

HOMEOSTATINE

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INTRODUCTION

Wrinkles are skin modifications produced by invagination of the skin tissue. Wrinkles result from a combination of intrinsic and extrinsic aging, the later caused by external factors.

HOMEOSTATINE is an active complex of two natural ingredients: from an Andean Tree and from a Marine Seaweed, developed through an innovative technological process, designed to prevent and reduce skin wrinkles.

DESCRIPTION

- Skin and wrinkles

Structural skin changes may affect either epidermal layers only, as it happens with superficial wrinkles, or the dermis, as it happens with deeper wrinkles (figure 1).

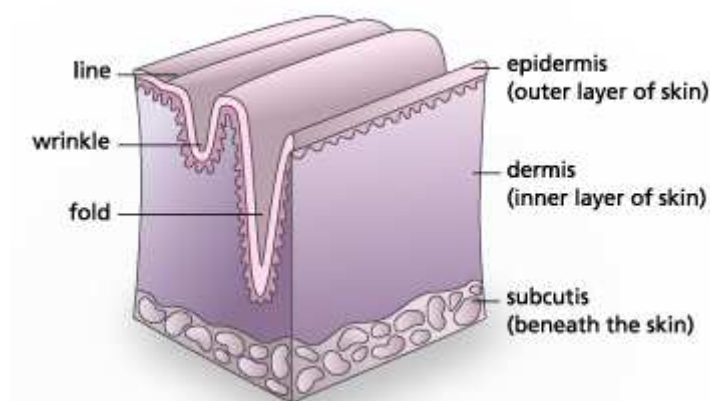


Figure 1. Wrinkles

Although wrinkles may occur in every part of the body, those appearing on the face and neck are the most important ones for cosmetics. Different factors are involved in the formation of wrinkles: natural skin aging, photo-aging and repeated facial movements (expression lines). However, all of these wrinkle-promoting factors share a physiological cause: **perturbations in the homeostasis of dermal extra-cellular matrix.**

- Homeostasis in the extracellular matrix

This concept refers to the balance between synthesis and degradation of extracellular matrix (ECM) components, which keeps the skin moisturized, firm, elastic and free of wrinkles. Such a balanced state, where keratinocytes and fibroblasts are metabolically efficient, ensures adequate quantity and quality of collagen fibers, elastic fibers and glycosaminoglycans (GAG) in the dermis.

The homeostasis situation, also involves the activity of matrix metalloproteinases (MMP), which renew the fibers, and of some cytokines, which regulate the activity of fibroblasts. The roles of each one of these components in homeostasis are briefly described below:

- Keratinocytes are typical epidermal cells. They release cytokines, which regulate the activity of fibroblasts.
- Fibroblasts are the most abundant cells in dermis. They synthesize and degrade the components of the extracellular matrix.
- Collagen fibers: these thick, long, unbranched fibers are the most abundant ones. Collagen makes up 70-75% of the dry weight of the skin (Freedberg IM, 2005) and gives the dermis its structural integrity and properties. Collagen fibers give firmness to the skin and help prevent wrinkles.

To the present, more than 20 different types of collagen molecules have been identified. The most useful types from the dermatological point of view are collagen I, III, IV and VII.

The amount of dermal collagen may be increased by stimulating collagen gene expression – like Tumor growth factor-beta (TGF- β) or retinoic acid do – or by reducing the synthesis/activity of collagenases and other MMP.

- Elastic fibers are synthesized in fibroblasts. These fibers are arranged in a continuing network from the Dermo-Epidermal Junction (DEJ) to the hypodermis, and have the important function of providing elasticity to the skin (Cordero, 1996). The elastic properties of skin are essential for prevention of wrinkles.
- Glycosaminoglycans are polysaccharides coming from fibroblasts, the most important one in adults being hyaluronic acid. GAGs are able to retain a large amount of water, thus modulating dermal water content. Well moisturized dermis is smooth and therefore less prone to wrinkles.
- Matrix metalloproteinases (MMP): these enzymes – mainly synthesized in fibroblasts – are in charge of degrading several macromolecules in the extracellular matrix (collagen, elastin, glycosaminoglycans and glycoproteins). Such degradation processes are naturally balanced with synthesis processes, so that normal renewal of dermal components occurs, thus preventing accumulation of old, non-functional fibers.

MMP activity in the dermis may be modulated by using inhibitors TIMP (Tissue Inhibitor of Metalloproteinases) or by regulating gene-expression. TGF- β inhibits the production of collagenases and other metalloproteinases in fibroblasts. Conversely, modulators like IL-1 or TNF- α stimulate MMP expression. Exposure to UV light induces the production of IL-1 in fibroblasts, consequently increasing the amount of MMP.

- Cytokines are involved in the regulation of the synthesis and degradation of different ECM components. Main cytokines are IL-1 and TGF- β .

In normal skin, keratinocytes release low constitutive levels of cytokines (IL-1 α and TGF- β), which regulate the metabolic activity of fibroblasts. The release profile for these mediators promotes balance between basal synthesis (TGF- β) and degradation (IL-1 α) of ECM components, thus contributing to homeostasis (figure 2). Under such balanced conditions, the skin shows good levels of firmness, elasticity and moisture, which keep it free of wrinkles.

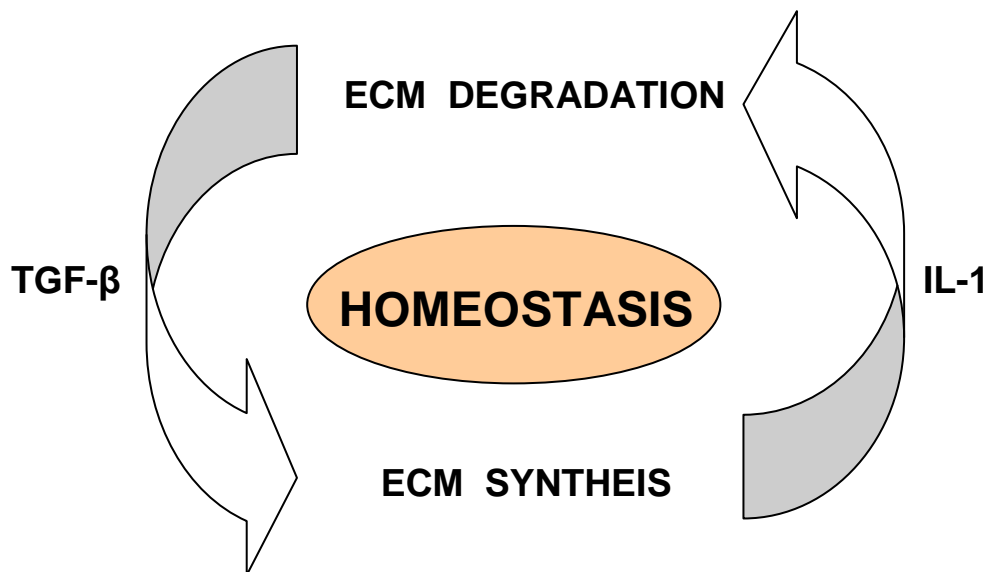


Figure 2. Homeostasis in the ECM

- *Perturbations of the homeostasis in ECM: wrinkles*

Intrinsic aging and external factors often alter homeostasis and result in a progressive imbalance towards the degradation of extracellular matrix components. The outcome is deterioration of the skin aspect, wrinkles, sagging, dryness and loss of elasticity.

Aging involves a number of physiological and functional changes, which contribute to produce an imbalance in the dermal extracellular matrix.

