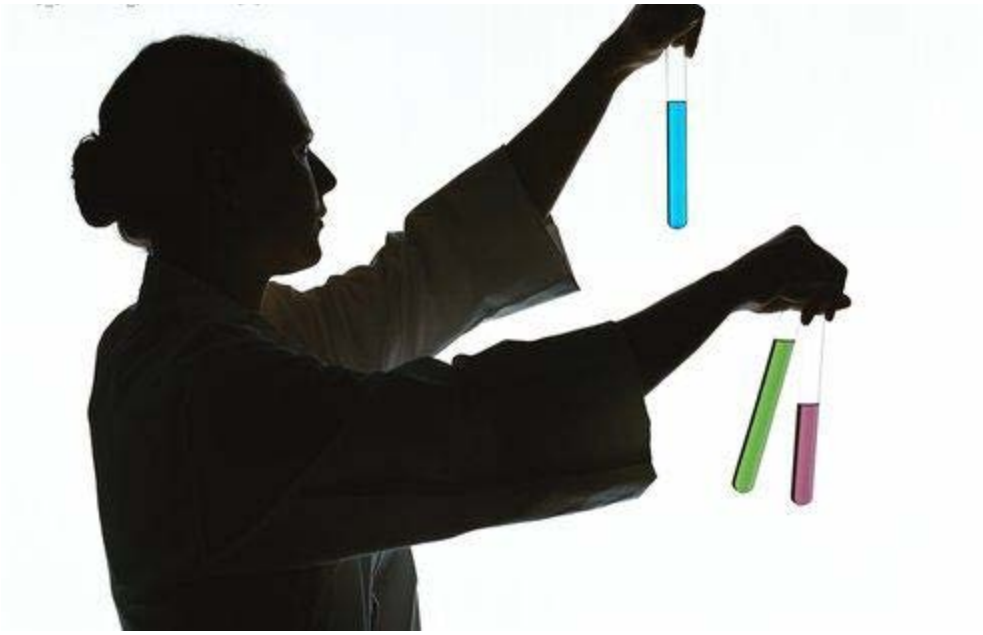
As Progeline™ acts on factors affecting the skin in the aging process, we also measured some other parameters involved in this process, such as the enzymatic activity of elastase and cutaneous metalloproteinases (MMPs). Progeline™ showed a dose-dependent reduction of MMP-1, MMP-3 and MMP-9, attesting to its efficacy in maintaining a healthy extracellular matrix.

In addition to having a reduced capability for producing collagen, aged fibroblasts produce weaker contractile forces than fibroblasts in young skin. This results in changes in the dermal architecture of the skin, ultimately leading to skin wrinkling and sagging. Progeline™ has demonstrated its capacity to improve the contraction of 3D collagen matrices in the presence of fibroblasts. This effect contributes to the strengthening of the dermal matrix and thereby reinforces the architectural framework of the skin.

Progeline™ defends the skin's collagen and elastin network against deleterious damages caused by aging. Designed for tired and loose skin, Progeline™ helps fight the symptoms of aging and restores firmness and elasticity to the skin.



EFFICACY STUDIES

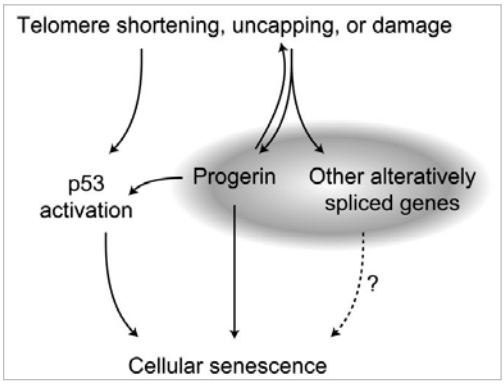


INHIBITION OF PROGERINE SYNTHESIS

INTRODUCTION

Beyond the modification of extracellular matrix macromolecules and molecules implicated in cell-matrix interactions, we have to consider the deregulation of the cellular machinery itself during aging.

In 2006, Mistelli et al. showed that progerin, a truncated form of nuclear Lamin-A, seems to be more abundant in the skin of older subjects (compared to younger skin) and so could be related to the "physiological" aging of the cutaneous tissue.[4] In 2011, Verdy et al. realized quantitative progerin measurements in *in vitro* cultures of fibroblasts issued from young and old donors, reinforcing the idea that progerin is overexpressed in skin during aging.[5] More recently, the finding that progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts[6] well exemplifies this type of cellular impairment and provides a solid basis for developing new and efficient anti-aging compounds.



PROTOCOL

Principle

Using an ELISA assay, we quantified progerin in "old" fibroblast cultures incubated in the absence or in the presence of Progeline™.

Biological material

Normal human fibroblasts issued from a 44-year-old Caucasian donor were cultured in a culture medium in a humidified incubator with a 5% CO_2/95% air atmosphere.

Tested molecules: Progeline™ was tested at different concentrations ranging from 0.0025% (10^{-8}M) to 0.025% (10^{-7}M). For that, a 10^{-4}M solution was prepared in DMSO/water (v/v 10/90) and diluted directly in the culture medium.

Progerin synthesis evaluation by specific ELISA

When the cells reached confluence, increasing concentrations of Progeline™ were added to the incubation medium. At the end of an incubation period of 96 hours, progerine was quantified in the cell lysate (after sonication) using a specific and sensitive ELISA (anti–human progerin antibody followed by the secondary antibody coupled with a peroxidase.

Peroxidase substrate was then added to enable the specific peroxidase reaction.

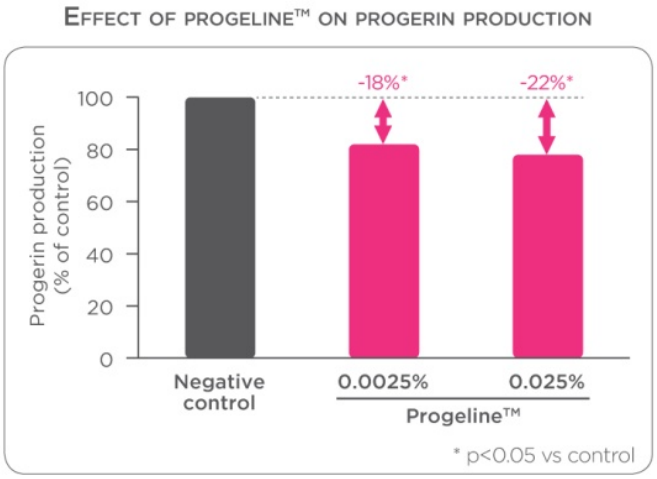
The colorimetric signal was analyzed (two wavelength readings of 450 and 540 nm) using an appropriate spectrophotometry plate reader (Victor V [PerkinElmer]). During calculations, the signal obtained at 540 nm was subtracted from that obtained at 450 nm.

Protein assay

In progerin expression studies, the total protein content of the cells was assessed in cell lysates (obtained by sonication) following the Bradford method.[7]

RESULTS

Effect of Progeline™ on progerin expression in fibroblasts issued from an aged subject:



Data came from an experiment conducted in triplicate (n=3) with cells issued from a 44-year-old subject.

*: Significantly different from the control condition ($p<0.05$; one-way ANOVA + all pairwise multiple comparisons using the Fisher's LSD method).

As shown in the figure, our peptide at 0.0025% and 0.025% concentrations was able to significantly reduce progerin production by fibroblasts: -18.0% ($p<0.05$) and -21.9% ($p<0.05$) reductions, respectively.

CONCLUSION

The results provide new information on Progeline™, which is able to fight aging by acting not only through "mechanical" pathways (inhibition of MMPs, etc.) but also through more "metabolic" ones.

