## Care Creations...



## **Beauty Creations**

The Passion for Beauty



## Science for beauty

People have always been preoccupied with fighting against aging. We have already been able to successfully mask the visible signs of skin aging for a long time, but the real revolution arrived with modern cosmetics, with which we no longer strive to only hide, but to actually prevent and repair the signs and effects of aging.

Advances in fundamental research enable us to better understand the mechanisms of skin aging and how these can be influenced.

Thus, the deleterious role of elastases and the dramatic long term consequences of glycation are well known today. More in-depth research has highlighted very important molecules such as FIBULIN-5 and EMILIN-1.

It was also found that in addition to protect what nature has given us, it is possible to counteract the negative effects of aging and to restore the skin's suppleness and firmness. To do all of this at once, however, seemed utopic.

## An overall solution

A true 4-in-1 solution for the skin, Elestan<sup>™</sup> preserves the skin's youth by providing protection and repair:

- Elastases are enzymes naturally present in the skin that regulate the degradation of elastin. The disturbance of this process with aging results in excessive degradation of elastin which causes a loss of skin elasticity. Thanks to its **anti-elastase** properties, Elestan<sup>™</sup> fights against the degradation of the
- elastic fiber network, thus preserving the skin's youth.
  In case the elastic fiber network is already damaged, Elestan<sup>™</sup> contributes to its
- In case the elastic liber network is already damaged, Elestan contributes to its recovery by stimulating the production of elastin.
- Elastic fibers are formed by the polymerization of tropoelastin monomers on specific microfibrils. EMILIN-1 is a glycoprotein that ensures an efficient binding of tropoelastin on the microfibrils and protects tropoelastin from degradation. It is also involved in the interaction between elastin fibers and FIBULIN-5.

FIBULIN-5 is a molecule that binds between tropoelastin and microfibrils, stimulating the deposition of tropoelastin onto the microfibrils. In addition, FIBULIN-5 binds to cross-linking enzymes, aiding elastic fiber assembly and correct alignment of the elastic fibers.

By stimulating the synthesis of elastin associated proteins, such as **EMILIN-1** and **FIBULIN-5**, Elestan<sup>M</sup> enables a correct organization of newly synthesized elastic fibers.

• Glycation is a well known phenomenon of skin aging, corresponding to the formation of covalent bonds between glucose molecules and proteins of the extra cellular matrix. These bonds induce a rigidification of the structural proteins of the skin, resulting in a loss of skin elasticity. Elestan<sup>™</sup> inhibits the rigidification of the skin's fibers caused by **glycation**.

Thanks to its overall action, Elestan<sup>™</sup> enables us to recover firmer and more elastic skin and significantly reduces the roughness of stretch marks.

## Definition / Composition

Elestan<sup>™</sup> is an extract from the leaves of *Manilkara multinervis*. Manilkara is a medium height tree with a rough fissured bark belonging to the Sapotaceae family. It is also called bullet-wood tree or sleeper wood and grows in the forests and savannas in Western Africa from Cameroon to Senegal. The bark and leaves of Manilkara are used in traditional African medicine.



Manilkara multinervis

Elestan<sup>™</sup> contains flavonoids and catechuic tannins amongst other components.

### INCI name

Elestan<sup>™</sup> LS 9913: Glycerin (and) Manilkara Multinervis Leaf Extract (and) Water. Elestan<sup>™</sup> LS 9879: Manilkara Multinervis Leaf Extract (and) Maltodextrin.

## Skin benefits

- Anti-aging:
  - protection against the effects of elastase,
  - enhancement of the synthesis of elastin.
- Stimulation of the synthesis of elastin associated proteins.
- Anti-glycation.
- Improvement of skin firmness and elasticity.
- Reduction of the roughness of stretch marks.

## Cosmetics use

- Anti-aging face care.
- Elasticity enhancing skin care.
- Skin care preventing aging and glycation.
- Firming body care.
- Prevention and correction of stretch marks.

