SUMMARY

Description Extract from *Leontopodium alpinum* obtained by elicited plant cell culture, fights against skin sagging, especially in the neck area, by protecting the mitochondrial network and the extracellular matrix from the effects of oxidative stress related to pollution and radiation.

INCI Name Glycerin (and) Leontopodium Alpinum Callus Culture Extract (and) Xanthan Gum

Proven cosmetic activity

In vitro

➢ ***Antioxidant effect of MAJESTEM™ (eq. 3%)***

- DPPH ..... **-73%** *(p<0.01)*
- Lipid peroxydation ..... **-60%** *(p<0.01)*
- Singlet oxygen ..... **-49%** *(p<0.01)*

➢ ***Anti-free radical effect of MAJESTEM™ (eq. 2%) and Leontopodic acid***

- ROS/DCFH ..... ⇨ **MAJESTEM™: -69%** *(p<0.01)*
  ⇨ Leontopodic acid (10 ppm): **-65%** *(p<0.01)*

➢ ***Revitalisation of the dermis in the presence of MAJESTEM™ (eq. 2%) after induced oxidative stress***

- Restoration of the mitochondrial network of fibroblasts, after UVB-induced oxidative stress ..... **+31%** *(p<0.05)*
- Reactivation of fibroblast contractile capacity, after H_2O_2 -induced oxidative stress ..... **+54%** *(p<0.01)*
- Decrease of MMP production by fibroblasts, after UVA-induced oxidative stress ..... ⇨ MMP-1:**-53%** *(p<0.01)*
  ⇨ MMP-7:**-56%** *(p<0.03)*
  ⇨ MMP-9:**-21%** *(p<0.04)*
- Restoration of TIMP synthesis by fibroblasts, after UVA-induced oxidative stress ..... ⇨ TIMP-1:**+170%** *(p<0.01)*
  ⇨ TIMP-2:**+141%** *(p<0.01)*
- Increase of thrombospondine-2 synthesis ..... **+57%** *(p<0.01)*
- Protection against Vitamin C oxidation, beneficial effect on collagens synthesis
  ⇨ Collagen I: (at 3h): **+58%** *(p<0.01); (*at 24h*):* **+111%** *(p<0.01)*
  ⇨ Collagen IV: (at 3h): **+45%** *(p<0,02); (*at 24h*):* **+72%** *(p<0.02)*

In vivo

Clinical studies for the evaluation of **MAJESTEM™**'s effect on the sagging neck skin, on the tear through and on the cutaneous relief of crow's feet. Volunteers applied a cream containing 2% **MAJESTEM™** on the face and the neck, and for some, a placebo (except the neck), for 6 weeks.

➢ **Study of the sagging neck skin and smoothing after 3 weeks (Method: image analysis)**

- Sagging surface (n=31) ..... **-10.6%** (*p<0.01 vs. T0*)

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➢ **Study of the tear through after 6 weeks (method: FOITS)**

- Circumference (n=21) ..... **-9%** (*p<0.01 vs. T0*)
- Volume (n=21)..... **-8.8%** (*p=0.082 vs. T0*)

➢ **Study of crow's feet wrinkle relief after 6 weeks (method: Silflo™ imprints)**

- Surface area occupied by deep wrinkles (n=34)..... ⇨ **-10.7%** (*p<0.05 vs. T0*)
  ⇨ UV panel*: **-16.8%** (*p<0.05 vs. T0*)
- Roughness (n=34) ..... ⇨ **-5.7%** (*p<0.05 vs. T0*)
  ⇨ UV panel*: **-9.1%** (*p<0.01 vs. T0*)
- Mean trough depth (n=34)..... ⇨ **-6.2%** (*p<0.05 vs. T0*)
  ⇨ UV panel*: **-10%** (*p<0.01 vs. T0*)

**: Panel of volunteers with photodamaged skin.*

➢ **Sensory assessment on 106 volunteers (Eurofins); favourable opinions after 3 weeks**

- Lasting skin hydration..... **74%** (*p<0.01*)
- Skin comfort ..... **83%** (*p<0.01*)
- Skin more plump ..... **58%** (*p<0.01*)
- Skin softness..... **86%** (*p<0.01*)
- Skin firmness and tonicity..... **67%** (*p<0.01*)
- Will use it again ..... **82%** (*p<0.01*)
- Overall satisfaction..... **79%** (*p<0.01*)

Recommended use:

➢ **General information:**

- Recommended pH : 3.00 - 6.00
- Incorporate **MAJESTEM™** at the end of the formulation at a temperature below 50°C.
  Solubility: water-soluble
- Compatibility with carbomer

Toxicology:

HETCAM
Neutral Red Releasing Method
Phototoxicity
SkinEthic test
HRIPT (100 volunteers)
Ames test
Micronucleus test on cultured human lymphocytes
Direct Peptide Reactivity Assay (DPRA)
Expert Toxicologist Certificate

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1. INTRODUCTION

The dictionary defines elegance as: "the aesthetic quality recognised in certain natural or man-made forms whose perfection arises from grace and simplicity". The concept of elegance dates back to Antiquity, when statues were sculpted in flattering poses that made traits shapely and displayed prominent muscles. At the time, the position of the head and the elegance of the neck were important beauty criteria.

A graceful woman (or man) needed to be especially watchful to maintain her carriage and posture. The proud way in which she held her head and her visible neck endowed her with grandeur. Audrey Hepburn and Grace Kelly were iconic actresses who perfectly embodied this majestic beauty. Even in the animal kingdom lions show their magnificence amongst the beasts by the way they carry their majestic head.

Unfortunately, the neck is one of the first areas affected by the signs of ageing, and especially by skin sagging (also known as ptosis). Although the facial skin also progressively droops with age, it is the skin of the neck, with its minimal structural support, that literally sags, creating an unsightly "chicken neck" appearance. Pulling the neck of the skin with the fingers demonstrates its flaccidity and lack of vitality - pulled skin keeps its shape instead of springing back and quickly reveals a person's real age. The connective structure of the skin breaks down with age and shows the more or less pronounced changes in the collagen fibres. The damaged dermis can no longer fight the force of gravity.

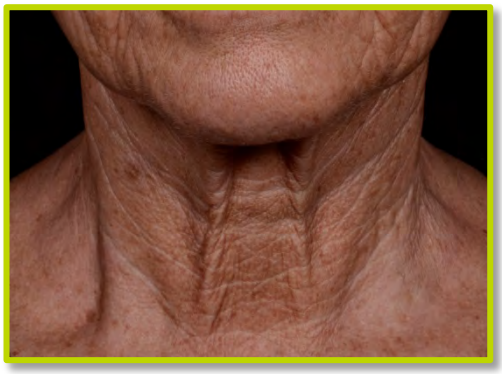


Figure 1: Signs of neck sagging and photoageing.

Neck flaccidity seems inevitable, affecting most of the general population. Only very few escape the phenomenon by going under the knife (neck lifting was the second most requested surgical intervention in the US in 2013) or because they are genetically lucky. At the same time, the facial skin becomes wrinkled and sags, especially around the eyes and on the cheeks. The shifting of the soft tissue of the cheeks leads to the appearance of the "tear troughs" and emphasises the nasolabial folds, making the face look tired and old. Many unsightly wrinkles of varying depth at the corner of the eyes intensify the appearance of ageing.

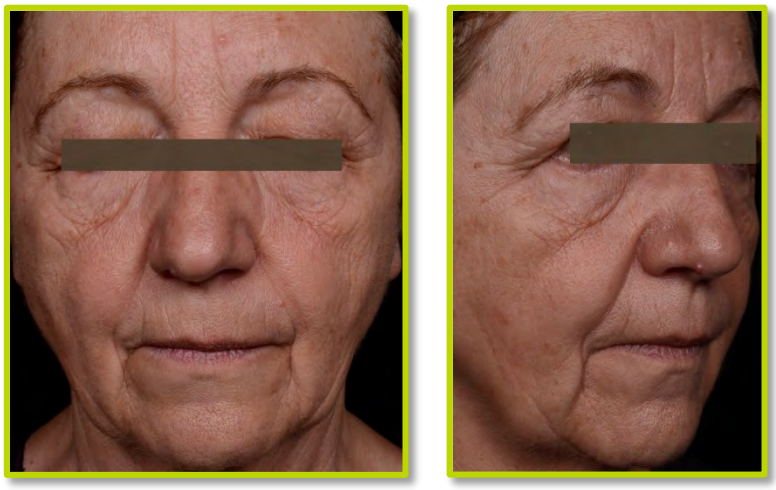


Figure 2: Tear trough changing the rounded shape of the cheek.

SKIN SAGGING: DESCRIPTION AND CAUSES

The decrease in skin dynamism and tension is exacerbated by many external factors that generate intense, repeated oxidative stress, such as urban pollution, diet quality, cigarette smoke, sun exposure and alcohol consumption etc. (HALLIWELL *et al.*, 1994).
Many authors have studied the causes of these phenomena and have demonstrated, to a certain extent, that it is not the cells that have aged, but rather, their surrounding environment. The decrease in the quality of the surrounding environment makes people believe that it is the cells that have aged, and that cellular homeostasis is altered. Therefore, not unlike elderly people who suffer due to a stagnant environment without stimulation, which is polluted and anxiety-provoking, these tissues age more quickly. In contrast, people who tend to look younger than they are often live in a stimulating environment in which they exercise and come into contact with younger people, even children. These people are dynamic, full of energy and adapt well.
We will now examine more closely the loss of skin tension caused by intense oxidative stress on various cellular structures.

Urban pollution and Reactive Oxygen Species (ROS)

The composition of the urban pollution mainly resulting from industrial and automotive discharge is now well understood. The primary pollutants are divided into two main groups: particles (such as $PM_{2,5}$ and PM_{10}) and gases (such as CO_2, CO, O_3, SO_2, NO, NO_2, NOx_2), including volatile organic compounds. The exact mechanisms behind ROS generation are not yet well-understood. However, it is suspected that fine particles penetrate the skin barrier through the highly lipophilic polycyclic aromatic hydrocarbons they contain. These molecules can bind to aromatic hydrocarbon receptors (AhR) found in keratinocytes and melanocytes (KRUTMANN *et al.*, 2014). It has been determined that $PM_{2,5}$ in urban pollution contains free radicals that remain in high concentrations of $1.3x10^{16}$ to $1.5x10^{17}$ radical/gram, which are the same concentrations found in cigarette smoke particles (GHELFI, 2011). Nitric oxide gas reacts quickly with oxygen to form the highly reactive peroxynitrite ion, which is known to attack DNA, proteins and lipids. Sulphur dioxide reacts with water to form sulphite and sulphate radical anions, which affect proteins. Ozone can react with certain unsaturated lipids to form free radicals through the breakdown of the ozonide anion. It also damages DNA (SINHA, 2013).
Free radicals stimulate the release of pro-inflammatory mediators by many skin cell types. The inflammatory reaction causes neutrophil and macrophage infiltration, which generates a surplus of free radicals, thereby establishing a vicious cycle. The oxidative stress can trigger reactions in all skin layers. These reactions cause genetic damage and activate the transcription factors and signals involved in cell differentiation or apoptosis (VALACCHI *et al.*, 2012).

