

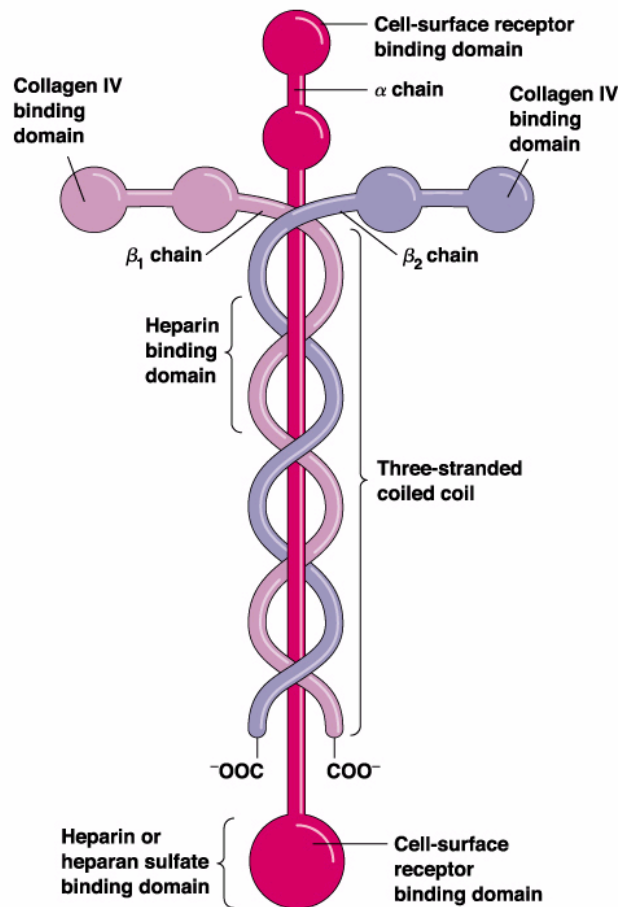
# SERILESINE<sup>®</sup> SOLUTION GC

## AN ADHESION SEQUENCE FROM LAMININ

CODE: P07-PD060

Date: May 2008

Revision: 0



## GENERAL DESCRIPTION

The study of the mechanisms of skin aging has traditionally overlooked an important area: the dermo epidermal junction (DEJ). This is the layer responsible for supporting the epidermis and for the communication between epidermal and dermal cells.

The DEJ is physically a basement membrane that separates the skin cells in the epidermis from the extracellular matrix (ECM) which lies below, in the dermis. This membrane is composed by two layers, the basal lamina, in contact with the cells, and the underlying reticular lamina, in contact with the ECM. The basal lamina is rich in collagen type IV, proteoglycans and the glycoproteins entactin and laminin. These molecules provide a structural network and bioadhesive properties for cell attachment.

Laminin is a glycoprotein of about 850 kDa and it is, after collagen, the most abundant protein in the ECM. However, while Collagen performs exclusively a structural role in the skin, Laminin is involved in functionalisation and activation of cells, in processes such as cell proliferation, migration and adhesion. These mechanisms are necessary to keep the normal balance of the skin and are essential for processes as important as wound healing, for instance.

Laminin only exists in basement membranes. It is composed of three very long polypeptide chains (alpha, beta and gamma) arranged in the shape of an assymetric cross and held together by disulfide bonds. The three chains exist as different subtypes which results in twelve different isoforms for laminin, of which the best well-studied is Laminin-1.

Keratinocytes recognise the binding domains on Laminin, particularly Laminin-5, by using their own integrin receptors, transmembrane proteins located on specific junction points called hemidesmosomes.

Lipotec has identified and synthesized a sequence from the alpha chain of Laminin: a hexapeptide named SERILESINE<sup>®</sup>. This peptide retains many of the characteristics of the native protein, and promotes cell adhesion and proliferation.

Certain features of the DEJ are altered by the aging process, such as the anchoring ability of keratinocytes, probably due to deficiencies in the expression of integrins as we age.

Laminin-5 synthesis has also been proved to decrease in aged skin. This causes a loss of contact between dermis and epidermis, and results in the skin losing elasticity and becoming saggy. The cohesion between dermis and epidermis is essential to maintain skin balance because it enables the transport of oxygen, nutrients and waste, contributing to the health of the epidermis.

This work will show that a synthetic hexapeptide from Laminin-1 is able to restore the skin's normal function by promoting synthesis of Laminin-5, stimulating keratinocyte and fibroblast proliferation, inducing a redensifying effect on the dermis, and an improvement in skin elasticity, compactness, tonicity and smoothness.

## PROPERTIES AND APPLICATIONS

- SERILESINE<sup>®</sup> SOLUTION GC improves cell adhesion by enhancing synthesis of Laminin-5
- Adhesion of cells to the basement membrane and among themselves provides firmness to the skin
- Increased contact between skin cells ensures correct nourishment and health
- SERILESINE<sup>®</sup> SOLUTION GC induces a significant increase in the dermis density, improving skin compactness

SERILESINE<sup>®</sup> SOLUTION GC can be incorporated in cosmetic formulations such as emulsions, gels, sera, etc., where restructuring and refirming of skin is desired.

## TECHNICAL INFORMATION

### PRODUCT SPECIFICATIONS

Code:	P07-PD060
Appearance:	Translucent solution
Colour:	Colourless
Active ingredient content:	0.05 % Hexapeptide-10

### PROCESSING AND DOSAGE

**SERILESINE<sup>®</sup> SOLUTION GC** can be incorporated at the final stage of the manufacturing product, provided the temperature is below 40 °C. Taking into consideration the concentration of peptide in **SERILESINE<sup>®</sup> SOLUTION GC**, it is recommended that 1 to 10% of the solution is present in the final formulation in order to obtain significant restructuring activity.

### STORAGE AND SHELF LIFE

Keep in a clean, cool and dark place. If product is stored as recommended it will remain stable for 24 months.

## **SAFETY**

The toxicological profile of SERILESINE<sup>®</sup> for cosmetic purposes was assessed *in vitro* and *in vivo*. A full toxicological report and a summary of all the safety tests performed are available on request.

### ***In vitro* tests**

#### **Citotoxicity test on human epidermal keratinocytes**

The results showed no signs of citotoxicity at the concentrations assayed.

#### **Mutagenicity test (Ames test)**

The results showed no mutagenical activity under the conditions assayed.

#### **Ocular Irritation (HET-CAM test)**

The product is potentially not irritating for the eyes.

#### **Ocular Irritation (NRU - Neutral Red Uptake test)**

The product is potentially not irritating for the eyes.

### ***In vivo* tests**

#### **Skin irritation (Patch Test)**

The test was performed on 25 human volunteers, aged 18 to 70, both sexes. SERILESINE<sup>®</sup> Solution was applied on the back, under an occlusive patch for 24 hours. Then, it is removed and cutaneous reactions are evaluated after 15 minutes, 1 hour and 24 hours. The skin is assessed separately for erythema and oedema in a scale from 0 (non existent) to 4 (serious). SERILESINE<sup>®</sup> scored 0 so it can be classified as non-irritant.