## Dosage / Solubility / Mode of incorporation

- Dose of use: Elestan<sup>™</sup> LS 9913: 2 - 3%. Elestan<sup>™</sup> LS 9879: 0.3 - 0.5%.
- 2. Solubility: Soluble in water, insoluble in oils.

#### 3. Mode of incorporation:

Elestan<sup>™</sup> LS 9913: is incorporated into the finishing process below 60°C, or at room temperature for cold processing.

Optimal pH: 4 - 7.

Elestan<sup>™</sup> LS 9879: dissolve at 10% w/w in water at room temperature for its introduction below 60°C during the finishing process or at room temperature for cold processing. Optimal pH: 3 - 7.

## Analytical characteristics

#### 1. Aspect:

Elestan<sup>™</sup> LS 9913: brown-red syrupy liquid with a weak odor.

Elestan<sup>™</sup> LS 9879: beige to pink-beige powder with weak characteristic odor.

- 2. Specifications: upon request.
- 2. Preservative: none.

Tolerance Good.

Efficacy Efficacy tests hereafter.

Storage In its original packaging, at 15 - 25°C.

\* Raw material certified by Ecocert Greenlife according to the Ecocert Standard for Natural and Organic Cosmetics available at http://cosmetics.ecocert.com: 100% of the total ingredients are from natural origin, 50% of the total ingredients are from Organic Farming.

## Efficacy tests

The efficacy of  ${\sf Elestan}^{\scriptscriptstyle \rm M}$  on the dermal elastic network was evaluated at both protection and repair levels.

#### Protection effects

- Anti-elastase activity on skin sections.
- Anti-glycation performance in tubo

#### **Repair effects**

- Synthesis of tropoelastin on fibroblasts.
- Synthesis of elastin associated proteins (FIBULIN-5 and EMILIN-1) on fibroblasts.

Finally, two clinical studies confirmed the beneficial effects of Elestan™; the skin is firmer and its tonicity is increased..

## Anti-elastase activity on human skin sections

#### Aim

To evaluate the anti-elastase activity of Elestan<sup>m</sup> on human skin sections.  $\alpha$ 1-antitrypsin ( $\alpha$ 1-AT) was used as positive control.

#### Background

Elastase is a serine protease responsible for the breakdown of elastin<sup>1</sup>. In the skin, the elastin network is damaged by elastase during intrinsic and photo-aging.

For anti-aging, fighting against elastin degradation due to elastase activity is a good way to prevent loss of skin elasticity.

#### Protocol

Human skin sections by cryostat Incubation with Elestan<sup>™</sup> + elastase (3 different concentrations)

for 2 hours at 20°C (α1-AT used as positive control)

Weigert staining to reveal elastic fibers

Pictures / image analysis Results expressed as % of occupation of elastic fibers in papillary and reticular dermis versus untreated control

Fig. 1 - Schema of protocol.



Fig. 2 - Illustration of protective effect of Elestan<sup>™</sup> against elastase degradation in papillary dermis.





#### Results

Elastase has induced destruction of elastic fibers in a dose dependent manner.

With the strongest dose of elastase almost all the elastic fibers have disappeared.

The addition of Elestan<sup>™</sup> significantly preserved the elastic fibers at at both the papillary and reticular dermis levels.

The anti-elastase efficacy of Elestan<sup>m</sup> is similar to  $\alpha$ 1-AT, a potent inhibitor of serine proteases.

#### Conclusion

In skin sections, Elestan<sup>™</sup> at 0.05% and 0.5% exhibited a potent antielastase efficiency.

Additional tests done *in tubo* with human leukocyte elastase also demonstrated an anti-elastase activity of Elestan<sup>M</sup>, with a half maximal inhibitory concentration (IC50) similar to a1-AT (data not shown).

<sup>1</sup> Hornebeck W et al.: Elastin and elastases. CRC Press, Boca Raton (Fla.), 1989

## Evaluation of anti-glycation effect (in tubo)

#### Aim

To evaluate the anti-glycation effect of Elestan<sup>™</sup> by measuring the level of Schiff's bases (glycation products) *in tubo*.

Aminoguanidine was used as positive control<sup>2</sup>.