ROS and mitochondrial dynamics

Over time, environmental oxidative stress leads to injury of the mitochondria, the cellular energy production units, and especially of the specific mitochondrial DNA. These lead to an increase in ROS, some of which diffuse throughout the cell (SCHROEDER *et al.*, 2008). Mitochondrial DNA codes for a small number of proteins that are involved in ATP production. Mitochondrial DNA mutations lead to a decrease in ATP production and oxygen consumption, the formation of proteases (such as MMP-1), which fragment collagen, and mitochondrial network imbalances. All of this contributes to premature ageing (BERNEBURG *et al.*, 2005).

Each cell of the body has energy-producing units that use environmental oxygen to manufacture energy for the cell; the number of these mitochondria vary with the energy needs of the tissue in question. Under normal physiological conditions, ROS serve as redox messengers that regulate intracellular signals. However, when in excessively high numbers, ROS cause irreversible cellular damage leading to mitochondrial apoptosis. Mitochondrial DNA is one of the prime targets of ROS. During the ageing process, an accumulation of mitochondrial DNA mutations, a malfunction of oxidative phosphorylation and a decrease in antioxidant enzyme expression lead to ROS overproduction. This imbalance creates a vicious cycle that is the basis for the mitochondrial ageing theory (WANG *et al.*, 2013). Hydrogen peroxide (H_2O_2), one of the ROS produced, also increases. It is highly oxidative and reacts with neighbouring mitochondrial components and constituents of the rest of the cell after diffusion.

Fibroblasts contain mitochondria that are associated in the form of 1 to 10 µm filaments. In contrast, in other cells and in particularly those with a high dividing potential, the mitochondria are in ovoid shape. However, both forms, i.e., isolated ovoids and networked filaments, coexist in each type of cell.

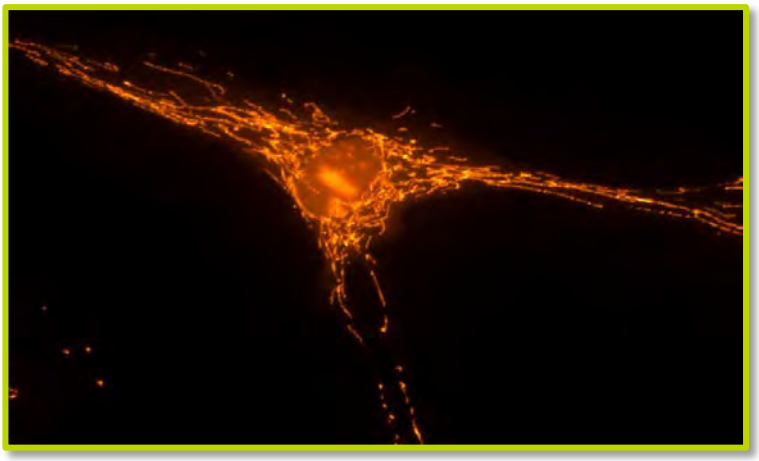


Figure 3: Mitochondrial network of a fibroblast tagged with MitoTracker® dye.

The preservation of the dynamism of passing from one state to the other is critical for maintaining mitochondrial function; an imbalance may lead to the appearance of ageing-related diseases (YOULE and VAN DER BLIEK, 2012). Although fission is essential for cellular division because it enables the pool of mitochondria to be distributed amongst daughter cells, fusion enables the genes and molecules needed to maintain cellular respiration to be grouped. The effect of fission is to isolate mitochondria damaged by ROS. These mitochondria will then be eliminated through mitophagy to prevent cellular accumulation. The loss of this mitochondrial process is a key cause of ageing because it increases the oxidative stress that leads first to an exacerbation of the mitochondrial injury, second to a reduction in fusion because it favours isolated, inactive forms and third to a 5 to 8% reduction in energy production per decade (GAZIEV *et al.*, 2014; FIGGE *et al.*, 2013, LANZA and NAIR, 2010; SAUNDERS *et al.*, 2013). It has also been observed that senescent cells are rich in the fusion form, which enables them to prolong their survival (MAI *et al.*, 2010).

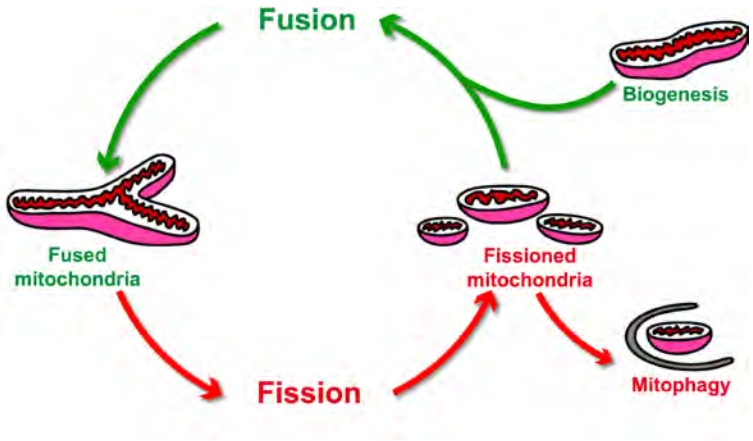


Figure 4: Mitochondrial cycle.

ATP, cytoskeleton and fibroblastic tension

Dermal fibroblasts are cells that are naturally under tension due to their attachment to the collagen fibres through adhesion molecules and thanks to their cytoskeleton. The cytoskeleton is made up of contractile bundles of actin-myosin "stress" fibres that make the tissue fibroblasts capable of contracting and exert pull on the extracellular matrix (EMC) that surrounds them or resist a mechanical force (SILVER *et al.*, 2003).

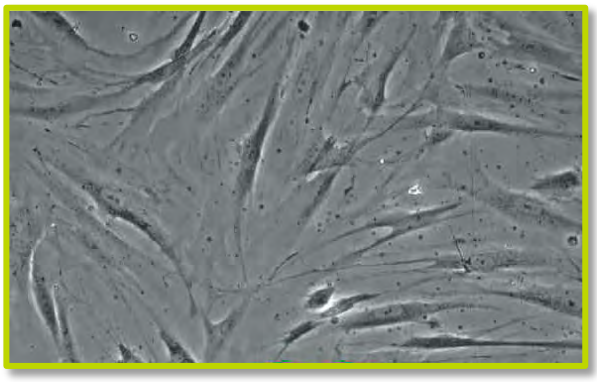


Figure 5: Fusiform fibroblasts

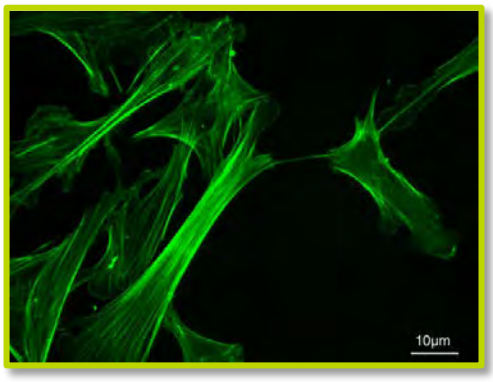


Figure 6: Cytoskeleton

Cytoskeletal contraction is a phenomenon that consumes tremendous energy in the form of ATP. When an oxidative stress leads to ATP depletion, the cytoskeleton undergoes reorganisation, becomes fragmented and forms aggregates or even disappears (HINSHAW *et al.*, 1988). The intracellular ATP level is critical for the dynamism of the cytoskeleton and its decrease inhibits actin polymerisation (SHELDEN *et al.*, 2002). The disappearance of actin fibres coincides with the internalisation of adhesion-type e-cadherin or integrin molecules, and then the dissolution of stress fibres is accompanied by a loss of adherence to the structure (BACALLAO *et al.*, 1994) since these fibres are associated with integrin-containing adhesion zones.

Concurrently, the actin can undergo direct oxidation by hydrogen peroxide. Cysteine and especially the methionine it contains are amino acids that are highly sensitive to oxidation (MILZANI *et al.*, 2000). Oxidative damage alters actin polymerisation kinetics, and subsequently, cytoskeletal integrity (DALLE-DONNE *et al.*, 2002).

The conformation of the actin cytoskeleton is dependent on the signals transmitted by the integrin molecules in response to environmental conditions, and in particular, to extracellular matrix fibres. In the presence of collagen fibres, the circular architecture of actin filaments is transformed to provide a unique structure of stress fibres associated with integrin molecules, which then form focal adhesion points

(WIESNER *et al.*, 2005). Fibroblast is interdependent with the extracellular matrix and under tension. Following oxidative stress, this entire architecture is disorganised.

Metalloproteinases and extracellular matrix

The dermis of an 80-year-old person contains four times more fragmented collagen than that of people 20 to 30 years of age (FISHER *et al.*, 2009). Studies have demonstrated that the key role of the loss of homeostasis in dermal fibroblasts is that with age, these fibroblasts produce more dermoproteases, the most active of which are matrix metalloproteinases (MMP) and, in parallel, less collagen-I. This imbalance in favour of MMPs explains the structural and functional decline of the skin, which provides less support and is less dynamic (VIERKÖTTER *et al.*, 2012).

MMPs are numerous (more than 20) and are produced in a latent form (pro-MMP) to avoid uncontrolled activity. Their main function is to break down matrix tissue to remodel it, release messengers trapped in this extracellular matrix or to reveal functional receptors at the surface of cells.

MMP-1, the predominant collagenase in fibroblasts, keratinocytes and macrophages (PIERCE *et al.*, 1996) can cleave type I collagen and fibronectin, two major structural proteins of the dermis. MMP-7 also cleaves fibronectin and decorin, while MMP-9 breaks down elastin and type IV collagen, a molecule that is crucial to the links between the epidermis and the dermis (NAGASE *et al.*, 2006). Moreover, it has been demonstrated that proteases can activate each other and lead to more extensive breakdown. Subsequently, MMP-1 is activated by kallikrein, certain elastases and even MMP-10. In return, the collagen cleaved by MMP-1 becomes a potential target of MMP-3 and MMP-9.

Their production increases with age, but acute or chronic oxidative stresses (e.g., microinflammation, ultraviolet and infrared rays, pollution, alcohol or cigarettes) amplify this phenomenon (NAGASE *et al.*, 2006). An excessive concentration of H_2O_2 due to age or UV-A and UV-B rays leads to an increase in MMP production through a protein complex called AP-1 which triggers the production of several MMPs (e.g., MMP-1, MMP-9). AP-1, which also increases with age, indirectly leads to the repression of the procollagen signal by TGF-β through the action of MMPs on its receptor and the destruction of the environment surrounding the cell (BRENNEISEN *et al.*, 1997; KARIN and SHAULIAN, 2001; HERNANDEZ GUERRERO *et al.*, 2006; FISHER *et al.*, 2009; SEO *et al.*, 2010).