Fibroblasts in aged skin show impaired proliferation and reduced collagen I and III production than young skin, as well as impaired response to growth factors and increased response to growth inhibitors (Phillips CL, 1994). Furthermore, aging reduces the production of MMP inhibitors and TIMP-2 (Thibodeau A, 2000). The overall effect is a modification of the synthesis/degradation balance towards the action of MMP.

The outcome is a clear reduction of dermal thickness, which drops to about 20% in the old age (Freedberg IM, 2005). Actually, adult skin loses almost 1% collagen per year. This situation worsens in post-menopausal women because low estrogen levels further impair collagen production (Schmid D, 2002). The number of elastic fibers also decreases since the onset of adult age. Aging also reduces the amount of glycosaminoglycans, especially hyaluronic acid, impairing the dermal moisture balance and the skin smoothness. Apart from the reduction of ECM components, the integrity of collagen, elastin and other dermal connective tissue components also become altered.

During the aging process, as a consequence of collagen reduction and perturbation (Chaudhuri RK, 2000), impaired dermal moisturizing (which results of reduced GAG levels) and elastic fibers perturbations (Freedberg IM, 2005), wrinkles and facial lines appear.

The most remarkable perturbation in the epidermis is the flattening of the dermo-epidermal junction, which leads to the disappearance of dermal papillae and rete ridge. As a consequence, the contact surface between both skin layers is reduced impairing communication and nutrients transport.

Skin injuries or other **external factors** such as UV radiation, toxins or microorganisms, induce the release of large amounts of cytokines, such as IL-1 α , IL-6, and TNF- α (Freedberg IM, 2005).

The interaction of IL-1 with fibroblasts stimulates the expression of MMP and impairs that of collagen, which leads to degradation of ECM components and reduction of dermal collagen (Wlaschek M, 1994). IL-6 in turn, induces the expression of MMP-1 and MMP-3, which also contribute to ECM degradation (Park CH, 2004).

Under continued exposure to such external agents, especially to sunlight, **photo-aging** occurs. The effects of photo-aging add up to those of intrinsic aging, so that exposed body areas may show large amounts of wrinkles. This happens mainly in the face and the neck.

In conclusion, processes which induce homeostasis imbalances in extracellular matrix components result in wrinkled, less elastic, loose and dry skin. If this happens, it is necessary to restore the homeostasis as soon as possible, in order to reduce the amount of wrinkles and recover the cosmetic properties of skin.

BOTANY AND CHEMISTRY

HOMEOSTATINE is a novel hydro-colloidal three-dimensional matrix of galactomannans of Andean origin, derived from the seeds of *Caesalpinia spinosa* (Mol.) O.Kuntze, which sequentially releases, a marine pentasaccharide derived from the algae *Enteromorpha compressa* (L.) Nees. This product helps recover and maintain homeostasis in the dermal extracellular matrix, which results in a reduced amount of wrinkles.



Figure 3. *Enteromorpha compressa* (L.) Nees

Enteromorpha compressa is a member of the class Chlorophyceae, also known as green algae, which are the largest algae group. In particular, this plant belongs to the order Ulvales, the most abundant algae in the flora of marine areas under stress conditions (sudden changes in salinity, temperature, nutrients concentration, etc.).

The chemical composition of *Enteromorpha* includes carbohydrates (48%), proteins (10-18%) and lipids (0.5-1.7%).

The extraction technology we have applied allows for selective separation of the oligosaccharides present in this alga.

Analysis of the resulting product by Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Nuclear magnetic resonance (NMR) indicated that the active fractions consisted in oligosaccharide mixtures, with the most abundant compound being a pentasaccharide (figure 4) made of five glucose residues; it has been identified as α -D-glucopyranosyl-(1 \rightarrow 6)-[α -D- glucopyranosyl-(1 \rightarrow 2)]- α -D- glucopyranosyl-(1 \rightarrow 6)- α -D- glucopyranosyl-(1 \rightarrow 4)-D-glucopyranose (figure 5).

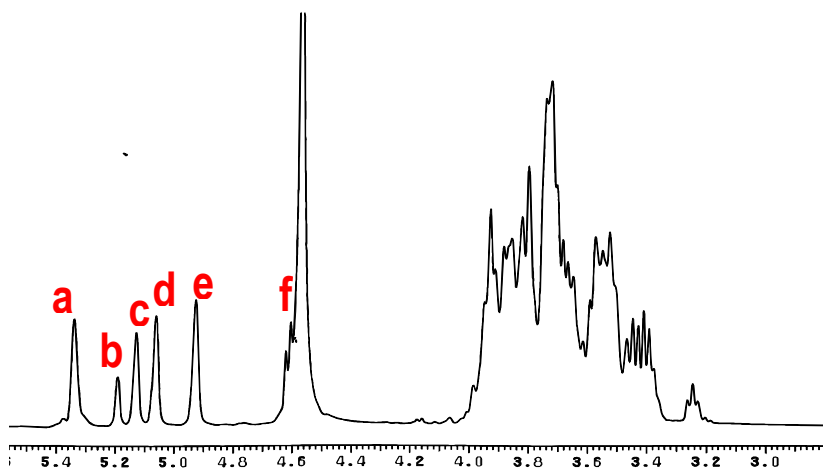


Figure 4. NMR

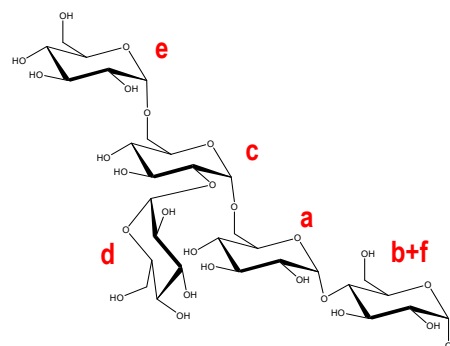


Figure 5. Pentasaccharide



Figure 6. *Caesalpinia spinosa*

Caesalpinia spinosa is commonly known as Tara. This species is native to Peru, although it has spread throughout Latin America. It grows wild in the Andean forests and valleys, at 1000-3100m altitude. Tara is a good species for reforestation since it endures dry climates and grows in sandy to stony soils.

Tara fruits are flat pods enclosing 4-7 seeds. Inside the hull of the seeds, two parts may be distinguished: the germ (embryo) and the endosperm (albumen). The endosperm is mainly composed of mannose- and galactose-polysaccharides called galactomannans.

Galactomannans are high molecular weight polysaccharides with **natural hydrating functions** within the seed, which prevent the embryo from drying up, due to their high water-uptake and water-retention capacities. Their structure consists of linear mannose chains connected to each other by glycosidic bonds, with randomly distributed side chains of galactose residues; the mannose:galactose ratio (Man:Gal) is 3:1. The regular distribution of galactose along the chains gives this molecule good **solubility** in water.

TECHNOLOGY AND STRUCTURE

The innovative structure of **HOMEOSTATINE**, which acts as a sequential molecule-release system, is the result of an innovative multi-capillary injection technology.

- Multi-capillary injection

In a first preparatory stage, the polysaccharides fraction (galactomannan) undergoes a humidification phase under controlled heating, until complete solubility.

The so formed pre-colloidal gel allows for the accurate incorporation of Enteromorpha pentasaccharide molecules. The galactomannan pre-gel circulates into a pipe under constant temperature conditions. At a certain point, the pentasaccharide concentrate is incorporated by means of a multi-capillary injection and the system is immediately cooled down. With the oligosaccharides injection, a critical concentration of solutes is reached that, in combination with the drastic temperature drop, induces galactomannan to form a three-dimensional matrix, which traps the smaller sugar molecules.

The pentasaccharide molecules are injected under continuous high-pressure conditions, thus ensuring homogeneous incorporation of the small molecules into the galactomannan matrix.

