Cosmetic Active Ingredients

MATRIXYL® 3000

sederma

ENGLISH

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MATRIXYL® 3000

SEDERMA Patents and publications: FR 2 975 904 ; WO 2012/164488 ;
WO 2005/048968 ; EP 1 686 957 ; EP 2 714 001 ; US 6,974,799;
JP 4256389 ; CN 1893911 ; KR101045658

RESTORES SKIN'S METABOLISM OF YOUTH THANKS TO THE POWER OF MATRIKINES

SYNOPSIS

Description: 100 ppm of Palmitoyl-GHK and 50 ppm of Palmitoyl GQPR (synthetic peptides) in a preservative-free hydroglycolic solution.

INCI Name: Glycerin (and) Water (Aqua) (and) Butylene Glycol (and) Carbomer (and) Polysorbate-20 (and) Palmitoyl Tripeptide-1 (and) Palmitoyl Tetrapeptide-7

China IECIC Listed

Demonstrated cosmetic activity

In vitro

➢ ***Study on senescence markers:*** New

On senescent fibroblasts :

- SA β-galactosidase (fluorescence) : - 56%, p<0.01
- Progerin (RNA) : - 27%, p<0.05 *vs*. lamin A (RNA) : + 4%, n.s.

➢ ***Study of de novo matrix synthesis by human fibroblasts:***

Synergistic effect of the combination of Pal-GQPR plus Pal-GHK

- Collagene I: +258% **MATRIXYL® 3000**
- Fibronectin: +164% **MATRIXYL® 3000**
- Hyaluronic acid: +179% **MATRIXYL® 3000**

➢ ***Gene activation study (DNA Array method).***

On SkinEthic® epidermis: 20 genes positively activated of which:

- EGF, PDGF, Rho, Rho GTP, α-catenin, for proliferation, migration
- Fibronectin, laminin, hemisdesmosomal protein, for cell installation
- VEGF, ephrin, for epidermal function

On fibroblasts: 15 genes positively activated of which:

- Procollagen, Lysyl oxidase, Fibronectin, MMP1, Tenascin, Syndecan, CD 44, for matrix synthesis and structuring

In vivo

➢ ***Tests on female panellists***

24 volunteers applied **MATRIXYL® 3000** twice daily, *vs*. placebo, for 2 months to compare **MATRIXYL®3000** *vs.* Placebo:

- reduction in main wrinkle depth (-19.9%) and volume (-23.3%),
- reduction in roughness (-16%),
- reduction in complexity (-16.2%), “lifting” parameter,
- decrease in the area occupied by deep wrinkles (>200µm) (-39.4%), -37% decrease in density,
- increase in skin tone (+15.5%).

➢ ***Tests on male panellists***

39 volunteers applied **MATRIXYL® 3000** twice daily, *vs*. placebo, for 2 months:

- mean reduction in principal wrinkle depth: 10.2%,
- mean reduction in principal wrinkle volume: 17.1%,
- reduction in the area covered by deep wrinkles (>200µm): 29.4%,
- decrease in the density of the principal sulci: 30.4%,
- decrease in roughness: 8.4%,
- increase in wrinkle spread angle: 5.4%

➢ ***Tests on female panellists***

28 volunteers applied **MATRIXYL® 3000** in a cream containing 3% **MATRIXYL® 3000**, compared to placebo for 2 months on half of their face and one of their forearms:

- SLEB analysis (by high resolution ultrasound):
  - ⇒ Increased SLEB density: on the internal surface of the forearm: +15.2%; (p<0.01 *vs*. placebo) and on the external surface +15.1% (p<0.01 *vs*. placebo)
  - ⇒ Reduced SLEB depth: on the internal surface of the forearm: -11%; (p<0.01 *vs*. placebo) and on the external surface -14.4% (p<0.01 *vs*. placebo)
  - ⇒ Improved SLEB depth: on the external surface of the forearm: gain of 5.5 years after 2 months.
- Analysis of dermal fibre structure (by confocal laser microscopy):
  - ⇒ Improvement in the structure of dermal fibres: +11.1% after 1 month and +13.9% after 2 months.

New

Ex vivo

➢ ***Study on skin explants***

Quantification of constituent, anchorage and cohesion proteins of the dermis and dermal-epidermal junction on skin explants (n=10, from 30 to 66 years):

- Treated explants MATRIXYL® 3000 vs. placebo :
  - ⇒ Collagen I: + 14.4%, p<0.01
  - ⇒ Collagen IV: + 6.4%, p<0.05
  - ⇒ Collagen VII: + 20.3%, p<0.01
  - ⇒ Collagen XVII: + 15.8%, p<0.01
  - ⇒ Nidogen I: + 14.5%, p<0.01
- Non-treated explants, **aged *vs*. young**:
  - ⇒ Collagen I: - 8%, p<0.2
  - ⇒ Collagen IV: - 11%, p<0.05
  - ⇒ Collagen VII: - 17%, p<0.01
  - ⇒ Collagen XVII: - 31%, p<0.01
  - ⇒ Nidogen I: - 15%, p<0.01

Recommendations for use:

- General details:
  - ⇒ recommended pH: 3.0 - 9.0
  - ⇒ Add MATRIXYL® 3000 to an emulsion preferably at between 25 and
  - ⇒ 50°C; depending on the type of formulations, temperatures of up to 80°C may be acceptable for a maximum time of 2 hours.
  - ⇒ Solubility: water soluble
- Recommended concentration for use: 3%

Toxicology:

Patch-test on humans
HET CAM
Neutral red test
HRIPT
Ames' test
Expert certification

CONTENT

05/2015/V9

1. INTRODUCTION

WOUND HEALING AND MATRIKINES

A concept born of the progress in our understanding of the mechanisms of skin repair after wounding [Professor MAQUART's team, France], "Matrikines" are peptide fragments whose sequence is generally less than or equal to 20 amino acids, derived from matrix proteolysis during cutaneous wound cleaning prior to healing.

Proteolysis of collagen, elastin and fibronectin fibers generates soluble peptides, veritable autocrine and paracrine messengers able to regulate, upstream, the sequence of events necessary for satisfactory wound healing [SIMEON *et al.*, 1999].

The hydrolysis products of the extracellular matrix, thus recycled as cell messengers, are generated and immediately available at the wound site: in consequence, the living tissue generates conditions conducive to fast healing with minimal energy expenditure.

Among the peptide sequences described as Matrikines are the hexapeptide VGVAPG [KAMOUN, 1995] derived from hydrolysis of elastin by elastase, the pentapeptide KTTKS [KATAYAMA,1993] derived from the proteolysis of α1-pro-collagen, the tripeptide GHK derived from the α2 chain of collagen I [MAQUART, 1990], and various other peptides derived from tropoelastin and laminin-5 [LOPES-MORATALLA, 1995].

All the peptides are able to exercise feedback control on the process of connective tissue renewal and cell proliferation, and are formed in larger quantities (during the process of skin repair) than under the normal circumstances of periodic tissue turnover.

However, with age and the progressive decrease in numerous cell functions, the systems become less effective. It will therefore be understood, for example, that protein modifications such as glycation disturb the cleavage recognition sites of the appropriate cleaning enzymes, thus slowing natural cutaneous turnover.
In that context, it is interesting to consider wrinkles as poorly repaired cutaneous lesions, hence the idea of restoring the dynamism of cell functions by topical application of matrikines.
The matrikine peptides may be incorporated in very effective cosmetic care products, provided that they are stabilized and rendered sufficiently fat-soluble for good cutaneous penetration.
The bio-mimetic characteristics of matrikines ensure a good safety profile positioning them favorably, relative to AHA and retinoids.

We selected two of our palmitoyl peptides that have already been widely studied and documented *in vitro* and *in vivo* and that have demonstrated very interesting synergistic properties in a new series of *in vitro* studies using fibronectin and hyaluronic acid.

The peptides selected are Pal-Glycyl-Histidyl-Lysine (Pal-GHK, **BIOPEPTIDE CL™** peptide) and Pal-Glycyl-Glutaminyl-Prolyl-Arginine (Pal-GQPR, **RIGIN™** peptide). The peptides have the following structures:

Figure 1: Pal GHK

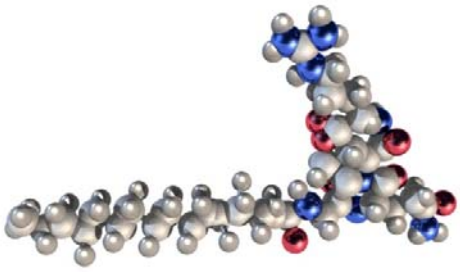


Figure 2: Pal GQPR

The results obtained *in vitro* and *in vivo* with the combination of the two peptides are presented herein.

2. REVIEW OF THE DATA ON PEPTIDE PAL-GHK
(SEDERMA BIOPEPTIDE CL™ dossier)

The biological activity, repairing the matrix tissue of the skin, of peptide Gly-His-Lys and certain of its derivatives, has previously been described in numerous reports and studies [MAQUART 1990, LINTNER 2000]. The results of those studies are summarized below.

2.1. Collagen and glycosaminoglycan synthesis

The studies were conducted on human fibroblast cultures. The main function of fibroblasts is production of the protein and glycoprotein components of the extracellular matrix, thus ensuring the cohesion and good maintenance of dermal connective tissue.

Table 1:
De novo collagen synthesis

Product	Concentration (µg/L)	^{3}H-Proline Incorporation	Collagen gain (%)
Pal-Gly-His-Lys	5800	3517	6,9
	2900	4996	51,9
	580	5348	62,6
	290	14842	351,3
	58	5948	80,8
Control	0	3289	0

The data generated showed an increase in collagen synthesis, up to 350%, and an increase in *de novo* glycosaminoglycan synthesis reaching +46%, for the concentration interval investigated (from 0.05 to 5ppm).

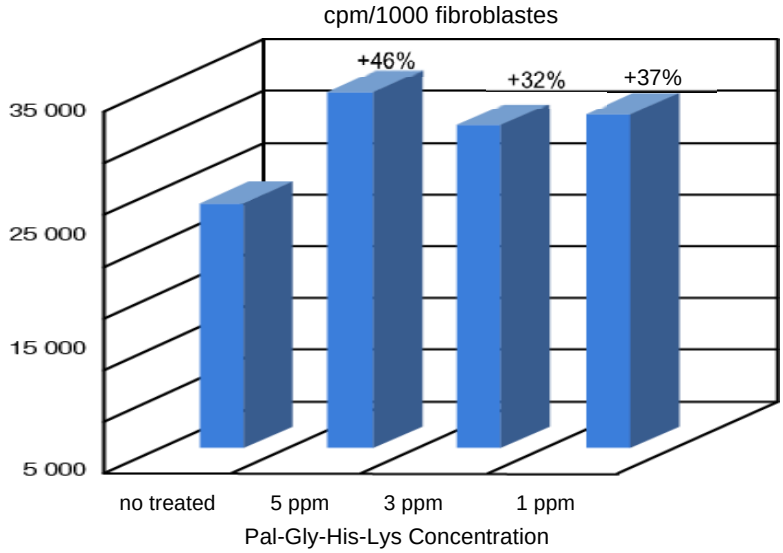


Figure 3: *de novo* GAGs synthesis

2.2. Collagen repair post-UVA irradiation

A dermal protective and repairing effect of Pal GHK on the collagen contained in the skin, post-UVA irradiation, was demonstrated [LINTNER and PESCHARD, 2000].
In those tests, the efficacy of the tripeptide Pal-Gly-His-Lys (5 ppm) was compared to that of retinoic acid (500 ppm), and the activities found to be equivalent.
Compared with the irradiated control (Photo 1), the biopsy specimens treated with 5% **BIOPEPTIDE CL™** (i.e. 5 ppm peptide Pal-GHK equivalent) and those treated with 500 ppm retinoic acid showed increased collagen fiber density in the presence of Pal-GHK (Photo 2) and a dermis with a high collagen content (relative to the non-irradiated control), with almost complete protection by retinoic acid at a concentration 100-fold higher than the Pal-GHK peptide.

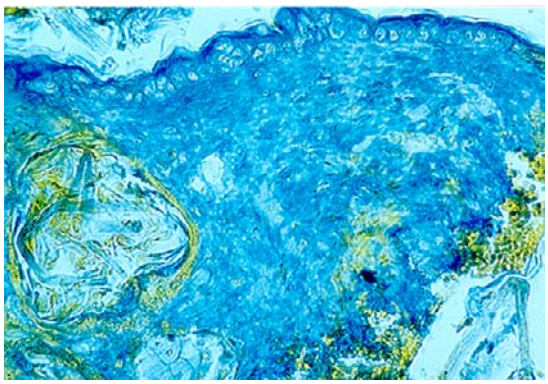


Photo 1: Skin section irradiated with UVA for one week.
The collagen fiber density is much lower than on a non irradiated skin (41% degradation) - (Magnification: x317)

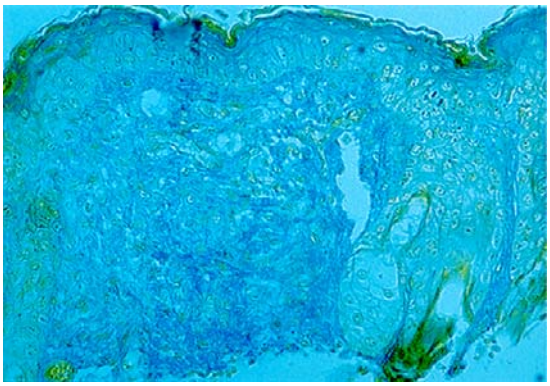


Photo. 2: Skin section after UVA irradiation and treatment with 5% **BIOPEPTIDE-CL** (i.e. 5 ppm Pal-GHK) for one week.
The collagen fiber density is greater than that for the UVA-irradiated sites. (Magnification: x317).