This leads to a loss of dermal density and resilience, to dermal thinning and to diminished dermal structural strength (HORNEBECK, 2003; FISHER *et al.*, 2009). In summary, MMPs lead to premature and exacerbated skin ageing. While dermal fibres in young skin are elongated, intact and dense, those of older skin are fragmented and less dense (Figure 7 on the next page; FLIGIEL *et al.*, 2003). This lower fibre quality reduces dynamic dermal tension, and interactions between the ECM and fibroblasts are reduced by 80%. These phenomena are observed with mutant cells that over express MMP-1. The actin cytoskeleton of these cells is disorganised or reduced in size and scope (DUFORT *et al.*, 2011; FISHER *et al.*, 2009; XIA *et al.*, 2013). Due to a lack of contact with the immediate cellular environment, this retraction is accompanied by tripled intracellular ROS production compared with fibroblasts that are linked to non-fragmented collagen (FISHER *et al.*, 2009).

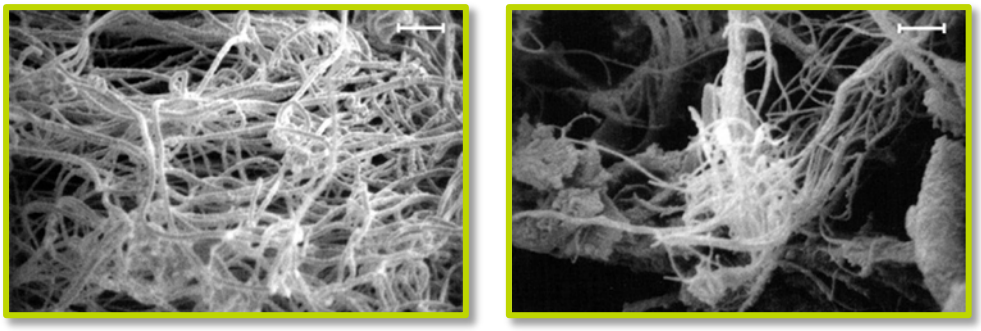


Figure 7: Appearance of young dermis (left) and aged dermis (right).

Three endogenous mechanisms protect the EMC, and therefore restore the dynamic characteristics of skin: thrombospondins, tissue inhibitors of metalloproteinases and anti-free radicals.

Thrombospondins (TSP) are matrix glycoproteins produced by the epidermis and the dermis. Their scientific interest is developing. When experimentally over expressed in mouse epidermis, TSP-1 effectively reduces the damage caused by pro-oxidising UVB radiation (YANO *et al.*, 2002). Interestingly, the authors observed a clear improvement in the organisation of collagen and elastin fibres of the underlying dermis as well as a decrease in skin wrinkle formation. This is reportedly related to the control of pro-angiogenic and protease-related factors. Its homologue, TSP-2, also potentially plays an important anti-ageing role. Mice that cannot produce this protein have a dermis with less organised, disordered collagen fibres, and their skin is more fragile when pulled. Their fibroblasts also produce artificial dermis layers that are less dense, have a lesser capacity to rebound and do not contract normally (KYRIAKIDES *et al.*, 1998; MACLAUCHLAN *et al.*, 2010). Moreover, the absence of TSP-2 is a pro-inflammatory factor (ADAMS and LAWLER, 2011).

Even if TSP levels in elderly mice are not yet conclusive, TSP-1 and TSP-2 seem to act on several key aspects of ageing, such as the inhibition of MMP activity, including MMP-9 activity (YANO *et al.*, 2002, MACLAUCHLAN *et al.*, 2010). The increase in TSP-1 production is also known to restore pro-collagen synthesis that has been diminished by UVB radiation. This restoration occurs through the activation of TGF-β, which is known for stimulating collagen synthesis (SOUCHELNITSKIY *et al.*, 2009; SEO *et al.*, 2010). In contrast, fibroblasts that cannot produce TSP-2 overproduce MMP-9. Therefore, it clearly appears that TSP expression, including by keratinocytes, represents an action that enhances dermal and skin quality and, in addition to TIMPs, limits ageing (YANO *et al.*, 2002).

MMPs are also naturally controlled in tissues by their TIMPs (**Tissue Inhibitor of MetalloProteinases**): this prevents a blind activity. It is the balance between TIMPs and MMPs that determines the level of MMP activity. TIMPs are small glycoproteins whose production is associated with a reduction in chronic MMP-related diseases and a decrease in UV-related damage. When associated with MMPs, TIMPs limit dermal matrix fragmentation. In turn, the dermis maintains its elasticity, which reduces the side effects of MMPs, namely pro-inflammatory cytokine production (YOKOSE *et al.*, 2012). In addition, TIMP-1 is considered an indicator of fibroblast and keratinocyte survival (HORNEBECK, 2003).

The decrease in TIMP-1 production is observed during UV radiation as well as during fibroblast senescence. This is evidenced by the increase in the activity of MMP, whose production increases in response to intracellular ROS accumulation. This demonstrates the primordial nature of a strategic rebalancing of MMP and TIMP in favour of the latter to prevent dermal fragmentation and fibroblast deregulation.

There are a certain number of endogenous enzymatic or non-enzymatic **anti-free radicals** (AR), such as glutathione, superoxide dismutase, catalase, alphalipoic acid and coenzyme Q10, etc. One of the most important of these antioxidants is **L-ascorbic acid**, or vitamin C, which acts in the intracellular or extracellular medium. At a physiological pH, the ascorbate ion predominates. Under the effect of ROS, ascorbate loses two electrons to form dehydroascorbic acid through the monodehydroascorbate radical, which has the characteristic of being relatively inert. It also enables neighbouring structures to avoid undergoing oxidative damage. Moreover, it works with tocopherol (vitamin E) as a co-antioxidant (BARRITA, 2013).

In addition to its antioxidant properties, vitamin C has many fundamental biological activities that preserve human health and help prevent ageing. It plays a role in tyrosine, tryptophan, folic acid and cholesterol metabolism, but one of its most important functions is collagen maintenance and formation (IQBAL *et al.*, 2004). L-ascorbic acid is an essential co-factor of lysyl and prolyl hydroxylase, the enzymes involved in the initial phases of procollagen chain synthesis. The L-ascorbic acid hydroxylises the lysyl and prolyl residues.

In this reaction, the role of vitamin C is to provide two hydrogen atoms to neutralise the O° radical formed by the use of an oxygen atom. Without integral, active vitamin C, the radical damages the tissues and vessels, which in extreme cases, leads to scurvy. This disease was well known by sailors in the past, but it is also exacerbated by metastases due to the breakdown of matrices and delayed healing in the elderly.

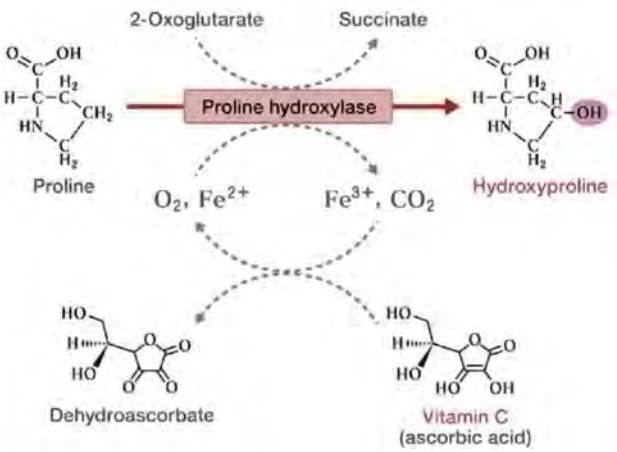


Figure 8: Role of vitamin C in collagen synthesis.

THE SEDERMA CONCEPT

SEDERMA offers the cosmetics industry an active ingredient that prevents the various signs of ageing associated with ptosis and the loss of skin tension, especially of the neck, cheeks and eyes.

MAJESTEM™

This is a natural cosmetic active ingredient that comes from edelweiss *(Leontopodium alpinum)* and is obtained using original, environmentally-friendly technology. The directed plant cell culture complies with current regulations. The activity of **MAJESTEM™** is based on its powerful antioxidant quality, which neutralises oxidative stress caused by environmental aggressors. Environmental aggressors attack various cell structures and harm the integrity of the extracellular matrix, and subsequently, of skin quality.

MAJESTEM™:

- reduces intracellular ROS overproduction, even under conditions of stress
- prevents mitochondrial network disorganisation and preserves the dynamic nature of this network
- reactivates fibroblast contractility
- stimulates TSP1 and TSP2 formation and revitalises the dermis
- reduces MMP formation
- increases the formation of TIMPs, which control MMPs
- preserves the collagen production system

PRESENTATION OF MAJESTEM™

MAJESTEM™ is obtained from the *alpinum* subspecies of *Leontopodium nivale,* which is generally known as Edelweiss, a plant with a name derived from the German edel (noble) and weiß (white). Edelweiss is also nicknamed “silver star” or “snow star”. This plant is found in a vast geographic area, but is limited in number in nature. It is even protected in certain countries. The initial leaf fragment was provided by a botanical garden, which helped avoid taking a plant from nature. It is this fragment that was cultivated and amplified using emerging, but robust HTN™ technology.

Leontopodium alpinum was first listed as *Gnaphalium* (Linnaeus’s plantarum species, 1753), and was then renamed with its current designation, which means lion's paw of the Alps. It grows above an elevation of 1500 metres in fairly hostile places (ravines and rocks that are cold and highly exposed to UV light). Subsequently, these plants are excellent at adapting to extreme conditions because they harbour a host of beneficial molecules and have protective hair on their flower and leaves. The plant is inedible, although it is non-toxic. However, brewing flowers in milk and adding honey is still used in Europe as a traditional remedy for stomach pains.

Figure 9: *Leontopodium alpinum*

DIRECTED PLANT CELL CULTURE: HTN™ TECHNOLOGY

For ages, human beings have been gathering plants from nature for nutritional and medicinal purposes. Humans then started cultivating the plant species that interested them. More recently, human beings mastered the culture of cells from small parts of plants, like leaf, root and stem fragments. This tool has opened the way to the production of biomass and molecules of interest (GRIZAUD *et al.*, 2012; MONDON *et al.*, 2014).

The advantages of this new technique are very important since this production, which is based on scientific methods, helps produce on-demand without dependency on unpredictable climates and seasons. Furthermore, this controlled method apparently respects the environment because it does not need pesticides, takes up little soil space, uses very little water and does not produce pollution. Rare, protected plant species are able to be cultured, and their benefits preserved, in this way.

For example, by exposing cultures to slight physical or nutritional variations, it is possible to modulate their metabolic processes and promote an increase in the synthesis of molecules of interest. *Leontopodium alpinum* has been cultured by using this directed technique. At the end of several trials, the final process reproducibly increases the quantity of a plant molecule with interesting cosmetic potential: leontopodic acid. **MAJESTEM**™ is rich of this molecule of interest (Figure 10).

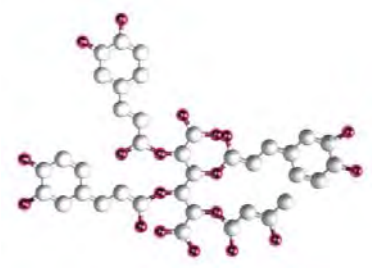


Figure 10: Leontopodic acid

PRELIMINARY TRIALS

Preliminary *in vitro* trials were conducted on various extracts of *Leontopodium alpinum.* The use of directed plant cell culture technology has generated the best production process, which led to **MAJESTEM**™.