#### Background

One of the causes of aging is the appearance of Advanced Glycation End Products (AGEs)<sup>3</sup>. The glycation products result from a non-enzymatic reaction between a sugar and a free amine group in proteins.

Glycated collagen and elastin fibers lose their ability to function normally. In addition, the glycated fibers can not be eliminated, which leads to skin aging.

#### Protocol

Solution with collagen type I ± glucose (1%)

Incubation with Elestan<sup>™</sup> for 3 weeks at 45°C (aminoguanidine used as positive control)

Measurement of the level of Schiff's bases by fluorescence at 430 nm (excitation at 350 nm)

Results expressed as % referring to the control with glucose in papillary and reticular dermis versus untreated control

Fig. 4 - Schema of protocol.

#### Results



Fig. 5 - Anti-glycation potential of Elestan<sup>™</sup> regarding Schiff's bases formation.

#### Conclusion

Elestan<sup>™</sup> clearly demonstrated an anti-glycation effect in a dose dependent manner.

<sup>&</sup>lt;sup>2</sup> HOU FF *et al.*: Aminoguanidine inhibits advanced glycation end products formation on β2-Microglobulin. Am Soc Nephrol, 9: 277-283, 1998

<sup>&</sup>lt;sup>3</sup> Jeanmaire C *et al*.: Glycation during human dermal intrinsic and actinic ageing: an *in vivo* and *in vitro* model study. Br J Dermatol, 145: 10-18, 2001

## Stimulation of tropoelastin gene expression (qRT-PCR on human dermal fibroblasts)

#### Aim

To evaluate the capacity of Elestan<sup>™</sup> to stimulate the expression of the gene coding for tropoelastin (ELN) in cultured human dermal fibroblasts.

Transforming growth factor beta 1 (TGF- $\beta$ 1) was used as positive control<sup>4</sup>.

#### Background

In the process of elastic fiber synthesis, elastin precursors (tropoelastin molecules) are deposited on microfibrils, aligned in an orderly way, and cross-linked by enzymes to form mature elastin<sup>5</sup>. The protein elastin is a major constituent of the elastic fibers and is responsible for the elastic properties. The loss of elasticity leads to the characteristics typically observed in aged skin.

#### Protocol



Fig. 6 - Schema of protocol.

#### Results



Fig. 7 - Upregulation of expression of gene coding for tropoelastin in human dermal fibroblasts.

#### Conclusion

Elestan<sup> $^{\text{M}}$ </sup> significantly increased, in a dose dependent manner, the expression of ELN gene coding for tropoelastin from cultured human dermal fibroblasts.

<sup>&</sup>lt;sup>4</sup> McGowan SE et al.: Exogenous and endogenous transforming growth factors-b influence elastin gene expression in cultured lung fibroblasts. Am J Respir Cell Mol Biol, 17: 25-35, 1997

<sup>&</sup>lt;sup>5</sup> Rosenbloom J et al.: Extracellular matrix 4: the elastic fiber. FASEB J, 7: 1208-1218, 1993

# Stimulation of (tropo)elastin protein synthesis (ICC on human dermal fibroblasts)

#### Aim

To evaluate the capacity of Elestan<sup>™</sup> to stimulate the synthesis of (tropo)elastin protein in cultured human dermal fibroblasts.

TGF- $\beta$ 1 was used as positive control<sup>4</sup>.

#### Background

The visualization of (tropo)elastin by ICC allows to confirm that the increase of ELN gene expression, observed by qRTPCR, results in an increase of the (tropo)elastin protein in human fibroblasts in culture.

### Protocol



#### Results



Fig. 9 - Visualization of (tropo)elastin by ICC. Stimulation of (tropo)elastin synthesis by human dermal fibroblasts in culture. TGF- $\beta 1 = 81.4\%$ .

#### Conclusion

Elestan<sup>™</sup> significantly increased, in a dose dependent manner, the synthesis of (tropo)elastin from cultured human dermal fibroblasts.

