PRO-COLL-ONE+®

Collagen I performance

Les incontournables SILAB

SILAB actives you can't perform without

A) General principles

Customers requirements are upgrated, searching for:

- **more visible efficacy,**
- **reliable and trustable molecules,**
- **safety,**
- **cost-effectiveness,**

The collagen is a well-identified target to fight against wrinkles.

Collagen I is the specific molecule to boost, thanks to SILAB's natural solution:

PRO-COLL-ONE+®:

✓ **the benchmark for the collagen I synthesis, obtained from soya**

PRO-COLL-ONE+®, collagen I performance

SILAB's works

Efficacy evaluation for reference molecules:

- Palmitoyl Pentapeptide-3
- Retinol

- *in vitro*: synthesis of collagen I through ELISA assay
- *in vivo*: anti-wrinkle effect by fringe projection

PRO-COLL-ONE+®:

- efficacy similar to those of synthetic molecules,
- is the natural solution to boost collagen I

Summary

Complete

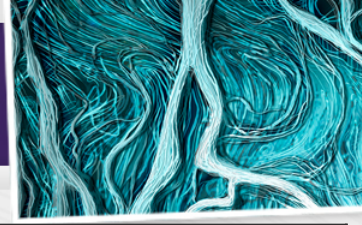
Performances of PRO-COLL-ONE+®

In vitro - Summary

Efficacy	Cells	Method	Marker	Results
Effect on collagen I synthesis	Normal human fibroblasts	ELISA	Collagen I	**PRO-COLL-ONE+® 0.25%:** ⇨ Stimulates the synthesis of collagen I by 1190% ⇨ Has an higher effect than that of palmitoyl pentapeptide -3
		Immunocytology		**PRO-COLL-ONE+® 1 ppm:** ⇨ Stimulates the synthesis of collagen I ⇨ Comparable visualization to that obtained with palmitoyl pentapeptide-3

Performances of PRO-COLL-ONE+®

In vivo - Summary



Panel	Area	Treatment time	Measurement	Method	Results / placebo
50 volunteers + 43 volunteers	crow's feet	56 days twice daily applications	Microrelief	Fringe projection	PRO-COLL-ONE+® 2%: ⇨ Reduces parameter Sa by 9.8% ⇨ Reduces parameter Sq by 9.5% Significantly smoothes the microrelief Comparable efficacy to synthetic molecules
			Wrinkles	Fringe projection	PRO-COLL-ONE+® 2%: ⇨ Reduces positive volume by 27 % ⇨ Reduces negative volume by 21.1% Significantly reduces wrinkles Comparable efficacy to synthetic molecules
			Subjective evaluation	Questionnaire	PRO-COLL-ONE+® 2%: ⇨ It smoothes the crow's feet ⇨ It is an anti-wrinkle Comparable efficacy to synthetic molecules

Les incontournables SILAB — SILAB actives you can't perform without

Technical part

B) Efficacy

In vitro studies

I. Effect of PRO-COLL-ONE+® on the synthesis of collagen I

Comparison with Palmitoyl Pentapeptide-3

I.1. Quantification by ELISA assay

I.2. Visualization by immunocytology

In vivo studies

II. Study of the anti-wrinkles properties of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

III. Subjective evaluation of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

I. Effect of PRO-COLL-ONE+® on the synthesis of collagen I

Comparison with Palmitoyl Pentapeptide-3

Principle

Objective: determine the capacity of PRO-COLL-ONE+® to boost the synthesis of collagen I, the major component of the dermis, in comparison with a synthetic peptide: the Palmitoyl Pentapeptide-3