Progeline™ decreases significantly the production of progerin, attesting its potential as anti-aging ingredient!

INHIBITION OF ELASTASE ACTIVITY BY PROGELINE™ IN HUMAN SKIN EXPLANT

INTRODUCTION

Proteolytic activity refers to the quantitative digestive action of a target protease on a protein (e.g., collagen, elastin, fibronectin) or glycoprotein (e.g., a proteoglycan or glycosaminoglycan) substrate. Elastin is the most important factor in tight and elastic skin and cannot be regenerated; loss of elastin in the skin system is irreparable. Elastase, one of the endogenous skin proteases, degrades skin elastin[8] in a natural and autoregulated process. However, aging, environmental factors, and UV radiation disturb this process and lead to an overdegradation of elastin through the overactivity of elastase. The development of an elastase inhibitor will protect elastic fibers from overdigestion by elastase.

OBJECTIVE

In order to evaluate the capacity of Progeline™ to decrease elastase activity, we evaluated the digestive capacity of elastase in the presence of natural elastin, in a human skin explant model. Elastin fibers are stained with orcein,[9] and the digestion of these fibers can be quantified by image analysis.

PROTOCOL

Skin Explant: Whole human skin from plastic surgery was used as a substrate for the experiment. The skin explant issued from a 48-year-old woman.

Inhibitor molecules: Progeline™ was tested from 0.025% to 0.25% concentrations in the presence of elastase at 500 mU/mL.

Treatment: Skin explants were pre-incubated in the presence or absence of Progeline™ or the reference inhibitor at different concentrations for 30 minutes. Human leucocyte elastase was then added for 3 hours before sections were rinsed and labelled with orcein. Each condition was effected in triplicate.

Elastin staining: Cryosections of the skin explants were stained with orcein in order to identify elastin fibers in red-brown.

Evaluation of elastase activity: The activity of elastase was measured by a decrease in elastin fiber degradation. The more elastase activity accelerates, the more elastin fibers will degrade.

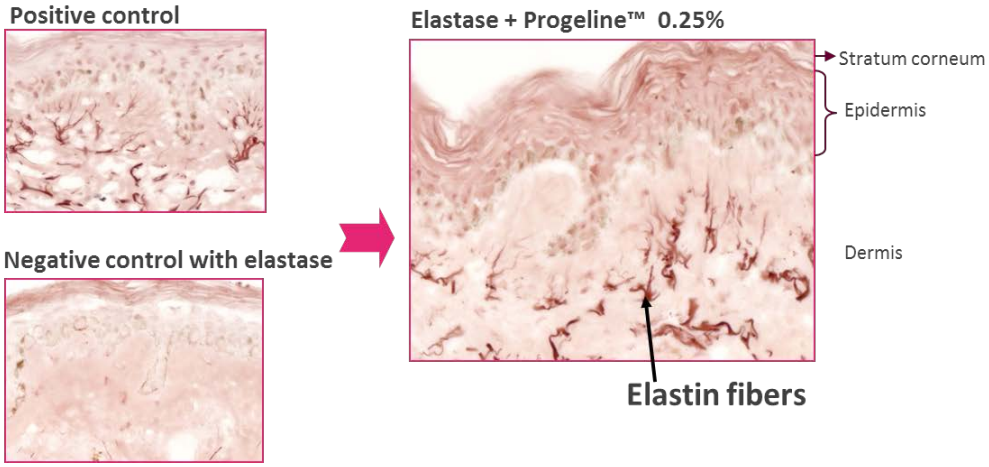
The quantification of elastase activity inhibition was carried out by image analysis on 2 different areas of the skin section. For each area, 10 pictures were analyzed. Results are given in terms of percentage of inhibition of the elastase activity. Dunn's test was used for statistical comparisons ($p<0.05$).

RESULTS

The **positive control** represents the untreated skin explant (without the addition of elastase and without inhibitors) in the presence of the medium alone.

The **negative control** represents the skin explant in the presence of elastase alone (500 mU/mL), without inhibitors.

Microscopic observations after specific labelling of elastin fibers (in red-brown)



After specific staining of the elastin fibers, we observed a red-brown colouration of the elastin fibers in the dermis of the untreated skin explant sections ("Positive control" image).

The addition of elastase to the skin sections led to vigorous digestion of the elastin fibers characterized by the total disappearance of the fibers in the dermis of the human skin ("Negative control" image).

Pretreatment of the human skin explant with Progeline™ induced a significant inhibition of elastase activity and averted the digestion of the elastin fibers ("Elastase + Progeline™" image).

Quantification of these results showed 100% inhibition of the elastase activity with Progeline™ at a 0.25% concentration level.

CONCLUSION

This experiment confirms the biomimetic activity of the elafin protease. Progeline™ is able to strongly and significantly inhibit elastase activity, thus improving the firmness and elasticity of the skin.

Progeline™ reduces elastase activity and protects elastin fibers

STIMULATION OF SYNDECAN BY HUMAN KERATINOCYTES

INTRODUCTION

Syndecans are transmembrane heparin sulfate proteoglycans that participate in the focal adhesion formation[10,11] and also play a very important role in skin homeostasis by acting as coreceptors for growth factors. Syndecans are also expressed, to a lesser extent, in aged keratinocytes[12] and aged skin.[13]

OBJECTIVE

The aim of this study was to determine the effects of Progeline™ on the stimulation of syndecan-1 synthesis in human keratinocytes.

PROTOCOL

Biological material

Human keratinocytes from cell line NCTC 2544 were cultured in Dulbecco's modified Eagle's medium (DMEM) in a humidified incubator with a 5% CO_2/95% air atmosphere.

Tested molecules

Progeline™ was added at 0.0025% and 0.00025% concentrations to the cell culture 24 hours after the cells were seeded into the Lab-Tek™. After an incubation period of 72 hours, the cells were fixed onto the Lab-Tek™. TGF-β was used as a positive control.

Syndecan-1 expression evaluation

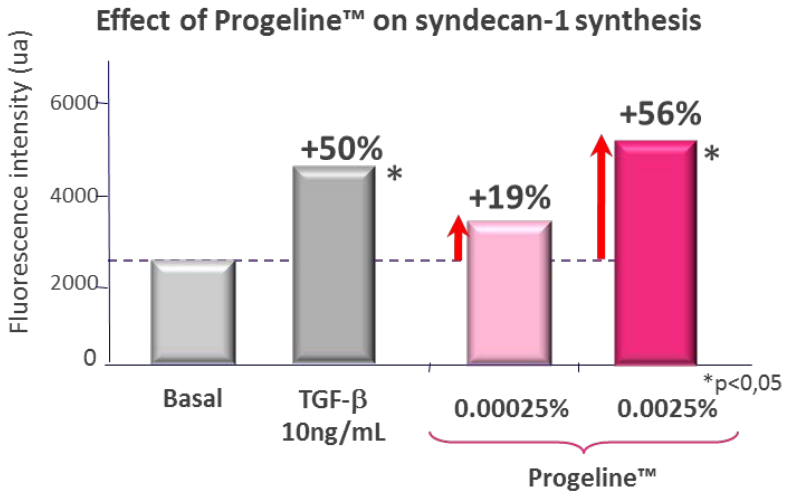
Treated or untreated cells were incubated with a specific monoclonal antibody for syndecan-1 proteins. Cells were then incubated with an FITC (green fluorescence) secondary antibody for 1 hour.

The fluorescence signal (em. 488 nm, exc. 520 nm) was then analyzed with an optical microscope and the dedicated software, ImageJ.