2.3. In vivo increase in skin thickness by ultrasonography

A clinical trial conducted on 23 subjects enabled demonstration of a significant increase in skin thickness (epidermis/dermis) following daily application of a cream formulation containing 4 ppm peptide Pal-GHK to the forearm for 4 weeks, *vs*. a placebo cream. The results are shown in the table below:

Table 2:
Time course of epidermal/dermal thickness (mm)

EPIDERMAL/DERMAL THICKNESS (mm)	BIOPEPTIDE™ CL (4%)		PLACEBO	
	T0	T28	T0	T28
Mean	1.26	1.31	1.25	1.25
	Statistical test for paired series			
Comparison T28 *vs*. T0	p<0.05 Significant difference		Non-significant difference	

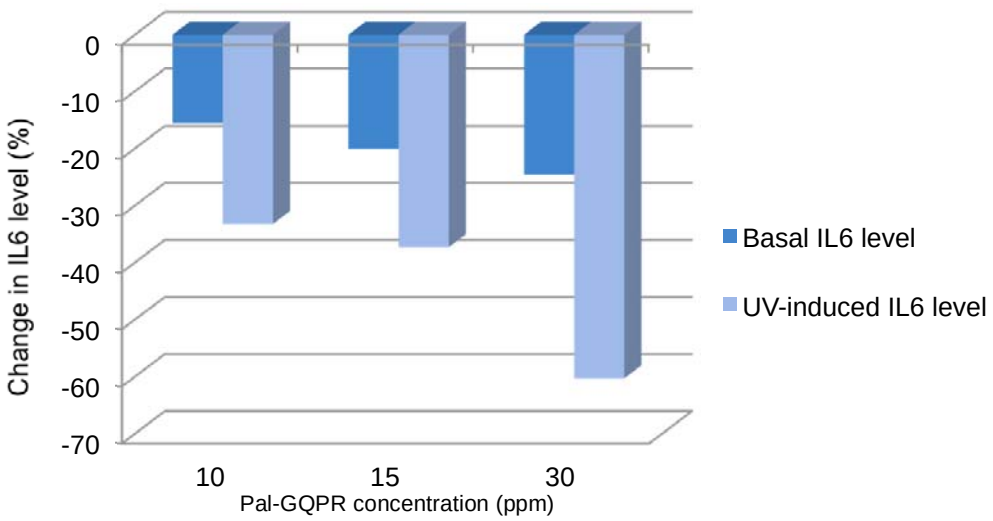
3. REVIEW OF THE DATA ON PEPTIDE PAL-GQPR

Tetrapeptide Gly-Gln-Pro-Arg is a natural fragment of immunoglobulin IgG endowed with various biological activities, immunomodulatory in particular. A number of *in vitro* and *in vivo* studies reported in **RIGIN™** and **EYELISS™** technical dossiers investigated those activities**.**

- ***Disequilibrium of cutaneous cytokines and ageing***

The equilibriums of cutaneous cytokines, particularly IL6, involved in the chronic inflammatory phenomena, have important consequences during the skin ageing process. This observation constituted the basis for research on a cosmetic peptide able to restore normal levels of cutaneous cytokines.

There is a strong correlation between the fall in DHEA with age and the increase in IL6. DHEA controls the circulating levels of the inflammatory cytokine. The objective to be achieved with peptide Pal-GQPR was therefore a reduction in IL6 levels in order to restore cutaneous cytokine equilibrium and enhance skin quality.
Peptide Pal-GQPR was shown to decrease IL6 secretion by keratinocytes in a basal setting and following exposure to 35 mJ/cm^2 of UVB irradiation.



IL6 level was also reduced in fibroblasts but the amplitude of the reduction was less since the basal secretion level of those cells is naturally lower

- ***Enhanced skin quality in vivo:***

A cutometric study was conducted on 17 subjects who applied a cream formulated with 15 ppm peptide Pal-GQPR to the face and neck for one month. A significant increase in firmness was observed with +19% for the face and +40% for the neck

Elasticity increased for both the face and neck, by 17% and 27%, respectively. The contralateral sides treated with placebo formulation did not show any significant improvement. Pal-GQPR also induced an increase in moisturisation (+24%).

Study of the skin surface (observation of the microdepression network) also showed that it was possible to obtain enhanced isotropy (+23%), a decrease in the deepest wrinkles (-56%) and an overall reduction in roughness (14%) after 15 days of application of the peptide. The set of changes yielded an image of a smoother rejuvenated skin.

4. *IN VITRO* DATA ON THE ACTIVITIES OF PAL-GHK AND PAL-GQPR

MATRIXYL® 3000 takes the Matrikine concept further by combining the tripeptide and tetrapeptide for a stronger anti-ageing reparative effect.

4.1. Effect of MATRIXYL® 3000 on senescence markers

The disease most often associated with skin ageing is "Progeria" (Hutchinson-Gilford Progeria Syndrome, or HGPS).

In this disease, lamin A, a protein necessary to provide structural support to the nuclear envelope, and thus involved in maintaining the stability of the nucleus and the structure of chromatin, is present in a truncated form called progerin.

However, the specific mechanism of the premature ageing that characterises this disease has not yet been determined. Lamins play a key role in skin regeneration. Lamins have been described as regulating the proliferation and differentiation of epidermal stem cells. Lamin dysfunction leads to a clear slowdown in cellular renewal, thereby triggering the ageing process (SCAFFIDI 2008).

Recent studies have demonstrated that progerin is also produced in small quantities in human cells and tissues (RODRIGUEZ 2009). However, the cause-effect relationship between progerin production and ageing in normal individuals has not yet been revealed. A recent study by CAO *et al*., (2011) suggest a synergistic relationship between the shortening of telomeres and progerin production in cellular senescence.

Cosmetic actives basing their anti-aging activity on the observation of pathological situations are rather seldom. The exceptional anti-ageing properties of **MATRIXYL® 3000** that have been repeatedly demonstrated *in-vitro* and *in-vivo* have led us to test the two-peptide combination of **MATRIXYL® 3000** on a model of replicative senescence to assess its effect on the accumulation of progerin induced by senescence. A well-known marker of cellular senescence was also studied: Senescence Associated β galactosidase (SA-β-GAL).

CULTURE PROTOCOL: REPLICATIVE SENESCENCE

Proliferative normal human fibroblasts (NHF) are cultivated in the presence of **MATRIXYL® 3000** (3% and 5% equivalents) on several cellular layers. Every 7 to 15 days, cells that have reached confluence are harvested by trypsination, counted and reseeded with the same cellular concentration. The culture is then monitored until the ability to proliferate is depleted, i.e., when the cells no longer divide and enter into an inactive state: replicative senescence.

a. Senescence Associated β galactosidase (SA-β-GAL)

PROTOCOL TO DETECT β-GALACTOSIDASE ACTIVITY

At the end of replicative senescence, SA-β-GAL cellular activity is assessed using an FDG (fluoresceine-di-β-galactopyranoside) substrate that, when cleaved by the enzyme, releases a quantifiable fluorescent form (YANG 2004). The fluorescence increases in proportion to the quantity of senescent cells.

RESULTS:

Table 3:

Study of SA-β–GAL activity after replicative senescence (n=4)

	Concentration	Mean activity relative to the SA-β-GAL (AFU/10^6 cells)	% variation *vs.* control
Control	-	34.08 ± 2.11	Reference
MATRIXYL® 3000	Eq. 3%	27.79 ± 3.38	-18%; p=0.08
	Eq. 5%	14.87 ± 2.38	-56%; p<0.01

As evidenced by the decrease in SA-β-GAL, treating fibroblasts with **MATRIXYL® 3000** clearly slows down cellular senescence.

b. Progerin

PROGERIN DETECTION PROTOCOL

At the end of replicative senescence, total RNA of cells treated with 3% and 5% of **MATRIXYL® 3000** are extracted and subjected to reverse transcription. Progerin expression is studied using quantitative PCR on these cDNA thanks to specific triggers (inspired by the research of RODRIGUEZ (2009) and CAO (2011)). The results are expressed as a ratio that represents the number of times the gene of interest is expressed with respect to an untreated control, weighted by the housekeeping gene (β-actin).

We also evaluated the expression relative to normal lamin A (non-mutated, non-truncated) to demonstrate specific progerin modulation in the presence of **MATRIXYL® 3000** and not modulation of all lamin forms.

Abnormal (HGPS) fibroblast cDNA is used as a positive detection control.

Ratio formula:

$$R = \text{Efficacy}^{-(\Delta Ct\ \text{product} - \Delta Ct\ \text{control})}$$

whereas: Efficacy = 1.95

ΔCt product = Ct (target gene)product – Ct (HKP)product

ΔCt control = Ct (target gene)control – Ct (HKP)control

HKP: "Housekeeping gene" or reference gene

A preliminary method development study enabled us to demonstrate that senescent fibroblasts have approximately 2.2 times as much progerin as young fibroblasts.

The results below show the variation in progerin after replicative senescence in fibroblasts in the presence of **MATRIXYL® 3000**.

RESULTS

Table 4:

Variation in progerin and normal lamin A expression after replicative senescence

		Ct β actin	Ct Progerin	Progerin ratio *vs.* β actin	Ct Normal lamin A	Ratio Normal lamin A *vs.* β actin
Control	-	16.36 ± 0.38	29.75 ± 0.51	1	19.91 ± 0.33	1
MATRIXYL® 3000	**3%**	16.69 ± 0.36	30.33 ± 0.41	0.84; p<0.05 **(-16%)**	20.28 ± 0.34	1.02; nsd
	5%	16.46 ± 0.16	30.38 ± 0.11	0.73; p<0.05 **(-27%)**	19.95 ± 0.21	1.04; nsd

Positive control = abnormal fibroblasts (HGPS): R= 132 for progerin and R= 0.47 for normal lamin A. nds = not significant difference.

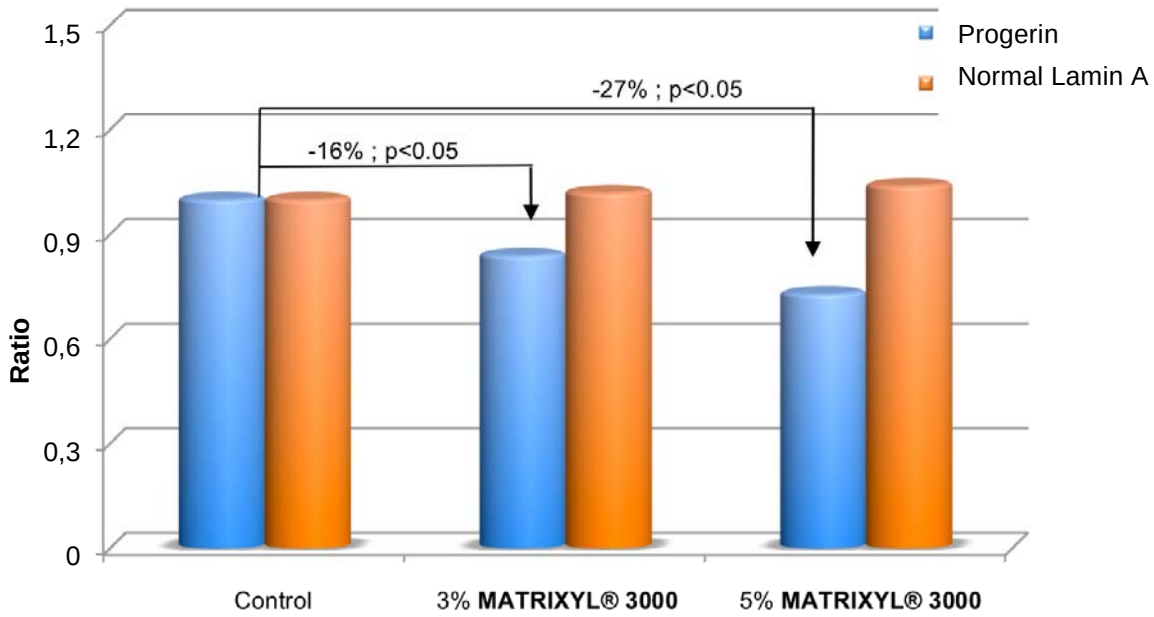


Figure 5: Variation in normal lamin A and progerin expression after replicative senescence of fibroblasts in the presence of **MATRIXYL® 3000**

Treating fibroblasts in replicative senescence by **MATRIXYL® 3000** seems to decrease progerin expression in a significant, dose-dependent manner. This diminished expression is specific to progerin because normal lamin A is not affected after **MATRIXYL® 3000** treatment.

N.B. The REUNERT (2012) study demonstrated that the severity of the illness of patients afflicted with HGPS is probably due to an increase in the ratio of truncated lamin (progerin) compared with normal lamin. This phenomenon also occurs during skin ageing, but to a much lesser extent, of course.

4.2. In vitro comparative study of the constituents of the matrix

The effects of various matrikines on stimulation of extracellular matrix reconstitution, with the matrikines used alone or in combination, were investigated.
An *in vitro* comparative study was conducted on the main connective tissue markers: *de novo* collagen I, fibronectin and hyaluronic acid synthesis.

PROTOCOL

Normal human fibroblasts (NHF) were cultured in appropriate DMEM medium in the presence of fetal calf serum.
When cell confluence had been obtained, the culture medium was replaced and the cells were incubated without serum but in the presence of the peptides under study for 72 hours. Each test was conducted in triplicate.

The following peptides were tested: Pal-KTTKS, Pal-GHK (Palmitoyl-Glycyl-Histidyl-Lysine), Pal-GQPR (Palmitoyl-Glycyl-Glutaminyl-Prolyl-Arginine) and a combination of the two.

The control media consisted in the culture medium alone or the culture medium plus a positive control product, in this case 10^{-6}% TGFβ.
The cultures were incubated in the presence of vitamin C and rising quantities of each peptide under study for 72 hours.
Matrix proteins (collagen I and fibronectin) were assayed by the ELISA method while hyaluronic acid was assayed by a colorimetric method.

RESULTS

The results presented below were mean values for n = 3 different tests.

Table 5:

De novo collagen I synthesis after NHF incubation for 72 hours

PRODUCT	Concentration	COLLAGEN I
TGFβ	10^{-6}%	102%
Pal-GQPR	0.5 ppm 1.5 ppm 2.5 ppm 3.5 ppm	-3% -1% -18% 57%
Pal-GHK	1 ppm 3 ppm 5 ppm 7.5 ppm	-3% -5% 3% 6%
MATRIXYL® 3000 Pal-GHK+Pal-GQPR	1% (1.5 ppm) 3% (4.5 ppm) 5% (7.5 ppm) 7.5% (11 ppm)	5% 35% 49% 258%

The expected results were obtained in the presence of TGFβ with 102% stimulation of collagen I synthesis.
A dose effect was also observed for the mixture of the 2 peptides Pal-GHK and Pal-GQPR of **MATRIXYL® 3000**.

It is highly remarkable to observe that the combination of the 2 peptides, Pal-GHK and Pal-GQPR, yielded synthesis stimulation values higher than those that would be expected on the basis of simple addition.