#### **Skin sensitisation (Hypoallergenicity)**

An HRIPT (Human Repeated Insult Patch Test) was performed on 50 volunteers. A 10% dilution in distilled water of SERILESINE<sup>®</sup> Solution was tested and the experimental area was the back. None of the subjects showed any reaction to the test product. SERILESINE<sup>®</sup> Solution may be considered as non-primary irritant and non-primary sensitiser to the skin.

# EFFICACY

## *In vitro*

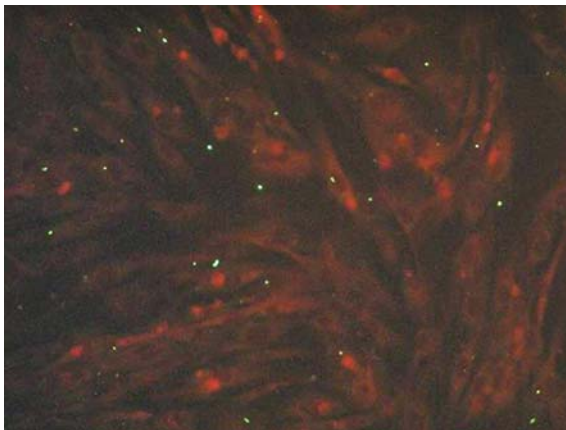
### ***Stimulation of Laminin-5 production (I)***

Immunostaining studies have been carried out in human fibroblasts (the natural producers of Laminin) in order to ascertain if SERILESINE<sup>®</sup> is able to enhance the expression of Laminin-5.

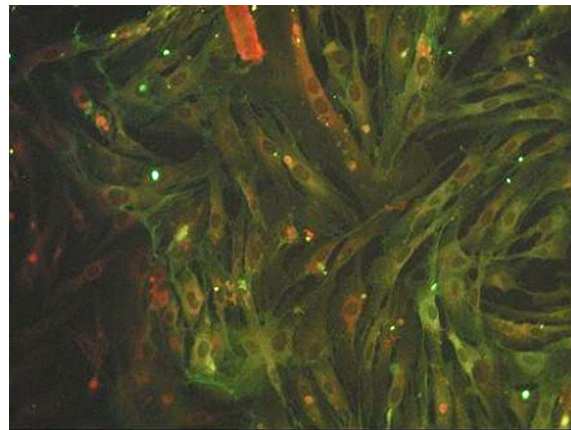
The test uses a primary (monoclonal) antibody that binds to Laminin-5, and a secondary antibody (polyclonal) that binds to the complex between protein and primary antibody. The secondary antibody is coupled to a fluorescent compound (FITC – Fluorescein Isothiocyanate).

FITC is illuminated with filtered light at 495 nm (absorption wavelength) and the light emitted by the dye is detected at 528 nm (emission wavelength).

Trypsinised fibroblasts were incubated for 72 hours in microplates with 0.05 mg/mL SERILESINE<sup>®</sup>, Minimal Essential Medium-Na-Piruvate and 5% FCS. Cells were then fixed and incubated with the primary antibody and then with the secondary antibody coupled to the dye (FITC). For the immunofluorescence analysis we used an Olympus fluorescence microscope (20x magnification).



Immunofluorescence – Laminin-5 expression in untreated human fibroblasts



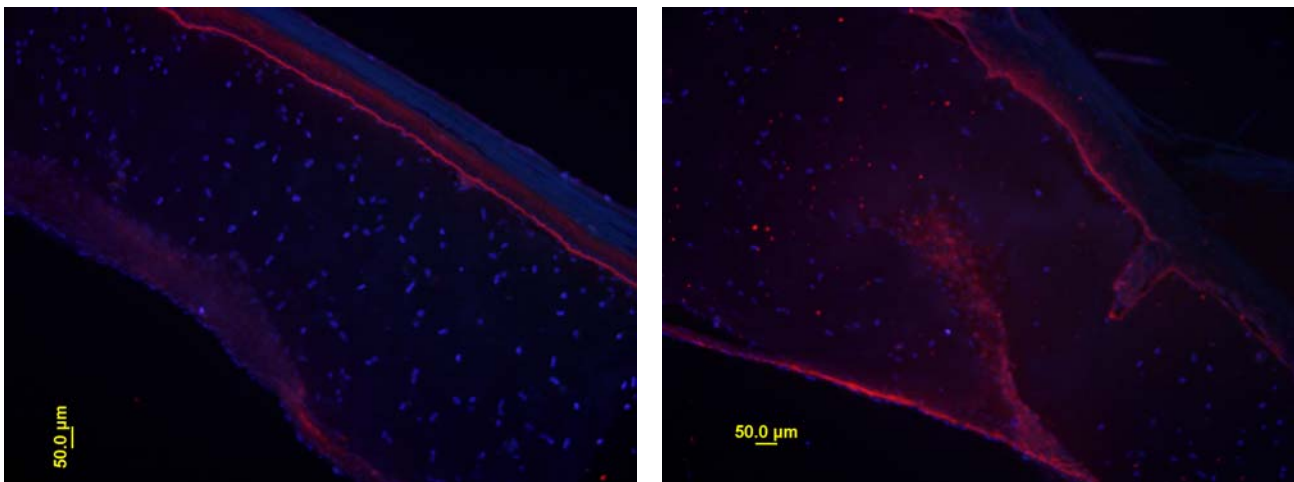
Immunofluorescence – Laminin-5 expression in treated human fibroblasts

**Figure 1.** Expression of Laminin-5

### **Stimulation of Laminin-5 production (II)**

The full thickness skin models exhibit in vivo-like morphological, metabolic, and growth characteristics which are uniform and highly reproducible. These skin-like tissues consists of organized basal, spinous, granular and cornified epidermal layers analogous to those found in vivo (1).

Human skin tissue models were used to monitor the levels of Laminin-5. EpidermFT full thickness skin model (supplied by Mattek Corporation, Ashland, MA, USA) consists of normal, human-derived epidermal keratinocytes (NHEK) and normal, human-derived dermal fibroblasts (NHFB) which have been cultured to form a multilayered, highly differentiated model of the human dermis and epidermis.

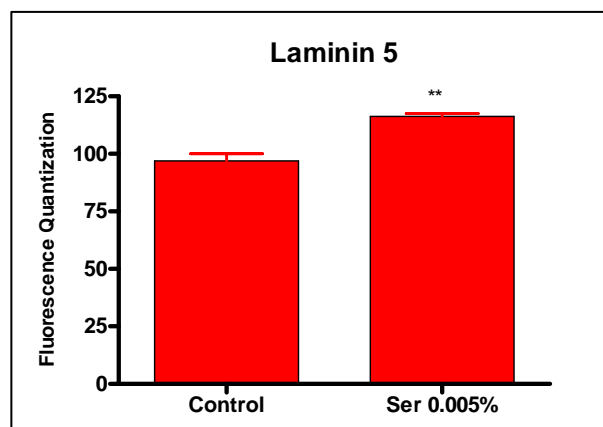


a) Control (untreated)

b) SERILESINE<sup>®</sup> 0.005%

**Figure 2.** Red Immunofluorescent stain for Laminin-5 in organotypic cultures

Fluorescent quantification by image analysis demonstrates that Laminin-5 is expressed 20% more in treated tissues than in controls (Figure 3).