We were able to detect a **powerful anti-free radical/antioxidant effect** on various oxidant compounds thanks to several spectrophotometric methods.

The DPPH radical is a molecule that is reduced in the presence of anti-free radicals. The loss of its blue colour can be followed. Concurrently, we used cell membrane models and followed the UVA-induced changes in their oxidation in the presence and absence of **MAJESTEM™**. Finally, the inhibition of the formation of singlet oxygen, one of the most powerful oxidants, was followed in the presence and absence of **MAJESTEM™**.

Table 1: Antioxidant effect of **MAJESTEM™** *(n=3)*, spectrophotometric measurements.

	DPPH	Variation (%)	Lipid peroxydation	Variation (%)	Singlet oxygen	Variation (%)
Control	0.560 ± 0.001	Reference	1.556 ± 0.025	Reference	37.67 ± 0.94	Reference
Eq. 0.7%	0.429 ± 0.007	**-23%**; *p<0.01*	0.612 ± 0.056	**-61%**; *p<0.01*	30.92 ± 0.78	**-18%**; *p<0.01*
Éq. 1.5%	0.308 ± 0.008	**-45%**; *p<0.01*	0.625 ± 0.023	**-60%**; *p<0.01*	25.12 ± 2.05	**-33%**; *p<0.01*
Éq. 3.0%	0.154 ± 0.004	**-73%**; *p<0.01*	0.617 ± 0.028	**-60%**; *p<0.01*	19.20 ± 1.04	**-49%**; *p<0.01*

We already discussed the fact that an excess of ROS in the cell leads to increased damage and directly impacts the future of cells and their ageing. **MAJESTEM™** was put in contact with human dermal fibroblasts (HDF) and the DCFH probe, which, once in the cell, fluoresces in contact with ROS. The fluorescence of the probe with **MAJESTEM™** was quantified and compared with the control. Concurrently, the effect of leontopodic acid was evaluated in the same way with a concentration corresponding to what was found in **MAJESTEM™** (10 ppm ⇔ 2%).

Table 2: Variation of intracellular ROS production in contact with **MAJESTEM™** *(n=3)*.

		ROS / DCFH (AFU*/10^4 cell.)	Variation (%)
Negative control		199166 ± 11993	Reference
MAJESTEM™:	Eq. 0.5%	54223 ± 3239	**-73%**; *p<0.01*
	Eq. 1.0%	62421 ± 2731	**-69%**; *p<0.01*
	Eq. 2.0%	61840 ± 4503	**-69%**; *p<0.01*
Negative control		182046 ± 11271	Reference
Leontopodic acid	1 ppm	85176 ± 3168	**-53%**; *p<0.01*
	5 ppm	52046 ± 2173	**-71%**; *p<0.01*
	10 ppm	63167 ± 2629	**-65%**; *p<0.01*

** AFU: Arbitrary Fluorescence Unit; no significant toxicity was observed versus the control.*

These results demonstrate that **MAJESTEM™** dramatically reduces intracellular ROS levels, an effect that can be directly related to the presence of leontopodic acid. In parallel, the cells were subjected to oxidative stress (H_2O_2) modelling the effect of ageing; this stress doubled numbers of intracellular ROS, while **MAJESTEM™** and leontopodic acid decreased these ROS by 70 à 80% (results not shown).
The results above encouraged us to think that **MAJESTEM™** could be of real interest in anti-ageing.

2. EFFICACY TESTING

2.1. *In vitro* studies

The trials below were conducted using experimental oxidative cellular ageing models (UVA, UVB or H2O2) to demonstrate, where applicable, the interest of **MAJESTEM™** in revitalising the dermis.

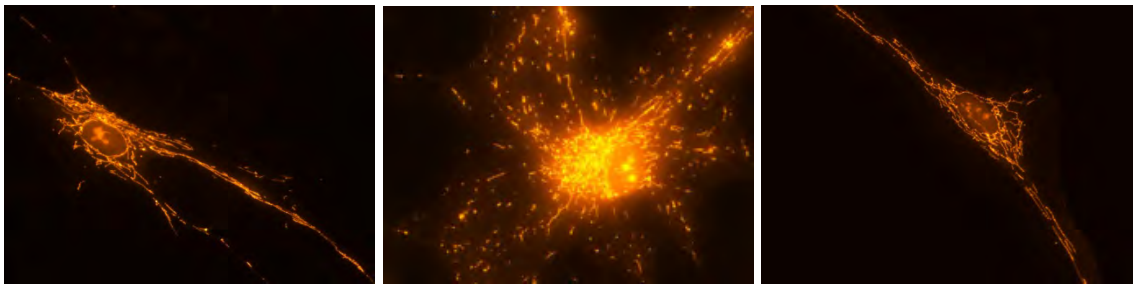
2.1.1. Cellular dynamism

a. Mitochondrial equilibrium

Principle

Young normal human fibroblasts under normal conditions have a typical spindle shape due to their role in maintaining tissue tension, their ability to bind to the matrix and their contractility. A radical attack generated by UVB leads to several functional and morphological modifications, like cellular retraction and swelling (MALORNI *et al.*, 1995). The preservation of the cell shape and the appearance of its mitochondrial network are therefore good indicators of cellular dynamism. HDFs in culture were stressed using UVB exposure to create an increase in intracellular ROS and a retracted phenotype. Just after irradiation, the cells were put back into their culture medium with **MAJESTEM**™ and fixed after 18 hours. The cells were then tagged using specific MitoTracker® dye and the network was quantified through photographs by first extracting network connections using a special IT tool.

Results



Control | UVB-radiated | UVB + 2% **MAJESTEM™**

Figure 11: Reparative effect of **MAJESTEM**™ on the mitochondrial network damaged by oxidative stress.

These photos demonstrate that exposure, even minor, of HDF to UVB causes them to retract and their mitochondrial network to become fragmented (fewer filament mitochondria, more small, round structures). Contact with **MAJESTEM**™, applied just after radiation, normalised the appearance of the cells, maintaining their non-stressed, spindle-like shape.

Table 3: Modulation of mitochondrial connections by HDF in the presence or absence of **MAJESTEM**™ *(n=28).*

	Mitochondrial connections (arbitrary units)	**Variation (%)**
Non-radiated	133 ± 45	Reference
UVB	104 ± 41	-22%; *p<0.02*
UVB + Eq. **2% MAJESTEM™**	136 ± 70	**+31%**; *p<0.05*

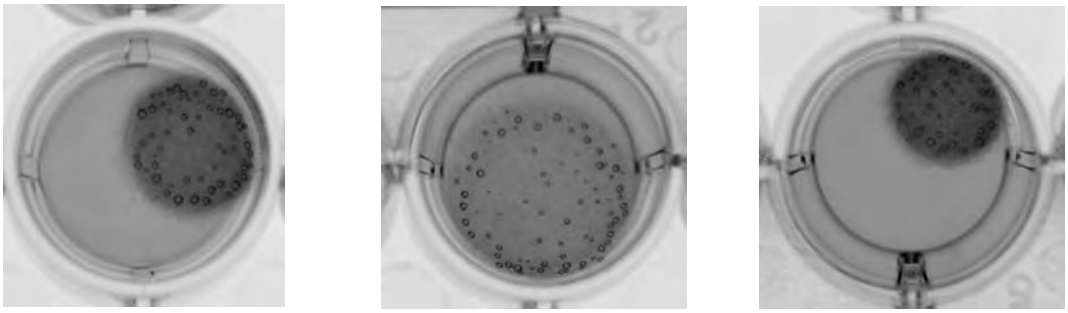
These results therefore demonstrate that the mitochondrial network is very disturbed several hours after UVB radiation, even if low-level radiation (22% fewer connections; *p<0.02*). Contact with **MAJESTEM™** applied after radiation maintains network and continuity in connections at the same levels as in non-radiated controls (+31%, *p<0.05 versus* UVB alone). **MAJESTEM™** therefore maintains cellular and mitochondrial dynamism.

b. Contraction of a modelled dermis

Principle

HDFs in culture were prematurely aged using H_2O_2. This was done to increase the intracellular concentration. The cells were then put back into their culture medium for 24 hours to potentiate the detrimental effects produced. Then, these cells were included in a dermal model (BELL *et al.*, 1979) and, after polymerisation, received **MAJESTEM™**. Dermal contraction was followed for four days, and photographic images were analysed to quantify differences.

Results



Control | H_2O_2-treated | H_2O_2 + 2% **MAJESTEM™**

Figure 12: Fibroblast contraction on the collagen lattice after oxidative stress (in the centre) and then in the presence of 2% **MAJESTEM™** (on the right).

Table 4: Modulation of the contraction of the dermal model by HDFs in the presence or absence of **MAJESTEM™** *(n=6)*.

	Contraction (arbitrary units)	**Variation (%)**	**Variation (%)**
Control without H_2O_2	5143 ± 1157	Reference	
H_2O_2	3459 ± 488	-33%; *p<0.01*	Reference
H_2O_2 + Eq. **1% MAJESTEM™**	4114 ± 346	-	+19%; *p<0.05*
H_2O_2 + Eq. **2% MAJESTEM™**	5319 ± 925	-	**+54%**; *p<0.01*

These results confirm that cellular contact with H_2O_2 creates intracellular oxidative stress resulting in decreased cellular contractility (-33%, *p<0.01*). Using **MAJESTEM™** provides a dose-dependent reduction in the detrimental effects of H_2O_2 on cellular dynamism (+54% contractility, *p<0.01*).

2.1.2. Reinforcement of the extracellular matrix

a. Reduction in stress-induced MMP synthesis

Principle

HDFs in culture were put in contact with **MAJESTEM™** for 24 hours, and then, after rinsing, the cell layers were exposed to UVA radiation to cause oxidative stress and increase the pool of MMP production by these cells (HERMANN *et al.*, 1993).

The cells were then put back into the culture medium in contact with **MAJESTEM™** for 24 hours. MMP production in the culture medium was followed using Multiplex / ELISA.

Results

Table 5: Variation of MMP production by oxidative stress in contact with **MAJESTEM™** *(n=4).*

	MMP-1 (pg/ml/10^6cell)	Variation (%)	MMP-7 (pg/ml/10^6cell)	Variation (%)	MMP-9 (pg/ml/10^6 cell)	Variation (%)
NR control	414 ± 34	Ref. 1	444 ± 50	Ref. 1	14.9 ± 0.6	Ref. 1
Control + UVA	1622 ± 47	+292%; **Ref. 2**	657 ± 196	+48%; **Ref. 2**	22.5 ± 3.1	+51%; **Ref 2**
UVA + Eq. 0.5%	942 ± 82	**-42%**; *p<0.01*	388 ± 136	**-41%**; *p<0.07*	18.8 ± 2.2	**-17%**; *nsd*
+ Eq. 1%	794 ± 55	**-51%**; *p<0.01*	266 ± 134	**-59%**; *p<0.02*	16.8 ± 1.9	**-25%**; *p=0.02*
+ Eq. 2%	762 ± 40	**-53%**; *p<0.01*	289 ± 159	**-56%**; *p<0.03*	17.8 ± 1.8	**-21%**; *p<0.04*

NR = non-radiated

These results demonstrate that oxidative stress applied to fibroblasts to model ageing did increase MMP-1 production (+292%; *p<0.01*), MMP-7 (+48%; *p=0.08*), MMP-9 (+51%; *p<0.01*) and MMP-10 (+873%; *p<0.01*, not shown).

