Matrixyl™ 3000

Synopsis

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

CTFA / INCI Name: Glycerin (and) Butylene Glycol (and) Aqua (water) (and) Carbomer (and) Polysorbate-20 (and) Palmitoyl Oligopeptide (and) Palmitoyl Tetrapeptide-3

♦ *<u>In vitro:</u>*

➢ **<u>Study of de novo matrix sythesis by human fibroblasts:</u>**

Synergistic effect of the combination of Pal-GQPR plus Pal-GHK
- Collegene 1:   +258% **MATRIXL™ 3000**
- Fironectin: +164% **MATRIXL™ 3000**
- Hyaluronic acid: +179% **MATRIXL™ 3000**

➢ **<u>Gene activation study</u>** (DNA Array method).

On SkinEthic® epidermis: 20 genes positively activated of which:
- EGF, PDGF, Rho, Rho GTP, ά-catenin, for proliferation, migration
- Fibronectin, laminin, hemisdesmoosomal 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

- *<u>In vivo:</u>*

Two groups of 24 volunteers to compare **MATRIXL™ 3000** vs. **MATRIXL™** and **MATRIXL™ 3000** vs. Placebo, respectively.
- Reduction in main wrinkle depth (-15%) and volume (-18%),
- Reduction in roughness (-14%),
- Reduction in complexity (-16%), "lifting" parameter,
- Decrease in the area occupied by deep wrinkles (>200µm) (-44%), -37% decrease in density,
- Increase in skin tone (+15%).

Toxicology (as per UNITIS Charter): Patch test on humans (10 volunteers)
HETCAM (ocular)
RIPT sensitization (50 volunteers)
Mutagenicity (Ames)
Expert certification

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 product of the extracellular matrix, thus recycled as cell messengers, is 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 a1-pro-collagen, the tripeptide GHK derived from the a2 chain of collagen 1 [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.

SEDERMA's in-depth knowledge of certain peptides from Matrikine family and the reference status of palmitoyl pentapeptide KTTKS (=MATRIXYL™) with regard to anti-wrinkle efficacy in vivo, led us to investigate for an even more marked effect on matrix turnover using certain combinations of peptides.

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-Histydyl-Lysine (Pal-GHK, **BIOPEPTIDE-CL** peptide) and Pal-Glycyl-Glutaminyl-Prolyl-Arginine (Pal-GQPR, **RIGIN**™ peptide).

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

2. <u>REVIEW OF THE DATA ON PEPTIDE PAS-GHK</u> (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 <u>Collagen and glycosaminoglycan synthesis</u>

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.
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 5 ppm).

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**

2.2 <u>Collagen repair post-UVA irradiation</u>
A dermal protective and repairing effect of Pal GHK on the collagen contained in the skin, post-UVA irradiation, was demonstrated [Lintner and Prechard, 2000].

In those tests, the efficacy of the tripeptide Pal-Gly-His-Lys (5ppm) was compared to that of retinoic acid (500ppm), and the activities found to be equivalent.

Compared with the irradiate control, the biopsy specimens treated with 5% B**IOPEPTIDE-CL** (i.e. 5 ppm peptide Pal-GHK equivalent) and those treated with 500ppm retionoic acid showed increased collagen fiber density in the presence of Pal-GHK 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.

2.3. In vivo increase in skin thickness by ultrasonography

A clinical trial conducted on 23 subjects enabled demonstration of a significant in crease in skin thickness (epidermis/dermis) following daily application of a cream formulation containg 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
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 and 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. NEW IN VITRO DATA ON THE ACTIVITIES OF PAL-GHK and PAL-GQPR

MATRIXL™ 3000 constitutes an improvement on and an alternative to **MATRIXL™**, the pentapeptide with sequence Pal-KTTKS of international renowned [Mas-Chamberlin et al., 2002, Lintner 2002, Robinson et al., 2002].

MATRIXL™ 3000 takes the Matrikine concept further by combining the tripeptide and tetrapeptide and for a stronger anti-aging reparative effect.

4.1 In vitro comparative study of the constituents of the extracellular matrix

The effects of various matrikines on stimulation of extracellular matrix reconstitution, with the matrikines alone or in combination, were investigated.

The reference matrikine consisted of the psptide, Palmitoyl-Lysyl-threonyl-threonyl-Lylyl-Serine (Pal-KTTKS = **MATRIXL™**), since that peptide had yielded excellent clinical results with regard to reduction of the wrinkles of crow's feet.
An in vitro comparative study was conducted on the main connective tissue markers: de novo collagen 1, fobronectin 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 $10^{-6}\%$ TGF β.
The cultures were incubated in the presence of vitamin C and rising quatities of each peptide under study for 72 hours.