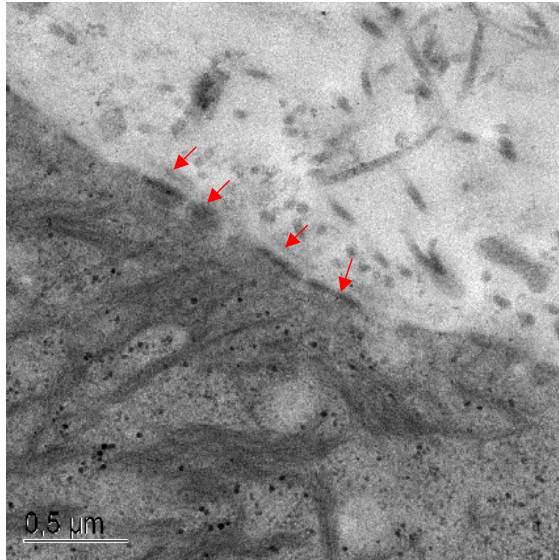


**Figure 3.** Fluorescence quantization of Laminin-5 expression in skin models untreated (control) or treated with SERILESINE<sup>®</sup> 0.005%. (\*\* P < 0.01).

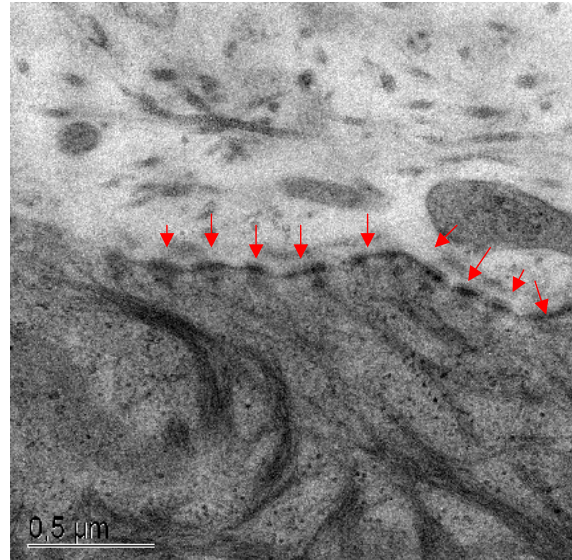


***Stimulation of hemidesmosome formation (I)***

Both proteins,  $\alpha 6$ -integrin and Laminin-5, form a cell structure called hemidesmosome, that can be seen by transmission electron microscopy as electron-dense plaques. SERILESINE<sup>®</sup> at 0.005% has proved to increase the number of hemidesmosomes (Figure 6), thus increasing cohesion between dermis and epidermis.



a) Control (untreated)



b) SERILESINE<sup>®</sup> 0.005%

**Figure 6.** Transmission electron microscopy observation of skin model sections

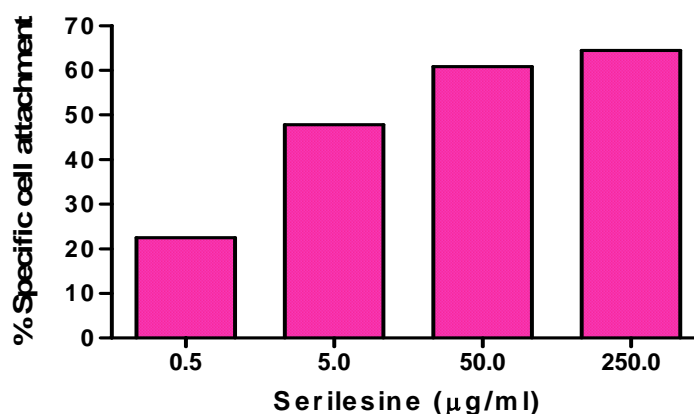
### **Specific keratinocyte adhesion**

Several microwells were coated with different concentrations of the peptide, and others were coated with BSA (Bovine Serum Albumin). Keratinocytes were added to the wells, and later washed to determine how many remained stuck to the wells. The amount of cells stuck to BSA was taken as a baseline (non specific binding). The binding of cells to SERILESINE<sup>®</sup> was specific and took place via specialised receptors. Using 10% SERILESINE<sup>®</sup> Solution in the final formula corresponds to 50 µg/mL peptide in the product, which increases cell adhesion 60%.

A microtiterplate was coated with 50 µl per well of the product SERILESINE<sup>®</sup> (0.5 - 250 µg/ml per well) in distilled water, by drying overnight at room temperature. Control wells to evaluate the non-specific cell adhesion were coated with 40 µM bovine serum albumin (BSA). All wells were then washed with 200 µl PBS and blocked for 1h with 1% BSA in PBS at 37°C.

Human Epidermal Keratinocytes were trypsinised, labelled for 30 min at 37°C in 5 µM Calcein-AM (Molecular Probes) and washed three times in Epilife medium. Labelled cells ( $4.5 \times 10^4$  /well) were added to the previously coated plate and incubated for 2h at 37°C in 5% CO<sub>2</sub> humidified air. Cell adhesion was assessed by measurement of calcein fluorescence before and after washing non-adhered cells. Percentage of adhesion was determined by dividing the fluorescence of adhered cells by the total fluorescence of cells added to each well. Cell adhesion promoted by the product was calculated respect non-specific cell adhesion to BSA.

Results show that the product SERILESINE<sup>®</sup> is able to promote keratinocyte adhesion in a dose dependent manner (*Figure 7*). The non-specific cell binding was taken as a baseline and the specific cell attachment to SERILESINE<sup>®</sup> was calculated as the relative excess percentage (compared to non-specific attachment). At the highest concentration of SERILESINE<sup>®</sup> (250 µg/ml) we found 65% more cells linked to the hexapeptide than cells linked to non-specific substrates (plastic and BSA). These results are indicative that SERILESINE<sup>®</sup> is recognised by specialised receptors on the surface of keratinocytes.



**Figure 7.** Keratinocyte adhesion

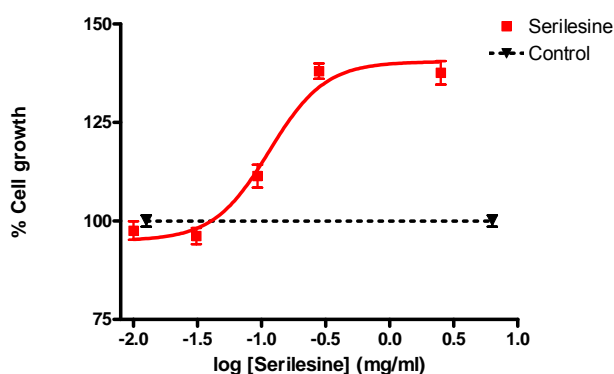
### In vitro keratinocyte proliferation

Cell proliferation is evaluated by a fluorescence-based cell viability method. Live cells are distinguished by the presence of intracellular esterase activity, determined by the enzymatic conversion of the nonfluorescent cell-permeant calcein-AM to the intensely fluorescent calcein, which is retained within live cells and imparts an intense green fluorescence.

Samples are tested at the following concentrations in Epilife medium without phenol red (*Cascade Biologics*): 2.5 mg/ml, 0.83 mg/ml, 0.28 mg/ml, 0.093 mg/ml, 0.031 mg/ml. Non-treated cells are used as controls.