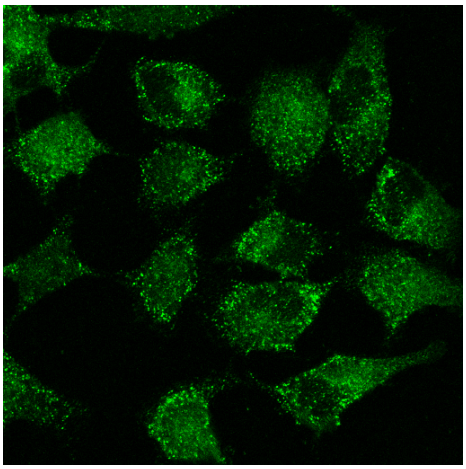
Student test was used for statistical comparison. * = significantly different from the basal condition

RESULTS

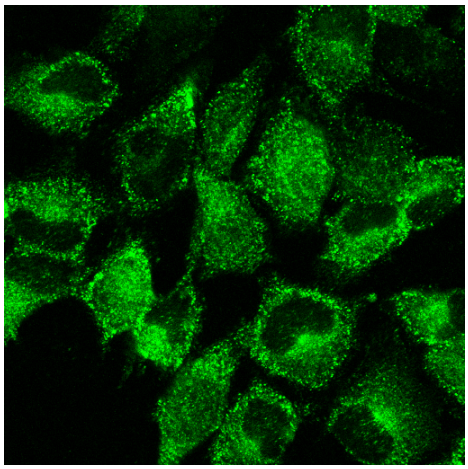
Results are expressed in terms of fluorescence intensity corresponding to the level of syndecan-1 expression in cells.



Illustrative images



Basal



Progeline™ 0.0025%

We clearly observed an increase in syndecan-1 expression in cells treated with Progeline™. The stimulatory effect of the peptide was about +19% and +56%; this effect is equivalent to that obtained with TGF-β, a well-known inductor of syndecan synthesis used as a reference product.

CONCLUSION

These results demonstrated the efficacy of Progeline™ in acting on cell matrix adhesion support. Since Wegrowski et al. in 2005, and more recently Oh et al. in 2011, have demonstrated that syndecan-1 production is lowered in keratinocytes[14] and in skin[15] during aging, our results emphasize the potential of Progeline™ as a good anti-aging agent.

Progeline™ stimulates syndecan synthesis for a better cell-matrix bonds for a maximal anti-sagging effect

INHIBITION OF MMP-1, MMP-3 and MMP-9 ACTIVITIES

INTRODUCTION

Matrix metalloproteinases (MMPs) form an important family of metal-dependent endopeptidases that represent the major family of enzymes responsible for the degradation of extracellular matrix (ECM) components. Collectively, MMPs are capable of degrading all ECM proteins.[16] MMPs are categorized based on their domain structures and their preferences for macromolecules substrates (see review by Nagase et al.[17]).

The MMP family has been found to have a great variety of activities.

- MMP-1 and MMP-8 were initially recognized for their ability to cleave native, interstitial collagens (type I and III), pro-MMP-2, and pro-MMP-9, and have since been shown to degrade other ECM proteins (like collagen type II, VII, X, entactin, aggrecan, proteoglycans, etc.).
- MMP-2 and MMP-9 were initially classified based on their degradative action on denatured collagens and type IV and V collagen and have since been demonstrated to cleave other ECM proteins (like fibronectin, laminins, elastin, aggrecan, entactin, etc.). In 1995, Ames et al.[18] showed that MMP-2 cleaves type I collagen.
- MMP-3 can participate in the activation of different inactive MMPs. The preferred substrates of this enzyme are collagen type III, IV, IX and X, gelatin, aggrecan, versican, fibronectin, laminins and elastin. MMP-3 like MMP-1 can fully degrade skin collagen and components of the elastic network.

In the skin, MMPs are produced by various cell types like keratinocytes, fibroblasts, macrophages and endothelial cells. They play an important role in skin biology, including cutaneous aging.[19] MMPs play an important role in the proteolytic remodelling of the extracellular matrix in various physiologic situations, including developmental tissue morphogenesis, tissue repair, and angiogenesis. On the other hand, MMPs play an important role in the excessive breakdown of connective tissue components in dermal photo-aging.

A comparison of MMP levels in young and old skin revealed that MMP-1, MMP-2, MMP-3 and MMP-9 are up-regulated in old skin.[20] It was also recognized that the UVB and UVA portions of sunlight are potent inducers of MMP activity. Thus, a comparison of naturally aged sun-exposed and sun-protected skin showed that MMP-1, MMP-2 and MMP-9 levels are increased in photo-aged skin *in vivo*.[21]

Chiang et al. recently demonstrated that UV irradiation increases MMP-1, MMP-3 and MMP-9 levels and then causes collagen and elastin degradation, leading to the formation of coarse wrinkles and sagging skin.[22]

PRINCIPLE

Purified human MMPs (MMP-1, MMP-3 or MMP-9) are activated in the presence of fluorescent specific substrate in order to quantify the activity using a fluorometer.

Progeline™ is tested for its capacity to inhibit the MMPs' activities (degradation of substrate).

PROTOCOL

Biological material

Human MMP-1 and MMP-9 were purified from natural sources (cell line THP1) as described by Shimokawa et al.[23]

For MMP-3, the catalytic domain was used for the assay. It was produced by recombinant methodology and purified by chromatography.

Calibrations of the enzymes were performed with a recognized MMP inhibitor (GM6001) to eliminate lot-to-lot variation.

The proteolytic activity of the MMPs was assayed by digesting a fluorescent substrate. In a typical assay, 10 µl of purified enzyme (10 ng), 5 µl of dissolved substrate (final concentration 10 µM) and 50 µl of a diluted solution of Progeline™ were mixed and completed with a buffer to yield a final volume of 75 µl.

All assays were performed in 96-well plates at 37°C and the reaction was initiated by the addition of substrate.

Tested molecules

Progeline™ was tested at different concentrations ranging from 0.001 to 0.75%.

A negative control (solvent vehicle) was included in order to determine the noise level.