Matrix proteins (collagen 1 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 3
De novo collagen 1 synthesis after NHF incubation for 72 hours

Product	Concentration	Collagen 1
TGF β	$10^{-6}\%$	102%
Pal-KTTKS	1ppm	10%
	2ppm	45%
	4ppm	84%
	8ppm	149%
Pal-GQPR	0.5ppm	-3%
	1.5ppm	-1%
	2.5ppm	-18%
	3.5ppm	57%
Pal-GHK	1ppm	-3%
	3ppm	-5%
	5ppm	3%
	7.5ppm	6%
MATRIXL™ 3000 Pal-GHK + Pal-GQPR	1% (1.5ppm)	5%
	3% (4.5ppm)	35%
	5% (7.5ppm)	49%
	7.5% (11ppm)	258%

The expected results were obtained in the presence of TGFβ, with 102% stimulation of collagen 1 synthesis.

A dose effect was also observed with peptide Pal-KTTKS (**MATRIXL™)** with respect to synthesis of this matrix macromolecule.
Similarly, a dose effect was observed with the combination of the 2 peptides, Pal-GHK and Pal-GQPR (**MATRIXL™ 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.

<u>Table 4</u>
De novo fibronectin and hyaluronic acid synthesis after NHF incubation
for 72 hours

Product	Concentration	Fibronectin	Hyaluronic Acid
TGF β	10^{-6}%	194%	132%
Pal-KTTKS	1ppm	17%	6%
	2ppm	27%	13%
	4ppm	64%	26%
	8ppm	119%	30%
Pal-GQPR	0.5ppm	2%	8%
	1.5ppm	8%	12%
	2.5ppm	26%	18%
	3.5ppm	47%	16%
Pal-GHK	1ppm	1%	5%
	3ppm	11%	25%
	5ppm	-2%	9%
	7.5ppm	5%	14%
MATRIXL™ 3000 Pal-GHK + Pal-GQPR	1% (1.5ppm)	3%	3%
	3% (4.5ppm)	18%	14%
	5% (7.5ppm)	64%	46%
	7.5% (11ppm)	164%	179%

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

Pal-KTTKS activated de novo synthesis of fibronectin (up to 119%) and hyaluronic acid (30%) with a dose effect. Pal-GQPR only stimulated fibronectin synthesis, and to a more moderate degree.

Combination of Pal-GQPR and Pal-GHK in **MATRIXL™ 3000** induced a synergy with 164% stimulation, i.e. greater stimulated than with Pal-KTTKS (119%).
With regard to hyaluronic acid, Pal-KTTKS stimulated de novo synthesis by 30 % with no clearly marked dose effect. The combination of the 2 peptides in **MATRIXL™ 3000**

enabled a 179% gain in stimulation vs. the values of 16% and 14% obtained with the peptides separately.

4.2. 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.

Pal-GQPR and Pal-KTTKS thus show very similar activation profiles.
The genes regulated in the same manner are those for functions associated with cell proliferation (PDGF associated protein and subunit, response factor ERF1), matrix remodeling (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-a-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 innervations in order to constitute a fully operational new tissue.

The profile characterized by the genes activated in fibroblasts showed that Pal-GHK stimulated numerous genes more strongly than Pal-KTTKS.

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 groth factor) and migration (tenascin, syndecan-4, FGF, Rho GDP) was also observed.

Conclusion on the gene activation profiles

When the functions that can be potentially activated y the Pal-GHK and Pal-GQPR combination are reviewed, a very complete profile emerges with respect to matrix remodeling 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 innervention.

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 **Comparison: MATRIXL™ 3000 vs. MATRIXL™ and vs. Placebo**

The clinical study had a duration of 2 months, since the clinical studies previously conducted with **MATRIXL™** had shown instillation of an already marked effect over that time period.

Forty-nine female volunteers (aged 39 to 74 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:

Panel 1:   randomized application of **MATRIXL™ 3000** vs. placebo
               (24 volunteers of mean age 56.1 years)

Panel 2:   randomized application of **MATRIXL™ 3000** vs. **MATRIXL™**
               (25 volunteers of mean age 55.6 years).

MATRIXL™ 3000, MATRIXL™ and the excipient were incorporated at a concentration of 3% in a cream formulation (cf. Appendix) 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 wrinkes (between 150 and 200 μm), the complexity (%) and density of the main wrinkles (μm/cm^2) and the roughness, now a conventional parameter.

For each parameter, mean values and the percentage change on the baseline value (T0) was calculated and appropriate statistical test 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 SEM474 Cutometer® fitted with a 2-mm probe.