By using this innovative technology, it is possible to produce the unique structure of **HOMEOSTATINE**, which determines its action mechanism and allows for prolonged pentasaccharide release.

The following scanning electronic microscopy images show the **HOMEOSTATINE** three-dimensional matrix (figures 7 and 8).

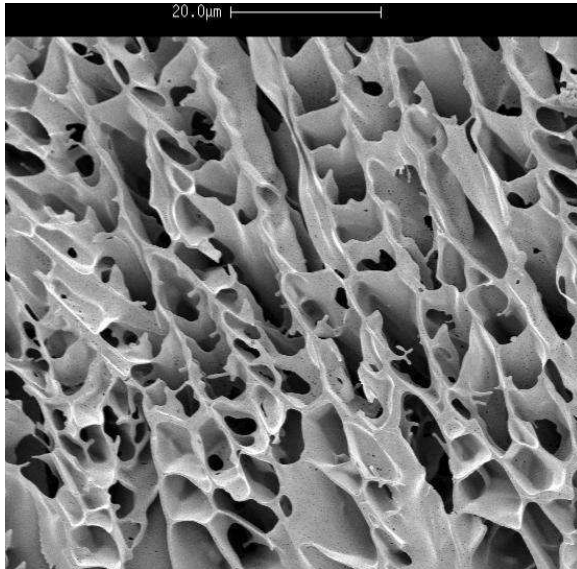


Figure 7.
HOMEOSTATINE: Tridimensional matrix

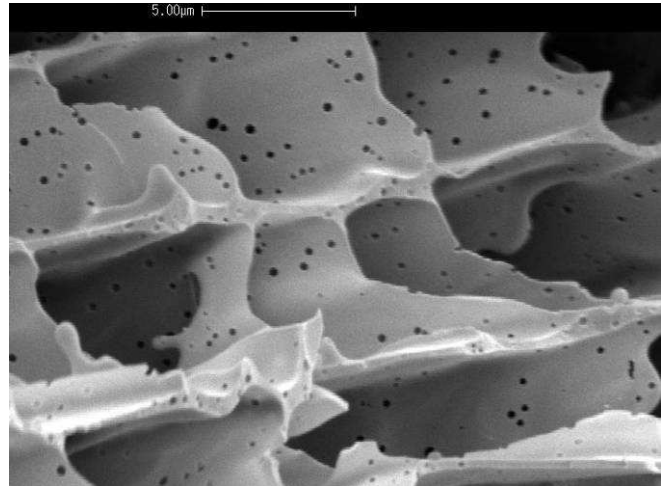


Figure 8.
HOMEOSTATINE : Tridimensional matrix

ACTION MECHANISM

HOMEOSTATINE activity is due to the combined action of its components: marine pentasaccharide (MPS) from *Enteromorpha compressa* and galactomannan from *Caesalpinia spinosa*. The action of **HOMEOSTATINE** is aimed at preventing and/or fighting skin wrinkles. To that end, this product acts on the following processes:

- Increases the production of dermal collagen and other ECM components in fibroblasts.
- Inhibits the synthesis of metalloproteinases (MMP)
- Inhibits the synthesis of pro-inflammatory mediators

Thus, **HOMEOSTATINE** applications on the skin trigger a number of beneficial effects aimed at restoring the **extracellular matrix homeostasis**. The final outcome is a rejuvenated, less wrinkled, firmer, more elastic and better moisturized skin.

In order to achieve this goal, **HOMEOSTATINE** components act at two different levels:

- **MPS**: these small-sized molecules penetrate the epidermis and reach the dermis, where they exert their effects.
- **Galactomannan**: these high molecular weight molecules cannot penetrate the skin, thus they remain on the surface of the horny layer, where they act as a **gradual MPS-release system**.

The hydro-colloidal MPS-containing matrix establishes a moisture continuum with the stratum corneum. In this way, a MPS concentration gradient from the surface to the epidermis is generated, which drives the movement of pentasaccharides along the gradient:

Once in the dermis, MPS act on the fibroblasts' metabolism in order to restore the extracellular matrix homeostasis. Their action mainly concerns two processes (figure 9):

- Stimulation of dermal ECM synthesis: the ability of **HOMEOSTATINE** to stimulate the synthesis of collagen I and III has been demonstrated in cultured fibroblasts (Study 1: *in vitro* efficacy). This action is probably due to an interaction with membrane receptors, much as it has been observed for other oligosaccharides (Jouandeaud M, 2004). Additionally, this stimulation also promotes the production of other ECM components.
- Inhibition of Metalloproteinases production: MPS inhibit IL-1 mediated stimulation of fibroblasts; thus it impairs directly the production of MMP and indirectly by reducing the expression of IL-6. Through this mechanism, excessive ECM degradation is prevented, which helps prevent and reduce wrinkles. This action also inhibits the production of inflammation mediators (such as IL-6, TNF- α and IL-1). Inhibition of IL-1 by **HOMEOSTATINE** has been demonstrated for in Study 2, under *In Vitro* Efficacy Assays.

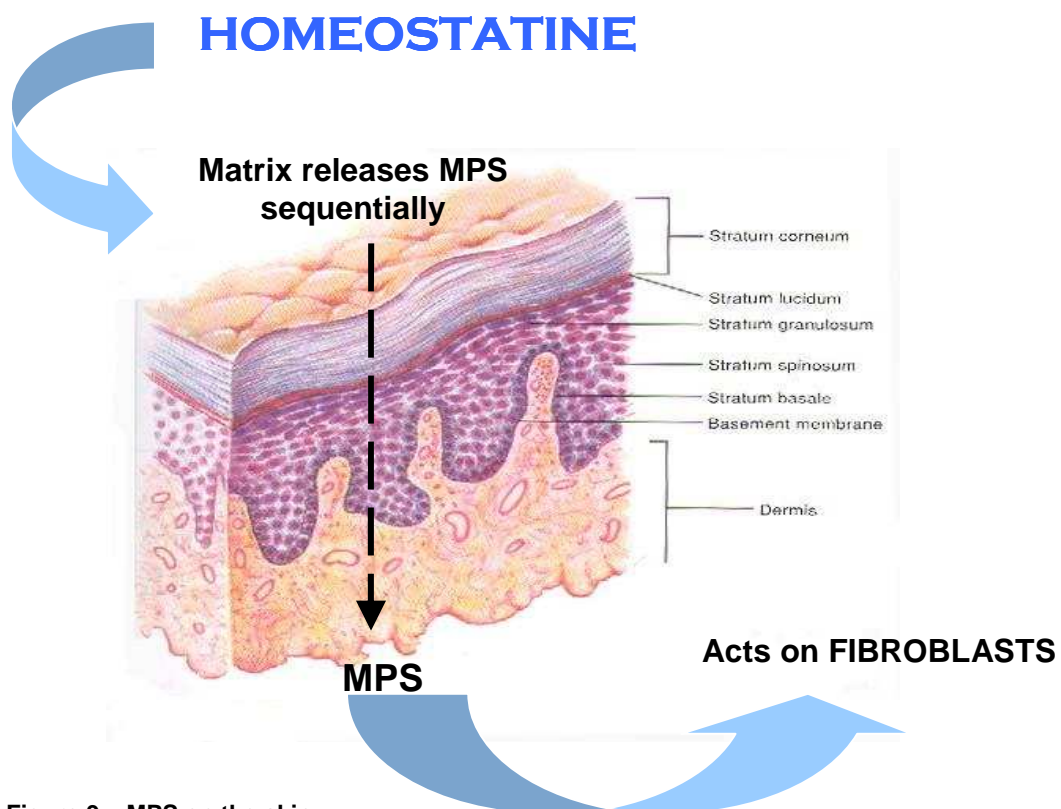


Figure 9 – MPS on the skin

IN-VITRO EFFICACY ASSAYS

- Effect on the synthesis of extracellular matrix proteins (collagen I and III)

This study was conducted on primary cultures of human dermal fibroblasts (HDF) using indirect ELISA (Enzyme Linked Immunoabsorbent Assay). Cells were incubated with the corresponding products (**HOMEOSTATINE** 0.1%, 0.2% and 0.4%, or TGF- β 20ng/ml as the positive control) for 72 hours. After termination of the treatments, cells were washed and incubated with the antibodies. The amount of collagen was measured with an ELISA reader at 492nm optical density. Results recorded for the synthesis of collagen I and III are shown in figures 10 and 11.