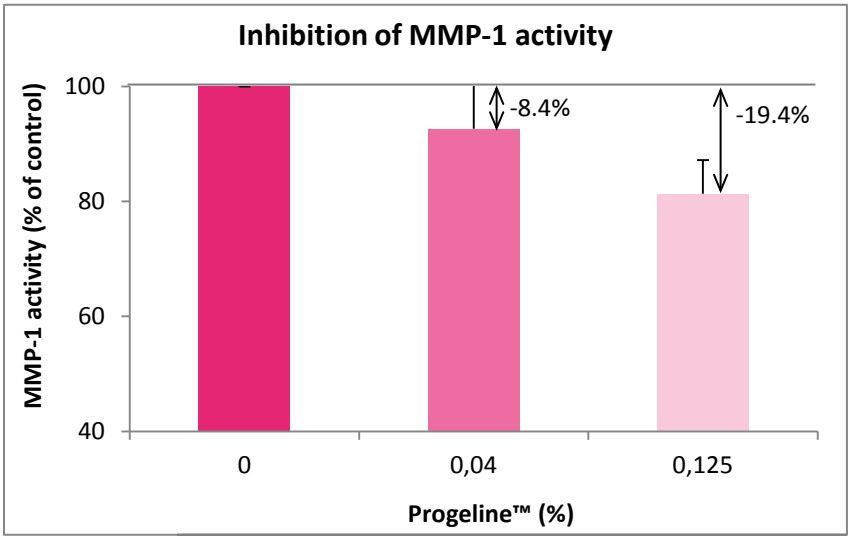
Evaluation of the activity

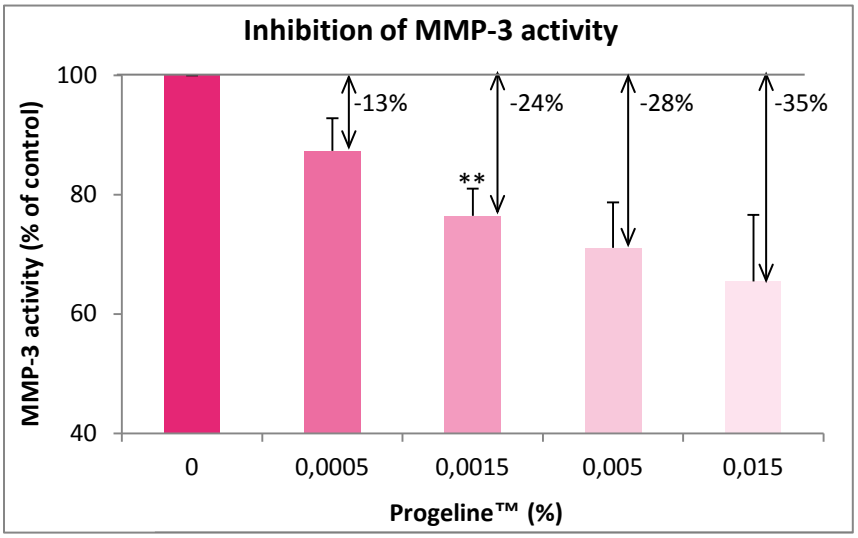
Fluorescence kinetic measurements (excitation 330 nm, emission 400 nm) were performed on a fluorometer (Polarion Tecan, NC, USA) after 20, 40 and 60 minutes. All measurements were performed twice.

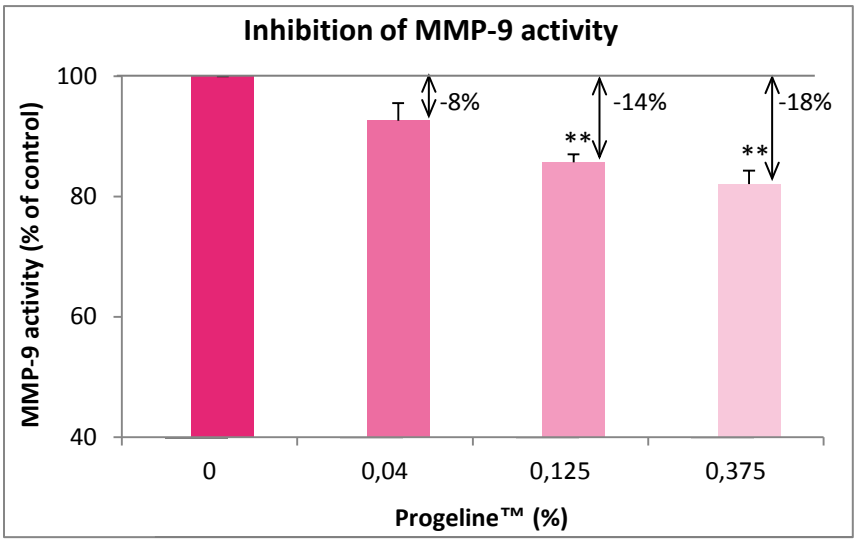
The inhibition was calculated by comparing the enzymatic activity with and without Progeline™.

RESULTS

Results are expressed in terms of fluorescence intensity and translated into inhibition percentage.







We observed a strong inhibition of MMPs activities in the presence of Progeline™ in a dose dependent manner. The inhibition of MMP-3 activity had the most powerful effect. This effect is important, as the MMP-3 participate in the activation of other MMPs.

CONCLUSION

Progeline™ decreases the activities of 3 specific MMPs and provides an efficient tool for preventing the excessive destruction of matrix proteins. Progeline™ not only inhibits the activities of the MMPs but may also interfere with the activation of the latent pro-MMP (via MMP-3 inhibition).

Progeline™ reduces ECM breakdown for maximal protection against sagging and loss of elasticity

CONTRACTION OF 3D COLLAGEN LATTICES BY HUMAN FIBROBLASTS

INTRODUCTION

In vivo fibroblasts are surrounded by an extracellular matrix (ECM) that profoundly influences the gene expression, morphology, growth, motility and differentiation of cells. Type I collagen is the most abundant protein in the ECM of the skin. The forces fibroblasts exhibit on the ECM are defined as cellular contraction. This attribute is especially important in the granulation tissue of wounds.[24,25] The reduction of collagen (type I and III) is a characteristic of chronologically aged skin and this feature is even more pronounced in photo-damaged skin. There is also strong evidence that the biological impact of elastic fiber modification is related to the loss of contractile capacity in fibroblasts, caused by the structural breakdown of the filament system.

In addition to having a reduced capability for collagen production, reflecting a breakdown of the elastic and filament dermis systems, fibroblasts from aged skin produce lower contractile forces than fibroblasts from young skin.[26] This results in changes in the dermal architecture of the skin, ultimately leading to skin wrinkling and sagging.

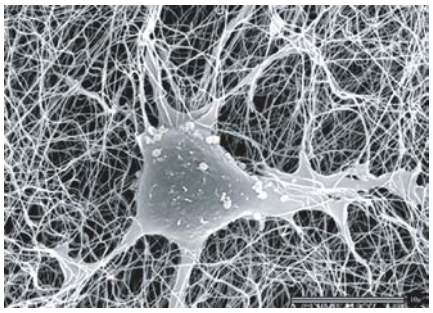
OBJECTIVE

The aim of this study was to determine the effect of Progeline™ on improving the contractile properties of normal fibroblasts in 3D collagen matrices.

PROTOCOL

Principle

When fibroblasts interact with the collagen matrix, their cells can penetrate into the substance of the matrix and become entangled in matrix fibrils.



Human fibroblasts interacting with a 3D collagen matrix.

Scanning electron microscopic image showing cells incubated in collagen. Cell extensions penetrate into the matrix and become entangled in collagen fibrils.

From Rhee and Grinnell, 2007.

The methodological approach involving collagen lattices is based on Bell et al.'s study[27] and was described by Delvoye et al.[28] for measuring contraction in fibroblasts.

Biological material

Type I collagen (2 mg/mL) was dissolved in a specific buffer and added to normal human fibroblasts in 24-well plates at 37°C for a 30–60 minute incubation period. The gels were then covered with a specific cell medium and incubated for another 4 hours.

Tested molecules

Progeline™ was then added at 0.025% or 0.0025% concentrations for 24 or 48 hours. A positive control was used for its capacity to contract 3D collagen matrices.