5.1.1. Results: MATRIXL™ 3000 vs. MATRIXL ™

Results

After 56 days, a very significant decrease in deep and intermediate wrinkles was obtained for the 2 half-faces treated with the emulsion containing 3% **MATRIXL™ 3000 or 3% MATRIXL™.**

Table 7
Evolution of the percentage area occupied by deep wrinkles (>200 μm) for
MATRIXL™ 3000 and MATRIXL™ after 56

% Area occupied by deep wrinkles (> 200 μm)	MATRIXL™ 3000			MATRIXL™	
	T0	T56		T0	T56
Mean	3.7±2.7	2.0±1.5		3.1±2.2	2.2±2.1
% Difference vs. T0	-44.9%			-27.7%	
	Statistical tests for paired series				
Comparison T56 v. T0	P<0.01			P<0.05	
Comparison MATRIXL™ 3000 vs. MATRIXL™	Non-significant difference				

The area occupied by deep wrinkles was reduced by **44.9%**, on average, over 2 months, for **MATRIXL™ 3000** and 27.7% for **MATRIXL™. The difference between T0 and T56 was very significant.** There was no significant difference between the two products.

An important decrease in the mean density of the wrinkles was observed for **MATRIXL™ 3000 and MATRIXL™ of -37% and -27.3%, respectively.** The differences vs. T0 were significant. However, the stronger trend observed with **MATRIXL™ 3000** was not statistically significant different from that observed with **MATRIXL™.**

Table 8
Evolution of the main wrinkle density for MATRIXL™ 3000 and MATRIXL™
after 56 days of application

Main wrinkle density ($\mu m/cm^2$)	MATRIXL™ 3000			MATRIXL™	
	T0	T56		T0	T56
Mean	2.7±1.8	1.7±1.1		2.9±2.3	2.1±2
% Difference vs. T0	-37%			-27.3%	
	Statistical tests for paired series				
Comparison T56 v. T0	P<0.01			P<0.05	
Comparison MATRIXL™ 3000 vs. MATRIXL™	Non-significant difference				

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 two months of application of each of the products.

The results obtained showed **a very marked and significant decrease in the mean depth of the wrinkle and its volume between the start and end of the study: -15.1% and -18.5%, respectively, for MATRIXYL™ 3000.**

For **MATRIXYL™,** the mean decrease in depth and volume was -9.85 and -14.7%, respectively. Both differences were very significant but less than those observed with **MATRIXYL™ 3000.**
As was previously the case, the between-individual variability does not enable a significant difference between the two products to be evidenced.

Table 9
Evolution of a main wrinkle mean depth for MATRIXYL™ 3000 and MATRIXYL™ after 56 days of application

Main wrinkle mean depth (µm)	MATRIXL™ 3000		MATRIXL™	
	T0	T56	T0	T56
Mean	73.0±8.3	62.0±12.3	67.4±11.5	60.8±12.6
% Difference vs. T0	-15.1%		-9.8%	
	Statistical tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	
Comparison MATRIXL™ 3000 vs. MATRIXL™	Non-significant difference			

Table 10
Evolution of a main wrinkle mean volume for MATRIXYL™ 3000 and
MATRIXYL™ after 56 days of application

Main wrinkle mean volume (mm^3)	MATRIXL™ 3000		MATRIXL™	
	T0	T56	T0	T56
Mean	0.37±0.12	0.30±0.10	0.33±0.11	0.28±0.10
% Difference vs. T0	-18.5%		-14.7%	
	Statistical tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	
Comparison MATRIXL™ 3000 vs. MATRIXL™	Non-significant difference			

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.

Table 11
Evolution of roughness for MATRIXYL™ 3000 and
MATRIXYL™ after 56 days of application

Roughness (µm)	MATRIXL™ 3000		MATRIXL™	
	T0	T56	T0	T56
Mean	26.7±5.5	22.8±4.1	26.5±6.0	23.7±6.0
% Difference vs. T0	-14.4%		-10.8%	
	Statistical tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	
Comparison MATRIXL™ 3000 vs. MATRIXL™	Non-significant difference			

Table 12
Evolution of complexity for MATRIXYL™ 3000 and
MATRIXYL™ after 56 days of application

Complexity (%)	MATRIXL™ 3000		MATRIXL™	
	T0	T56	T0	T56
Mean	3.7±1.6	3.1±1.2	3.8±1.9	3.3±1.7
% Difference vs. T0	-16.6%		-12.7%	
	Statistical tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	
Comparison MATRIXL™ 3000 vs. MATRIXL™	Non-significant difference			

The two criteria, roughness and complexity showed a very significant decrease with **MATRIXYL™ 3000 and MATRIXYL™.**
The smoothing effect was, however, more marked with MATRIXYL™ 3000 (-14.4 and -16.6%) than with MATRIXYL™ (-10.8% and – 12.7%) but no statistically significant difference was evidenced.