Method

Model: cell culture

Cells: normal human fibroblasts

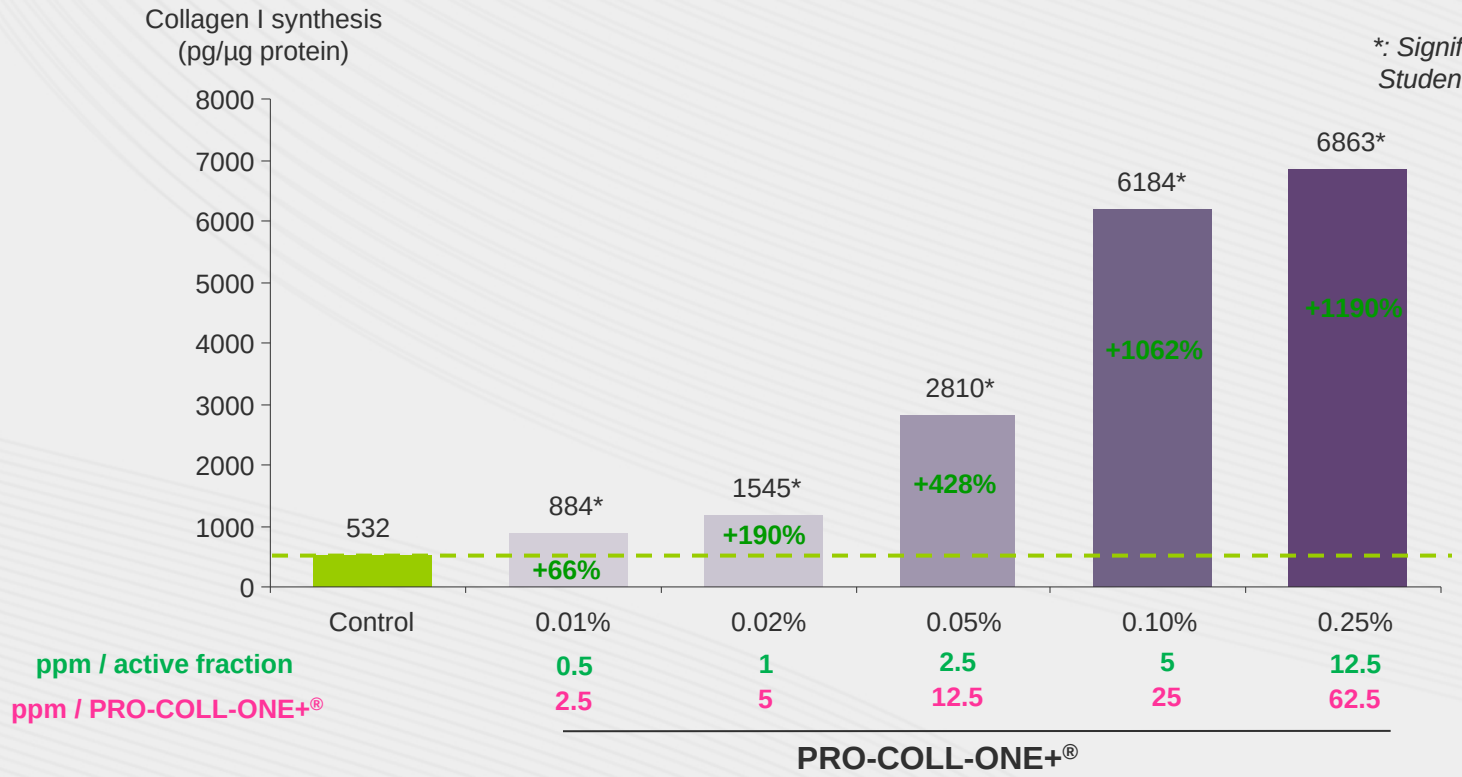
Method:
1. quantification by ELISA assay
2. visualization by immunocytology

Products: PRO-COLL-ONE+® at 0.01%, 0.02%, 0.05%, 0.10% and 0.25%
synthetic peptide: Palmitoyl Pentapeptide-3 at 0.5%, 1% and 2%

Treatment time: 24 hours

Results

1. Quantification by ELISA assay



**: Significant results according to Student's t test (P < 0.05), n = 6*

Effect of PRO-COLL-ONE+® on the synthesis of collagen I.