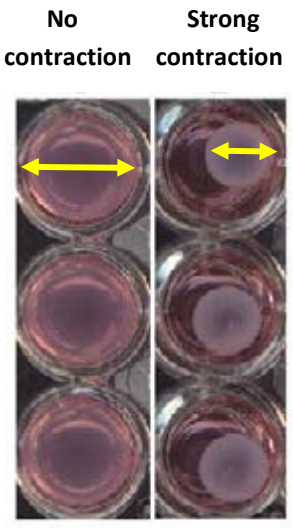
Evaluation of the activity

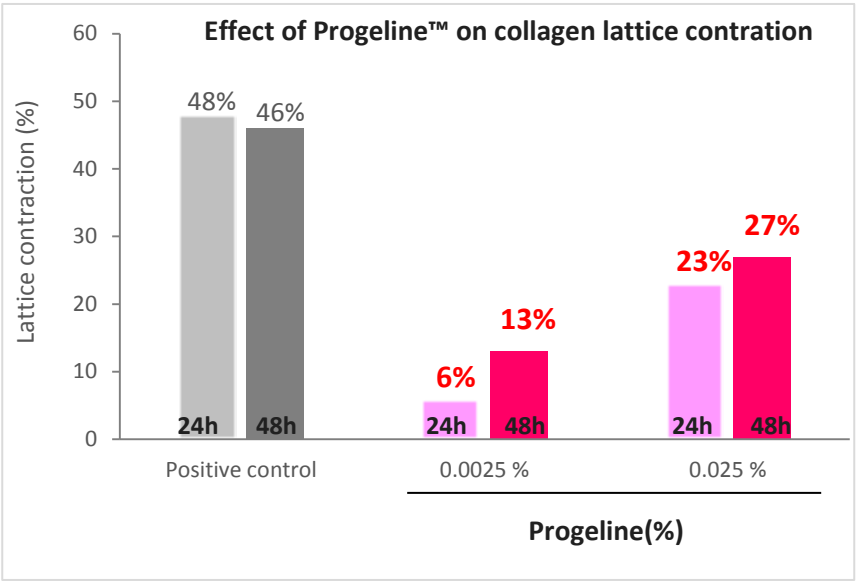
To examine the contraction capacity of the 3D collagen matrix by fibroblasts, the dishes were placed on top of a transparent metric ruler on a dark background. The average of the major and minor axes was taken as the diameter.

For each experiment, 3 matrices were examined. The average of the diameters of the 3 matrices was taken.

RESULTS

The 3D collagen lattice is illustrated hereafter, following 24 and 48 hours incubation periods with or without a contractile product.





The incubation of Progeline™ in a 3D collagen matrix in the presence of fibroblasts induces a strong contractile reaction in the fibroblasts. This effect is dose and time dependent.

CONCLUSION

Progeline™ improves the ability of fibroblasts to contract 3D collagen matrices. This effect contributes to the strengthening of the dermal matrix and thereby reinforces the architectural framework of the skin.

Progeline™ induces collagen contraction to restore tone and firmness to the skin

CLINICAL STUDIES

EVALUATION OF ANTI-SAGGING EFFECT

In vivo fringe projection of the jawlines

OBJECTIVE

The objective of this study was to evaluate the *in vivo* efficacy of Progeline™ in the reduction of sagging and slack skin observed at the jawline level.

PROTOCOL

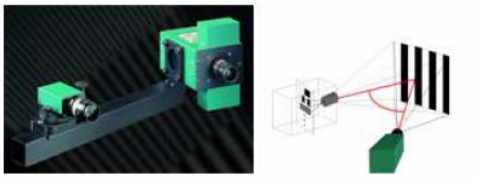
Subjects: 10 healthy volunteers aged 54 to 64 years (mean age=59) were included in this study.

Test conditions: For 56 days the volunteers applied an emulsion cream containing either 2% of Progeline™ or a placebo. The trial cream and the placebo cream were applied randomized split-face and split-neck twice a day in the morning and evening.

Clinical evaluation

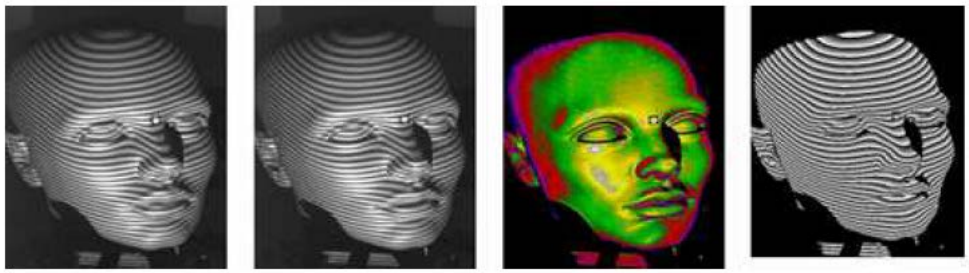
At days 0, 28 and 56, the jawline volume of each volunteer was measured using an *in vivo* fringe projection profilometric technique.
This technique, adapted to the analysis of the morphology of the face, allows us to evaluate the decrease in facial ptosis after treatment. The measurements are taken directly from the subjects, *in vivo*, at each measurement time. This technique allows the direct and immediate acquisition of the morphology of this region in 3 dimensions.

The system includes a measurement sensor containing a halogen projector coupled with a high resolution CCD camera (field of view: 360 x 210 x 270 mm), linked to the acquisition software Optocat.



Fringe projection sensors

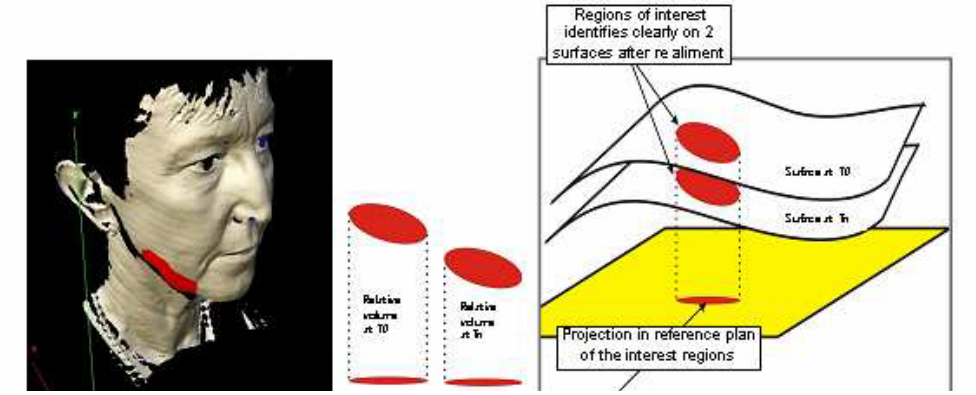
The lighting system, connected to a video camera, permits the projection of a sequence of lattice lines (fringes) onto the object. Three-dimensional information about the object is calculated from the deformation of the fringes on the object's surface and recorded by the digital camera.



Acquisition methodology

One acquisition is carried out on each half of the face.

Analysis of these 3D acquisitions consists of matching the envelope of one half of the face, obtained at different times of the kinetics (T28 and T56 days), to the envelope determined at the initial time (T0).



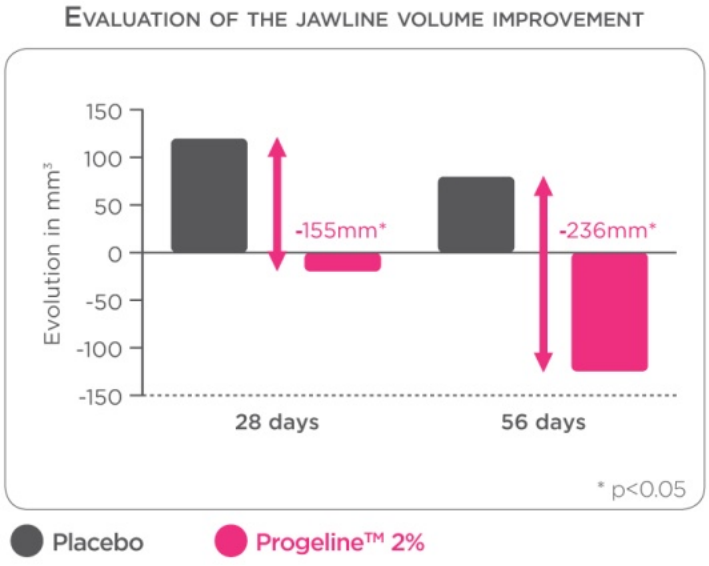
A **B** **C**

A: Definition of the regions of interest for all the acquisitions of a given subject after realignment.