# Stimulation of FIBULIN-5 synthesis (ICC on human dermal fibroblasts)

#### Aim

To evaluate the capacity of Elestan<sup>™</sup> to stimulate the synthesis of FIBULIN-5 from cultured human dermal fibroblasts.

TGF- $\beta$ 1 was used as positive control.

#### Background

FIBULIN-5 is an extra-cellular glycoprotein involved in elastic fiber formation by promoting deposition and crosslink of tropoelastin onto microfibrils<sup>6</sup>.

With aging, FIBULIN-5 may become truncated (cut short due to certain enzyme actions) and accumulates. Such truncated FIBULIN-5 cannot associate with microfibrils. Recovering functional FIBULIN-5, which acts as an organizer molecule for elastogenesis, is a good way to counterbalance the deleterious effects of aging on the elastic fiber network.

#### Protocol

Dermal fibroblasts isolated from human skin

Seeding fibroblasts in a growth medium

Incubation for 3 days at 37°C

Exchange of the growth medium by a medium containing Elestan<sup>™</sup> (TGF-β1 used as positive control)

Incubation for 5 days at 37°C

Immunocytochemistry (ICC) technique Evaluation of FIBULIN-5 synthesis

Fig. 10 - Schema of protocol.

#### Results



Fig. 11 - Visualization of FIBULIN-5 by ICC. In presence of Elestan<sup>™</sup>, FIBULIN-5 synthesis is increased compared to control. TGF- $\beta$ 1 = 80%.

#### Conclusion

Elestan<sup>™</sup> significantly increased, in a dose dependent manner, the synthesis of FIBULIN-5 from cultured human dermal fibroblasts.

<sup>6</sup> Hirai M et al.: Fibulin-5/DANCE has an elastogenic organizer activity that is abrogated by proteolytic cleavage in vivo. J Cell Biol, 176: 1061-1071, 2007

## Stimulation of EMILIN-1 synthesis (ICC on human dermal fibroblasts)

#### Aim

To evaluate the capacity of Elestan<sup>™</sup> to stimulate the synthesis of EMILIN-1 (Elastin Microfibril Interface-Located proteIN-1) from cultured human dermal fibroblasts. Vitamin C was used as positive control.

#### Background

EMILIN-1 is an extra-cellular matrix glycoprotein (located at the elastin-microfibrils interface) playing an important role in elastogenesis.

EMILIN-1 binds elastin and FIBULIN-5, and the association of FIBULIN-5 with elastin is altered in the absence of EMILIN-1.

Lack of EMILIN-1 leads to formation of abnormal elastic fibers7.

Boosting EMILIN-1 is an appropriate way to have a correct organization of neosynthesized elastic fibers.

#### Protocol

Dermal fibroblasts isolated from human skin Seeding fibroblasts in a growth medium Incubation for 1 day at 37°C

Exchange of the growth medium by a medium containing Elestan<sup>™</sup> (Vitamin C used as positive control)

Incubation for 2 days at 37°C

Immunocytochemistry (ICC) technique Evaluation of EMILIN-1 synthesis

Fig. 12 - Schema of protocol.

#### Results



**Fig. 13** - Visualization of EMILIN-1 by ICC. Elestan<sup>TT</sup> increased the synthesis of EMILIN-1 from cultured human dermal fibroblasts. Vitamin C = 34.33%.

#### Conclusion

Elestan  $^{\scriptscriptstyle\rm M}$  significantly increased, in a dose dependent manner, the synthesis of EMILIN-1 from cultured human dermal fibroblasts.

7 ZANETTI M: Emilin-1 deficiency induces elastogenesis and vascular cell defects. Mol Cell Biol, 24, 638-650, 2004

## Anti-aging effect (clinical test) Standard method - Cutometer

#### Aim

To evaluate, *in vivo*, the anti-aging activity of an emulsion containing 0.5% Elestan<sup>™</sup> in comparison to placebo emulsion, by measurement of skin biomechanical properties using Cutometer.