Table 6:

De novo fibronectin and hyaluronic acid synthesis after NHF incubation for 72 hours

PRODUCT	Concentration	FIBRONECTIN	HYALURONIC ACID
		Synthesis stimulation	
TGFβ	10^{-6}%	194%	132%
Pal-GQPR	0,5 ppm	2%	8%
	1,5 ppm	8%	12%
	2,5 ppm	26%	18%
	3,5 ppm	47%	16%
Pal-GHK	1 ppm	1%	5%
	3 ppm	11%	25%
	5 ppm	-2%	9%
	7,5 ppm	5%	14%
MATRIXYL® 3000	1% (1.5 ppm)	3%	3%
Pal-GHK+Pal-GQPR	3% (4.5 ppm)	18%	14%
	5% (7.5 ppm)	64%	46%
	7.5% (11 ppm)	164%	179%

For the two tests, the positive control, TGFβ, induced 194% stimulation of fibronectin synthesis and 132% stimulation of hyaluronic acid synthesis.

Pal-GQPR only stimulated fibronectin synthesis, and to a more moderate degree. Combination of Pal-GQPR and Pal-GHK in **MATRIXYL® 3000** induced a synergy with 164% stimulation.

With regard to hyaluronic acid, the combination of the 2 peptides in **MATRIXYL® 3000** enabled a 179% gain in stimulation *vs*. the values of 16% and 14% obtained with the peptides separately

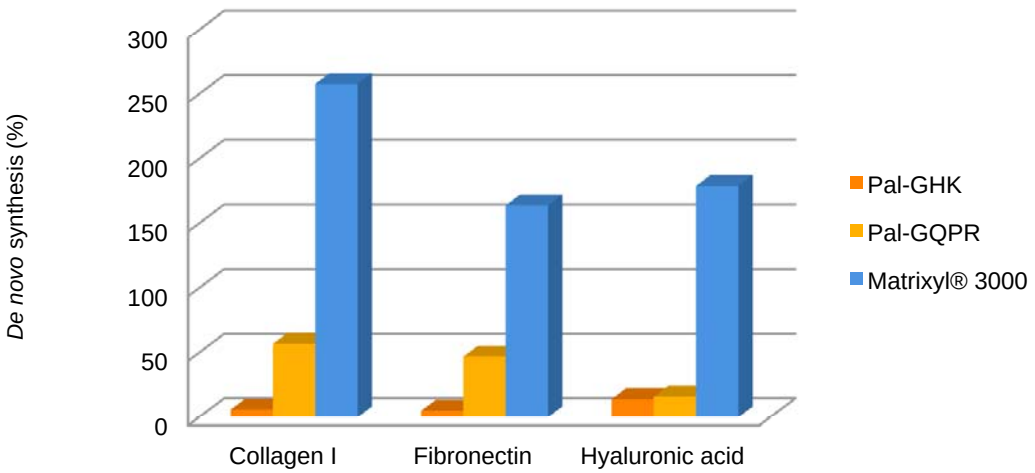


Figure 6: Maximum stimulation study (%)

4.3. DNA array study of epidermal and dermal gene regulation

The recently developed methods of molecular biology enable access to intracellular, functional and morphological changes induced by the substances to which the cell layer (fibroblasts or keratinocytes) or tissue (epidermis and synthetic epidermis) are exposed.
It is thus possible to define the profile of the method of action of a substance in terms of the genes activated or repressed in comparison with a control cell culture or tissue.
The gene activation profile of peptides Pal-GHK and Pal-GQPR was thus determined using a bank of 450 genes.
The comparative table below shows the genes up-regulated in reconstructed epidermis (Skin-Ethic® 3D-model) and those up-regulated in fibroblasts in a confluent cell layer.

Table 7:
Change in gene expression in the presence of various peptides

EPIDERMIS	**Percentage *vs*. control**	
	Pal-GQPR	**Pal-GHK**
Fibronectin receptor b ; integrin β_1 et α_4	206%	
Urokinase inhibitor (plasminogen inhibitor)	187%	
EGF response factor (=ERF1)	209%	
PDGF associated protein	175%	
PDGF subunit A	185%	
Rho HP1	209%	
90 HSP	177%	
Metallothioneins (MT1/H/L/I/R)	179%	
Granulocyte chemotactic protein GCP-2	278%	
Hemidesmosomal plaque protein	188%	
Neuromedin B receptor	187%	
Integrin α_6 precursor	181%	
Vascular endothelial growth factor (VEGF) receptor	187%	
Ephrin type-B receptor precursor	162%	
Epidermal filaggrin precursor		-53%
Fibronectin receptor beta subunit		-50%
Cytokeratin 10		-50%
Vascular endothelial		-40%
Alpha-catenin		178%
Laminin receptor binding protein		155%
Syndecan, Heparine Sulphate Proteoglycan Core protein		155%
Binding transcription factor; nucleoside diphosphate kinase		170%
EGF response factor		179%
Chemotactic protein		213%

The genes regulated in the same manner are those for functions associated with cell proliferation (PDGF associated protein and subunit, response factor ERF1), matrix remodelling (urokinase inhibitor, metallothioneins, lysyl oxidase), cell migration (HSP 90, Rho and GTPase) and cell attachment (fibronectin receptor)

Pal-GQPR also induced marked expression of a gene coding for chemotactic protein CGP-2, which recruits cleaning cells prior to wound healing, and the VEGF and ephrin receptor genes, which create conditions conducive to setup of cutaneous microvascularization and innervation, rendering the newly

synthesized epidermis fully operational (integrin-α-6 for keratinocyte installation on the basal lamina and hemidesmosomal plaque protein for cohesion of the corneocytic layer).

Pal-GHK activated rather less genes but its profile was more specifically oriented toward keratinocyte anchoring (alpha-catenin and laminin receptor) and differentiation (keratin 10). In addition, Pal-GHK increased the synthesis of extracellular matrix (syndecan, heparin sulfate glycoprotein).

It is thus clear that the combination of Pal-GHK and Pal-GQPR affords a complete profile of activated genes which contribute to cell proliferation, cleaning and turnover of the extracellular matrix, and anchoring of new cells for epidermal reconstruction. In addition, the combination summons the genes required for satisfactory vascularization and innervation in order to constitute a fully operational new tissue.

The profile characterized by the genes activated in fibroblasts (cf. table on the following page) showed that Pal-GHK stimulated numerous genes.

In particular, all the functions associated with *de novo* matrix synthesis were strongly expressed with:
TIMP1 (tissue inhibitor of metalloproteinase 1 precursor), procollagen 3, fibronectin precursor, syndecan and tenascin precursors and, lastly, cross-linking of the newly formed fibers by lysyl oxidase.
A pronounced effect on the markers of cell proliferation (cysteine fibroblast growth factor) and migration (tenascin, syndecan-4, FGF, Rho GDP) was also observed.

Table 8:
Change in gene expression in the presence of Pal-GHK

FIBROBLASTS	**Percentage *vs.* control**
	Pal-GHK
Alpha1 catenin	179%
CD44 antigen precursor	161%
Procollagen 3	186%
Fibronectin precursor	147%
Fibronectin receptor beta subunit	181%
Lysyl-Oxidase	155%
Tissue inhibitor of metalloproteinase 1 precursor	188%
Tenascin precursor	159%
Syndecan-4 precursor	161%
Tissue-type plasminogen activator precursor (TPA)	157%
Cysteine-rich fibroblast growth factor receptor	153%
Monocyte Chemoattractant protein	227%
RHO-GDP- dissociation inhibitor	146%
Tumor necrosis factor receptor	175%
90-kDa heat-shock protein beta	156%

CONCLUSION ON THE GENE ACTIVATION PROFILES

When the functions that can be potentially activated by the Pal-GHK and Pal-GQPR combination are reviewed, a very complete profile emerges with respect to matrix remodelling by *de novo* synthesis, proliferation of both keratinocytes and fibroblasts. This phenomenon is associated in a structuring of all the intercellular and inter-fibrillar connections accompanied by preparation of the newly formed tissue for *de novo* vascularization and innervation.

The combination of the two peptides activates complementary genes, arguing in favor of very good efficacy in a product designed to repair wrinkles.

5. *IN VIVO* STUDIES OF THE REJUVENATING EFFECT: ANTI-WRINKLE AND TONING EFFICACY

5.1. Comparaison de MATRIXYL® 3000 versus placebo

The clinical study had a duration of 2 months, 24 female volunteers (aged 42 to 67 years) free from a history of allergy, and skin lesions, and receiving no medicinal treatment liable to interfere with the results of the study were included in the protocol.

The anti-ageing effect was assessed using several methods:

- profilometry and image analysis
- photography
- cutometry

PROTOCOL

The cream application sites were crow's feet. Application was randomized, left side and right side of **MATRIXYL® 3000** *vs*. placebo. (Panellists: 24 volunteers, mean age: 56.1 years).

Panellists: 24 volunteers

MATRIXYL® 3000 and the excipient were incorporated at a concentration of 3% in a cream formulation (cf. Annex 1) that the subjects applied morning and night for 2 months, to the exclusion of all other anti-wrinkle, reparative, restructuring or regenerating products.

➢ ***Profilometry***

Cutaneous relief impressions were obtained by application of Silflo® gel to the crow's feet at the corners of the eyes. The gel polymerizes *in situ* and constitutes a “negative” impression of the irregularities of the skin surface following detachment.
The irregularities or wrinkles were automatically analyzed as digital images (HITACHI CCTV camera, Mountains Map software, version 3.0) following illumination of the impressions with light at a low angle of incidence (halogen light at 25°).

The parameters generated included the number of wrinkles, the mean or maximum depth of one of the main wrinkles (µm), the volume of one of the main wrinkles (mm^3), the percentage area occupied by deep wrinkles (>200 µm) or intermediate wrinkles (between 150 and 200 µm), the complexity (%) and density of the main wrinkles ($\mu m/cm^2$) and the roughness, now a conventional parameter.

For each parameter, mean values and the percentage change on the baseline value (T0) were calculated and appropriate statistical tests used for analysis (Student's t tests in the event of homogeneity of variance, T0 *vs*. T56, and Wilcoxon's tests for paired series in the event of non-homogeneity).

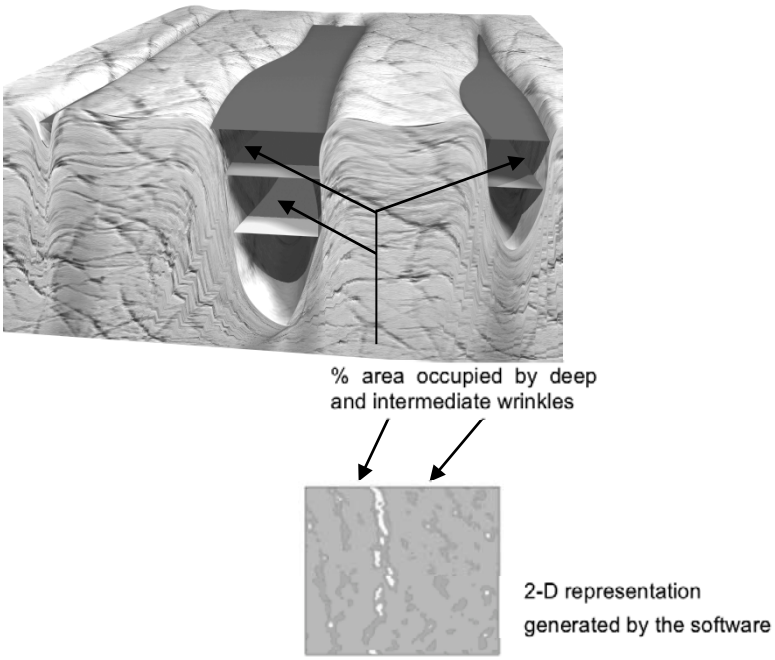
➢ ***Photographs***

A Coolpix 990 camera was used. Standardization of the photographs was ensured by positioning the volunteers using a chin and forehead support system.
Lighting was generated by a source at fixed position and at a constant distance from the object. Low angle of incidence lighting can be used to ensure optimum imaging of wrinkles and crow's feet.

➢ ***Cutometry***

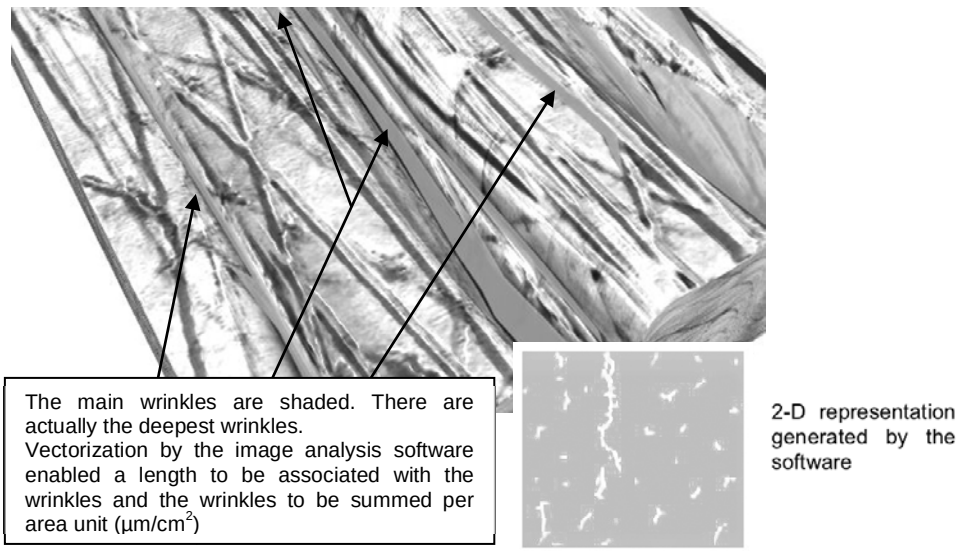
The cutometric determinations were conducted using a Courage and Khasaka SEM 474 Cutometer® fitted with a 2-mm probe
The following diagrams illustrate the significance of the determinations conducted:

a. Percentage area occupied by deep wrinkles (>200 µm)



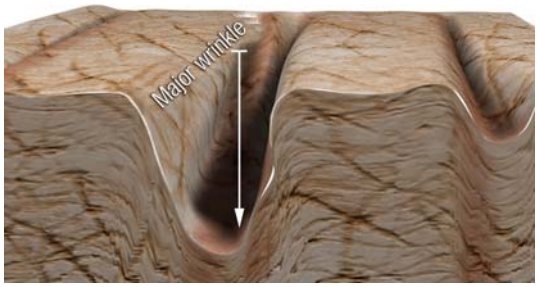
b. Density of the main wrinkles

This parameter reflects the length of the deepest wrinkles. The sum divided by the analysis area defines the density per area unit (µm/cm^2).