B-C: Volume calculation between the region of interest at the surface of the skin and its projection in the reference plane.

RESULTS

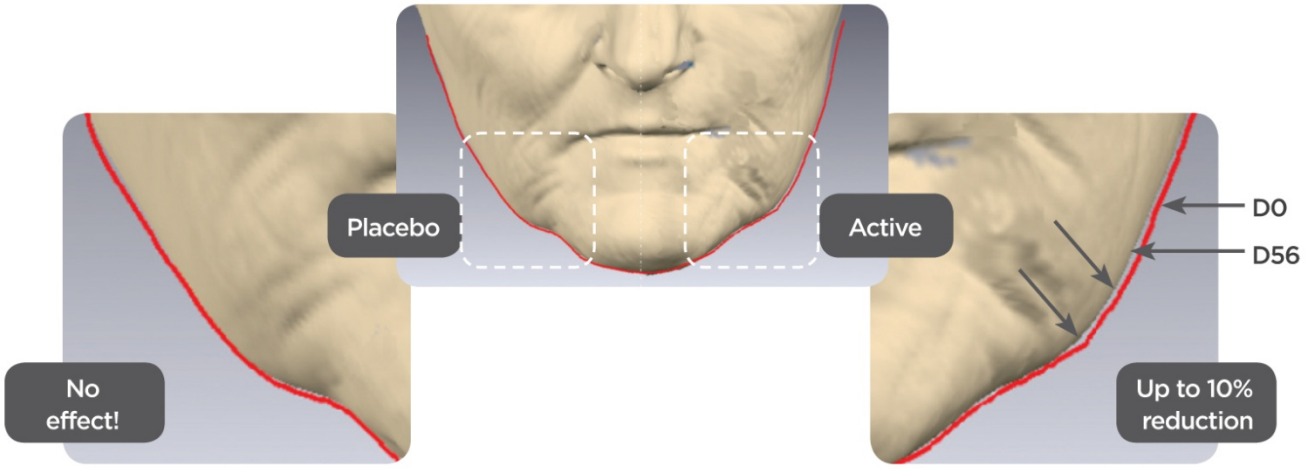
Results are expressed in terms of the evolution of the jawline volume (mm^3) after 28 days and 56 days of the placebo- and Progeline™-treated sides.



As shown, Progeline™ significantly reduces the volume of the jawline in healthy volunteers after 4 and 8 weeks of treatment: -0.6% and -3.4%, respectively; whereas the placebo-treated half of the face shows no effect or, even worse, sagging.

The volume reduction reaches up to 493 mm^3 (-10%) after only 28 days of application.

Illustrative digital photographs



CONCLUSION

By reducing the jawline volume of volunteers applying Progeline™, we clearly demonstrate that this peptide plays a major role in the reduction of the signs of aging, especially those relating to the sagging and slackness effect observed when the dermis becomes unstructured and disorganized.

Progeline™ significantly reduces the jawline volume after only 28 days

EVALUATION OF FIRMNESS AND ELASTICITY

Cutometry

OBJECTIVE

The objective of this study was to evaluate the *in vivo* efficacy of Progeline™ on the skin's mechanical properties: firmness, elasticity and viscoelasticity.

PROTOCOL

Subjects: 13 healthy volunteers ranging in age from 54 to 66 years (mean age=60) were included in this study.

Test conditions: For 56 days the volunteers applied an emulsion cream containing either 2% Progeline™ or a placebo. The trial cream and the placebo cream were applied randomized spit-face and split-neck twice a day in the morning and evening.

Clinical evaluation

At days 0 and 28, the cutaneous firmness and elasticity of every volunteer's **cheeks** (one treated with the placebo, the other treated with the cream containing Progeline™) were measured with a cutometer (SEM575, Courage & Khazaka).

The principle of this technique is based on the creation of negative pressure on the skin's surface and on the measurement of vertical movement on the induced skin. This movement is determined by an optical system (composed of an infrared diode, two prisms and a light sensor). The quantity of light detected by the sensor is proportional to the movement of the skin's surface.

Measurement of the vertical movement on the induced skin was performed to permit the determination of 3 main parameters:

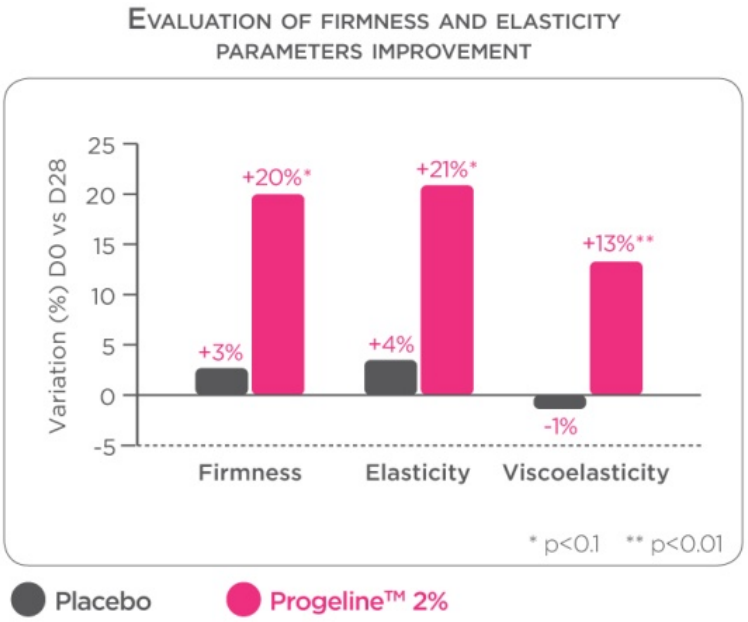
- Firmness = firmness, tightness, tonicity;
- Elasticity = capacity of the skin to stretch;
- Viscoelasticity = capacity of the skin to go back to its original state.

Illustrative digital photographs of the face

This technique consists of obtaining high resolution photographs of ¾ of the face on both sides, in completely reproducible lighting conditions.

RESULTS

Results are presented in terms of percentage of variation of the 3 parameters between the beginning (T0) and after 28 days of treatment with either the placebo or Progeline™.



As shown, Progeline™ improves cutaneous **firmness (+20%)**, cutaneous **elasticity (+21%)** and cutaneous **viscoelasticity (+13%)**.

Improvement up to:

93% of cutaneous elasticity;
82% of cutaneous firmness;
42% of cutaneous viscoelasticity.

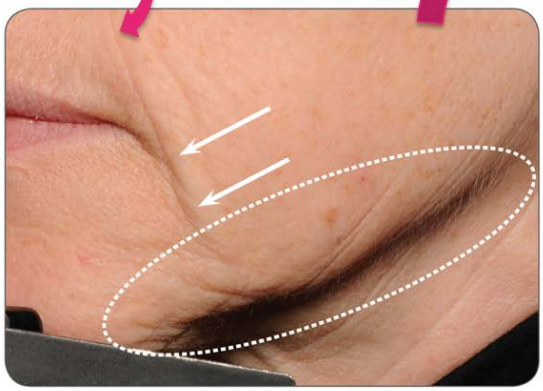
Progeline™ significantly increases skin firmness, elasticity and viscoelasticity, attesting its efficacy on skin slackening and sagging