Human Epidermal Keratinocytes (HEKa, *Cascade Biologics*) were grown until confluence in Epilife medium (*Cascade Biologics*) supplemented with specific growth factors for keratinocytes. Human Epidermal Keratinocytes were trypsinised and  $1 \times 10^4$  cells/well were seeded at 96-well plates. After 24 h incubation at 37° C in 5% CO<sub>2</sub> humidified air, fresh medium was added with scalar dilutions of tested product. Non-treated cells (only medium) were used as controls. Cells were incubated for an additional 48 h at 37° C in 5% CO<sub>2</sub> humidified air. At this time, well medium was replaced with 100 µl of 0.4 µM calcein-AM (*Molecular Probes*) in Epilife medium without phenol red (*Cascade Biologics*) following the method described by Lynch *et al* [4]. The fluorescence was read at  $\lambda_{exc}=485$  nm and  $\lambda_{em}=530$  nm in a microtiter plate reader (1420 VICTOR2, EG&G Wallac). The proliferation was calculated as:  $T/C \times 100$ , where T represents the absorbance of test wells and C that of the control wells.

As shown below, the product SERILESINE<sup>®</sup> has shown a stimulatory effect on keratinocyte cell growth, after 48 hours. Since the control was taken as 100%, the highest dose of the hexapeptide induced an increase in keratinocyte proliferation of 37.6%.



Dose (mg/ml)	% Cell growth
2.5	137.60
0.83	138.00
0.29	111.40
0.093	96.18
0.031	97.55
Controls	99.99

**Figure 8.** Keratinocyte proliferation

Keratinocyte adhesion and proliferation are related mechanisms since keratinocytes must be attached to the basal membrane in order to divide.

### In vitro fibroblast proliferation

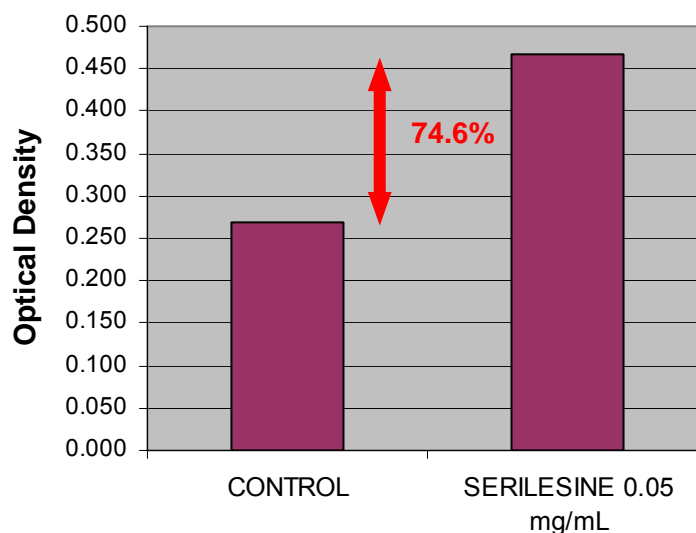
Cell viability is evaluated through an MTT assay. The key component is 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or MTT, which is yellowish in solution. Mitochondrial dehydrogenases of viable cells cleave its tetrazolium ring, leading to the formation of purple crystals which are insoluble in aqueous solution. The crystals are redissolved in acidified isopropanol and the colour in the resulting purple solution is quantified spectrophotometrically. An increase or decrease in cell number results in a proportional change in the amount of formazan formed, indicating the degree of inhibiting or stimulating cell growth.

Trypsinised fibroblast from human skin are incubated for 48 hours in microplates with SERILESINE® (0.05 mg/ml), Minimal Essential Medium-Na-Piruvate and 5% Fetal calf Serum (FCS). After the incubation with the tested substance and after medium substitution with fresh medium and MTT, cells are incubated for 3 hours at 37 °C. Then cells are washed to eliminate MTT excess. The fluorimeter reading is performed at 540 nm wavelength.

A culture of fibroblasts was treated with 0.05 mg/mL SERILESINE® and results were measured after 48 hours. Results are given as optical density (O.D.), that is proportional to cell number:

	CONTROL	SERILESINE® 0.05 mg/ml
Mean value (O.D.)	0.268	0.468
Stddev (O.D.)	0.030	0.017

### Cell growth



**Figure 9.** Fibroblast proliferation

After 48 h, incubation with the hexapeptide induced a 74.6% increase in fibroblast proliferation.

## Ex vivo

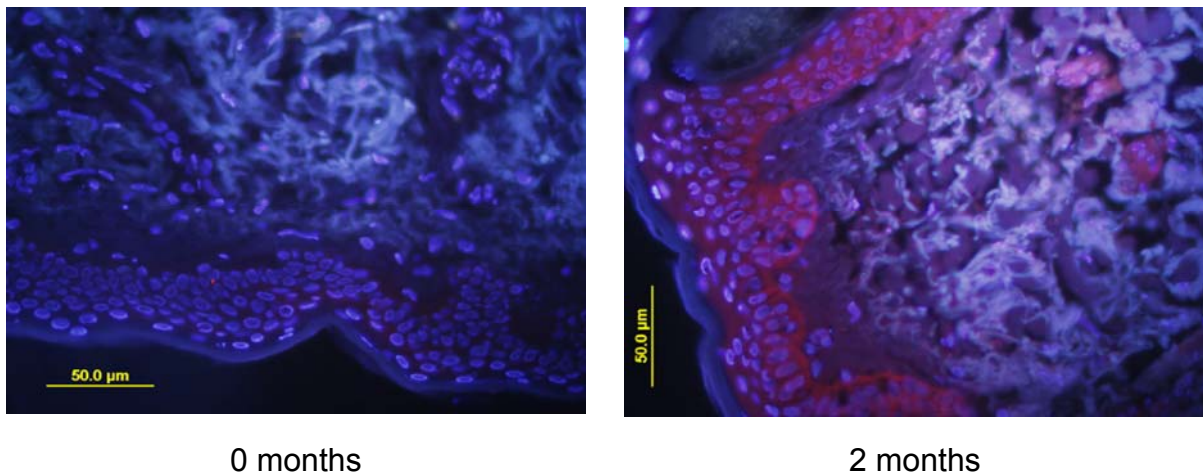
### Histochemical study of human skin biopsies

SERILESINE<sup>®</sup> was tested in order to determine its effects on the basement membrane proteins and on hemidesmosome formation. This test is a replicate of the *in vitro* test, this time using a panel of volunteers.

Laminin-5 and  $\alpha 6$ -integrin expression was determined by immunohistochemistry in skin biopsies of three patients, before and after a two-month treatment with a cream containing 0.005% SERILESINE<sup>®</sup>. Hemidesmosome formation was observed by Transmission Electron Microscopy with the same study conditions.

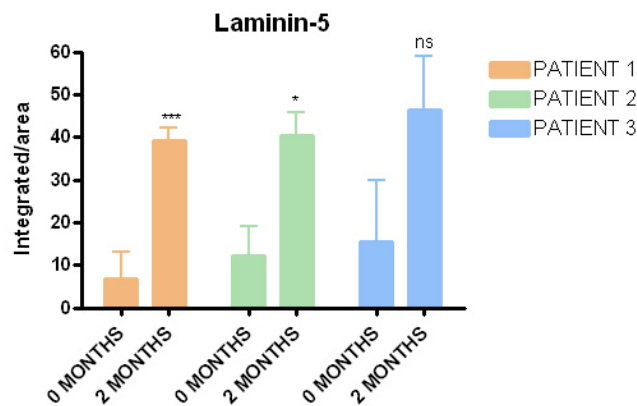
### Stimulation of Laminin-5 production (III)

SERILESINE<sup>®</sup> showed to increase Laminin-5 synthesis in the basement membrane zone of the biopsies.