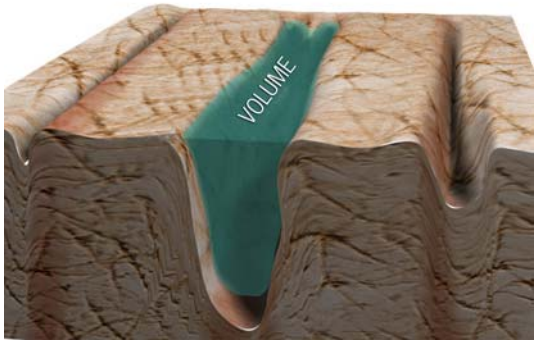


c. Variation in the mean depth and mean volume of the wrinkles

The two parameters reflect the evolution of wrinkle amplitude from baseline (T0) to the end of the 2 months of application of each of the products



Mean depth of a main wrinkle



Wrinkle volume (mm^3)

d. Roughness and complexity

Roughness is undoubtedly the most widely used parameter since it is generated by the various software packages available on the market.

Roughness enables an overall approach to the flatness of the surface by characterizing it using a **mean amplitude value for cutaneous relief** (the resultant of all the depressions and elevations).

The **complexity**, a very similar concept, **compares the total area developed by the cutaneous relief to the area of a plane surface**. The evolution of the complexity between T0 and T56 yields the percentage change toward a perfectly smooth surface of the skin.

RESULTS

The study showed that, in the absence of the cosmetic active substances in the cream, the excipient cream only induced a minor improvement in skin surface. The differences *vs*. T0 were all non-significant.

Table 9:

Comparison of the effects of **MATRIXYL® 3000** *vs*. placebo after 56 days (2 months)

PARAMETERS	MATRIXYL® 3000	PLACEBO
% area occupied by wrinkles > 200 µm	-39.4**	+4.3 n.s.
Wrinkle density	-32.9**	-9.9 n.s.
Roughness	-16.0**	-1.4 n.s.
Complexity	-16.2**	+4.2 n.s.
Mean volume of a main wrinkle	-23.3**	-8.7*
Mean depth of a main wrinkle	-19.9**	-3.2 n.s.

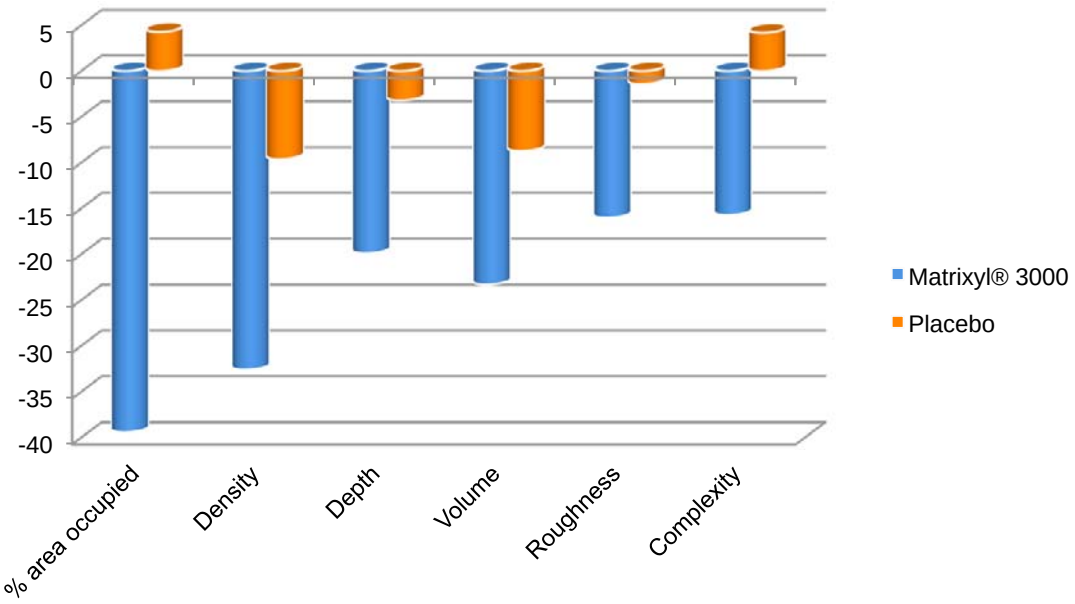
n.s. : non-significant

* : significant ($p \leq 0.05$)

** : highly significant ($p \leq 0.01$)

RESULTS

The data are illustrated by the following plot:



5.2. Cutometric study of MATRIXYL® 3000 versus placebo

The measurements were conducted on a clearly defined site for each volunteer so as to conduct half-face comparisons of the effects of **MATRIXYL® 3000** *vs.* placebo.
Each measurement was made in triplicate and the mean value was taken into account in the comparison of T0 *vs*. T56 days for all the volunteers.

Two parameters were analyzed:

- Raw elasticity Ua/Uf: this parameter describes the return to the baseline situation after induced stretching of the skin and should ideally be 1.
- Tone Ur: this parameter reflects the immediate retraction of the skin when stretching stops.

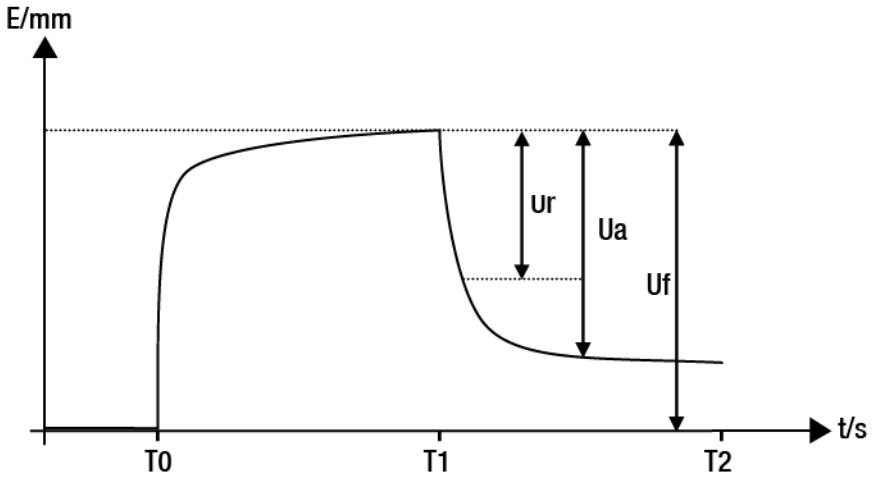


Table 10:

Evolution of the raw elasticity for **MATRIXYL® 3000** and placebo after 56 days of application (mean values for n=24 volunteers)

RAW ELASTICITY	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
Mean	0.482 ± 0.06	0.508 ± 0.07	0.488 ± 0.07	0.508 ± 0.09
% difference *vs*. T0	**+5.5%**		**+4.1%**	
	Statistical test for paired series			
Comparison T56 *vs*. T0	p<0.06 Significant difference		Non-significant difference	

Table 11:

Evolution of the skin tone for **MATRIXYL® 3000** and placebo after 56 days of application (mean values for n = 24 volunteers)

TONE (%)	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
Mean	0.113 ± 0.02	0.130 ± 0.03	0.120 ± 0.02	0.127 ± 0.02
% difference *vs*. T0	**+15.5%**		**+6.5%**	
	Statistical test for paired series			
Comparison T56 *vs*. T0	p<0.01 Significant difference		p<0.01 Significant difference	

The results clearly show an effect on the quality of elasticity and skin firmness procured by daily application of the product, **MATRIXYL® 3000**, *vs*. placebo, which yielded no significant improvement at the end of the study

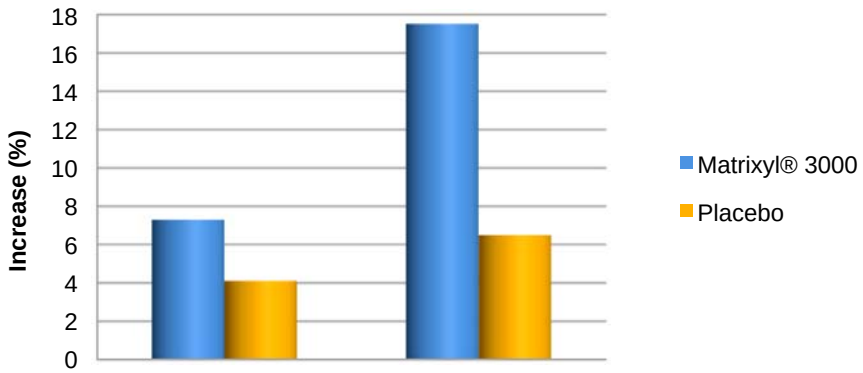


Figure 7: Cutometric study

CONCLUSION

In short, the results generated by the clinical studies show excellent anti-wrinkle efficacy for **MATRIXYL® 3000** after 2 months of daily application: reduction in the volume (-23,3%) and depth of deep wrinkles (-19,9%) giving rise to smoother skin (-16% roughness) and a “lifted” surface (-16.2% complexity) which, in the cutometric study, was reflected in an improvement in tone (+15.5%).

The percentage decrease in the number and amplitude of the wrinkles on the analyzed surface was very strong (-44%). This effect was also correlated with the visible difference between T0 and T56, as illustrated by the photographs.

6. *IN VIVO* STUDIES OF THE ANTI-WRINKLE EFFICACY ON MALE VOLUNTEERS

LABORATORY SEDERMA, OCTOBER - NOVEMBER 2006

MASCULINE COSMETIC CARE

The masculine beauty market is thriving and specific care products are beginning to rival the conventional products associated with shaving providing that the 2005 /2004 growth of 29.8% (ECM, market report, March 2006) and 50% for the major French brands is confirmed in 2007 and in coming years.

The demand for masculine specific facial products is based on the “moisturizing-nourishing” and “anti-aging” dimensions since men are changing and male attitudes towards beauty are progressing: men seek well-being by frequenting spas and also taking care of their skin and appearance. These are dimensions of the new male attitude in which look and presentation are associated with professional performance.
Among candidates with equivalent qualifications, the one with a balanced, groomed and radiant look will be recruited and all the more so as the candidate becomes more “senior”. Beauty care is now a male secret weapon for seducing and convincing.

For the male market, in order to remain faithful to beauty care products they must be, above all, of high performance
For that reason, SEDERMA selected a potent complex of matrikins (peptides naturally occurring in the skin), which restructures the skin in depth, from its broad range of anti-wrinkle care products.

PROTOCOL

The clinical trial was conducted on a panel of 39 male volunteers of mean age 54.5 ± 6 years over a 2-month period. The anti-wrinkle effect was assessed by means of profilometry and image analysis

INCLUSION CRITERIA

Male volunteers aged between 40 and 64 years presenting with marked wrinkles in the form of crow's feet, with no history of adverse reactions to cosmetics and not having used a cosmetic product with the same aim for at least 4 weeks.
Volunteers undertaking not to deliberately expose themselves to UV radiation (artificial UV radiation or vacations in the sun) throughout the duration of the study and not taking part in any other cosmetic test of the same type. The volunteers were only to apply the products supplied by SEDERMA (except for their usual shaving products) to their faces.

STUDY TYPE AND DURATION

The study had a blind, hemi-facial design. Following randomization, the product, **MATRIXYL® 3000**, and placebo were each applied to one side of the face.
The product, **MATRIXYL® 3000**, consisted of a 4% active substance concentration in a cream-gel formula. It was compared to a placebo consisting of the active product excipients (Appendix 2).
The product was applied twice daily around the eyes and over the superior part of maxillary region for 56 days.

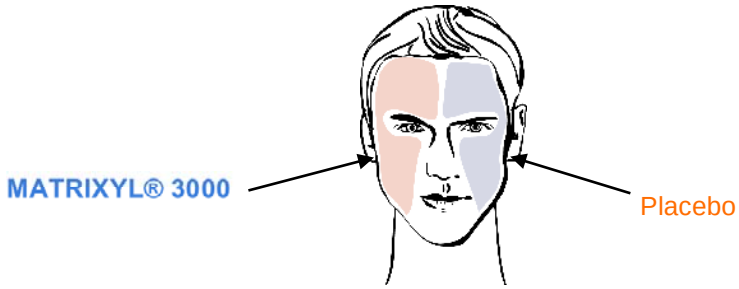


Figure 8: Hemi-facial product application

T0 — T56 days

SILFLO® — **SILFLO®**

Photographs — **Photographs**

➢ ***Profilometry***

The impressions of skin relief were obtained by applying SILFLO® gel to the wrinkles in the corners of the eyes. The product polymerizes *in situ* and, after detachment, a "negative" impression of the irregularities of the skin surface is obtained.

The irregularities, the wrinkles, were subjected to automated digital-image analysis (HITACHI CCTV camera, Mountain Maps software) under low angle of incidence illumination (halogen light at 40°).

Several parameters were thus generated, including the depth of the principal wrinkles (µm), the volume of the principal wrinkles (mm^3), the area covered by the deep wrinkles (>200µm), the density of the principal wrinkles ($µm/cm^2$) and the roughness. All the foregoing parameters are now conventional. In addition, a wrinkle spread angle parameter was computed.

For each of the parameters, the means and percentage differences *vs*. T0 were computed. Appropriate statistical tests (Student's t test for paired series if the variances were equal, T0 *vs*. T56, or Wilcoxon's test for paired series if the variances were different) were conducted.

➢ ***Photographs***

The photographs were taken using a Nikon digital camera. Standardization of the photographs was ensured by positioning the volunteers using a chin and forehead rest system placed on a photographic bench.

Lighting was supplied by a fixed-position system at a constant fixed distance from the objective. Low-incidence illumination was used to enhance wrinkle and small wrinkle imaging.

RESULTS

SAFETY: The product, applied for 2 months, was well tolerated.

a. Area occupied by deep wrinkles (200µm)

Table 12:

Change in the area occupied by deep wrinkles (>200µm), **MATRIXYL® 3000** *vs*. placebo, after 56 days

AREA OCCUPIED BY DEEP WRINKLES (>200µm) (%)	**MATRIXYL® 3000**		**PLACEBO**	
	T0	T56	T0	T56
Mean	0.88 ± 0.96	0.62 ± 0.61	0.66 ± 0.76	0.70 ± 0.69
% change on T0	**-29.4%**		**+5.1%**	
	Statistical test for paired series			
T56 *vs*. T0	p<0.01 Significant difference		NS	
MATRIXYL® 3000 *vs*. placebo	p<0.05 Significant difference			

On average, the area occupied by deep wrinkles was reduced by 29% over 2 months for MATRIXYL® 3000. The difference, T0 *vs*. T56, was very significant.