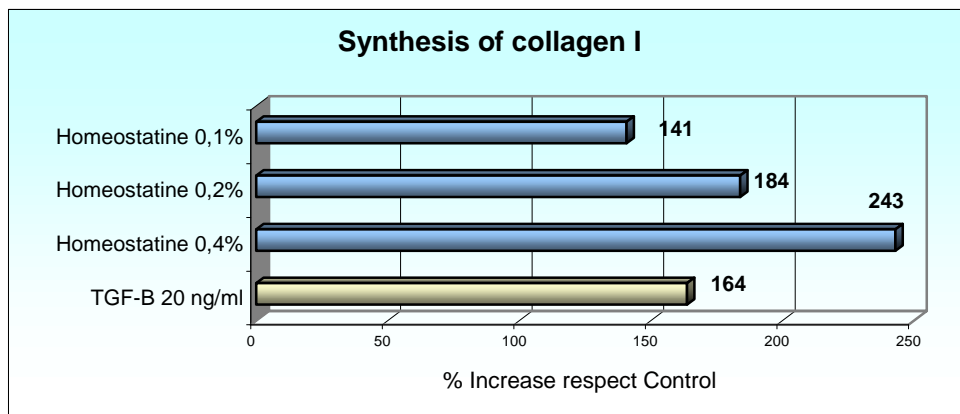


Figure 10. Synthesis of Collagen I

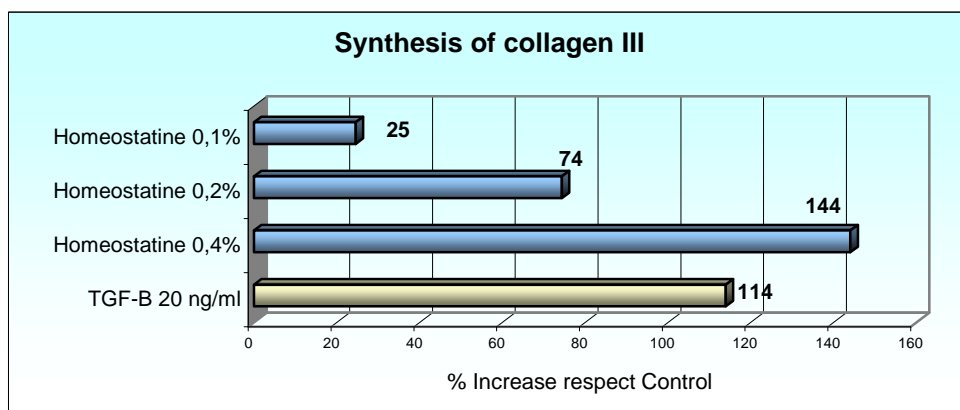


Figure 11. Synthesis of Collagen III

Results clearly showed that **HOMEOSTATINE** is a powerful activator of the synthesis of collagen I and III. The effects of this product were completely dose-dependant; the smallest tested concentration induced more than 100% collagen I synthesis; the effects on the collagen III synthesis were slightly weaker, although all the three tested concentrations activated it.

- Inhibition of IL-1 α stimulation of fibroblasts

This assay evaluated the inhibitory action of **HOMEOSTATINE** on IL-1 α stimulation of fibroblasts. We measured IL-6 and IL-8 release in a human IL-1 α activated fibroblasts culture.

HOMEOSTATINE 0.2% was directly dissolved in the culture medium. The free radicals scavenger and antioxidant agent pyrrolidine dithiocarbamate (PDTC) 50 μ M was used as the positive control.

Cells were treated with IL-1 α (1ng/ml) for 24 hours, with or without the tested products. After termination of the incubation period, the production of IL-6 and IL-8 was assessed by ELISA. Additionally, the amount of protein in each well was determined by the Pierce's BCA method.

Figure 12 and figure 13 show IL-1 α -induced production of IL-6 and IL-8 respectively.

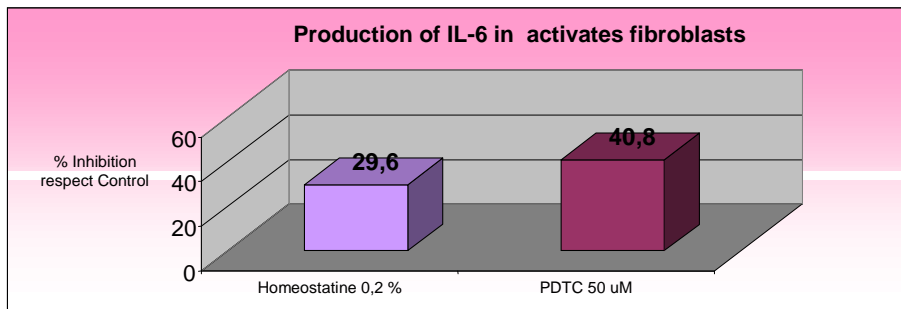


Figure 12. Production of IL-6 in activates fibroblasts

IL-1 α -induced activation of fibroblasts remarkably increased the production of IL-6. In this situation, **HOMEOSTATINE** acted as a powerful inhibitor of IL-6 production, reducing it by 30%.

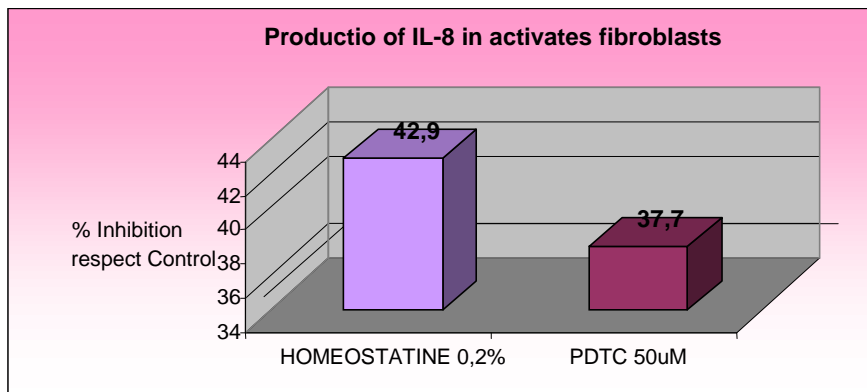


Figure 13. Production of IL-8 in activates fibrobalsts

IL-1 α -induced activation strikingly increased the production of IL-8. In this case, **HOMEOSTATINE** blockade of IL-1 α activation resulted in more than 40% inhibition of IL-8 synthesis.

HOMEOSTATINE appears to be a powerful inhibitor (35-40%) of IL-1 α -induced stimulation. This action reduces the production of metalloproteinases by fibroblasts, which would in turn reduce degradation of skin collagen and other extracellular matrix components. In this way, formation of wrinkles may be prevented. Furthermore, reducing the production of cytokines, impairs the amplification of inflammatory responses.