**Figure 10.** Red Immunofluorescent stain for Laminin-5 in skin biopsies of Patient 1

The fluorescence quantification was calculated by image analysis with Metamorph software. For all patients, Laminin-5 expression is extremely higher after two months of SERILESINE<sup>®</sup> treatment, with a **305%** average increase (Fig. 11).

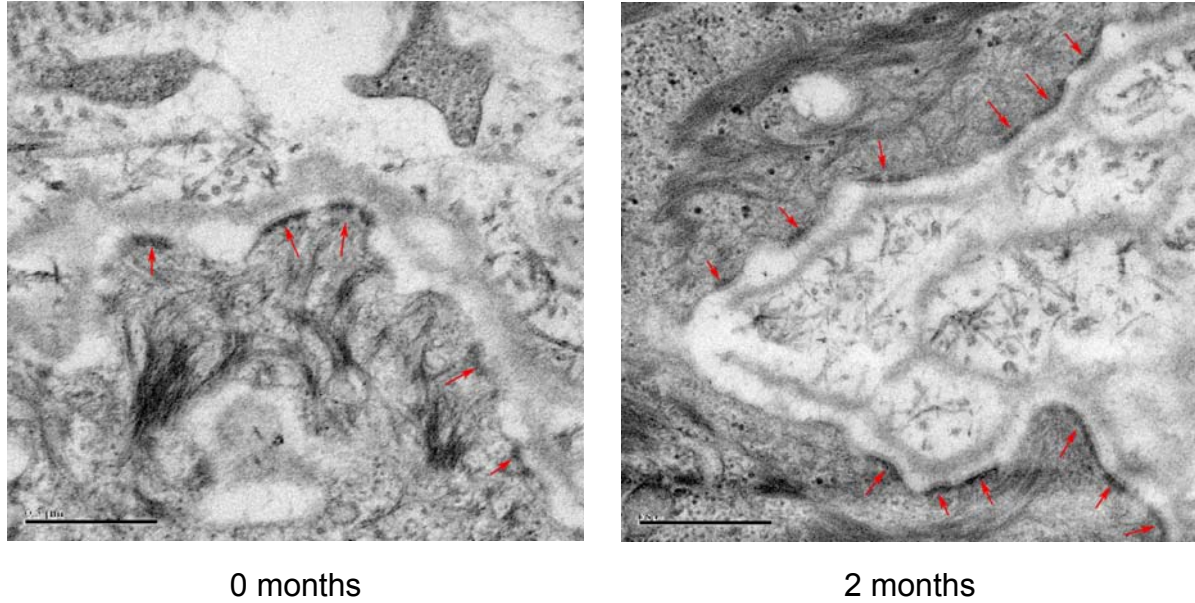


**Figure 11.** Fluorescence quantification of Laminin-5 expression in skin biopsies (\* P<0.001; \*\* P<0.05; ns: no significance)



**Stimulation of hemidesmosome formation (II)**

Hemidesmosomes of at least three different fields of each sample were counted. The samples treated with SERILESINE® showed to qualitatively increase the number of hemidesmosomes, compared to the untreated samples (Fig. 14).



**Figure 14.** Transmission electron microscopy observation of hemidesmosomes in skin biopsies of Patient 2

The results of this *ex vivo* test confirm the benefits of SERILESINE® in increasing the levels of basement membrane proteins and also validate our *in vitro* testing.

## ***In vivo***

### **In vivo evaluation of skin appearance**

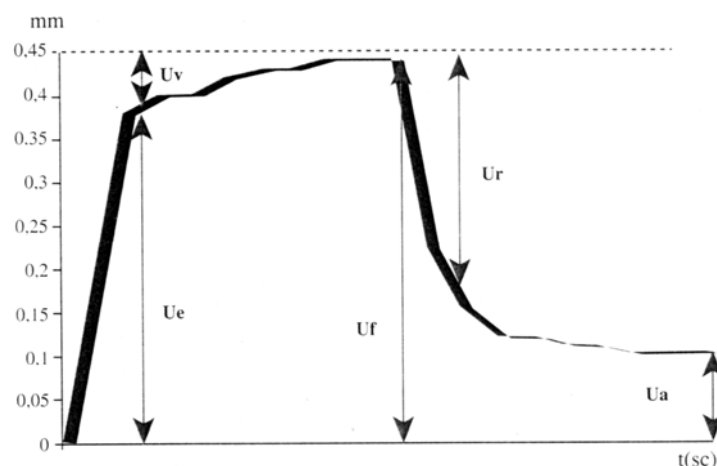
SERILESINE<sup>®</sup> has been tested *in vivo* on a group of 20 female volunteers, aged 18 to 70. A cream containing 0.005% SERILESINE<sup>®</sup> was applied twice a day during 60 days on the hip and thigh area. Several parameters were measured instrumentally or visually and physically evaluated by a dermatologist.

### **Skin elasticity**

The elasticity measurements are performed with a Cutometer<sup>®</sup>. For this test, a suction cycle of 1 second at 500 mBar followed by a release cycle of one second was selected.

In elastometric measurement, the skin surface is aspirated from the depression induced by the machine into the aperture of the elastometer's measuring probe. The depth of the skin penetration inside the probe is measured by an optic sensor. Cutaneous elasticity reflects the skin's potential capacity (measured in mm) for retraction.

A graph is obtained that represents the deformation curve of skin undergoing aspiration, and includes two components (see the figure).



- An elastic component ( $U_e$ ), which corresponds to the part of the curve that rises rapidly, and is reversible to the deformation;
- A plastic component ( $U_v$ ), which corresponds to the part of the curve that rises slowly and is not completely reversible to the deformation.

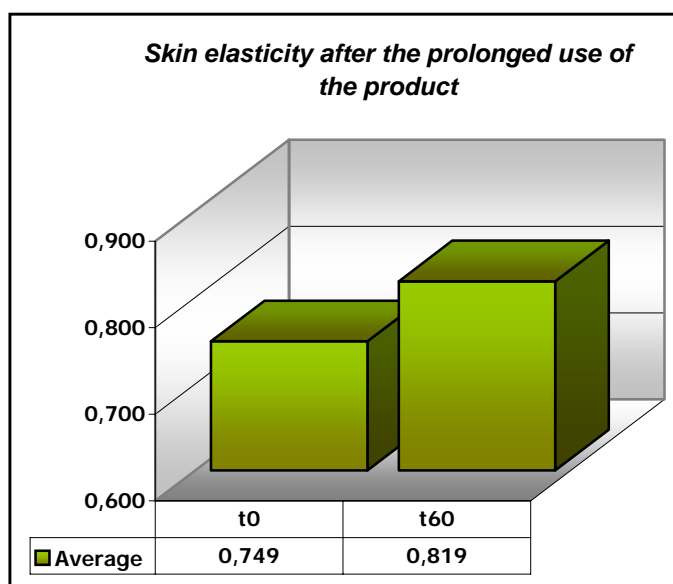
The total distension of the skin obtained at the end of an aspiration cycle is defined as skin extensibility ( $U_f$ ). During the releasing phase, the quantity of deformation remaining in the skin can be observed ( $U_a$ =residual deformation). Cutaneous elasticity is defined as the ratio:

$$\text{Elasticity} = \frac{U_f - U_a}{U_f}$$

It represents the recovery degree of the maximum deformation reached, whose values range between 0 and 1 (maximum elasticity).