No change was observed on the placebo side. A significant difference in favor of MATRIXYL® 3000, *vs*. placebo, was observed.

b. Density of the principal wrinkles

Table 13:

Time course of principal wrinkle density,
MATRIXYL® 3000 *vs*. placebo, after 56 days of application

PRINCIPAL WRINKLE DENSITY ($\mu m/cm^2$)	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
Mean	3.92 ± 3.32	2.73 ± 1.91	3.66 ± 2.88	2.94 ± 1.85
% change on T0	**-30.4%**		**-19.7%**	
	Statistical test for paired series			
T56 *vs*. T0	p<0.01 Significant difference		NS	
MATRIXYL® 3000 *vs*. placebo	Non-significant difference			

A marked decrease in the mean density of wrinkles was observed with **MATRIXYL® 3000** (-30.4%). The decrease *vs*. T0 was significant. On the placebo side, a more moderate and non-significant reduction was observed (-19.7%; NS).

c. Variation of mean depth and mean volume of wrinkles

Table 14:

Change in mean depth of the principal wrinkle,
MATRIXYL® 3000 *vs*. placebo, after 56 days of application

MEAN DEPTH OF THE PRINCIPAL WRINKLE (µm)	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
Mean	93.9 ± 40.7	84.3 ± 43.1	81.36 ± 24.2	81.5 ± 26.4
% change on T0	**-10.2%**		**+0.2%**	
	Statistical test for paired series			
T56 *vs*. T0	p<0.01 Significant difference		NS	
MATRIXYL® 3000 *vs*. placebo	p<0.01 Significant difference			

Table 15:

Change in the mean volume of the principal wrinkle,
MATRIXYL® 3000 *vs*. placebo, after 56 days of application

MEAN VOLUME OF THE PRINCIPAL WRINKLE (mm^3)	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
Mean	0.270 ± 0.12	0.224 ± 0.9	0.254 ± 0.10	0.247 ± 0.12
% change on T0	**-17.1%**		**-2.7%**	
	Statistical test for paired series			
T56 *vs*. T0	p<0.01 Significant difference		NS	
MATRIXYL® 3000 *vs*. placebo	p<0.01 Significant difference			

The results obtained showed a **very noteworthy and significant decrease in the mean depth of the wrinkle and its volume between the start and end of the study. The differences were -10.2 and -17.1% for MATRIXYL® 3000.**

For the **placebo, there was no improvement** in terms of a decrease in mean depth or volume.

As previously observed, a very marked significant difference in favor of **MATRIXYL® 3000** was observed.

d. Change in roughness

Roughness is undoubtedly the most widely used parameter since it is generated by all the relevant software packages on the market.
Roughness enables an overall assessment of the flatness of a surface by characterizing the **mean amplitude of the cutaneous relief irregularities** (the resultant of all the peaks and troughs).

Table 16:
Change in roughness,
MATRIXYL® 3000 *vs*. placebo, after 56 days of application

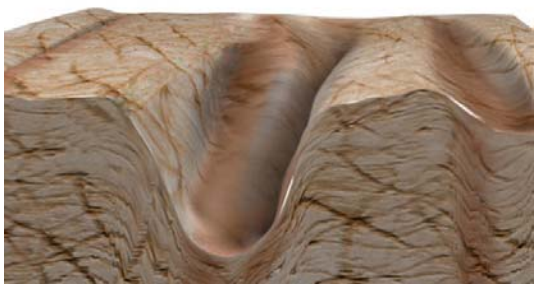
ROUGHNESS (µm)	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
Mean	29.8 ±8.3	27.3 ± 6.5	28.4 ±7.3	27.8 ± 5.9
% change on T0	**-8.4%**		**-2.2%**	
	Statistical test for paired series			
T56 *vs*. T0	p<0.01 Significant difference		NS	
MATRIXYL® 3000 *vs*. placebo	p<0.05 Significant difference			

It will be observed that the roughness decreased significantly on the **MATRIXYL® 3000** side while there was no significant change on the placebo side.

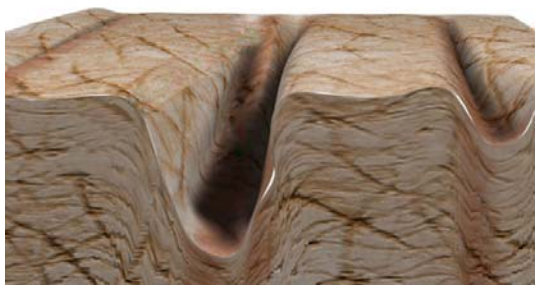
The smoothing effect was significant for MATRIXYL® 3000 *vs*. the placebo.

e. Wrinkle spread

In the visual perception of a wrinkle, not only the depth but also the wrinkle spread act in a complementary manner and yield an impression of either a deep wrinkle or a less marked wrinkle.
For equal depths, a wrinkle with a narrow sulcus will allow less light to enter it than a wrinkle with a broad sulcus, as shown in the diagram on the following page.



Open wrinkle



Closed wrinkle

The wrinkle spread angle may thus be defined. This parameter enables enhanced reflection of the visual effect of the wrinkle. The more open the wrinkle, the less marked it appears to be.

SEDERMA measured the change in the spread angle of the principal wrinkle, T0 *vs*. T56 days, on the **MATRIXYL® 3000** and placebo sides.

Table 17:

Change in wrinkle spread, **MATRIXYL® 3000** *vs*. placebo, after 56 days of application

WRINKLE SPREAD (°)	MATRIXYL® 3000		PLACEBO	
	T0	T56	T0	T56
	100.9 ± 11.7	106.3 ± 17.2	99.7 ± 15.3	99.0 ± 14.7
% change on T0	**+5.4%**		**-0.7%**	
	Statistical test for paired series			
T56 *vs*. T0	p<0.05 Significant difference		NS	
MATRIXYL® 3000 *vs*. placebo	p<0.05 Significant difference			

After 56 days of application of **MATRIXYL® 3000**, the principal wrinkle was more open than at T0: +5%.

On the placebo side, no significant change was observed.
The wrinkle spread parameter was significantly in favor of **MATRIXYL® 3000.**

SUMMARY

Table 18:

Comparison of the effects of **MATRIXYL® 3000** and placebo at time point 56 days (2 months)

PARAMETERS	MATRIXYL® 3000	PLACEBO
Area occupied by deep wrinkles (> 200 µm)	-29,4**	+5,1 n.s.
Density of the principal wrinkles	-30,4**	-19,7*
Mean depth of the principal wrinkle	-10,2**	0,2 n.s.
Mean volume of the principal wrinkle	-17,1**	-2,7 n.s
Roughness	-8,4**	-2,2 n.s.
Wrinkle spread angle	+5,4*	-0,7 n.s

n ns : non-significant

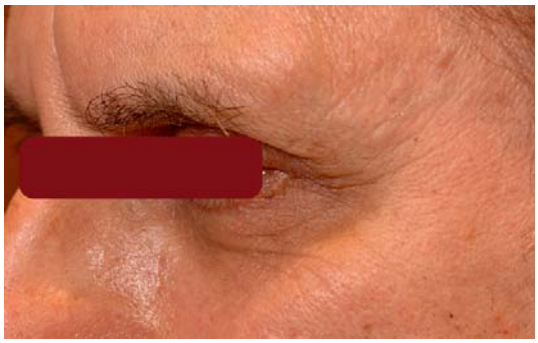
* : significant *vs*. T0 (p≤0.05)

** : highly significant *vs*. T0 (p≤0.01)

The excellent effect in terms of reduction of crow's foot wrinkles is illustrated by the following photographs:

MATRIXYL® 3000 side: T0

MATRIXYL® 3000 side: after 2 months



placebo side: T0

placebo side: after 2 months

7. REPARATION

7.1. *In vivo* studies on papillary dermis - study on female panel (LABORATORY SEDERMA, SEPTEMBER - NOVEMBER 2010)

PRINCIPLE

A further study of the efficacy of **MATRIXYL® 3000** was conducted on a panel of 28 female volunteers with aged skin in order to demonstrate improvement in the upper dermal fibres.

The dermis consists of a deep part and a superficial part called the papillary dermis, which is in contact with the epidermis via the dermo-epidermal junction (DEJ). The papillary dermis is a good indicator of a person's age and sun exposure. Its components are repeatedly exposed to ultraviolent light degradation and forces making them fragment and making the dermis fragile.

This effect has been seen histologically; sections of skin from highly light-exposed or elderly people exhibit finer and more amorphous dermal structures than those in younger or sun-protected people. Skin ultrasound has confirmed the histological findings, components which are thinner returning fewer echoes, thereby forming a poorly echogenic band beneath the DEJ called the SLEB (Subepidermal Low Echogenic Band) (cf. §7.1.a.). The latest generation confocal microscopy now has three lasers which can visualise and analyse the structure of the fibres in this area and allow studies to be carried out on this area.

We therefore used these two complementary methods in order to study the superficial area of the dermis:

- SLEB analysis by **high resolution 50MHz ultrasound**
- Analysis of the structure of fibres in this area by **Confocal Laser Microscopy**

PROTOCOL

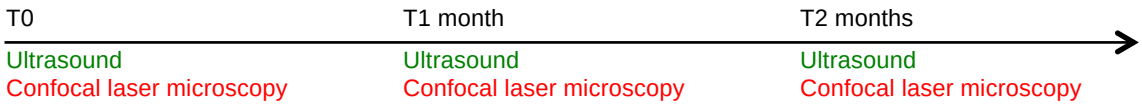
SPECIFIC INCLUSION CRITERIA FOR STUDIES

Women were selected based on an age of between 50 and 75 years old and on prior ultrasound examination of the skin to confirm the existence of a sufficiently large anechogenic area of dermis to be visualised and demonstrating a certain degree of ageing. The volunteers were required to follow a wash-out period using the test placebo for 15 days before measurements began.
Subjects were required not to make any change to their hormone status during the 3 months before the test and during the test (no change in contraceptive, replacement or curative hormone therapy) and they were only allowed to use the cosmetics provided during the study.

TYPE AND DURATION OF STUDY

This clinical study was conducted blind using non-invasive measurements on 28 volunteers between 51 and 72 years old (average age 59 years old) who were randomised and who applied cream containing 3% **MATRIXYL® 3000** (cf. Annex 3), to half of their face and one of their forearms. The placebo cream was applied controlaterally. Both creams were applied by twice daily, by massaging, for 2 months.

The study is summarised in the diagram below.



Statistical tests used the Student *t* test or a non-parametric Wilcoxon test, if needed, both on paired series

SAFETY

The clinical study was conducted under medical supervision and demonstrated that product tolerability by the volunteers was excellent.

a. Analysis of SLEB by high resolution ultrasound

When ultrasounds hit a tissue in the human body, they are reflected and return a signal or “echo”. The intensity of the echoes is translated by the ultrasound instrument into grey scale levels to construct a reliable anatomical indicator of the area being investigated. The software in the instrument can also allocate colours depending on the intensity of the echoes.

Beginning from histological observations of elastosis, DE RIGAL *et al.*, (1989) demonstrated the relationship between ageing and the development of a low echogenic band in the sub-epidermal part of the skin (papillary dermis). They called this structure SLEB (*Subepidermal Low Echogenic Band*). Several studies subsequently confirmed this finding and demonstrated the involvement of light-induced ageing in its development and severity (GNIADECKA, 2001; SANDBY-MOLLER and WULF, 2004, QUERLEUX *et al.*, 2009). With ageing, the SLEB increases in depth and becomes less echogenic, indicating a fall in density of the thickest fibres.
Our study therefore set out to assess changes in depth and density of the SLEB on a site which is not extensively exposed to sunlight (internal surface of the forearm) and on a more exposed site (external surface)

A Dermascan C (Cortex) ultrasound equipped with a 50 MHz frequency probe was used to obtain images approximately 6 mm wide and 3 mm thick with a resolution of 25 x 60 µm. A sequence of 100 successive images was recorded over 4 cm. Five representative images were extracted and analysed by image analysis. The SLEB is traced accurately over a width of 5.5 mm allowing its depth (in µm) and density (in Grey Scale Levels or GSL) to be calculated automatically.

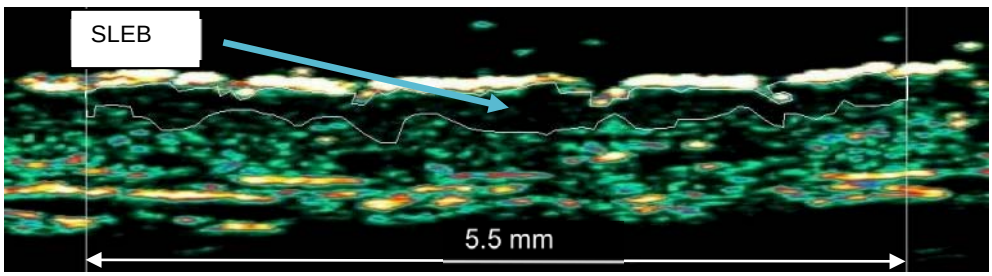


Table 19:

Changes in thickness and densification of the SLEB after applying **MATRIXYL® 3000** 3% or placebo.
(**Internal** surface of forearm; 28 volunteers, n= 5 replicates)

	SLEB thickness (µm)		**SLEB densification (GSL)**	
	T0	**T 2 months**	**T0**	**T 2 months**
Placebo	171 ± 20	173 ± 20	19.67 ± 1.91	18.93 ± 2.31
% change *vs*. T0 Significance		1.2% *Dns*		-3.8% *p<0.05*
MATRIXYL® 3000	**176 ± 30**	**159 ± 20**	**18.88 ± 2.67**	**21.03 ± 3.11**
% variation *vs*. T0 Significance Maximum Responders		**-9.8%** ***p<0.01*** **→ -23%** **93%**		**+11.4%** ***p<0.01*** **→ +44%** **68%**
Change (**MATRIXYL® 3000** *vs*. placebo) Significance		**-11%** *p<0.01*		**+15.2%** *p<0.01*

The results show that applying **MATRIXYL® 3000** 3% produces a significant fall in SLEB depth of -5.5% (cf. Annex 4; *p<0.01*) compared to placebo from one month of application, associated with a +7.8% increase in density (*p<0.01*). Remarkably, these results are increased by a factor of 2 after applications for two months, reaching -11% depth and +15% density in this area (*p<0.01* in both cases).