Cellular contact with (eq. 2%) **MAJESTEM™** greatly reduced MMP-1 induction (-53%, *p<0.01*), MMP-7 (-56%, *p<0.03*), MMP-9 (-21%, *p<0.04*) and MMP-10 (-59%, *p<0.01*, not shown). Subsequently, **MAJESTEM™** limits the formation of the proteases that fragment the dermal extracellular matrix.

b. Increase in TIMP synthesis

Principle

In line with the previous trial, TIMP production was followed using Multiplex / ELISA methods in the culture medium.

Results

Table 6: Variation of oxidative stress-induced TIMP production in contact with **MAJESTEM™** *(n=4)*.

	TIMP-1 (ng/ml/10^6cell)	Variation (%)	TIMP-2 (ng/ml/10^6 cell)	Variation (%)
Non-radiated control	162.0 ± 3.8	Ref. 1	48.9 ± 1.1	Ref. 1
Control + UVA	34.5 ± 9.0	-79%; **Ref. 2**	10.3 ± 2.4	-79%; **Ref. 2**
UVA + Eq. **1% MAJESTEM™**	87.6 ± 17.6	**+154%**; *p<0.01*	22.1 ± 2.9	**+115%**; *p<0.01*
UVA + Eq. **2% MAJESTEM™**	93.0 ± 2.5	**+170%**; *p<0.01*	24.8 ± 0.9	**+141%**; *p<0.01*

	TIMP-3 (ng/ml/10^6 cell)	Variation (%)	TIMP-4 (pg/ml/10^6 cell)	Variation (%)
Non-radiated control	35.8 ± 2.6	Ref. 1	367 ± 17	Ref. 1
Control + UVA	16.2 ± 3.2	-55%; **Ref. 2**	294 ± 45	-20% ; **Ref. 2**
UVA + Eq. **0.5% MAJESTEM™**	26.1 ± 5.3	**+61%**; *p<0.02*	388 ± 35	**+32%**; *p<0.02*
UVA + Eq. **1% MAJESTEM™**	28.4 ± 3.7	**+75%**; *p<0.01*	402 ± 11	**+37%**; *p<0.01*

These results demonstrate that oxidative stress as a model for fibroblast ageing decreased TIMP-1 production, as expected (-79%, *p<0.01*), TIMP-2 (-79%, *p<0.01*), TIMP-3 (-55%, *p<0.01*) and TIMP-4 (-20%, *p<0.03*).
Cellular contact with (eq. 2%) **MAJESTEM**™ reactivated TIMP-1 induction (+170%, *p<0.01*), TIMP-2 (+141%, *p<0.01*) and with (eq. 1%) **MAJESTEM™**, TIMP-3 (+75%, *p<0.01*) and TIMP-4 (+37%, *p<0.01*). Subsequently, **MAJESTEM**™ is a strong inducer of MMP inhibitors.

c. Protection of the collagen production system

Principle

We saw that vitamin C is a co-factor of the enzyme prolyl-hydroxylase, and it recycles the enzyme and restores its collagen-organising activity.

Aqueous solutions of vitamin C left in ambient air quickly break down under the effect of oxygen. In our trial, we prepared culture media containing vitamin C so as to stimulate quality collagen production (on demand). These media were left for 3 or 24 hours to enable the vitamin C to oxidise before coming into contact with the cells. Concomitantly, identical media received **MAJESTEM**™ in addition to evaluate its abilities to protect vitamin C. These media were put into contact with HDF for three days, and then levels of collagen I and IV produced by the cells were determined by ELISA.

Results

Table 7: Variation of collagen I and IV production by HDF with vitamin C solutions of varying levels of degradation, effect of **MAJESTEM**™ *(n=3)*.

Collagen-I (ng/10^6)	3h	Variation (%)	24h	Variation (%)
Control	2389 ± 609	Ref. 1	1692 ± 433	Ref. 1
Control + Vitamin C	3154 ± 401	+32%; **Ref 2**	1564 ± 173	-7.5%; **Ref 2**
Vitamin C + Eq.**1% MAJESTEM™**	3883 ± 927	**+23%**; *nsd*	2689 ± 234	**+72%**; *p<0.01*
Vitamin C + Eq. **2% MAJESTEM™**	4970 ± 474	**+58%**; *p<0.01*	3306 ± 652	**+111%**; *p<0.01*

Collagen-IV (ng/10^6)	3h	Variation (%)	24h	Variation (%)
Control	7.2 ± 1.4	Ref. 1	5.7 ± 1.5	Ref. 1
Control + Vitamin C	12.2 ± 1.1	+68%; **Ref 2**	6.5 ± 1.0	+14%; **Ref 2**
Vitamin C + Eq.**1% MAJESTEM™**	13.7 ± 1.2	**+13%**; *nsd*	9.7 ± 0.8	**+50%**; *p<0.02*
Vitamin C + Eq. **2% MAJESTEM™**	17.6 ± 2.2	**+45%**; *p<0.02*	11.2 ± 1.6	**+72%**; *p<0.02*

Vitamin C promotes collagen I synthesis (+32%) *versus* non-supplemented cases. The presence of the equivalent of 1% and 2% **MAJESTEM**™ in this solution better protects vitamin C from oxidative stress at 3 hours and 24 hours, as demonstrated first by increases in synthesis at 3 hours (respectively +23%, *nsd* and +58%, *p<0.01*). Likewise at 24 hours, there is an observed increase in collagen I production (respectively +72%, *p<0.01* and +111%, *p<0.01*).

Vitamin C promotes collagen IV synthesis (+68%, *p<0.01*) compared with non-supplemented cases. The presence of the equivalent of 1% and 2% **MAJESTEM™** in this solution reveals an increase in collagen IV synthesis at 3 hours (respectively +13%, *nsd* and +45%, *p<0.02*). At 24 hours, there was an observed improved production of collagen IV (respectively +50%, *p<0.02* and +72%, *p<0.02*).

MAJESTEM™ therefore protects vitamin C from accelerated degradation by the oxygen in the aqueous media, thereby promoting more sustainable synthesis of collagen-I and collagen-IV by promoting the recycling of prolyl and lysyl hydroxylase.

d. Thrombospondin synthesis

Principle

As indicated earlier, TSP production by keratinocytes acts on skin quality by affecting the control of procollagen and protease factors (YANO *et al.*, 2002). Normal human keratinocytes (NHK) in culture were put in contact with **MAJESTEM™** or leontopodic acid for 48 hours. Leontopodic acid at 5 ppm, 10 ppm and 50 ppm corresponds to what is contained in 1%, 2% and 10% **MAJESTEM™**. TSP-1 and -2 synthesis were evaluated using ELISA in the cell culture media.

Results

Table 8: Modulation of TSP-2 production by NHK
in the presence or absence of **MAJESTEM™** or leontopodic acid (*n=3*).

		TSP-2 (ng/10^6 cell)	Variation (%)
Control		5.87 ± 0.28	Reference
Eq. **1% MAJESTEM™**		7.57 ± 1.37	**+29%**; *nsd*
Eq. **2% MAJESTEM™**		9.20 ± 0.32	**+57%**; *p<0.01*
Control		5.85 ± 0.34	Reference
Leontopodic acid:	5 ppm	8.49 ± 0.53	**+45%**; *p<0.01*
	10 ppm	9.77 ± 0.58	**+67%**; *p<0.01*
	50 ppm	11.13 ± 0.73	**+90%**; *p<0.01*

These results demonstrate that the equivalent of 2% **MAJESTEM™** induces significant TSP-2 production in NHK (+57%, *p<0.01*) and that this effect is closely related to the presence of leontopodic acid since we obtain a significant dose effect from 5 to 50 ppm (+67%, *p<0.01* for 10 ppm). Similar results were obtained with TSP-1 (not shown). We saw that this TSP increase strengthened the effect of TGF-β, limited MMP activity and ensured a sound collagen architecture, the results of which were an improvement in skin qualities.

2.2. *In vivo* studies (APRIL-MAY 2014)

The evaluation of **MAJESTEM**™ efficacy was conducted on the face:

1. A series of studies demonstrated the action of **MAJESTEM**™ on sagging neck skin, on tear troughs and on crow's feet depth (SEDERMA, France).
2. In addition, a study revealed the effects perceived by a large panel of consumers following the use of a treatment containing **MAJESTEM**™ (EUROFINS, France).

Principle

The first series of studies was conducted by including up to 34 women of a mean age of 59 years [41 - 71 years], presenting several visible signs of ageing on the neck and face. The effect of **MAJESTEM**™ was evaluated on three distinct sites on the face:

- On the **neck** with a measurement of the sagging surface of this area by standardised photographic image analysis, as well as by an expert evaluation of these same photographs.
- On the **tear troughs** with an innovative measurement of the surface area by fringe projection.
- On the **crow's feet wrinkles** with a measurement of the depth through negative imprint analysis.

For the neck, a panel of 31 people of a mean age of 60 years [45 - 71 years] was selected based on sagging and a lower surface quality of the skin in this area.
For the tear trough, 21 people aged over 55 years (mean age 64 years) [56 - 71 years] were included on the basis of the visible extent of this surface.
For this wrinkle study, the entire panel was included (n=34). From this panel, we also selected 19 women who indicated that they frequently exposed themselves to the sun. Therefore, these people theoretically had more damaged skin. For this series of 19 people, the mean age was 62 years [56 - 74 years].

Protocol

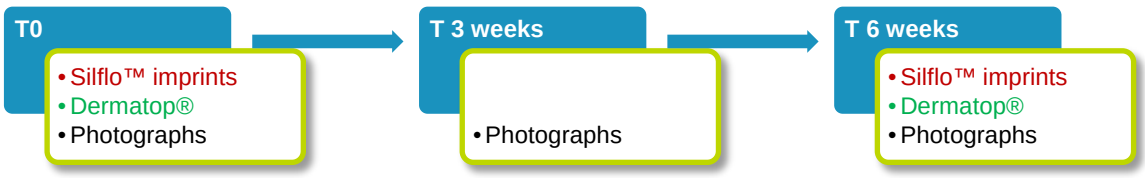
Specific inclusion criteria

Volunteers needed to have definite sagging neck skin and a clearly visible fold in the area of the so-called tear trough. Moreover, they needed to have clearly visible crow's feet wrinkles. The women had to have had hormonal consistency in the three months preceding the test as well as during the test. Sederma required a seven-day washout period during which the volunteer could only use a simple moisturising cream on the face. During the test, subjects were asked to only expose themselves moderately to the sun and only use the provided products.

Study types and duration

The study was a simple blind on the neck and the face. Each volunteer applied a 2% **MAJESTEM**™ cream and a placebo cream to the other side, except for the neck, where for practical reasons, volunteers only applied 2% **MAJESTEM**™ cream (see formulas in the Appendix). Both creams were massaged into the skin twice daily for 6 weeks.

The study synopsis is shown in the diagram below.