Before and after pictures

D0

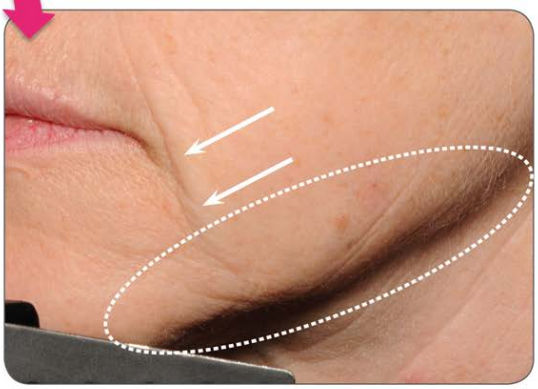
Slight improvement

Progeline™

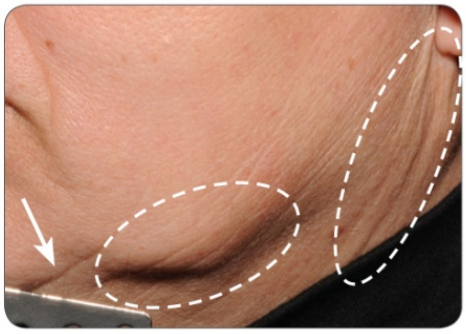


D28

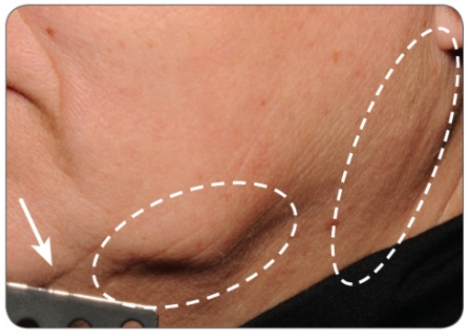
Major building effect over time!



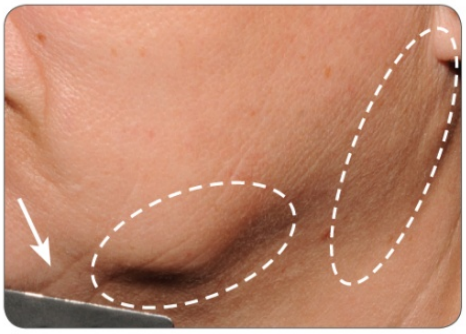
D56



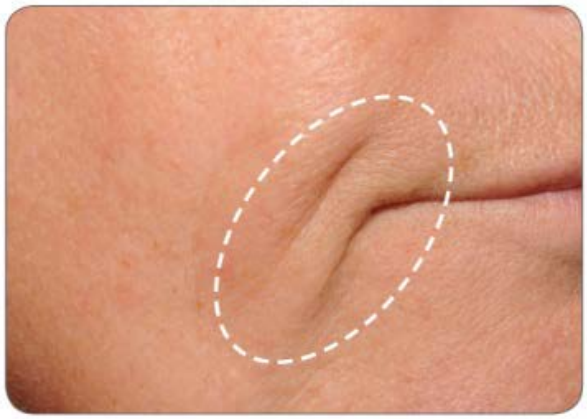
D0



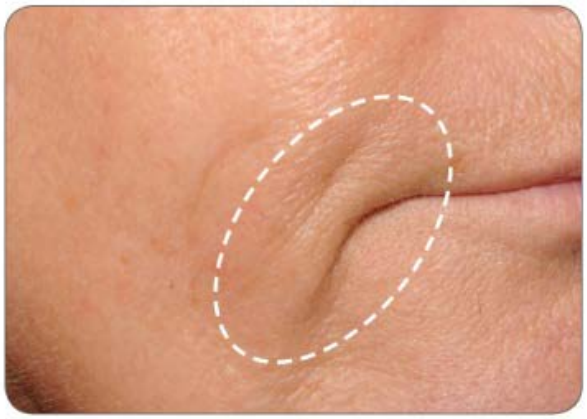
D28



D56



D0



D28

CONSUMER EVALUATION

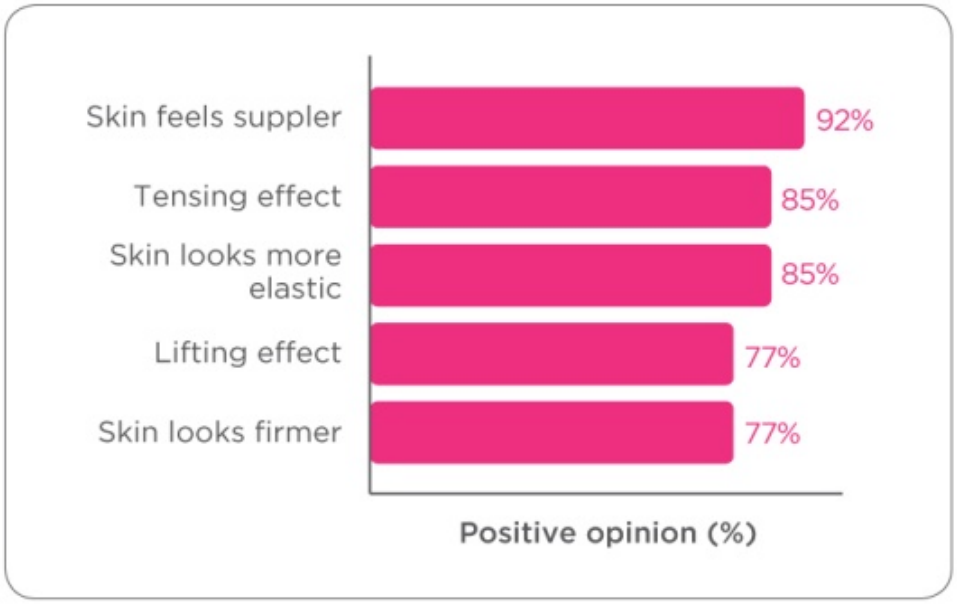
OBJECTIVE

The objective of this study was to evaluate consumer perception of Progeline™ as a firming, anti-sagging product following 56 days of treatment.

Subjects were asked, at the end of the study (D56), to fill out a self-evaluation questionnaire comprising closed and open questions in order to evaluate their overall opinion and feelings on the effectiveness of the product.

RESULTS

CLOSED QUESTIONS



OPEN QUESTIONS

Open questions promote spontaneous positive or negative comments, underlining the product's principal characteristics.

Spontaneous positive opinions—"the product has a":

- Firming effect
- Skin-smoothing effect
- Skin-lifting effect

CONCLUSION

Progeline™ is a 3 amino acids peptide biomimetic of elafin, an elastase inhibitor produced by keratinocytes. Progeline™ decreases the synthesis of progerin, a new aging biomarker involved in skin cellular senescence. By acting directly on the senescence process, Progeline™ clinically improves the signs associated with skin maturation—sagging and slacking—for a complete remodelling effect.

Progeline™ is thus a sophisticated and high-tech product that fights the aging process and creates youthful and healthier looking skin.

COSMETIC APPLICATIONS

- Anti-aging
- Firming care
- Remodelling skin care
- Alternative to surgery
- Post-pregnancy care
- Skin care for mature skin
- Men's care
- Body care

RECOMMENDATION FOR USE

Progeline™ is easy to use and has excellent stability.

Progeline™ should be incorporated at the end of the manufacturing process, at a temperature below 40°C.