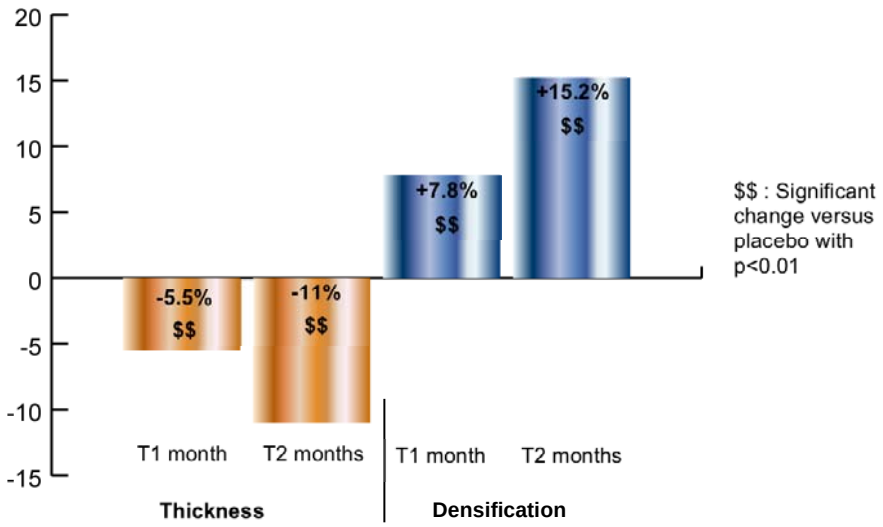


Figure 9: Change in thickness and density of the SLEB at T1 month and T2 months compared to placebo

Measurements taken from the external forearm, i.e. on a light-exposed area, confirm these good results.

Table 20:

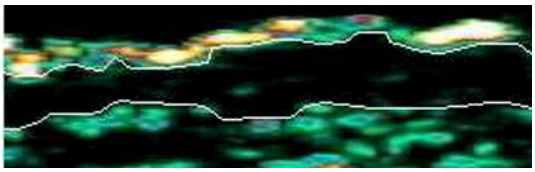
Change in thickness and densification of the SLEB after applying **MATRIXYL® 3000** 3% or placebo; (**External** surface of forearm; 28 volunteers, n= 5 replicates)

	SLEB depth (µm)		SLEB density (GSL)	
	T0	**T 2 months**	**T0**	**T 2 months**
Placebo	195 ± 30	204 ± 30	16.25 ± 2.87	15.67 ± 2.62
% change *vs*. T0 Significance		4.6% *Dns*		-3.6% *Dns*
MATRIXYL® 3000	193 ± 40	174 ± 30	16.57 ±4.74	18.48 ± 3.58
% variation *vs*. T0 Significance Maximum Responders		**-9.8%** ***p<0.01*** **→ -33%** **86%**		**+11.5%** ***p<0.01*** **→+45%** **82%**
Change (**MATRIXYL® 3000** *vs*. placebo) Significance		**-14.4%** p<0.01		**+15.1%** p<0.01

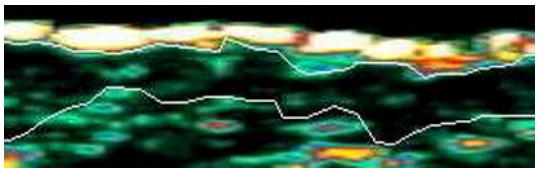
Consistent with published findings, the external surface of the volunteers' forearms which is exposed to sunlight deteriorates more than the internal surface, which is better protected from the sun. The SLEB is thicker (195 µm versus 171 µm for the internal surface) and less dense (GSL of 16.2 compared to 19.7 for the internal surface).

Applications of **MATRIXYL® 3000** for 2 months has a very similar effect to the effect seen on the protected surface, with a significant improvement in depth (-14.4%) and density (+15.1%), both of which were significant *p<0.01*.

Light-induced age-related damage was repaired as the features of the SLEB were equivalent to those of skin which had not suffered U.V. damage.
This improvement is illustrated in the example below:

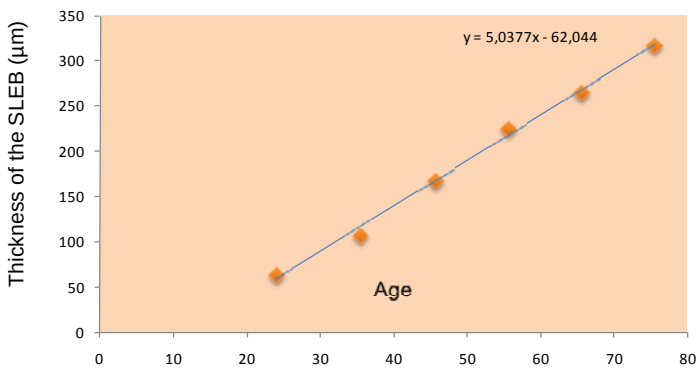


T0 – external forearm: volunteer 1



T2 months – external forearm: volunteer 1

Some published data on approximately 400 volunteers of different ethnic origins (below; QUERLEUX *et al.*, 2009), the equation **y = 5.04x -62** expressing the change in SLEB depth as a function of age was calculated to be:



Using this equation and our data (cf. Annex 4), the improvements in depth obtained with **MATRIXYL® 3000** can be converted into a theoretical age gain, an indicator which is easier to understand.

Table 21:

Conversion of change in SLEB thickness into age gain following applications of **MATRIXYL® 3000** or placebo to the arm (external surface *vs.* internal surface)

Age gain	**SLEB internal surface**		**SLEB external surface**
	T 1 month	**T 2 months**	**T 2 months**
Placebo	+0.8 year	+0.4 an	+1.8 years
MATRIXYL® 3000	**-1 year**	**-3.4 years**	**-3.7 years**
Change **MATRIXYL® 3000** *vs.* placebo	-1.8 years	-3.8 years	-5.5 years

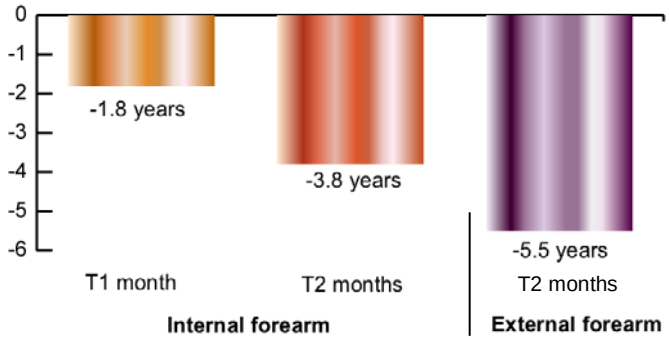


Figure 11: Visualisation of age gain at T1 month and T2 months

b. Analysis of dermal fibre structure by confocal laser microscopy

Only four years after the pioneering work by NEW *et al.*, (1991) and CORCUFF *et al.*, (1993), an *in vivo* confocal mono-laser microscope was marketed under the name Vivascope 1000 (Lucid). This had long been a reference product in its field and a key improvement was made, producing the Vivascope 1500, the multi-laser instrument used in this study (see figure 12).

Figure 12: Vivascope 1500

The Vivascope 1500 uses three laser beams (785 nm, 658 nm and 445 nm) reflected differently depending on the refractory index of the structures they hit. Keratins, melanin and collagens in particular appear white on a grey scale level image.

The Vivascope can firstly “illuminate” a specific point in the skin (focusing illumination) and, in parallel, accurately detect the returning light (focusing detection). This device is called “confocal” for “conjugate focal planes”.

The main feature of the instrument is that it produces a high-definition, horizontal and vertical image in order to obtain a genuine real-time biopsy of the skin, entirely non-invasively. The instrument therefore allows horizontal *in situ* “sections” of the skin (500 µm x 500 µm) to be obtained with a horizontal resolution of 1.25 µm and vertical resolution of less than 5 µm.

Depending on the laser used, the sections can be made every 3 to 5 µm to a depth of approximately 150 µm (see figure 13).

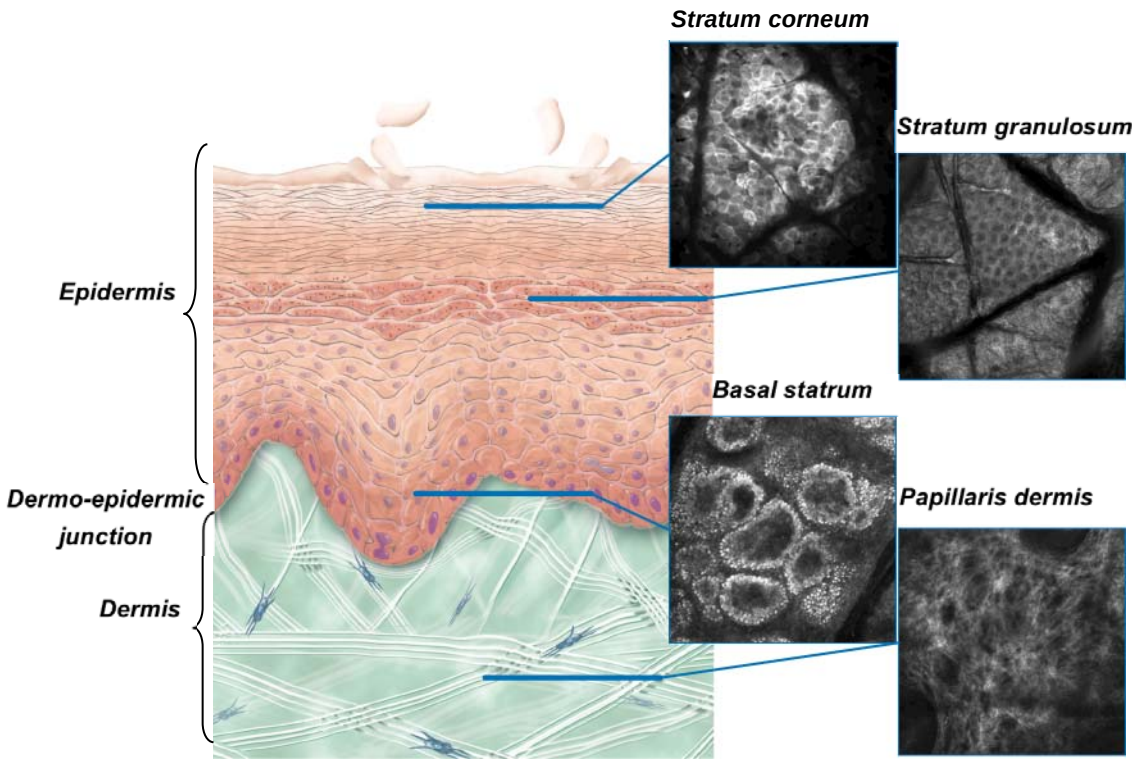
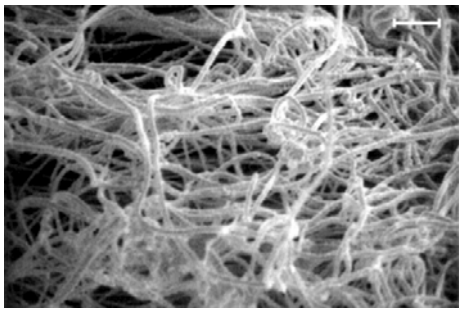


Figure 13: Vivascope 1500 study areas

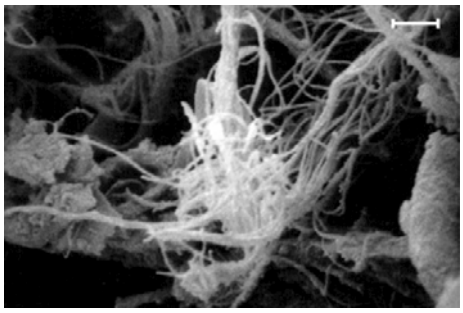
This instrument literally allows us to look into the skin without damaging it and has led to numerous publications particularly in the diagnosis of pigmented and non-pigmented inflammatory and neoplastic lesions (BENVENUTO-ANDRADE *et al*., 2007; CALZAVARA-PINTON *et al*., 2008).

Research has also been conducted on healthy skin (GONZALEZ and GILABERTE-CALZADA, 2008) in pigmentation (DECLERCQ *et al*., 2008), pores (SUGATA *et al*., 2008), ageing (SAUERMANN *et al*., 2002; 2004; NEERKEN *et al*., 2004), light-induced ageing (GAMBISLHER *et al*., 2006), capillaries (HEGYI *et al*., 2009) and depth of skin layers (ROBERTSON K. and REES J; 2010).

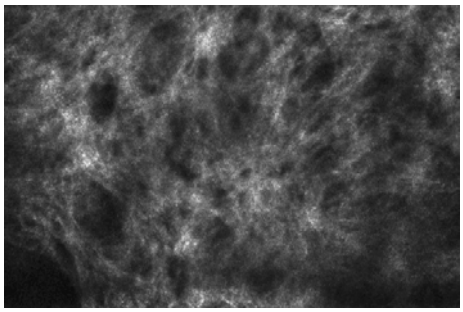
The images on the following page illustrate the parallel findings between scanning electron microscopy (or SEM; FLIGIEL *et al*., 2003) and confocal microscopy (SEDERMA). Aged dermal fibres lose their filamentous appearance and organisation into networks taking on a blurred fluffy appearance, indicating fragmentation and degradation.



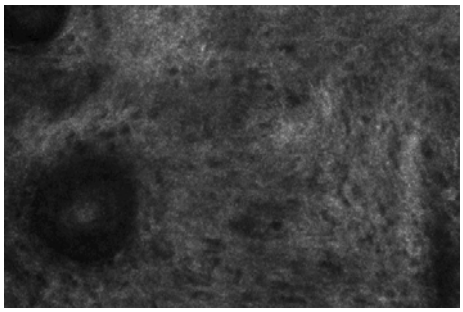
Young dermis:
Scanning Electron Microscope



Elderly dermis:
Scanning Electron Microscope



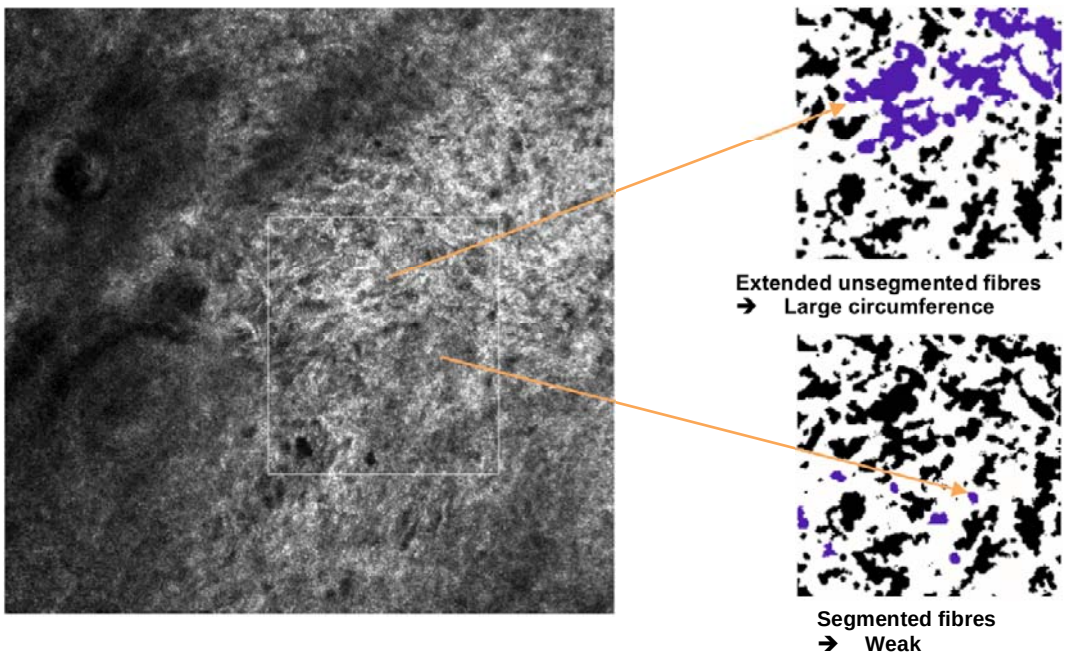
Young dermis:
Confocal microscope



Elderly dermis:
Confocal microscope

The study examined changes in structure of the superficial dermis of the face in the outer corner of the eye, where depth varies between people (mean depth: 70 µm) after applying **MATRIXYL® 3000**. Analysis of the images obtained revealed the extent of degradation and fragmentation of the papillary dermis depending on the person's age and ultra violet exposure.