Statistical testing was performed using the Student's *t* test or, if needed, a Wilcoxon signed-rank test. The tests were conducted on paired series. For the self-evaluation, a Chi^2 test was used.

2.2.1. Study of the sagging neck skin and smoothing

The test of **MAJESTEM™**'s efficacy on the neck involved measuring the sagging part of the neck by analysing standardised photographic images and having an expert evaluation performed of these photographs.

Photos were taken at T0 and T3 weeks using a photographic apparatus (Orion Concept, France) equipped with a high definition digital camera, special lighting and a system for immobilising volunteers. The position of the face, the photographic parameters and the lighting were standardised and controlled so that they could be reproduced over time.

The sagging neck surface area was measured on a profile photo that underwent image analysis. Fixed anatomical landmarks (e.g., scars, marks) were used to precisely define the neck surface contours, including the sagging surface area. Then, a more realistic surface was used for calculations (30% of the initially measured surface area).

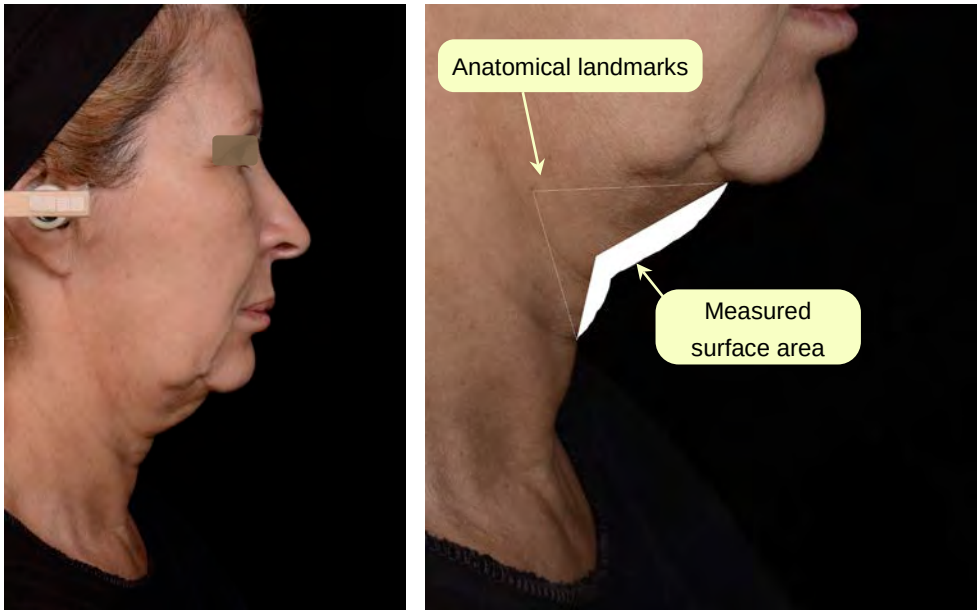


Figure 13: Measurement of the sagging neck skin surface area.

Results

Table 9: Variation of the sagging neck surface area after **MAJESTEM™** application *(n=31 volunteers).*

	Surface (mm^2)	
	T0	**T3 weeks**
Mean ± SD	101.2 ± 40	**90.5 ± 42**
Variation *vs*. T0 (%)	reference	**-10.6%**
Significance *vs.* T0		*p<0.01*
Maximum		-56%
Responders		77%

These results, and the photographs below, clearly demonstrate a decrease after three weeks of the sagging neck skin surface area; this decrease was approximately 11% (*p<0.01*) *versus* the surface area measured at T0. At the same time, there was a high response rate (77%).

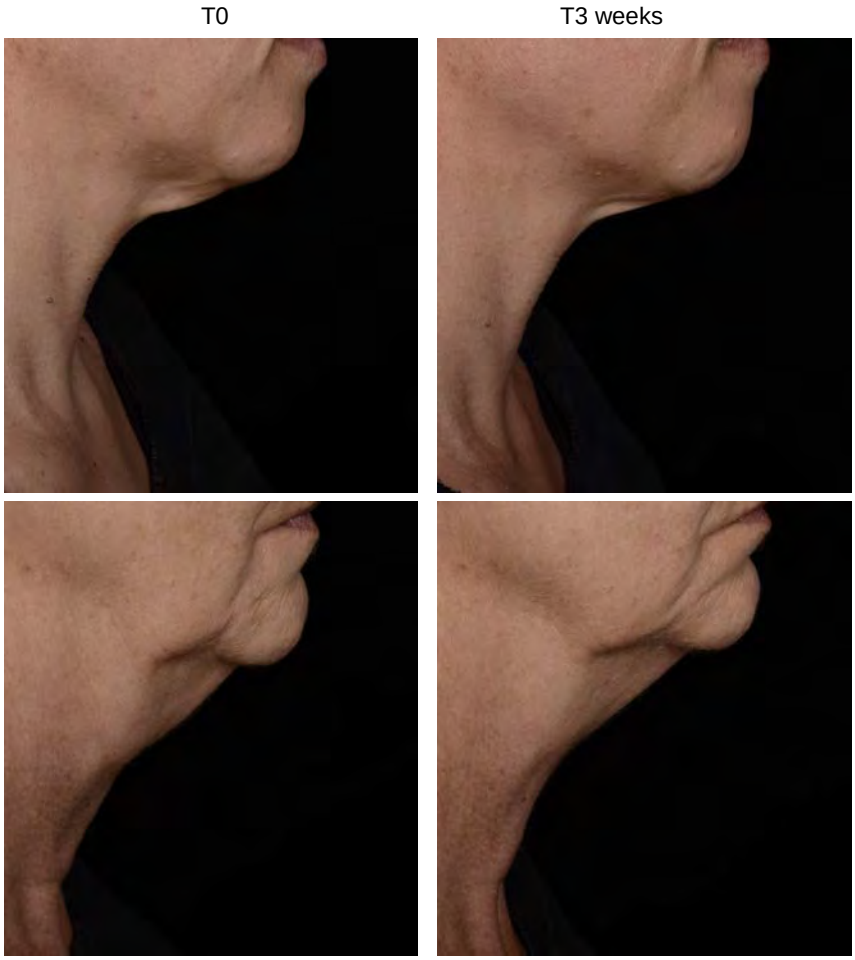


Figure 14: Visualisation of the effect of **MAJESTEM™** on the sagging neck skin on two volunteers.

The effect of smoothing on the neck skin was evaluated by a panel of seven expert judges who viewed the photos of 28 volunteers with neck folds. The experts found a smoothing effect in 56% of the cases after six weeks (Figure 15).

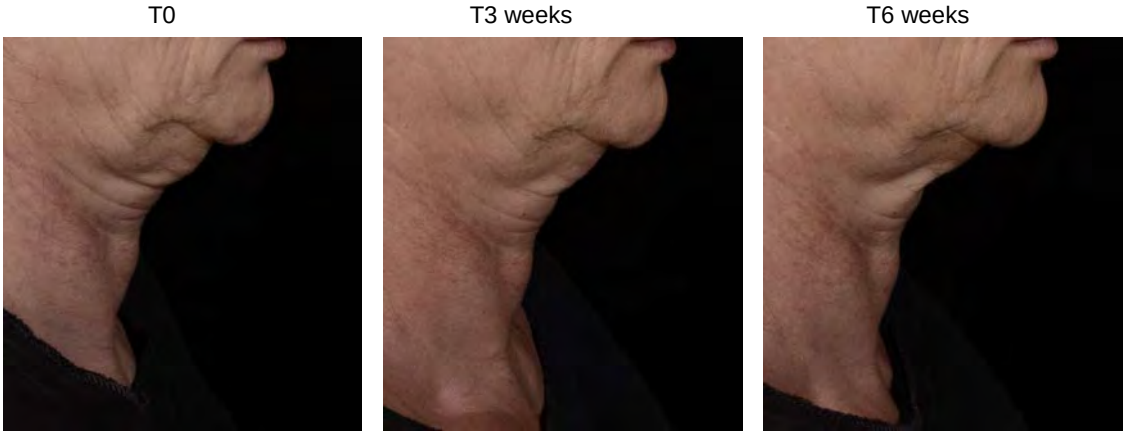


Figure 15: Visualisation of the effect of **MAJESTEM™** on neck smoothing after 3 and 6 weeks.

2.2.2. Study of the depth of the tear trough

The tear trough is the line through which tears move before rolling down the cheeks. This suborbital line continues from the lower eyelid to the crest of the cheek. It becomes significantly more marked and wider with age (CAMP *et al.*, 2011). The three factors that are generally considered to exacerbate tear troughs are:

- the decline in facial adipose tissue
- the force of gravity
- skin slackening with a loss in elasticity and a shifting of the soft tissues.

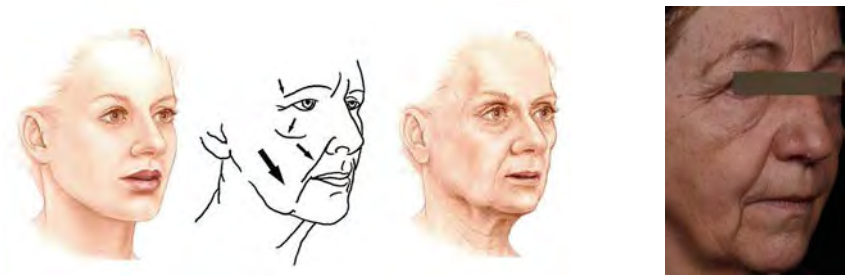


Figure 16: A loosening of the soft tissues and the appearance of the tear trough with age.

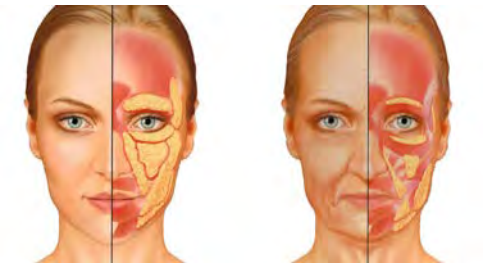


Figure 17: A decline in facial adipose tissue mass with age.

There are several, highly invasive solutions to attenuate this flaw, such as surgery, botulinum toxin injections and hyaluronic acid fillers (SHARAD, 2012).

To test the efficacy of **MAJESTEM™** on this site and adapt it to this zone, we used contactless FOITS (Fast Optical *In vivo* Topometry System). This system is based on optically analysing the fringe projection of the skin area being studied, which was the tear trough in this case. The device used (Dermatop, Eotech, France) is comprised of a projector and camera apparatus that form a specific angle, thereby providing triangulation. The study of the deformation of the fringes by the raised surface of the area enables a 3D image of the surface of the skin to be reconstructed (Figure 18).

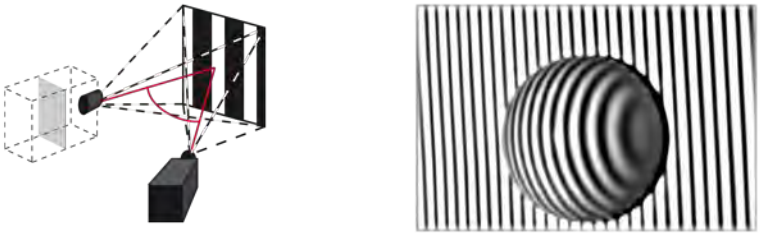


Figure 18: The principle behind the fringe projection technique. The projector and the camera are placed according to the triangulation angle (left). Deformation of the fringes projected onto the surface under consideration (right).