- Effect on the production of PGE₂

The cyclooxygenase enzyme (COX) is present in most of the tissues, where it catalyzes the formation of inflammation mediators such as prostaglandins (PGE₂) from arachidonic acid. COX inhibition reduces the synthesis of these mediators and consequently, impairs the generation of inflammatory processes. Fibroblasts are known to produce baseline levels of PGE₂ mainly through the action of constitutive cyclooxygenase (Cox-1).

This study was conducted on primary cultures of human dermal fibroblasts (HDF) by using an immunoassay technique. **HOMEOSTATINE** 0.1% was directly dissolved in the culture medium. Indomethacin 10 μ M was used as the positive control.

The treatments (**HOMEOSTATINE** and positive control) were applied for 18-24 hours. The production of PGE₂ was assessed using a commercially available enzyme immunoassay kit (Amershan Biosciences). Additionally, the amount of protein in each well was determined by the Pierce's BCA method. Results were expressed as PGE₂ pg/total protein mg. Percent inhibition was calculated by comparing with basal controls. Results are shown in figure 14.

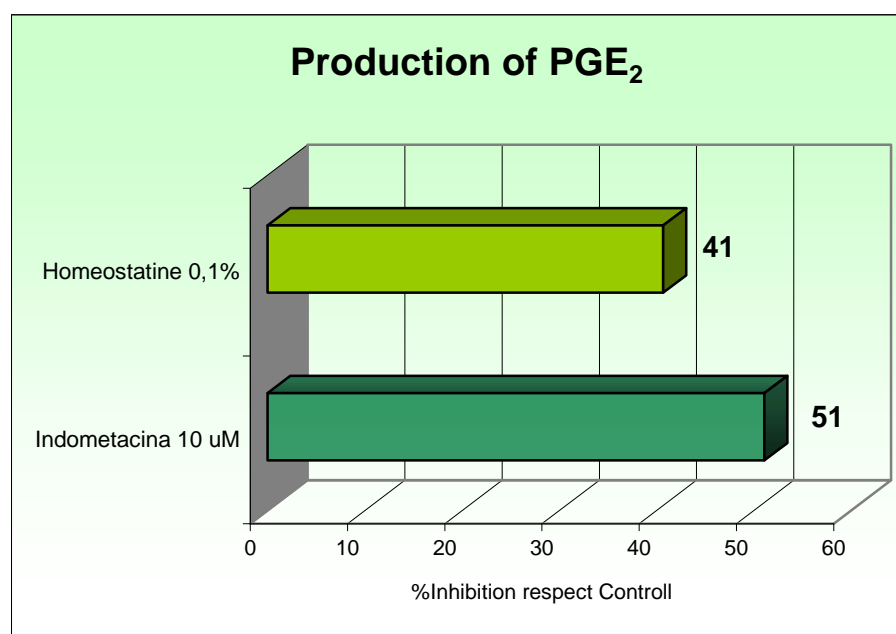


Figure 14. Production of PGE₂

As it can be observed, the cyclooxygenase inhibitor indomethacin reduced baseline production of PGE₂ by more than 50%. Under the present experimental conditions, **HOMEOSTATINE** also acted as an anti-inflammatory agent, since it inhibited baseline PGE₂ synthesis by 40%.

IN-VIVO EFFICACY ASSAYS

- Evaluation of the anti-wrinkle effect

The anti-wrinkle effects of **HOMEOSTATINE** were evaluated by FOITS (Fast Optical In vivo Topometry of human Skin). This technique allows for *in vivo* quantitative analysis of the skin surface topography by using an optical system (projection unit, measurement sensor, high resolution digital camera and software) designed to measure surface micro-relief (figure 15).

The data recorded by this optical system on a 12 cm² surface yielded a “main wrinkle” value (SPt) and an “average area rugosity” (SPa) value, which were entered into a software designed to reconstruct the image of the analyzed surface.

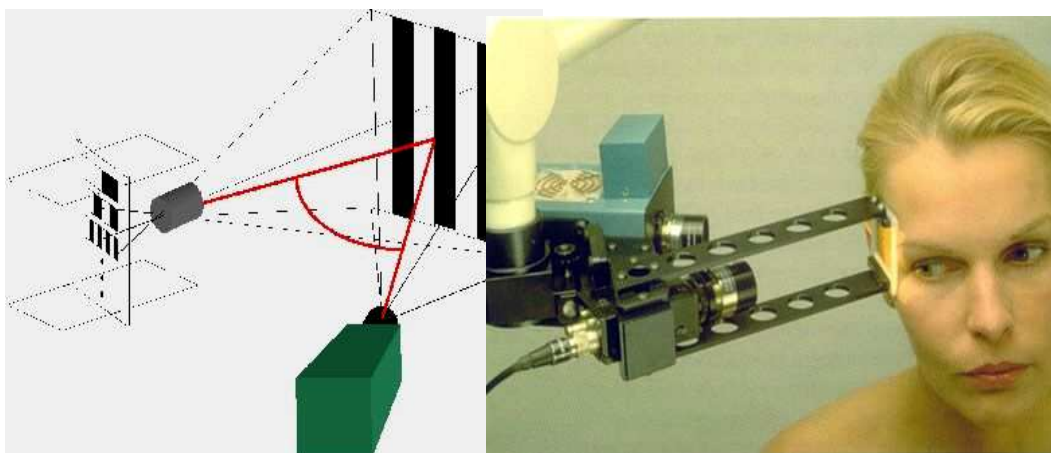


Figure 15. FOITS Test (Fast Optical In vivo Topometry of human Skin).

This assay was conducted on the crow's feet area of the face of 12 female volunteers, aged 44-58 years. The volunteers applied a **HOMEOSTATINE** 2%-containing cream on one side of the face and a placebo-cream on the other side, twice a day for 8 weeks. Measurements were made at the beginning (T0) and at the end (T56) of the treatment.

Results recorded for all the 12 volunteers, expressed as % variation in the main wrinkle (SPt) and average rugosity (SPa) values, are shown in figure 16. It can be clearly observed that **HOMEOSTATINE** remarkably reduced the amount and depth of facial wrinkles, even more than vitamin C. Reduction of SPt indicates that this product acts on deep wrinkles. Improvement of average rugosity (SPa) indicates an action on facial lines and wrinkles that makes the skin surface smoother. Placebo slightly reduced rugosity, most probably due to its moisturizing effects.

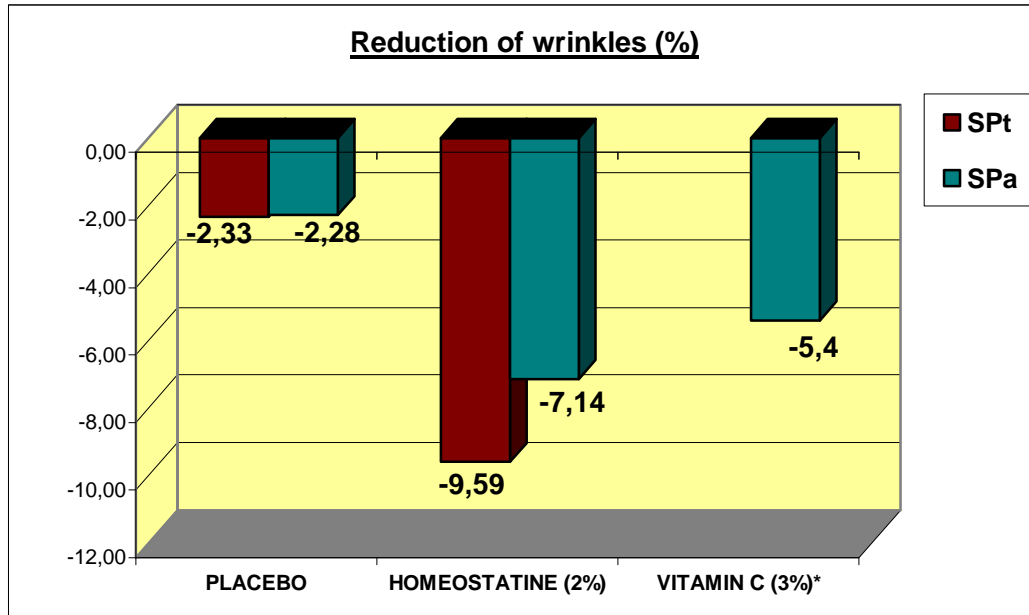


Figura 16. Reduction of wrinkles with HOMEOSTATINE, Vitamin C and Placebo.
 SPt = Main wrinkle - SPa= Average rugosity