The images obtained were analysed by Confoscan software (Orion concept) which isolates and measures the size (circumference) of fibres. The circumference increases with whole unsegmented fibres.

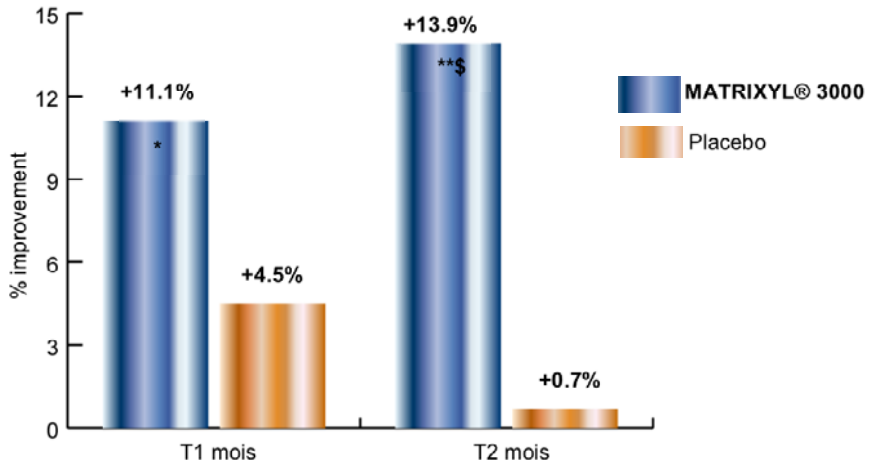


An anti-ageing product acting on this part of the dermis should therefore reduce segmentation of fibres and increase their circumference.

Table 22:

Change in fragmentation of dermal fibres after application of **MATRIXYL® 3000** 3% or its placebo to the faces of 28 volunteers (n=3 measurements/volunteer)

	Mean fibre circumference at a depth of approx. 70 µm (pixels)	
	T0	**T 2 months**
Placebo	192.9 ± 36.3	194.3 ± 33.7
% change *vs.* T0 Significance		0.7% *Dns*
MATRIXYL® 3000	**179.1 ± 25.3**	**204.1 ± 45.4**
% change *vs*. T0 Significance Maximum Responders		+13.9% *p<0.01* → +54% 71%
Change (**MATRIXYL® 3000** *vs.* placebo) Significance		+13.2% *p<0.05*



* or **: Significant difference *versus* T0 with p<0.05 or p<0.01.

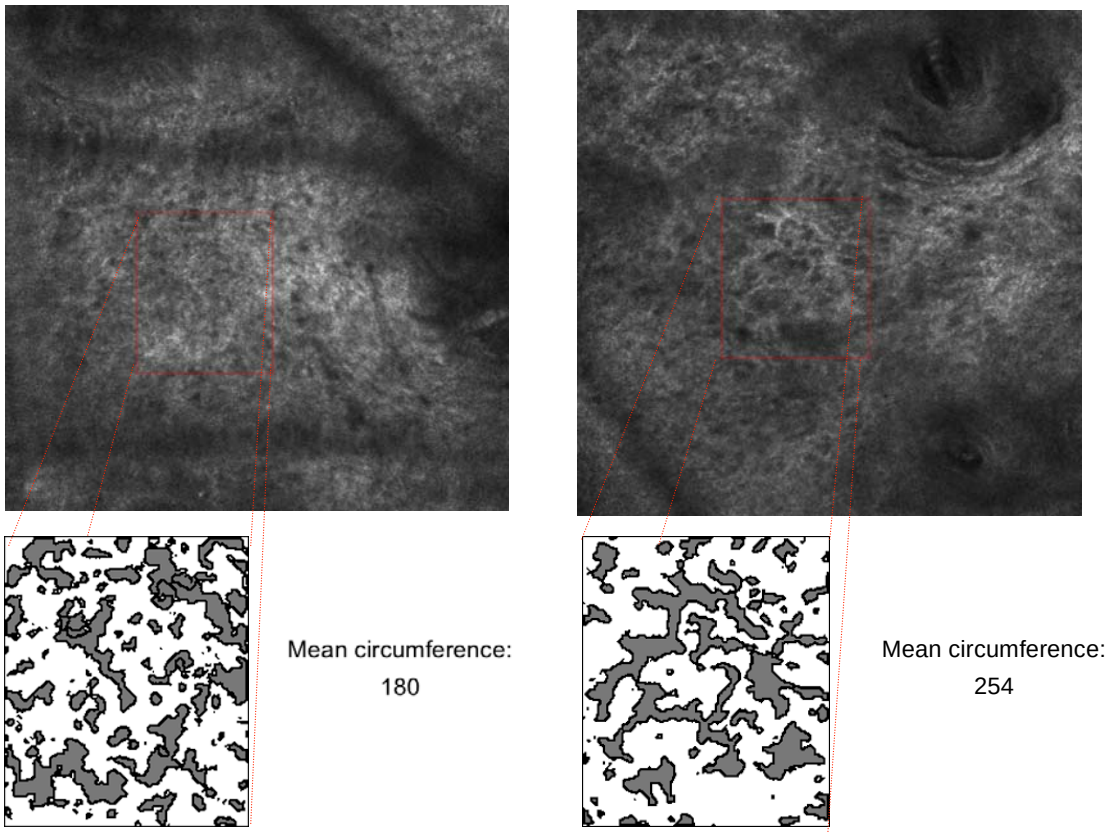
$: Significant difference *versus* placebo with p<0.05.

Figure 14 : Change in fragmentation of dermal fibres at T1 month and T2 months

After application of **MATRIXYL® 3000** 3%, the dermal fibre structure was greatly improved with a significant increase in their circumference of +11.1% at T1 month (see Annex 4) and +13.9% at T2 months. In parallel, the placebo caused little or no change in this parameter.

The good results measured *in situ* with the Vivascope clearly indicate that **MATRIXYL® 3000** acted precisely at the level of the skin dermal fibres to restore an improved structure.

The improvements measured are illustrated in the images on the following page.



T0 T2 months

Application of a cream containing 3% of **MATRIXYL® 3000** for 2 months improved the quality of the papillary dermis, a structure which is very considerably degraded by U.V. irradiation. After treatment, the characteristics of the papillary dermis were similar to those of a papillary dermis 5.5 years younger.

7.2. *EX VIVO* studies - Dermal rejuvenation (SEDERMA, SEPTEMBER - OCTOBER 2012)

The extracellular matrix (ECM) of the skin is comprised of many proteins and polysaccharides that endow the skin with resistance and elasticity. In addition to providing the support structure for cells, the ECM regulates cellular functions through cellular adhesion and provides a transport system for nutrients and waste (HWANG 2011).
The epidermis, which protects the body from external damage and drying, is anchored to the dermis through a complex network of molecules that form the dermal-epidermal junction (DEJ). The composition of the DEJ is modified quantitatively and qualitatively with age and photoageing.
Collagen I and III synthesis diminishes with age, and even more so with photoaging (FLIEGIEL, 2003). Collagens VII and IV are the two main components of the anchoring filaments. They are produced by keratinocytes and fibroblasts and interact with neighbouring proteins to create a solid anchoring framework between the epidermis and the dermis.
Collagen XVII is another component of anchorage filaments. Studies have shown the importance of collagen in cellular matrix adhesion. Mutations in collagen change the capacity of tissue to resist external shear stress. In addition to its role in maintaining dermal-epidermal junction integrity, it is clear that collagen XVII is involved in transmembrane signal transduction and in regulating keratinocytic differentiation (ZILLIKENS 1999).
Nidogen-1 (or entactin) is a key element of basal membrane supramolecular assembly. Nidogen binds to laminins and collagen IV, thereby forming complex structures that provide stability to the basal membrane.

PROTOCOL

Abdominal skin explants were obtained from 10 Caucasian women aged 30 to 66 (mean age 49 ± 14 years). For studies on DEJ changes with age, two groups with 5 members each were defined: 36 ± 6 years and 61 ± 5 years.
For the immunofluorescence study on the effect of age on the macromolecules of interest, the skin explants were frozen in liquid nitrogen and kept at a temperature of -80°C until they were used.
For the study of the **MATRIXYL® 3000** effects, explants from 10 donors received a five-day course of daily topical application of a cream containing 3% of **MATRIXYL® 3000** or the corresponding placebo. At the end of the culture period, the skin samples were frozen in liquid nitrogen and kept at a temperature of -80°C until they were used.

A 7-µm slice of each explant was then cut using a cryomicrotome. The sections were then labelled using antibodies specific to the macromolecules of interest.

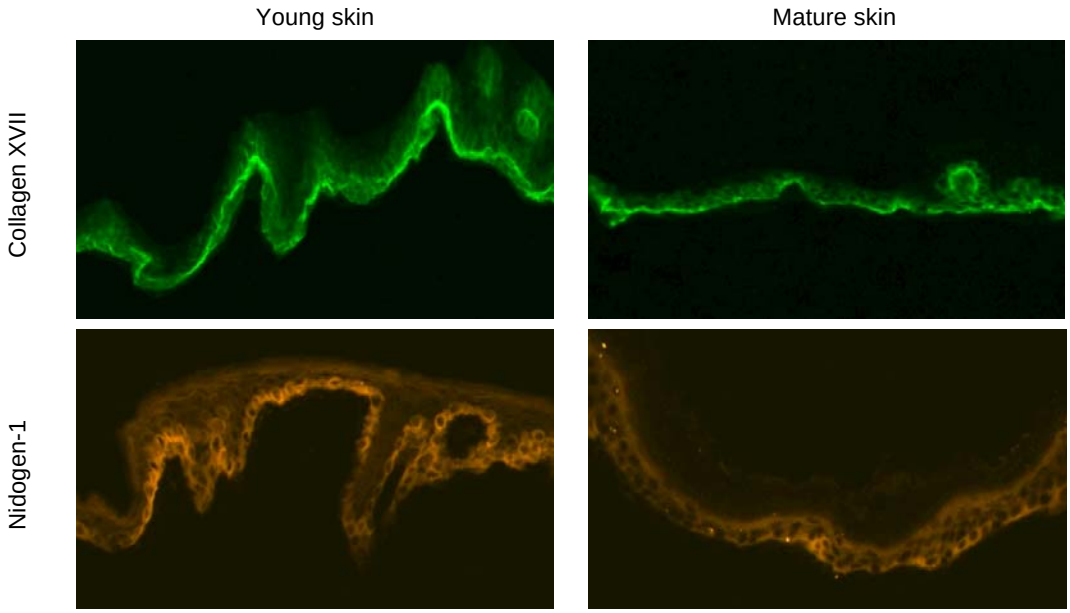
RESULTS:

Table 23: Variation in DEJ macromolecules/papillary dermis with age;
effect of **MATRIXYL® 3000** on these components 5 days after topical application.

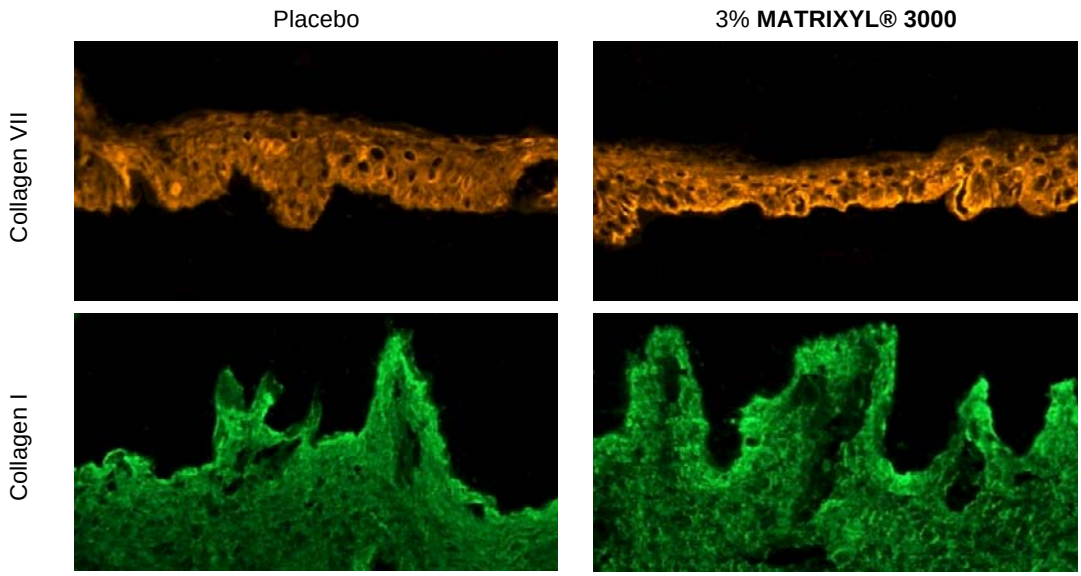
	Collagen I	Collagen type IV	Collagen type VII	Collagen type XVII	Nidogen-1
Variation with age (in %)	(↓) -8%; p<0.2	(↓) -11%; p<0.05	(↓) -17%; p<0.01	(↓) -31%; p<0.01	(↓) -15%; p<0.01
Placebo (AFU)	20.80 ± 4.02	10.51 ± 2.95	12.56 ± 2.01	4.64 ± 1.05	5.09 ± 2.55
MATRIXYL® 3000 3% (AFU)	23.79 ± 2.96	11.18 ± 2.28	14.99 ± 3.75	5.37 ± 2.39	5.83 ± 4.04
Variation (%) *vs.* Placebo	(↑) +14.40%; p<0.01	(↑) +6.4%; p<0.05	(↑) +20.30%; p<0.01	(↑) +15.84%; p<0.01	(↑) +14.49%; p<0.01

AFU: Arbitrary Fluorescence Unit; (↑) = increase (↓) = decrease

Below are two examples (Collagen XVII and Nidogen) of variations with age. The photos below also demonstrate a decrease in DEJ undulation with age.