#### Protocol Evaluated parameters

28 female volunteers, 45-60 years old, with a loss of elasticity on the face Twice daily randomized application on the face for 56 days

Placebo emulsion



Emulsion containing 0.5% Elestan<sup>™</sup>

Measurements of the biomechanical properties of the skin on the temples by Cutometer SEM 575 (Courage & Khazaka) before treatment (D0) and after 56 (D56) days of the treatment

Fig. 14 - Protocol of the clinical evaluation of the anti-aging effect.

#### - Extensibility parameters:

Ue: immediate extensibility = immediate distension of the skin within the first 0.1 s of suction. Uf: maximal extensibility = final distension of the skin at the end of suction.

Increase of these parameters can be interpreted as an improvement of skin suppleness.

#### - Recovery parameters:

Ur: immediate elastic recovery = immediate relaxation of the skin within the first 0.1 s after the end of suction.

Ua: total deformation recovery = capacity of the skin to return to the initial state.

Increase of these parameters can be interpreted as an improvement of skin tonicity.

#### Results



Fig. 15 - Evolution of the biomechanical properties after 56 days of treatment.

### Conclusion

A significant increase of extensibility parameters (Ue and Uf) with concomitant improvement of recovery parameters (Ur and Ua) was obtained after 56 days of treatment with Elestan<sup>™</sup>, while no significant evolution was observed after placebo treatment.

## Anti-aging effect (clinical test) Innovative method - Dynaskin

#### Aim

To evaluate, *in vivo*, the anti-aging activity of an emulsion containing 0.5% Elestan<sup>™</sup> in comparison to placebo emulsion, by measurement of skin biomechanical properties using an innovative method, Dynaskin.

#### Method

Dynaskin combines two technologies: the creation of a skin deformation by an airflow and a 3D technique of fringe projection for its quantification (Orion concept and Eotech).

#### **Evaluated parameters**

- volume of the depression created by the airflow (mm<sup>3</sup>),
- maximal depth of the depression created by the airflow (mm).

The anti-aging activity is translated by a diminution of these parameters that corresponds to an increase of skin tonicity and firmness.

#### Protocol

29 female volunteers, 45-60 years old, with a loss of elasticity on the face Twice daily randomized application on the face for 56 days

Placebo emulsion



Emulsion containing 0.5% Elestan™

Measurements of the depression created by the airflow on the cheeks by Dynaskin before treatment (D0) and after 56 days of treatment (D56)

Fig. 16 - Protocol of the clinical evaluation of the anti-aging effect.

#### Results



Fig. 17 - Evolution of the skin depression parameters after 56 days of treatment.



Fig. 18 - 3D illustration of skin deformation during the depression - Dynaskin.

#### Conclusion

A significant reduction of both tested parameters was obtained after 56 days of treatment with Elestan<sup>™</sup> at 0.5%, while no significant evolution was observed for placebo treatment. The skin firmness and skin tonicity were significantly improved comparatively to placebo formulation: volume of depression by 10% and maximal depth by 5%.

## Anti stretch mark efficacy

#### Type I collagen synthesis



Ex vivo

On dermal fibroblasts culture Evaluation of cellular protein level (Bradford's method) and type I collagen synthesis (ELISA technique)

#### Elestan<sup>™</sup> specifically enhances Collagen I production.



Ex vivo

On fibroblasts from biopsies of recent stretch marks.

Synthesis of GAGs measured by colorimetric tests (Blyscan<sup>™</sup> Sulfated GAG assay).

#### Elestan<sup>™</sup> specifically enhances GAGs production.



#### Elastin synthesis

Ex vivo

On fibroblasts from biopsies of recent stretch marks.

Synthesis of elastin measured by colorimetric tests (Fastin<sup>™</sup> Elastin assay).

#### Elestan<sup>™</sup> specifically enhances Elastin production.

#### Clinical study



The skin is distinctly smoothed at the level of the stretch marks.



In vivo Study

29 subjects women recent post-partum stretch marks on the belly. Twice daily application for 12 weeks of an emulsion containing 0.5% Elestan<sup>™</sup> or placebo. Evaluation of skin roughness (Primos Pico, 3D evaluation) after 3 months of treatment and macrophotographs.

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