By comparing the topographical reconstructions, it is possible to appreciate the improvements occurred in the volunteers' facial wrinkles. By way of an example, figure 17 shows the changes in facial wrinkles for one of the volunteers, where the brown color intensity indicates depth. Reduction in wrinkles size and depth can be observed for the **HOMEOSTATINE**-treated area, while no change can be noticed for the placebo-treated area.

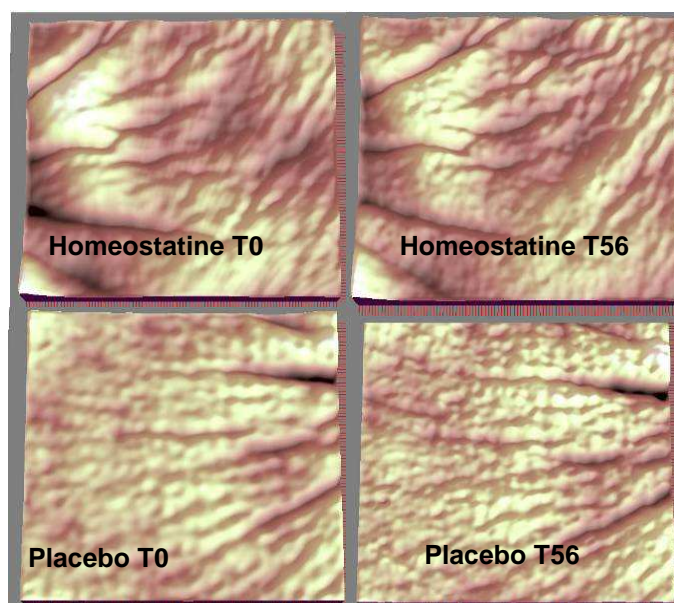


Figure 17. Topographical reconstructions of wrinkles evolution

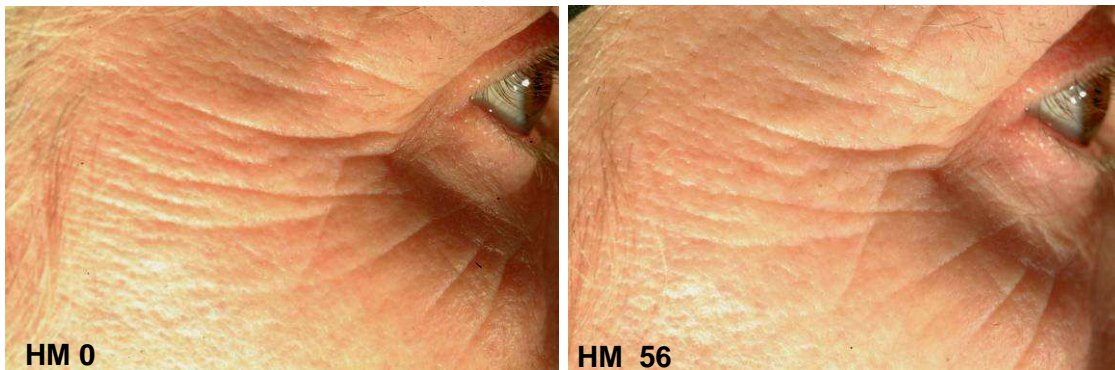


Figure18. Pictures of the HOMEOSTATINE-treated area

- Evaluation of skin thickness

One of the most typical effects of aging is the loss of dermal collagen and other ECM components, which produces skin-thinning and loss of some physical properties.

The present study was conducted on 13 female volunteers, aged 44-60 years. A **HOMEOSTATINE** 2%-containing cream or a placebo-cream were applied on the forearms of the volunteers for 56 days. Skin thickness was measured with 20MHz ultrasound imaging. Figure 19 shows the comparison of results recorded at the beginning and at the end of the study.

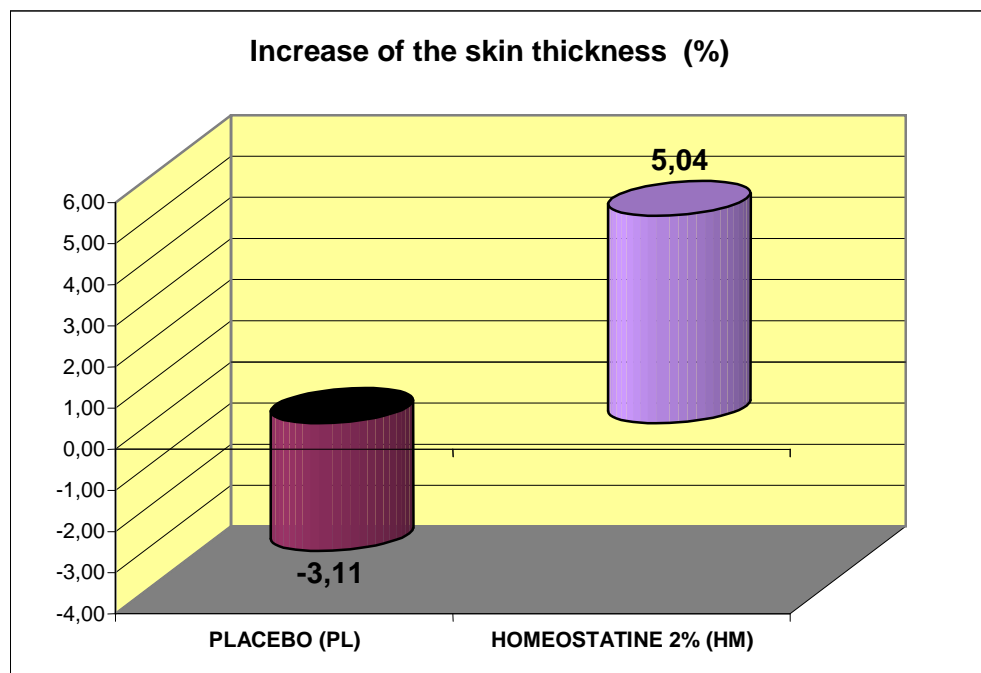


Figure 19. Increase of the skin thickness with HOEMOSTATINE and Placebo

These results showed that **HOMEOSTATINE** remarkably increased skin thickness. Figure 20 shows an example of such skin improvement in one of the volunteers.