RECOMMENDED DOSAGE:

- Intensive treatment: 2%

- Body care: 1%

- Preventive care: 0.5–2%

FORMULATIONS

Formulation used in the clinical study

Phase	Commercial name	INCI name	%
A	Demineralized water	Aqua	qsp
	Carbopol® Ultrez-10	Carbomer	0.15
	Butylen glycol	Butylen Glycol	3.00
B	Neommekkin M16	Phenoxyethanol (and) Methylparaben (and) Ethylparaben (and) Butylparaben (and) Propylparaben (and) Isobutylparaben	0.8
	Glycerin	Glycerin	2
C	Cutina HVG	Hydrogenated vegetable 2.00 Consistency factor glycerides	5
	Lanette 16	Cetyl Alcohol	1
	Span™ 80	Sorbitan Oleate	0.5
	LABRAFAC CC	Caprylic/Capric Triglyceride	5
	SCHERCHEMOL MM ESTER	Myristile myristate	3
	TWEEN 60	Polysorbate 60	2
	SHEA BUTTER	Butyrospermum Parkii	1
	DM FLUID 350 CS	Dimethicone	0.04
D	FRAGRANCE	Parfum	0.02
	Progeline™	Trifluoro acetyl tripeptide-2	2
	Sodium hydroxide 10%	Sodium Hydroxide	0.66

Since the trifluoroacetyl-tripeptide-2 is composed of hydrophilic natural amino acids, it is very easy to use thanks to its full solubility in aqueous medium, glycerin, glycol and ethanol.

Also, as established by laboratory tests, the trifluoroacetyl-tripeptide-2 is stable at pH values ranging from 3.0 to 6.5 and is compatible with most types of emulsifiers and thickeners.

In order to maximize it stability in formula post addition at the end of the process (35-40°C) is recommended.

LA SPIRIT CREAM

	INGREDIENTS	INCI NAME	%
A	Deionised Water	Water	77.10
	Dermofeel™ PA-12	Sodium Phytate	0.10
	Chlorphenesin	Chlorphenesin	0.30
	Phenoxyethanol	Phenoxyethanol	0.80
	Granlux® AOX-GL	Glycerin (and) Picea Abies Extract (and) Alcohol	1.00
	Biophilic™ H	Hydrogenated Lecithin (and) C12-16 Alcohols (and) Palmitic Acid	4.00
B	Dermorganics GMS-SE	Glyceryl Stearate SE	1.00
	Mirasil DM350	Dimethicone	4.00
	Dekanex 2006 FG	Hydrogenated Polydecene	3.00
	Phytosqualan	Squalane	2.00
	Lecigel™	Sodium Acrylates Copolymer (and) Lecithin	1.50
C	Adipofill™	Water (and) Propanediol (and) Ornithine (and) Phospholipids (and) Glycolipids	2.00
	Progeline™	Glycerin (and) Water (and) Dextran (and) Trifluoroacetyl Tripeptide-2	2.00
	Exo-H™	Butylene Glycol (and) Alteromonas Ferment Filtrate	1.00
D	Douceur 3246	Fragrance	0.20

REFERENCES

[1] Rankin J, Ellard S (2006) The laminopathies: a clinical review. Clinical Genetics 70: 261–274.

[2] Worman HJ, Bonne G (2007) "laminopathies": a wide spectrum of human diseases. Experimental Cell Research 313: 2121–2133.

[3] McClintock D, Ratner D, Lokuge M, Owens D.M, Gordon L.B, Collins F.S, Djabali K. The Mutant Form of Lamin A that Causes Hutchinson-Gilford Progeria Is a Biomarker of Cellular Aging in Human Skin. Journal Pone. 2007 Dec e1269.

[4] Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. Science. 2006 May 19;312(5776):1059-63.

[5] C. Verdy, J-E. Branka and N. Mekideche, Quantitative assessment of lactate and progerin production in normal human cutaneous cells during normal ageing: effect of an Alaria esculenta extract. Int. J. Cosmet. Sci., 3, 1-5 (2011).

[6] K. Cao, C. Blair, D. Faddah, J. Kieckhaefer, M. Olive, M. Erdos, E. Nabel and F. Collins, Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. J. Clin. Invest., 121, 2833-2844 (2011).

[7] M. Bradford, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal. Biochem., 72, 248-254 (1976).

[8] Homsy R., Pelletier-Lebon P., Tixier J.M., Godeau G., Robert L., Hornebeck W. Characterization of human skin fibroblasts elastase activity. 1988. *J. Invest. Dermatol.* 91(5): 472-477.

[9] Molero M.L., Del Castillo P., Stockert J.C. Fluorescence reactions of orcein: new applications for an old stain. 1986. *J. Microsc.* 144 (Pt 1): 45-53.

[10] Carvallo L, Muñoz R, Bustos F, Escobedo N, Carrasco H, Olivares G, Larraín J. Non-canonical Wnt signaling induces ubiquitination and degradation of Syndecan4. *J Biol Chem.* 2010. 17; 285(38): 29546-29555.

[11] Bass MD, Humphries MJ. Cytoplasmic interactions of syndecan-4 orchestrate adhesion receptor and growth factor receptor signalling. Biochem J. 2002 . 15;368(Pt 1): 1-15.

[12] Vuillermoz B, Wegrowski Y, Contet-Audonneau JL, Danoux L, Pauly G, Maquart FX. Influence of aging on glycosaminoglycans and small leucine-rich proteoglycans production by skin fibroblasts. *Mol Cell Biochem.* 2005. 277(1-2): 63-72.

[13] Oh JH, Kim YK, Jung JY, Shin JE, Chung JH. Changes in glycosaminoglycans and related proteoglycans in intrinsically aged human skin in vivo. *Exp Dermatol.* 2011. 20(5): 454-456.

[14] Wegrowski Y, , Danoux L, Contet-Audonneau J, Pauly G, Maquart FX. Decreased syndecan-1 expression by human keratinocytes during skin aging. *J. Invest. Dermatol.* 2005. 125, A5.

[15] Oh JH, Kim YK, Jung JY, Shin JE, Chung JH. Changes in glycosaminoglycans and related proteoglycans in intrinsically aged human skin in vivo. Exp Dermatol. 2011. 20(5): 454-456.

[16] Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. J Periodontol. 1993 May;64(5 Suppl):474-84

[17] Nagase H, Fields GB. Human matrix metalloproteinase specificity studies using collagen sequence-based synthetic peptides. Biopolymers. 1996;40(4):399-416.

[18] Aimes RT, Quigley JP. Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4- and 1/4-length fragments. J Biol Chem. 1995 Mar 17;270(11):5872-6.

[19] Kähäri VM, Saarialho-Kere U. Matrix metalloproteinases in skin. Exp Dermatol. 1997 Oct;6(5):199-213.

[20] Rittié L, Fisher GJ. UV-light-induced signal cascades and skin aging. Ageing Res Rev. 2002 Sep;1(4):705-20.

[21] Chung JH, Seo JY, Choi HR, Lee MK, Youn CS, Rhie G, Cho KH, Kim KH, Park KC, Eun HC. Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. J Invest Dermatol. 2001 Nov;117(5):1218-24.

[22] Chiang HM, Lin TJ, Chiu CY, Chang CW, Hsu KC, Fan PC, Wen KC. Coffea arabica extract and its constituents prevent photoaging by suppressing MMPs expression and MAPK pathway. Food Chem Toxicol. 2010 Nov 4.

[23] Shimokawa K, Nagase H. Purification of MMPs and TIMPs. Methods Mol Biol. 2001;151:275-304.

[24] Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971 May 15;27(5):549-50

[25] Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol. 1994 Feb;124(4):401-4.

[26] Pageon H, Bakala H, Monnier VM, Asselineau D. Collagen glycation triggers the formation of aged skin in vitro. Eur J Dermatol. 2007. 17(1):12-20. Epub 2007 Feb 27.

[27] Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc Natl Acad Sci U S A.* 1979 Mar;76(3):1274-1278.

[28] Delvoye P, Wiliquet P, Levêque JL, Nusgens BV, Lapière CM. Measurement of mechanical forces generated by skin fibroblasts embedded in a three-dimensional collagen gel. J Invest Dermatol. 1991. 97(5): 898-902.