The objectification of **MAJESTEM™** was performed using Aeva software (Eotech,France) by analysing the data on the volume of the tear trough generated by measuring its circumference (Figure 19). At the same time, the volume parameter of this trough was obtained using the same technique.

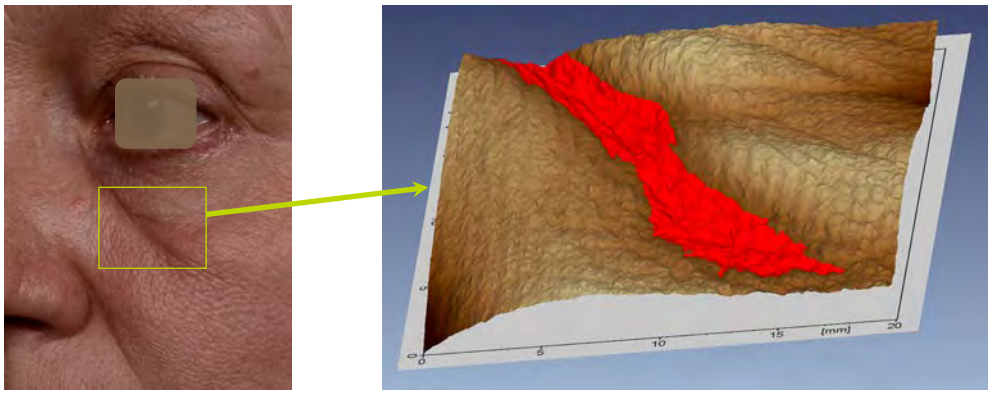


Figure 19: 3D extraction of the tear trough area.

Results

Table 10: Variation of the length and volume of the tear trough after applying **MAJESTEM™** *(n=21 volunteers).*

	Circumference (mm)				**Volume (mm^3)**			
	2% MAJESTEM™		Placebo		**2% MAJESTEM™**		Placebo	
	T0	**T6**	**T0**	**T6**	**T0**	**T6**	**T0**	**T6**
Mean ± SD	55.6 ± 15.1	50.6 ± 14.0	51.6 ± 13.0	51.6 ± 14.1	6.8 ± 5.2	6.2 ± 4.9	7.4 ± 6.9	7.8 ± 6.7
Variation *vs.* T0 (%)		**-9%**		0.0%		**-8.8%**		+5.4%
Significance *vs.* T0		***p<0.01***		*dns*		***p=0.082***		*nsd*
Maximum		-33%				-84%		
Responders		86%				62%		
Significance *vs.* placebo		***p<0.01***				***p<0.01***		

nsd: non-significant difference.

The photographs below illustrate the effect of **MAJESTEM™** between T0 and T6 weeks.

T0 T6 weeks

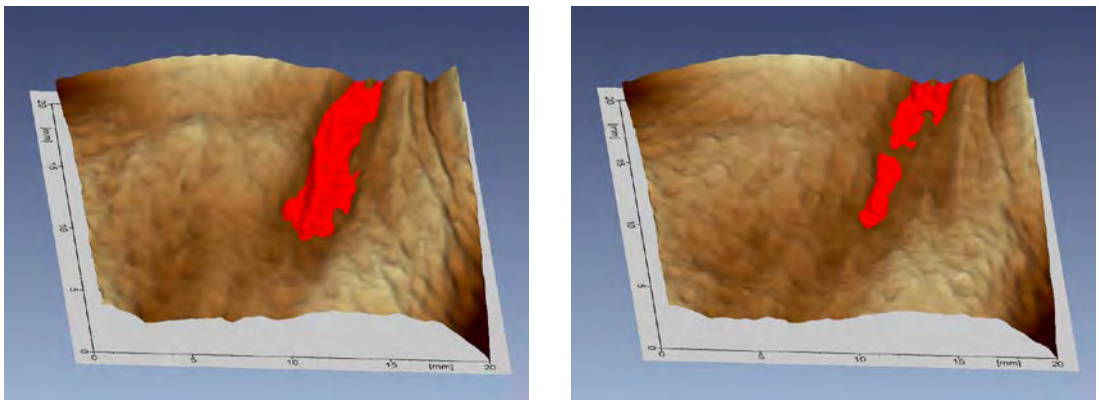


Figure 20: 3D acquisition of the tear trough area before and after 6 weeks.

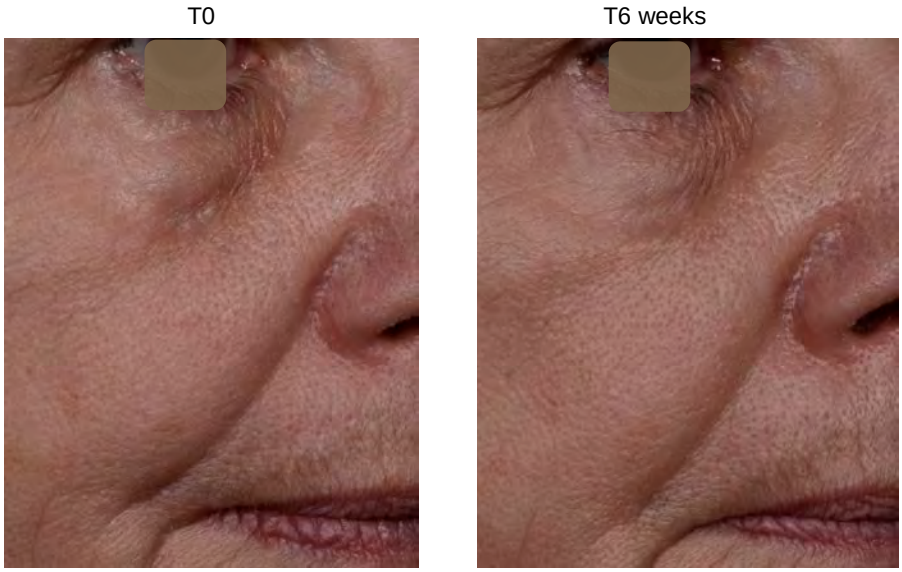


Figure 21: Visible improvement in the tear trough and the nasolabial fold after 6 weeks.

The results demonstrate that the application of **MAJESTEM**™ leads to a 9% reduction in the volume of the tear trough (measured in circumference), and that this reduction is significant *versus* placebo (*p<0.01*). On the other side, where the placebo was applied, there was no significant change. Furthermore, the volume of the tear trough for these people, who were over the age of 55, decreased by 8.8%, which was a significant reduction (*p<0.01*) *versus* placebo.

2.2.3. Study on crow's feet wrinkles

For each time, a negative imprint was performed of the skin using a Silflo™ silicone polymer on the crow's feet on both sides of the face (a placebo side and a 2% **MAJESTEM**™ side. These imprints were then analysed by the Visioline® VL650 (Courage-Khasaka) station, which uses the shadow technique. Briefly, the wrinkle imprint is lit by a low-angle LED light (35°) that generates shadows behind raised surfaces. The size of the shadow is proportional to the height of the wrinkle. MountainsMap® (Digital Surf, France) software was used to quantify the raised surfaces.

Silflo™ imprinting | Acquisition | Analysis software | 3D representation and parameters

Figure 22: Technique for obtaining 3D representations of raised skin surface.

Three parameters representative of the different characteristics of raised surfaces were analysed: The percentage of the surface occupied by deep wrinkles (>150 µm), overall roughness (Ra) and mean wrinkle depth.

Results

Table 11: Variation in the relief of wrinkles after **MAJESTEM™** application *(n=34 volunteers).*

	Surface area occupied by deep wrinkles >150 µm (%)				Roughness (in µm)				Mean wrinkle depth (in µm)			
	2% MAJESTEM™		Placebo		2% MAJESTEM™		Placebo		2% MAJESTEM™		Placebo	
	T0	T6	T0	T6	T0	T6	T0	T6	T0	T6	T0	T6
Mean	3.28	2.93	3.44	3.82	48.7	45.9	50.0	50.8	98.5	92.4	101.7	101.6
SD	2.04	1.85	2.17	2.22	12.2	11.5	13.1	12.9	23.8	22.4	25.6	24.6
Variation *vs.*T0 (%)		**-10.7%**		11%		**-5.7%**		1.6%		**-6.2%**		-0.1%
Significance *vs.* T0 Maximum Responders		***p<0.05*** -75% 50%		*nsd*		***p<0.05*** -39% 56%		*nsd*		***p<0.05*** -40% 65%		*nsd*
Significance *vs.* placebo		***p<0.01***				***p<0.05***				***p<0.05***		

These results demonstrate that **MAJESTEM™** generates a decrease in the surface area occupied by deep wrinkles, overall roughness and mean wrinkle depth of 10.7%, 5.7% and 6.2% respectively. At the same time, an applied placebo did not significantly change these parameters. For the three relief parameters, the effect of **MAJESTEM™** was significantly positive compared with that of the placebo (*p<0.01* or *p<0.05*, depending on the case).

Influence of sun exposure (photoageing)

From the preceding panel, we selected a "UV" coded panel using a questionnaire on sun exposure history and habits; theoretically, these people had more damaged skin and more signs of photoageing caused by intense oxidative stress, which has a similar effect to that of pollution (HALLIWELL *et al.*, 1994, VALACCHI *et al.*, 2012). The specific results of the "UV" panel are detailed below.

Table 12: Variation of wrinkle depth following **MAJESTEM™** application.
(UV Panel, *n=19 volunteers*).

	Surface area occupied by deep wrinkles >150 µm (%)				Roughness (in µm)				Mean wrinkle depth (in µm)			
	2% MAJESTEM™		Placebo		2% MAJESTEM™		Placebo		2% MAJESTEM™		Placebo	
	T0	T6	T0	T6	T0	T6	T0	T6	T0	T6	T0	T6
Mean	3.63	3.02	3.39	3.78	51.4	46.7	50.1	51.0	104.2	93.8	102.1	102.2
SD	2.24	2.11	2.01	2.20	12.7	12.5	12.2	12.6	25.0	24.7	23.9	24.8
Variation *vs.*T0 (%)		**-16.8%**		11.5%		**-9.1%**		1.8%		**-10%**		0.1%
Significance *vs.* T0		***p<0.05***		*nsd*		***p<0.01***		*nsd*		***p<0.01***		*nsd*
Significance *vs.* placebo		***p<0.01***				***p<0.01***				***p<0.01***		

The application of **MAJESTEM™** seems to be more intense in this very targeted "UV" panel. It provides stronger, more significant effects on the wrinkles of these volunteers (between +3% and +6% more on average). It is interesting to observe that the placebo effect is strictly identical in both panel types. These observations reinforce the interest in **MAJESTEM™** for repairing skin damage caused by intense oxidative stress. An illustration of the effects is presented on the next page.