The experimental measurements, as well as the statistical analysis performed on the data, are shown below.

<i>Skin elasticity after the prolonged use of the product</i>		
<i>Vol. Ref</i>	t0	t60
01LF	0.829	0.903
02MA	0.808	0.818
03TG	0.800	0.850
04FA	0.706	0.800
05SS	0.862	0.994
06DM	0.667	0.714
07GR	0.706	0.724
08SA	0.850	1.000
09FS	0.625	0.772
10AA	0.806	0.905
11CS	0.895	0.923
12MG	0.647	0.818
13IS	0.865	0.865
14PE	0.688	0.714
15SS	0.765	0.765
16CR	0.704	0.886
17SP	0.688	0.714
18DM	0.667	0.772
19DL	0.692	0.714
20NM	0.714	0.720
<i>Average</i>	0.749	0.819
Dev. STD	0.839126	0.0968066
Test t student	sign 99% (val. est. + -2.81)	3.6072292 yes



Skin elasticity, as measured with a Cutometer<sup>®</sup>, is significantly increased after 60 days.

### Skin compactness

A dermatologist evaluated skin compactness by focusing on parameters such as skin density and uniformity and assigned a score to enable statistical analysis:

Grade 1 – insufficient compactness,

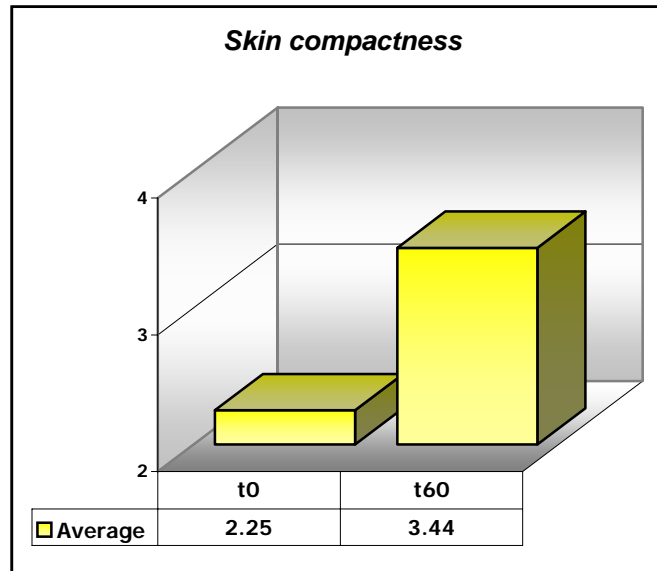
Grade 2 – sufficient compactness,

Grade 3 – fairly good compactness,

Grade 4 – good compactness

The scoring table of the dermatologist is enclosed, as well as the statistical analysis performed on the data. Since the measurements are non parametric (which means we don't know the underlying distribution of the variable measured), they are analysed using Friedman's Test.

Vol. Ref	Skin compactness	
	t0	t60
01LF	Grade 2	Grade 3
04FA	Grade 2	Grade 4
05SS	Grade 3	Grade 4
06DM	Grade 2	Grade 4
08SA	Grade 3	Grade 4
10AA	Grade 3	Grade 4
11CS	Grade 2	Grade 3
12MG	Grade 2	Grade 3
13IS	Grade 2	Grade 3
14PE	Grade 1	Grade 3
15SS	Grade 3	Grade 4
16CR	Grade 3	Grade 4
17SP	Grade 2	Grade 2
18DM	Grade 2	Grade 4
19DL	Grade 2	Grade 3
20NM	Grade 2	Grade 3
<b>Average Grade</b>	<b>2.25</b>	<b>3.44</b>



Several volunteers were not valid for evaluation because their initial value was already Grade 4 and they affected the statistical analysis.

Skin compactness, according to the dermatologist's evaluation, is increased after 60 days.

Applying Friedman's test, the data for the intermediate timepoints (15 days and 30 days) is determined to be non significant and is eliminated from the test. The data for the final timepoint is significant:

IC 95% values > 1.07695899	t60-t0
	1.19
	<b>YES</b>

### Skin tonicity

A dermatologist evaluated skin tonicity by evaluating skin consistency and assigned a score to enable statistical analysis:

Grade 1 – insufficient tonicity,

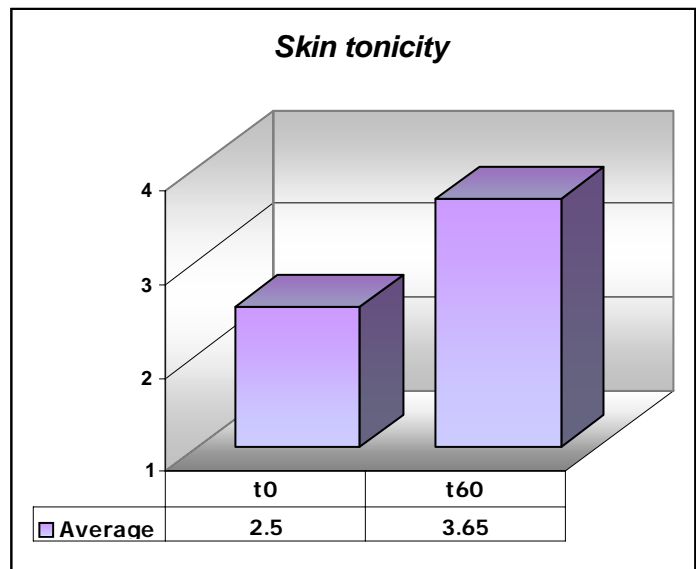
Grade 2 – sufficient tonicity,

Grade 3 – fairly good tonicity,

Grade 4 – good tonicity

The experimental measurements, as well as the statistical analysis performed on the data, are shown below. Since the measurements are non parametric they are analysed using Friedman’s Test.

Vol. Ref	Skin tonicity	
	t0	t60
01LF	Grade 2	Grade 4
02MA	Grade 3	Grade 4
03TG	Grade 4	Grade 4
04FA	Grade 2	Grade 4
05SS	Grade 4	Grade 4
06DM	Grade 2	Grade 4
07GR	Grade 4	Grade 4
08SA	Grade 3	Grade 4
09FS	Grade 3	Grade 3
10AA	Grade 3	Grade 4
11CS	Grade 2	Grade 4
12MG	Grade 2	Grade 3
13IS	Grade 2	Grade 4
14PE	Grade 1	Grade 4
15SS	Grade 3	Grade 3
16CR	Grade 3	Grade 3
17SP	Grade 2	Grade 3
18DM	Grade 2	Grade 3
19DL	Grade 2	Grade 4
20NM	Grade 2	Grade 3
<b>Average grade</b>	<b>2.50</b>	<b>3.65</b>



Several volunteers (3) presented an initial grading of 4, but their exclusion did not affect the statistical analysis so they were taken into account for the calculation of averages.