Sample photos after applying 3% of **MATRIXYL® 3000**



8. OVERALL CONCLUSION

Matrikines, small endogenous peptides derived from matrix proteolysis, are cell messengers able to regulate the sequence of events required for skin repair (wound healing).
To a certain degree, wrinkles may be considered localized defects due to deficient repair related to the ageing of the cutaneous functions of tissue repair and turnover.

With the matrikine combination, it is possible to recreate conditions conducive to cell and matrix turnover.
The two peptides, Pal-GHK and Pal-GQPR, combined in the product MATRIXYL® 3000, showed a complementary gene activation profile with stimulation of protein remodelling (urokinase, TIMP1, lysyl oxidase, tenascin, syndecan), and cell proliferation, migration and installation (EGF, PDGF, Rho, α-catenin, laminin, fibronectin). Moreover, epidermal function progressed toward microvascularization and innervation (VEGF and ephrin).

***In vitro*, the two peptides showed synergistic effects on the synthesis of collagen I, fibronectin and hyaluronic acid.**

A clinical trial enabled comparison of the benefits obtained with MATRIXYL® 3000 *vs.* placebo, thus validating the synergistic approach adopted by combining the two peptides.

After 2 months of daily application of MATRIXYL® 3000 (3% formula), the following points were observed:

- **reduction in the mean depth of the main wrinkle (-19.9%) and in its volume (-23.3%)**
- **reduction in roughness (-16%) and complexity (-16.2%), a surface "lifting" parameter,**
- **decrease in the area occupied by deep wrinkles (>200 µm) (-44%), giving rise to a decrease in density (-32.9%),**
- **increase in skin tone (+15.5%).**

The difference in the beneficial actions of the two products is to be related to the expected synergistic effect. Synergy was observed *in vitro* for the mixture of Pal-GQPR and Pal-GHK matrikines and suggests an even more positive activity after longer term application of MATRIXYL® 3000.
The male cosmetic market is growing fast in response to a change in modern man's behavior and mentality. Modern man has clearly identified his needs, which are more than just toiletry and shaving care. He wishes to look younger and more radiant and is in search of anti-aging care that will enhance his seductiveness and help him affirm himself professionally.
For a man, preference is given to natural products with strong performances. And, above all, he is looking for simplicity and efficiency: with a complex of matrikins (peptides naturally present in the skin, resulting from skin repair processes) it is possible to recreate favourable conditions leading to tissue renewal. Therefore, this kind of product seems well appropriate to this concern.

In MATRIXYL® 3000, peptides Pal-Glycyl-Histidyl-Lysine and Pal-Glycyl-Glutamyl-Prolyl-Arginine are combined for their synergistic effect on the reconstitution of matrix proteins and in order to strengthen tissue cohesion.

After 2 months of twice daily application of a 4% MATRIXYL® 3000 formulation by a male panel of mean age 54.5 years, the following were demonstrated vs. placebo (excipient):

- **a 29.4% decrease in the area occupied by deep wrinkles (>200µm), as evidenced by a 30% decrease in wrinkle density.**
- **a 10% reduction in the mean depth of the principal wrinkle with a 17% mean reduction in overall volume.**
- **an 8.4% reduction in roughness, a parameter indicative of surface smoothing.**
- **a 5.4% increase in wrinkle spread angle rendering the wrinkle less visibly perceptible.**

The placebo-treated side showed no significant improvement for any of the above parameters.

A new series of tests carried out by SEDERMA in 2010 and 2012 have demonstrated MATRIXYL® 3000's efficacy on repairing skin damage caused by age through two complementary approaches: protection of the papillary dermis against photo-ageing on the one hand and reversion of the chronological ageing on the other hand.

The papillary dermis, situated just below the dermis/epidermis junction, is a tissue more fragile than the reticular dermis, which is situated even deeper. It particularly undergoes U.V. radiation attacks which cause more damage. So, ageing alters its functions and characteristics in a more noticeable way.

Today, there are only a few *in vivo* methods which can estimate its characteristics: the Subepidermal Low Echogenicity band (SLEB) and the confocal microscopy.

These methods were used to measure the effects of the application of a cream containing 3% MATRIXYL® 3000 on the repair of the papillary dermis which had and had not undergone premature ageing.

We note a significant improvement in thickness just after one month (-5.5/placebo) and in density (+7.8/placebo) of the SLEB. This improvement is confirmed after two months:

- **SLEB thickness: -11%/placebo on the unexposed dermis and -14.4%/placebo on the exposed dermis.**
- **SLEB density: +15.2%/placebo on the unexposed dermis and +15.1%/placebo on the exposed dermis.**

Based on published studies with reference to the variation in SLEB thickness according to age, it is possible to deduce that using MATRIXYL® 3000 for two months provides a gain of 5.5 years on the ageing of the papillary dermis.

The confocal laser microscopy produces an image of the inside of the skin in a non invasive way. The fibre structures of the damaged papillary dermis by ageing reveal a disrupted structure, clustered and fragmented.

After a two month treatment and image analysis on the external eye corner, it clearly appears that MATRIXYL ® 3000 helps to reduce the fibre fragmentation and notably supports the reconstruction of the papillary dermal fibre network:

- **Fragmentation of the papillary dermal fibres: -13.9%/T0.**

MATRIXYL ® 3000 is an effective cosmetic active in the repair of the damage due to ageing and U.V. attacks in the papillary dermis.

By regulating the expression of senescence markers, and in particular the progerin in comparison to its normal form (the lamin A), MATRIXYL® 3000 contributes to its ability to reverse chronological ageing.
Ex vivo studies have shown that with age the synthesis of constituent, anchorage and cohesion, proteins of the dermis and dermal-epidermal junction (Collagen I, Collagen IV, Collagen VII, Collagen XVII and Nidogen I) significantly decreased whereas these syntheses are significantly stimulated with MATRIXYL® 3000 vs. placebo. With Matrixyl®3000, ageing skin tends to behave like young skin.

In an anti-wrinkle treatment for both men and women,
we recommend to use MATRIXYL ® 3000 at 3%
to enhance skin's smoothness and radiance.

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10. ANNEXES

ANNEX 1

Clinical study on female panelists:
MATRIXYL® 3000 (Ref. A) vs. Placebo (Ref. B)

Starting materials	INCI	% Ref. A	% Ref. B
Phase 1			
Demineralized water	Water (Aqua)	q.s. 100	q.s. 100
Ultrez 10	Carbomer	0.15	0.15
Phase 2			
Glycerin	Glycerin	3.50	3.50
Phase 3			
Brij S2 SS	Steareth-2	0.40	0.40
Brij S10 SO	Steareth-10	1.20	1.20
Crodafos CES	Cetearyl Alcohol (and) Dicetyl Phosphate (and) Ceteth-10 Phosphate	4.00	4.00
DC 345	Cyclopentasiloxane (and) Cyclohexasiloxane	2.00	2.00
Laurocapram	Laurocapram	2.50	2.50
Crodamol OSU	Diethylhexyl Succinate	7.00	7.00
Preservatives		q.s.	q.s.
Phase 4			
Potassium sorbate	Potassium Sorbate	0.10	0.10
Phase 5			
NaOH 30%	Sodium Hydroxide	0.30	0.30
Phase 6			
MATRIXYL® 3000		3.00	-
Placebo*		-	3.00
Phase 7			
Fragrance		q.s.	q.s.

* Placebo: **MATRIXYL® 3000** excipient

ANNEX 2

Clinical study on male panelists:
(**MATRIXYL® 3000** ref. SED 0608573A / Placebo ref. SED 0608573B)

Starting materials	INCI Name	Suppliers	Placebo (%)	MATRIXYL® 3000 (%)
Phase 1				
Demineralized water	Water (Aqua)		qsp 100	qsp 100
Ultrez 10	Carbomer		0.25	0.25
Phase 2				
Glycerin	Glycerin		3.50	3.50
Phase 3				
Brij S2 SS	Steareth-2	CRODA	0.40	0.40
Brij S10 SO	Steareth-10	CRODA	4.00	4.00
Crodafos CES	Cetearyl Alcohol (and) Dicetyl Phosphate (and) Ceteth-10 Phosphate	CRODA	2.00	2.00
DC 345	Cyclopentasiloxane (and) Cyclohexasiloxane		2.50	2.50
Laurocapram	Laurocapram		7.00	7.00
Crodamol OSU	Diethylhexyl Succinate	CRODA	1.20	1.20
Preservatives				
Phase 4				
Potassium Sorbate	Potassium Sorbate		0.10	0.10
Phase 5				
NaOH 30%	Sodium Hydroxide		0.40	0.40
Phase 6				
MATRIXYL® 3000	(voir synopsis)	SEDERMA	-	4.00
Placebo*			4.00	-
Phase 7				
Fragrance	Fragrance		0.10	0.10

* Placebo: **MATRIXYL® 3000** excipient

OPERATING PROCEDURE:

(Laboratory-scale preparation)

1. Weigh the ingredients of phase 1 together and allow to swell without stirring for 20 minutes.
2. Incorporate phase 2 in phase 1.
3. Heat phases 1+2 in a water-bath at 75°C.
4. Weigh the ingredients of phase 3 together. Heat phase 3 in a water-bath at 75°C and blend thoroughly.
5. Incorporate phases 1+2 in phase 3 under stirring (Staro v=30%)
6. Blend thoroughly. Add phase 4.
7. Neutralize by adding phase 5 at about 55°C and blend thoroughly.
8. Incorporate phase 6 at about 45°C and blend thoroughly.
9. Incorporate the fragrance, phase 7, at about 35°C and blend thoroughly.

ANNEX 3

Clinical study on female panelists (on papillary dermis)

Starting materials	INCI Name	Suppliers	Placebo (%)	MATRIXYL® 3000 (%)
Phase 1				
Demineralized water	Water (Aqua)		qsp 100	qsp 100
Ultrez 10	Carbomer		0.25	0.25
Phase 2				
Glycerin	Glycerin		3.50	3.50
Phase 3				
Brij S2 SS	Steareth-2	CRODA	0.40	0.40
Brij S10 SO	Steareth-10	CRODA	4.00	4.00
Crodafos CES	Cetearyl Alcohol (and) Dicetyl Phosphate (and) Ceteth-10 Phosphate	CRODA	2.00	2.00
BRB CM 56	Cyclopentasiloxane (and) Cyclohexasiloxane		2.50	2.50
Laurocapram	Laurocapram		7.00	7.00
Crodamol OSU	Diethylhexyl Succinate	CRODA	1.20	1.20
Preservatives				
Phase 4				
Potassium sorbate	Potassium Sorbate		0.10	0.10
Phase 5				
NaOH 30%	Sodium Hydroxide		0.40	0.40
Phase 6				
MATRIXYL® 3000	(seesynopsis)	SEDERMA	-	3.00
Placebo*			3.00	-

* Placebo : Excipient de **MATRIXYL® 3000**

Operating procedure:

(Laboratory preparation)

1. Weigh together the phase 1 ingredients and leave to swell without mixing for 20 minutes.
2. Incorporate phase 2 into phase 1.
3. Heat phase 1+2 in a water bath at 75°C.
4. Weigh the phase 3 ingredients together. Heat phase 3 in a water-bath at 75°C, homogenise well.
5. Incorporate phase 1+2 into phase 3, mixing with Staro v=30%.
6. Homogenise well and add phase 4.
7. Neutralise by adding phase 5 at approximately 55°C and homogenise well.
8. Incorporate phase 6 at around 45°C and homogenise well.
9. Add fragrance at around 35°C with phase 7 and homogenise well.

ANNEXE 4

	Thickness of the SLEB (µm)			Densification of the SLEB (NG)		
	T0	**T 1 month**	**T 2 months**	**T0**	**T 1 month**	**T 2 months**
Placebo / internal face	171 ± 20	175 ± 20	173 ± 20	19.67 ± 1.91	18.95 ± 2.40	18.93 ± 2.31
MATRIXYL® 3000 / internal face	**176 ± 30**	**171 ± 20**	**159 ± 20**	**18.88 ± 2.67**	**19.65 ± 2.34**	**21.03 ± 3.11**
% change **MATRIXYL® 3000** *vs*. T0) Significativity Maximum Responders		-2.8% *p<0.05* → -17% 68%	**-9.8%** ***p<0.01*** **→ -23%** **93%**		4.1% *p<0.05* → +32% 61%	**11.4%** ***p<0.01*** **→ +44%** **68%**
% variation **MATRIXYL® 3000** *vs*. placebo) Significativity		-5.5% *p<0.01*	**-11%** ***p<0.01***		+7.8% *p<0.01*	**+15.2%** ***p<0.01***
Placebo / external face	195 ± 30	*nd*	204 ± 30	16.25 ± 2.87	*nd*	15.67 ± 2.62
MATRIXYL® 3000 / external face	**193 ± 40**	*nd*	**174 ± 30**	**16.57 ± 4.74**	*nd*	**18.48 ± 3.58**
% change **MATRIXYL® 3000** *vs*. T0) Significativity Maximum Responders		*nd*	**-9.8%** ***p<0.01*** **→ -33%** **86%**		*nd*	**11.5%** ***p<0.01*** **→ +45%** **82%**
% variation **MATRIXYL® 3000** *vs*. placebo) Significativity		*nd*	**-14,4%** ***p<0,01***		*nd*	**+15,1%** ***p<0,01***

	Mean circumference of fibres at a depth of approximately 70µm (pixels)		
	T0	**T 1 month**	**T 2 months**
Placebo	192.9 ± 36.3	201.5 ± 38.3	194.3 ± 33.7
% change *vs.* T0 Significativity		4.5% *Dns*	0.7% *Dns*
MATRIXYL® 3000	**179.1 ± 25.3**	**199.0 ± 39.4**	**204.1 ± 45.4**
% change **MATRIXYL® 3000** *vs*. T0) Significativity Maximum Responders		+11.1% *p<0.05* →+64% 64%	**+13.9%** ***p<0.01*** **→ +54%** **71%**
% variation **MATRIXYL® 3000** *vs*. placebo) Significativity		+6.6% *p=0.27*	**+13.2%** ***p<0.05***

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