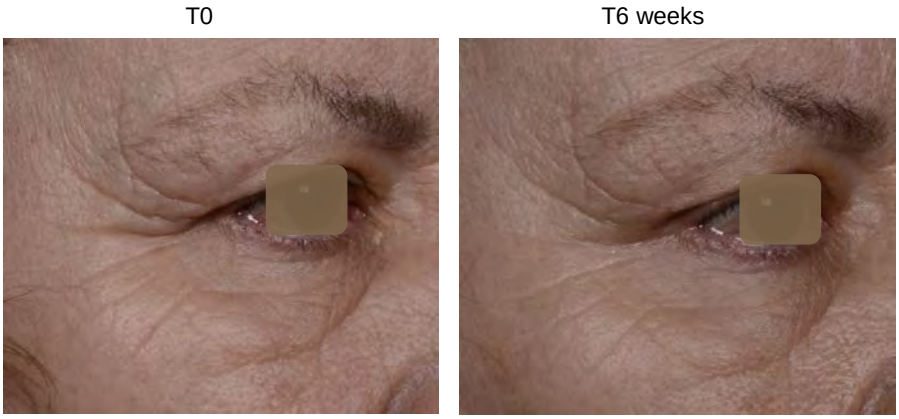


Figure 23: Effect of **MAJESTEM™** on the crow's feet wrinkles after 6 weeks.

2.3. Perceived effect of a panel of 106 volunteers (EUROFINS, France)

Principle and protocol

The evaluation of **MAJESTEM™**'s efficacy was conducted on a panel of 106 women of a mean age of 54 years [40 - 70 years], who stated having deep or shallow facial into the skin and neck skin wrinkles. The 2% **MAJESTEM™** cream (see the formula in the appendix) was massaged twice daily for 3 weeks. The evaluation of the cosmetic qualities and the action of the product after 3 weeks of use was performed using a self-assessment questionnaire.

For each statement, the volunteers needed to select one of the following:

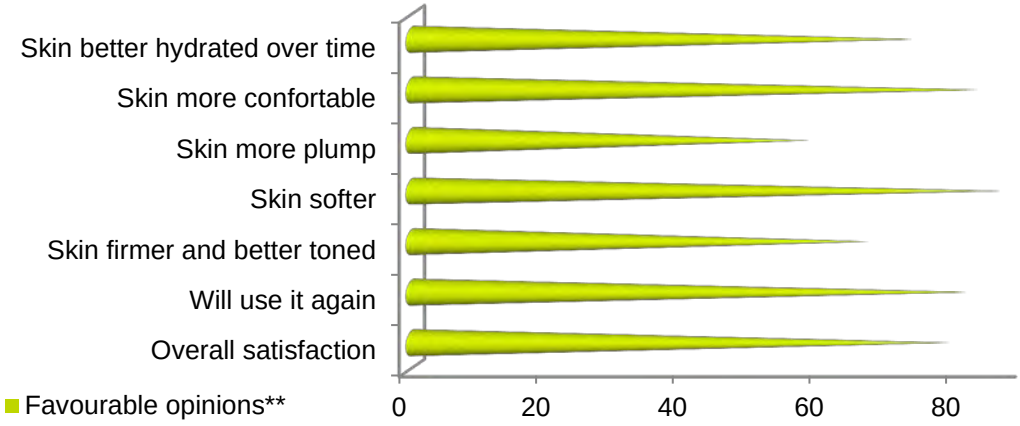
- Totally agree
- Agree
- Neither agree nor disagree

Grouped together as "positive opinions"

- Disagree
- Totally disagree

Grouped together as "negative opinions"

Furthermore, volunteers needed to specify if they were satisfied with the product (rating of 0 to 10, only ratings of 7 to 10 were considered as satisfactory), and whether they would be willing to continue to use the product.



*** Significant variation versus negative opinions with p<0.01 (Chi² test)*

Figure 24: Percentage of favourable opinions after applying **MAJESTEM™** *(n=106)*.

The results were clearly positive and demonstrated a high rate of satisfaction (79%) associated with a high proportion of people ready to reuse the product (82%). This seems to be the result of an overall effect on the skin (comfort, softness and hydration) as well as a more specific action on the signs of ageing (increase in firmness and plumpness of the skin).

3. CONCLUSION

Skin sagging of the face and neck, i.e., the shifting of soft tissues under the effects of gravity, is a major sign of ageing that deteriorates beauty and elegance; an important beauty criteria is how one carries their head with their smooth, graceful neck.

The loss of skin tension is mainly due to extracellular matrix changes and fibroblast damage. Under the attack of free radical-generating environmental oxidants, such as atmospheric pollutants and UV light rays, fibroblasts experience an imbalance in mitochondrial network dynamism and a breakage in their anchoring to extracellular matrix fibres. They are no longer capable of ensuring fibre tension, and subsequently, skin tonicity.

MAJESTEM™ is an active cosmetic ingredient that comes from cell cultures of edelweiss (*Leontopodium alpinum*). It has powerful antioxidant properties that effectively neutralise the free radicals generated by environmental aggressions:

- DPPH: -73% (at 3%)
- Lipid peroxidation: -60% (at 3%)
- Singlet oxygen: -49% (at 3%)
- Reactive Oxygen Species: -69% (at 2%)

The mitochondrial network, which provides energy (ATP) to cells, and especially to the cytoskeleton, is constantly shifting between fusion and fission, and is a target preferred by free radicals. Oxidative stress interferes with this balance, and generates some physiological events, such as the shift of the mitochondrial network to a fission state, a grouping of mitochondria around the nucleus, a disorganisation of the cytoskeleton accompanied by a rounding of the fibroblasts due to the loss of cytoskeletal tension and ECM anchor rupture. Thanks to an innovative test using the MitoTracker® tag, we were able to demonstrate that **MAJESTEM™** could restore mitochondrial dynamism (Mitochondrial connections: +31%, *p<0.05*, at 2%) and restore optimal fibroblast tension (fibroblast contractility: +54%, *p<0.01* up to 2%).

Concurrently, 2% **MAJESTEM™** ensures the integrity of the extracellular matrix on the one hand and reduces stress-induced MMP production: MMP-1 (-53%, *p<0.01*), MMP-7 (-56%, *p<0.03*), MMP-9 (-21%, *p<0.04*) and MMP-10 (-59%, *p<0.01*, not shown), and on the other hand stimulates TIMP production: TIMP-1 (+170%, *p<0.01*), TIMP-2 (+141%, *p<0.01)*.

Moreover, **MAJESTEM**™ stimulates collagen synthesis process by protecting and supporting vitamin C activity, essential enzymatic cofactor in the collagen I and collagen IV synthesis (+111%, *p<0.01* and +72%, *p<0.02;* at 2% respectively). Finally, **MAJESTEM™** stimulates the synthesis of thrombospondin-2 (TSP-2: +57%, *p<0.01*, at 2%), a glycoprotein known to strenghten collagen fibres architecture and organisation.

MAJESTEM™ is the first cosmetic active, extracted from *Leontopodium alpinum*, which presents a visible lifting effect on the sagging neck and face skin.

LIFTING OF THE SAGGING NECK SKIN

In only three weeks, **MAJESTEM™** tightens sagging skin under the chin which visibly retracts and firms. The sagging surface of neck skin is reduced by -10.6% *(p<0.01)* up to -56%. In the long term, expert judges found a lifting effect after six weeks associated with a pronounced smoothing of neck folds for most volunteers.

LIFTING OF THE CHEEKS

In six weeks, **MAJESTEM**™ enhances cheeks by visibly reducing the tear trough (hollow from inner corner of the eye and extends to the top of the cheek). The hollow volume is reduced by -8.8% *(p=0.082)* up to -84% and the circumference is reduced by -9% *(p<0.01)* up to -33%.
While the results of the placebo side are not significant.

SMOOTHING OF THE CROW'S FEET WRINKLES

In six weeks, **MAJESTEM**™ smoothes and lifts wrinkles around the eye with greater efficacy on skin damaged by the sun. The results of the placebo side are not significant.

- Wrinkle area: -10.7%, *p<0.05* up to -16.8%*, *p<0.05*
- Wrinkle depth: -6.2%, *p<0.05* up to -10%*, *p<0.01*
- Roughness: -5.7%, *p<0.05* up to -9.1%*, *p<0.01*

(: Panel of volunteers with photodamaged skin)*

A consumer test conducted on a panel of 106 volunteers, mean age 54 years [40-70 years], with visible signs of ageing shows that within three weeks, **MAJESTEM™** provides majestic comfort to the skin (83%, *p<0.01*) and makes the skin soft and toned respectively for 67% *(p<0.01)* and 86% *(p<0.01)* of volunteers. The majority of panellists felt their skin more plump and hydrated and would therefore intend to continue to use the product.

MAJESTEM™ lifts the skin for a majestic beauty.

It is recommended to formulate **MAJESTEM** ™ at 2%.

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5. ANNEX

Formula of products used for *in vivo* evaluations.

Raw materials	INCI Name	Suppliers	Product (%) Placebo	Product (%) Active
Phase A				
H_2O	Water		qsp 100	qsp 100
Optasense G83	Carbomer	CRODA	0.30	0.30
Phase B				
Brij S2-SS -(RB)	Steareth-2	CRODA	0.40	0.40
Brij S10-SO -(RB)	Steareth-10	CRODA	1.20	1.20
Crodafos CES-PA -(RB)	Cetearyl Alcohol (and) Dicetyl Phosphate (and) Ceteth-10 Phosphate	CRODA	4.00	4.00
Crodacol CS90-PA -(RB)	Cetearyl Alcohol	CRODA	1.50	1.50
Laurocapram	Laurocapram		2.50	2.50
Cyclopentasiloxane (and) Cyclohexasiloxane	Cyclopentasiloxane (and) Cyclohexasiloxane		2.00	2.00
Crodamol OSU-LQ -(RB)	Diethylhexyl Succinate	CRODA	7.00	7.00
Phase C				
Glycerin	Glycerin		4.00	4.00
Octanediol	Caprylyl Glycol		0.50	0.50
Phase D				
Phenoxyethanol	Phenoxyethanol		qs	qs
Phase E				
Potassium sorbate	Potassium Sorbate		qs	qs
Phase F				
H_2O	Water		4.00	4.00
NaOH 30%	Sodium Hydroxide		0.40	0.40
Phase G				
Excipient of **MAJESTEM™**			2.00	-
MAJESTEM™	(see synopsis)	SEDERMA	-	2.00
Phase H				
Perfume		Expressions Parfumées	0.10	0.10

Operating procedure:
(Laboratory preparation)

Step 1. Weigh phase A and let it swell without stirring for 30 minutes.
Step 2. Heat phase A to 75°C in a bain-marie.
Step 3. Weigh phase B and heat to 75°C in a bain-marie. Mix well.
Step 4. Weigh phase C and melt at a temperature of 45°C. Mix well.
Step 5. Put phase D into already-cooled phase C and mix.
Step 6. Pour phase C+D into phase A under staro at a speed of 500 rpm. Homogenise well.
Step 7. Add phase B into phase A+C+D under staro at a speed of 1000 rpm.
Step 8. Extemporaneously add phase E into the preceding phase under staro stirring at a speed of 1000 rpm. Homogenise well.
Step 9. Add phase F. Homogenise well.
Step 10. Add phase G. Homogenise well.
Step 11. Add phase H. Homogenise well.

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