Skin tonicity, according to the dermatologist’s evaluation, is increased after 60 days.

Applying Friedman’s test, the data for the intermediate timepoints (15 days and 30 days) is determined to be non significant and is eliminated from the test. The data for the final timepoint is significant:

IC 95% values > 1.07695899	t60-t0
	1.15
	<b>YES</b>

### Skin smoothness

A dermatologist evaluated skin smoothness by focusing on the absence of scales and assigned a score to enable statistical analysis:

Grade 1 – insufficient smoothness,

Grade 2 – sufficient smoothness,

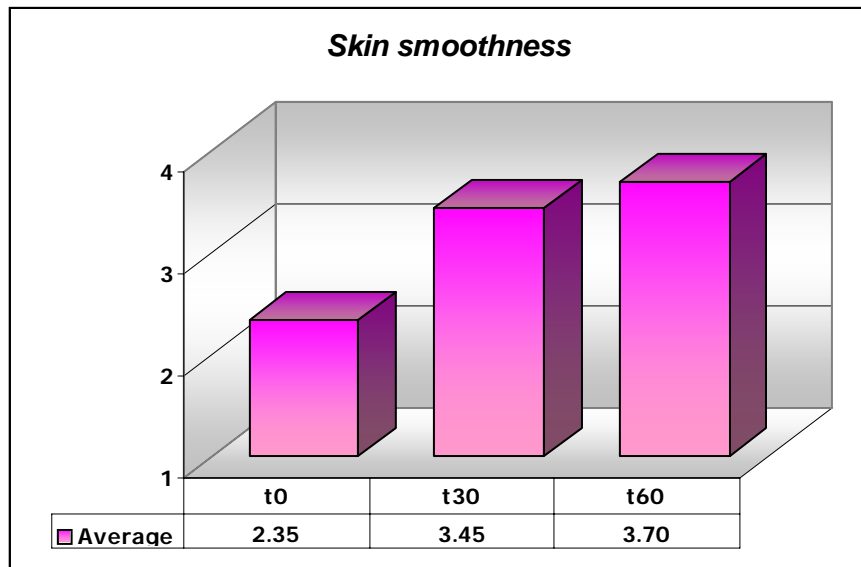
Grade 3 – fairly good smoothness,

Grade 4 – good smoothness

The experimental measurements, as well as the statistical analysis performed on the data, are shown below. Since the measurements are non parametric they are analysed using Friedman’s Test (timepoint “15 days” resulted non significant and is eliminated).

<i>Vol. Ref</i>	<i>Skin smoothness</i>		
	t0	t30	t60
01LF	Grade 3	Grade 4	Grade 3
02MA	Grade 3	Grade 4	Grade 4
03TG	Grade 2	Grade 4	Grade 4
04FA	Grade 2	Grade 4	Grade 4
05SS	Grade 3	Grade 4	Grade 4
06DM	Grade 2	Grade 4	Grade 4
07GR	Grade 3	Grade 4	Grade 4
08SA	Grade 3	Grade 4	Grade 4
09FS	Grade 3	Grade 3	Grade 4
10AA	Grade 3	Grade 3	Grade 4
11CS	Grade 2	Grade 2	Grade 3
12MG	Grade 2	Grade 3	Grade 3
13IS	Grade 3	Grade 4	Grade 4
14PE	Grade 2	Grade 4	Grade 4
15SS	Grade 2	Grade 4	Grade 4
16CR	Grade 2	Grade 3	Grade 4
17SP	Grade 2	Grade 2	Grade 3
18DM	Grade 2	Grade 3	Grade 3
19DL	Grade 1	Grade 2	Grade 3
20NM	Grade 2	Grade 4	Grade 4
<b>Average grade</b>	<b>2.35</b>	<b>3.45</b>	<b>3.70</b>

IC 95% values > 1.07695899	t30-t0	t60-t0
	0.45	1.35
	<b>YES</b>	<b>YES</b>



Skin smoothness, according to the dermatologist's evaluation, is increased after 30 days.

#### **Evaluation of the dermis density (10% SERILESINE<sup>®</sup> Solution)**

A panel of 20 female volunteers aged 55 to 64 (average 62) used a cream containing 10% SERILESINE<sup>®</sup> Solution twice daily during 54 days. The volunteers applied a placebo cream on one side of the face and the cream containing SERILESINE<sup>®</sup> on the other.

Measurements were made using the high frequency echograph Dermascan C<sup>®</sup> 2D. The measurement principle is that of the echograph: an ultrasound beam is emitted by piezo-electric ceramics. This beam is partially reflected by the interface separating two mediums of different ultrasound impedance. At the interface between two types of tissue, the wave will be refracted, and part of the wave will be reflected back and detected by the apparatus. How much is reflected depends on the densities of the respective tissues. The ultrasound used is equipped with a 20 MHz probe, which is applied directly to the skin. A contact gel provides homogeneous diffusion of the signal.

This method allows the bi-dimensional visualization of the skin on the epidermis and dermis. It is also possible to measure skin thickness (epidermis and dermis) and evaluate dermis density. Therefore, the effect of an anti-aging product can be evaluated. The accuracy of this method is estimated to be 2%.

An image analysis software is used to calculate dermis density. An increase in the dermis density characterises a redensifying effect of the tested product.