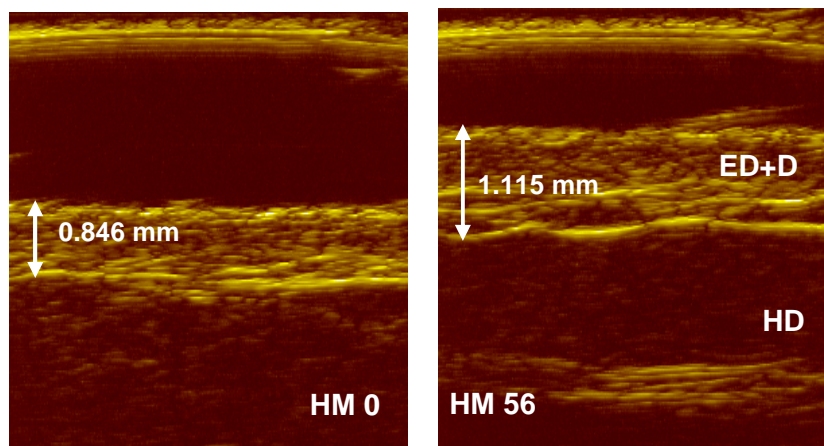


Figure 20. Improvement of skin thickness with **HOMEOSTATINE**
ED=Epidermis - D=Dermis - HD=Hipodermis

Skin thickness is mainly determined by the amount of collagen (Schmid D, 2002). Thus, it can be stated that the *in vivo* recorded 8.15% skin thickness increase corroborates the *in vitro* recorded results for the synthesis of collagen I and III in fibroblast cultures (study I, *in vitro*).

- Histological evaluation of skin

The present study was designed to evaluate the histological effects of **HOMEOSTATINE** on the extracellular matrix components and the skin structures.

Three volunteers applied a **HOMEOSTATINE** 2%-containing cosmetic product and a placebo-product on the abdomen for 56 days. Biopsy samples were taken from each volunteer: one sample before the treatment (T0) and two samples after the treatment: PL 56, from the placebo-treated area and HM 56, from the **HOMEOSTATINE**-treated area.

Biopsy tissue samples were stained for histological evaluation by a specialist in anatomical pathology.

- Visualization of the Dermo-Epidermal Junction (DEJ)

Epidermal undulations have been qualitatively assessed by hematoxylin-eosin staining.

As it can be observed in figure 21, **HOMEOSTATINE**-treated skin shows improved DEJ undulation and larger dermal papillae. This result may be due to an increase in collagen VII and IV, which are synthesized in keratinocytes.

Additionally, a slight increase in the size of papillary dermis can be observed, probably also due to a larger amount of collagen VII.

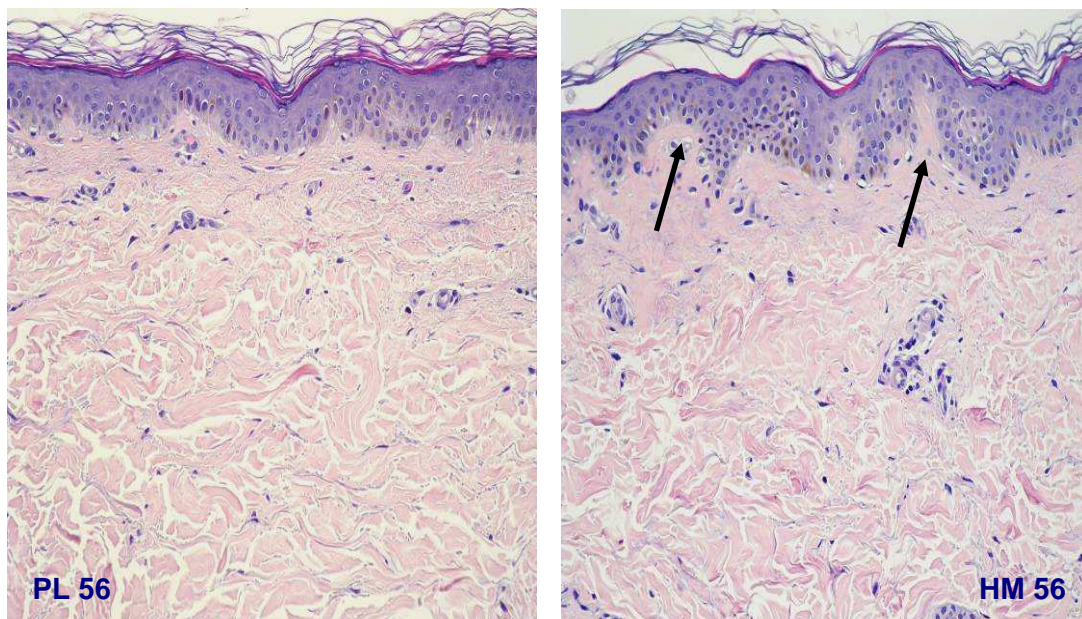


Figure 21. DEJ of the skin treated with HOMEOSTATINE and Placebo

Increased number and size of dermal papillae results in a larger epidermis-dermis contact surface, which promotes nutrient supply to the epidermis and a better communication between both skin layers. Such improvements help preventing and reducing wrinkles.

- Visualization of elastic fibers:

Orcein staining allowed for a more accurate qualitative evaluation of density, fragmentation and orientation of the elastic fibers.

The results of this staining technique (figure 22) clearly showed a larger amount of elastic fibers and a remarkable reduction of fiber fragmentation for **HOMEOSTATINE**-treated skin. Furthermore, fibers have recovered their correct spatial disposition and parallel arrange in the reticular dermis.

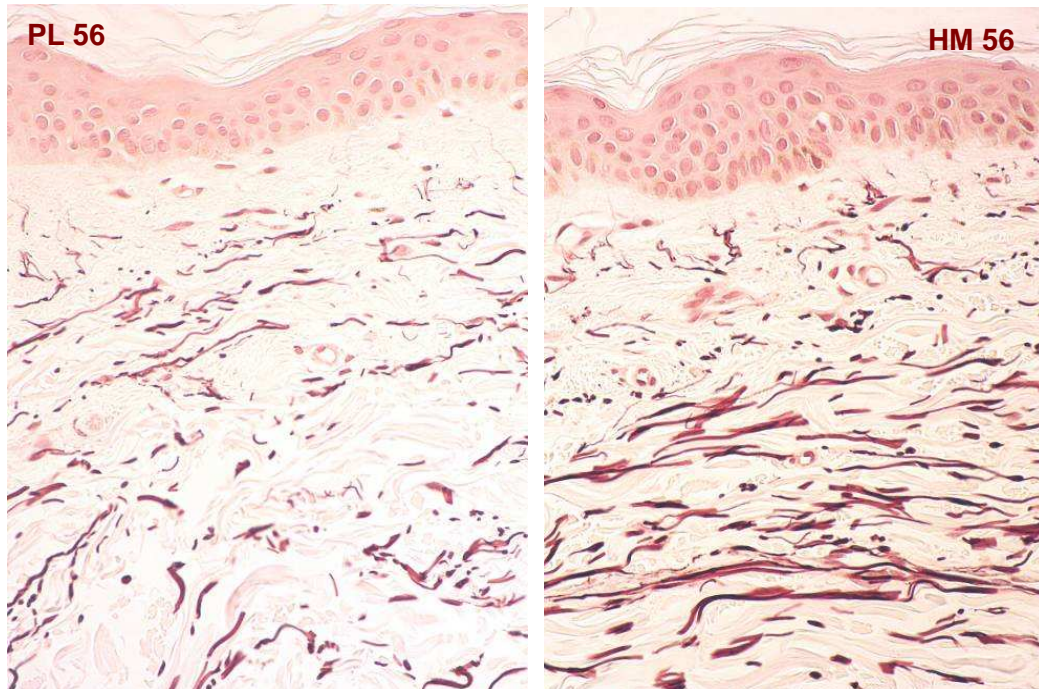


Figure 22. Elastic fibers (red) in the skin treated with HOMEOSTATINE and Placebo

Such improvement of the elastic fibers is essential for skin elasticity recovery and maintenance. This is a further key factor in maintaining a wrinkle-free skin.