Two months of application of MATRIXYL™ 3000 or MATRIXYL™ resulted in a very significant reduction in all the prameters characcherizing wrinkle depth and skin surface condition.

MATRIXYL™ 3000 is clearly shown to be somewhat superior to MATRIXYL™ without the difference being statistically significant.

When an efficacy ratio taking into account the percentage changes obtained with the two products is calculated, systematically superior values are observed: **MATRIXYL™ 3000** is 1.3 to 1.6 fold superior to **MATRIXYL™** over the 2 month period. However, this trend in the numerical differences observed in a population of 23 volunteers does not enable statistically significant differences to be evidenced.

The reparative effect of the two matrikine-containing products concepts was visually evident for the volunteers.

5.1.2. Comparison: MATRIXYL™ 3000 vs. placebo

All the parameters stated above were investigated. The volunteers applied **MATRIXYL™ 3000** cream to one half-face and the exipient cream to the other.
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 difference vs. T0 was all non-significant.

Results

Table 13
Comparison of the effects of MATRIXYL™ 3000 vs.
placebo after 56 days (2 months)

PARAMETERS	MATRIXYL™ 3000	PLACEBO
% area occupied by wrinkles >200µm	-39.4**	+4.3n.s.
Wrinkle density	-32.6**	-9.6n.s.
Roughness	-16.0**	+1.4n.s.
Complexity	-15.7**	+4.2
Mean volume of a main wrinkle	-23.3**	-8.7*
Mean depth of a main wrinkle	-19.9**	-3.2n.s.

5.2. Cutometric study of MATRIXYL™ 3000 vs. MATRIXYL™ and vs. 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. **MATRIXYL™** and **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.
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Table 14
Evolution of the raw elasticity for MATRIXYL™ 3000 and
MATRIXYL™ after 56 days of application
(mean values for n = 24 volunteers)

Raw elasticity	MATRIXL™ 3000		MATRIXL™	
	T0	T56	T0	T56
Mean	0.495±0.07	0.540±0.07	0.476±0.05	0.525±0.05
% Difference vs. T0	+9.1%		+10.4%	
	Statistical tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	

Table 15
Evolution of skin tone for MATRIXYL™ 3000 and
MATRIXYL™ after 56 days of application
(mean values for n = 24 volunteers)

Tone (%)	MATRIXL™ 3000		MATRIXL™	
	T0	T56	T0	T56
Mean	0.124±0.03	0.148±0.03	0.125±0.02	0.143±0.03
% Difference vs. T0	+19.5%		+15.2%	
	Statistical tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	

The positive evolutions for raw elasticity and tone were significant for **MATRIXYL™ 3000** and **MATRIXYL™**, with **MATRIXYL™ 3000** showing slight but non-significant superiority.

Table 16
Evolution of the raw elasticity for MATRIXYL™ 3000 and
placebo after 56 days of application

Raw Elasticity	MATRIXL™ 3000		MATRIXL™	
	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 tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	

Table 17
Evolution of the skin tone for MATRIXYL™ 3000 and
placebo after 56 days of application

Tone (%)	MATRIXL™ 3000		MATRIXL™	
	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 tests for paired series			
Comparison T56 v. T0	P<0.01 Significant difference		P<0.05 Significant difference	

On the second population of subjects, the results again 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.

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 (-18%) and depth of deep wrinkles (-15%) giving rise to smoother skin (-14% roughness) and a "lifted surface (-16% complexity) which, in the cutometric study, was reflected in a improvement in tone (+15%).

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.   <u>OVERALL CONCLUSION</u>

Matrikines, small endogenous peptides derived from matrix proteolysis, are cell messerngers 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 remodeling (urokinase, TIMP1, Iysyl oxidase, tenascin, syndecan), and cell proliferation, migration and installation (EGF, PDGF, Rho, ά-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 1, fibronectin and hyaluronic acid.

A clinical trial conducted in 2 groups of 24 subjects enabled comparison of the benefits obtained with **MATRIXYL™ 3000** vs. **MATRIXYL™** and **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 (-15%) and in its volume (-18%)**

- **reduction in roughness (-14%) and complexity (-16%), a surface "lifting parameter,**
- **decrease in the area occupied by deep wrinkles (> 200 μm) (-44%), giving rise to a decrease in density (+15%).**

While the difference between MATRIXYL™ 3000, and **MATRIXYL™** was not statistically significant, the enhanced efficacy of the former product was illustrated by a 1.3- to 1.6-fold greater improvement in the profilometric parameters of crow's feet.

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, vs. Pal-KTTKS, and suggest an even more positive activity after longer term application of **MATRIXYL™ 3000.**