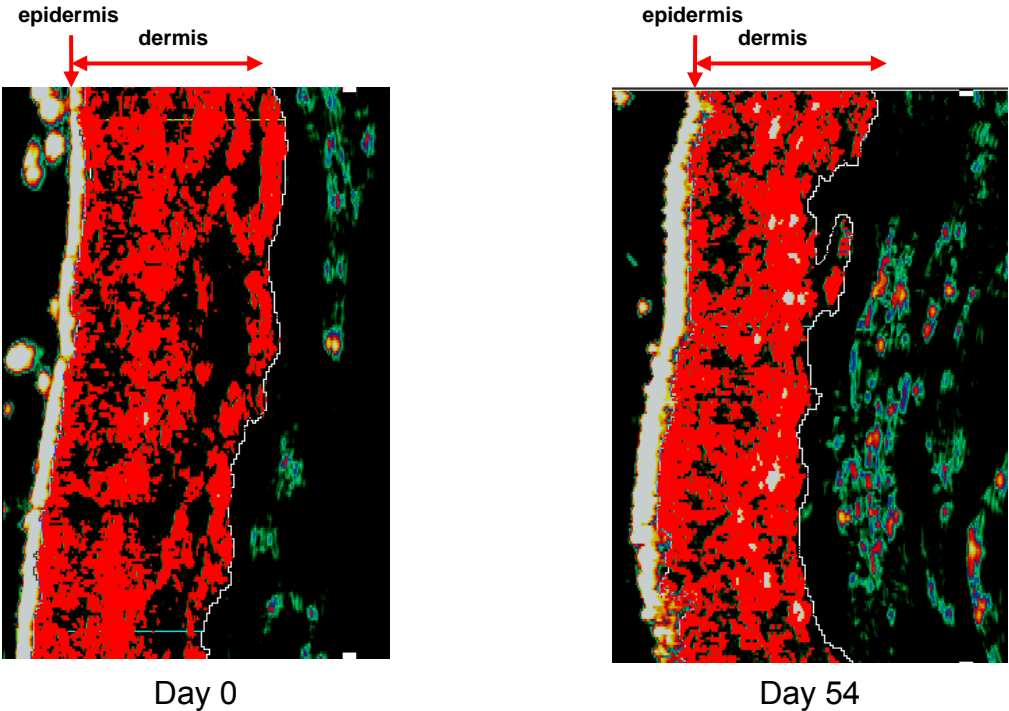
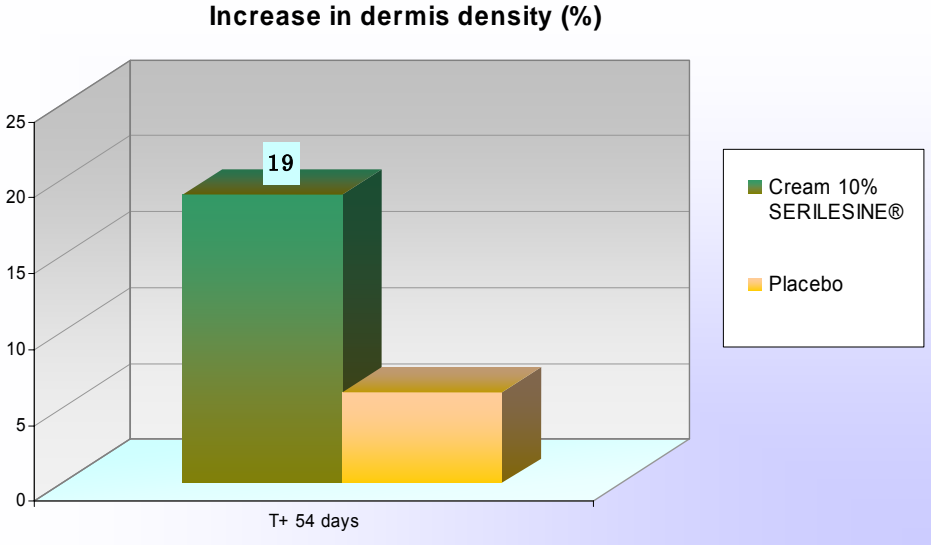


Figure 15. Example of obtained image for volunteer #11

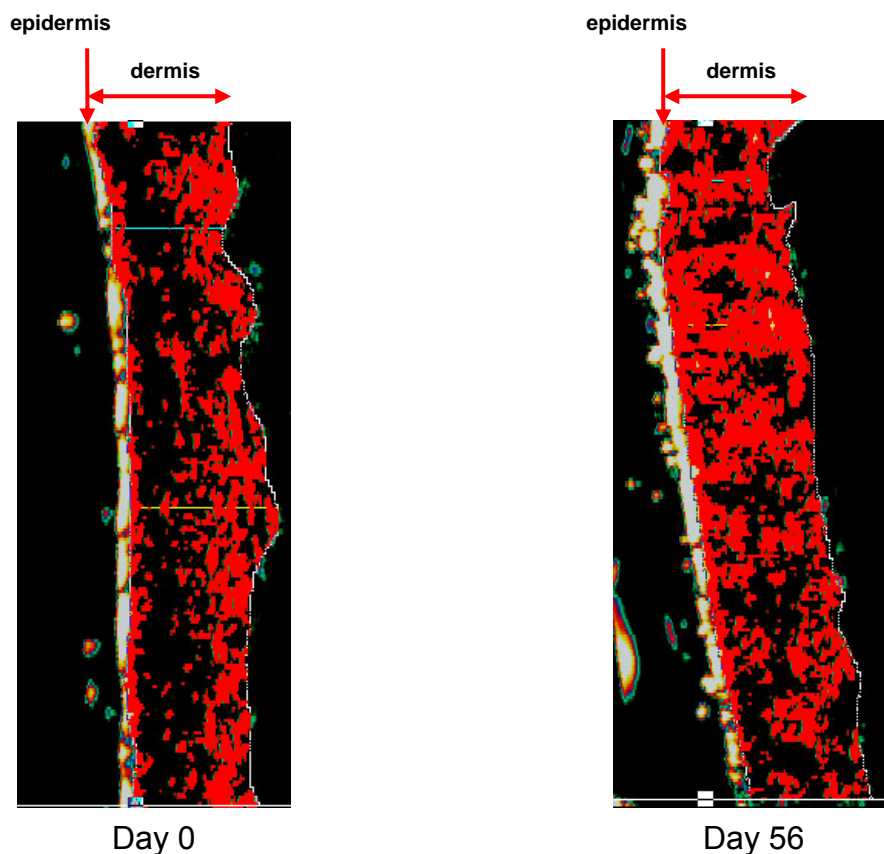
After 54 days, the cream containing SERILESINE® had induced a long-term **redensifying effect of 19%**, compared to the measure at Day 0. The placebo did not induce a significant redensifying effect.



### Evaluation of the dermis density (1% SERILESINE<sup>®</sup> Solution)

A panel of 20 female volunteers aged 47 to 65 (average 56) applied a cream containing 1% SERILESINE<sup>®</sup> Solution on the face twice daily during 56 days. Measurements of dermis density were made using the high frequency echograph Dermascan C<sup>®</sup> 2D. An increase in the dermis density characterises a redensifying effect of the tested product.

After 56 days a significant increase was observed: the cream containing 1% SERILESINE<sup>®</sup> had induced a **long-term redensifying effect of 6%**.



**Figure 16.** Example of obtained image for volunteer #22

## CONCLUSIONS

The adhesion between epidermal cells and the basement membrane starts to decrease from the age of 30. This loss of contact is related to the structural and functional changes associated with aging.

SERILESINE<sup>®</sup> is able to fight the degeneration of the dermo-epidermal junction caused by aging. It replenishes this layer of the skin, boosting synthesis of Laminin-5 and improving proliferation of keratinocytes and fibroblasts. The new synthesis of Laminin-5, a protein that is decreased in mature skin, returns the skin to a youthful stage.

In the first place, SERILESINE<sup>®</sup> stimulates the synthesis of Laminin-5, favouring the cohesion between dermis and epidermis, which improves the elasticity and tonicity of the skin.

Secondly, the hexapeptide increases skin cell proliferation and adhesion, and induces a significant increase in the dermis density, which macroscopically results in an improvement in skin compactness and smoothness, due to an increase in the number of cell layers and their binding strength.

These findings suggest that SERILESINE<sup>®</sup> is a good candidate to be considered for cosmetic formulations with restructuring activity.

## GENERAL PRODUCT INFORMATION

Trade name	SERILESINE <sup>®</sup> SOLUTION GC
Product code	P07-PD060

## INGREDIENTS

INCI name	CAS No	EINECS No
WATER (AQUA)	7732-18-5	231-791-2
GLYCERIN	56-81-5	200-289-5
HEXAPEPTIDE-10	N.L. <sup>a</sup>	N.L. <sup>a</sup>
CAPRYLYL GLYCOL	1117-86-8	214-254-7

<sup>a</sup> Not Listed

*Note: Graphs and photographs are available for customer use provided that the final product contains the same concentration of active as the formulations in our tests. Customers must request written permission for use of the graphic material and/or ingredient tradenames to Lipotec. Customers are responsible for compliance with local and international advertising regulations.*

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