- Visualization of GAG:

Staining preparations with colloidal iron stains glycosaminoglycans in blue. We quantified the presence of glycosaminoglycans in the dermis. Hyaluronic acid is known to be the most abundant one.

This staining technique revealed largest amounts of glycosaminoglycans for **HOMEOSTATINE**-treated skin (figure 23).

Especially important is the glycosaminoglycan increase observed in the reticular dermis, because it means greater water-retention capacity in a deep skin layer.

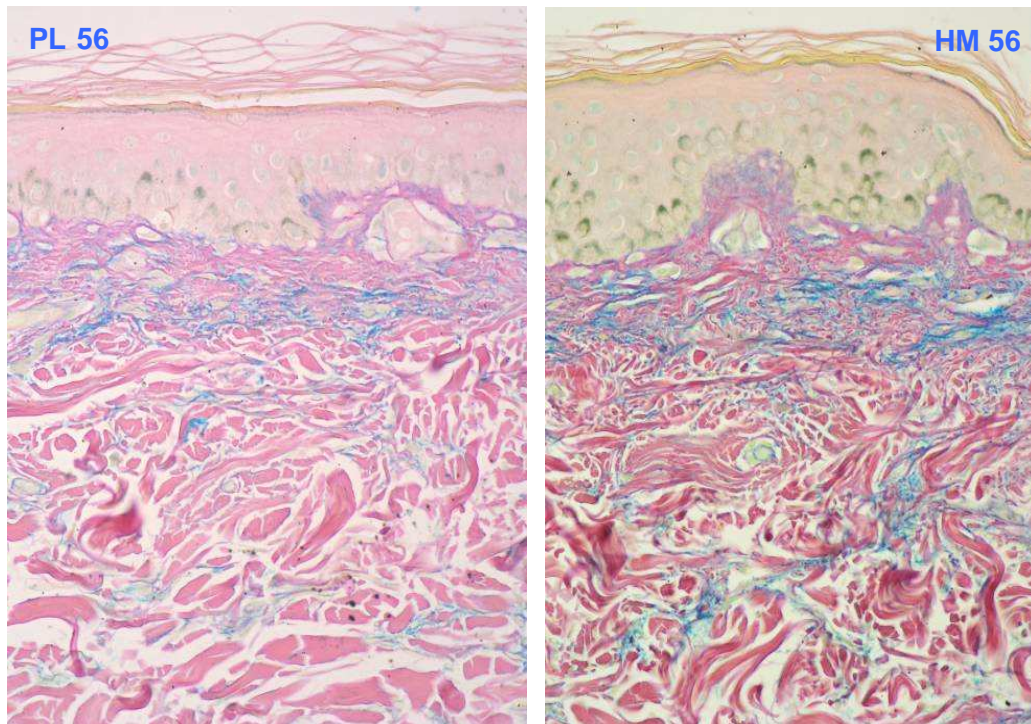


Figure 23. GAG (blue) in the skin treated with HOMEOSTATINE and Placebo

Better moisturized dermis is smoother, which contributes to a firmer, wrinkle-free skin.

COSMETIC PROPERTIES AND APPLICATIONS

HOMEOSTATINE is a natural cosmetic ingredient, which combines the activity of both of its components - the marine pentasaccharide (MPS) from *Enteromorpha compressa* and the galactomannan from *Caesalpinia spinosa* – to produce a remarkable anti-wrinkle effect.

The action of MPS is focused on maintaining or recovering homeostasis of the skin extracellular matrix. The main outcome is a reduction of wrinkles and a better moisturized, firmer and more elastic skin. Besides these properties, **HOMEOSTATINE** has anti-inflammatory effects.

Table 1 shows **HOMEOSTATINE** cosmetic properties based on the results of the above explained assays.

PROPERTY	EFFICACY	
	<i>IN VITRO</i>	<i>IN VIVO</i>
ANTI-WRINKLES	COLLAGEN I: + 243 % COLLAGEN III: + 144 % IL-1 α INHIBITION: 30-40 % PGE ₂ INHIBITION: 22-40 %	MAIN WRINKLE: -7.26 % AVERAGE RUGOSITY: - 4.87 % SKIN THICKNESS: + 8.15 % IMPROVEMENT DEJ IMPROVEMENT ELASTIC FIBERS IMPROVEMENT GAG
RESTORE ECM HOMEOSTASIS (FIRMING, ELASTIC AND MOISTURIZING)	COLLAGEN I: + 243 % COLLAGEN III: + 144 % IL-1 α INHIBITION: 30-40 %	SKIN THICKNESS: + 8.15 % IMPROVEMENT DEJ IMPROVEMENT ELASTIC FIBERS IMPROVEMENT GAG

Table 1. HOEMOSTATINE cosmetic properties

- *Cosmetic applications*

On the basis of the cosmetic properties described in table 1, **HOMEOSTATINE** may be used in a number of cosmetic applications.

- **Cosmetic products for the treatment of first wrinkles:** aimed at prolonging the healthy aspect of skin and preventing wrinkles.
- **Cosmetic products for aged skin:** besides reducing wrinkles, it helps fighting flaccidity, loss of elasticity and dryness.

RECOMMENDED CONCENTRATION

APPLICATION	CONCENTRATION
TREATMENT OF THE FIRST WRINKLES	2.0 – 3.0 %
TREATMENT OF AGED SKIN	2.0 – 5.0 %

Table 2. Concentrations of use

ANALYTICAL METHODS

- Determination of oligosaccharides

Determination of oligosaccharides was carried out by High Performance Liquid Chromatography (HPLC) using a Beckman System Gold chromatographer with refraction index detector (Beckman, Model 166); mobile phase was acetonitrile:water (60:40, v/v); stationary phase was a Spherisorb-5-NH₂ (250 x 4,5 mm Ø) column; flow rate was 1.0 mL/min; sample injection volume was 20µL.

- Thin Layer Chromatography (TLC)

Sample preparation: 4g of sample were dried and re-suspended in 3ml water; mobile phase was a butanol:ethanol:water mixture (50:50:30); stationary phase was a 20cm x 20cm silica gel plate; sample load, 10 µl. Detection: diphenylamine reagent (dissolve 2.4g diphenylamine + 2.4g aniline-HCl in 200mL methanol and add 20mL phosphoric acid 86%). Sprinkle the plate with 10mL reagent, heat at 105° for 5 min and watch under visible light.

- Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is a technique based on the capacity of some atoms' nuclei to absorb radio frequency waves, when under the effects of a magnetic field. The most often used ones are those with spin= ½ such as ¹H and ¹³C. A signal is then generated, which is detected by a receptor and analyzed by a computer.

On applying a magnetic field to a molecule, an electromagnetic momentum is induced, which opposes the applied field thus producing a mild “shielding” effect. The degree of shielding depends on the electron density in every region of the molecule. The shielding effect and the resonance frequency determine a signal shift. Such a shift is independent of the instrument used and has characteristic values for every functional group.

TOXICOLOGICAL PROFILE

The safety of **HOMEOSTATINE** as a cosmetic ingredient has been studied by a specialist in toxicological risk. Data from toxicological assays and the toxicological profile of the **HOMEOSTATINE** components have been evaluated.

A Patch-Test study conducted under dermatological control with 10 human volunteers revealed good skin tolerance for **HOMEOSTATINE**.

It was finally concluded that the use of **HOMEOSTATINE** as a cosmetic ingredient of formulations for human use under normal or reasonable conditions involves no

- irritative skin intolerance
- systemic toxicity
- skin sensitization

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