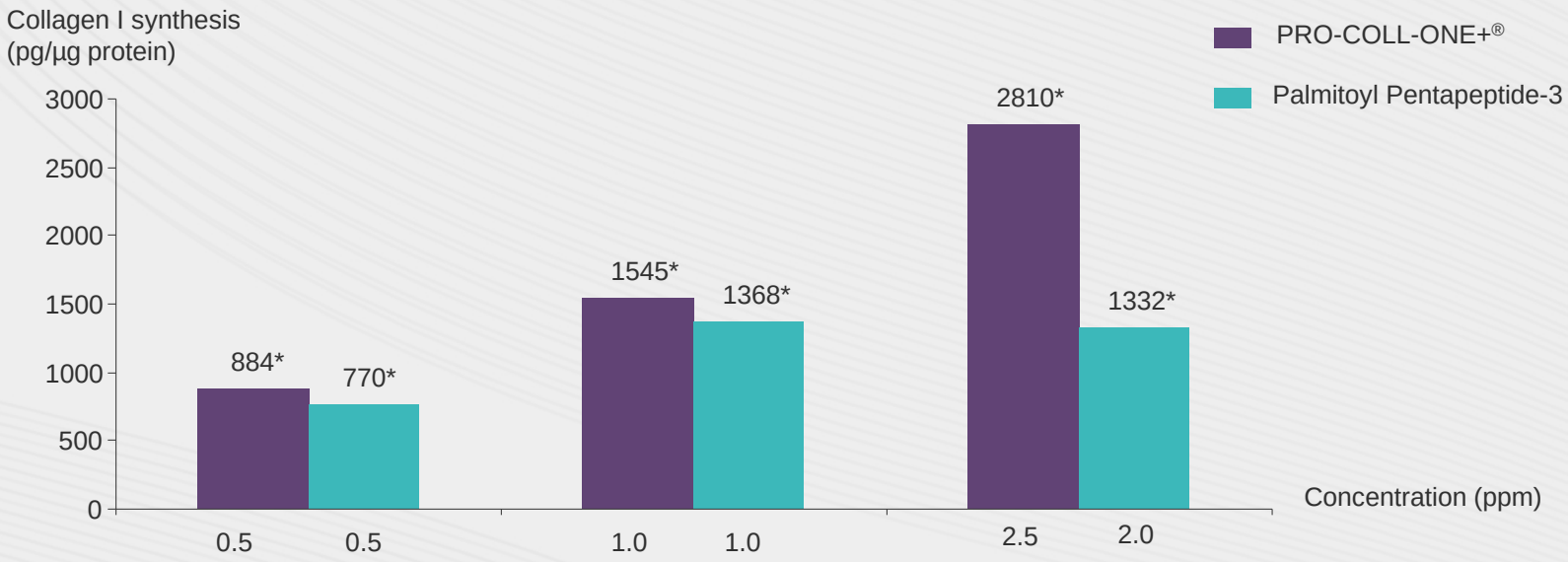
Tested at 0.25% on normal human fibroblasts, PRO-COLL-ONE+® significantly stimulates the synthesis of collagen I by 1190%. This effect is dose-dependent.

I. Effect of PRO-COLL-ONE+® on the synthesis of collagen I

Comparison with Palmitoyl Pentapeptide-3

Results

1. Quantification by ELISA assay



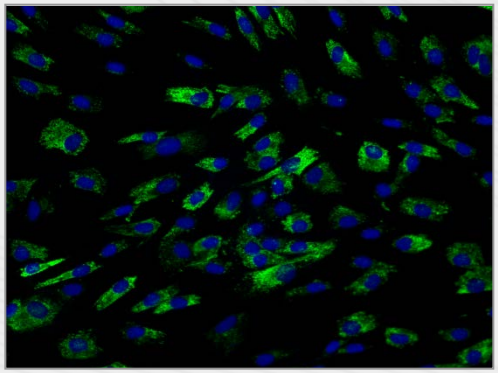
**: Significant results according to Student's t test (P < 0.05)*

Effect of PRO-COLL-ONE+® on the synthesis of collagen I.
Comparison with palmitoyl pentapeptide-3.

Results

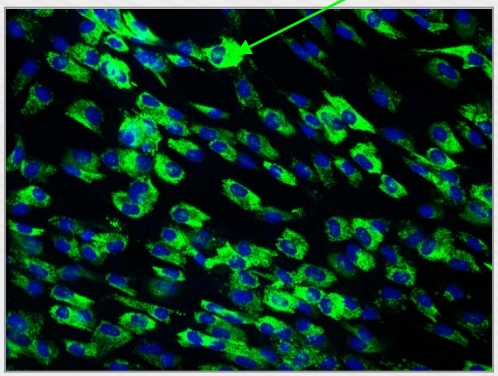
2. Visualization by immunocytology

Synthesis of collagen I
(fluorescence intensity)



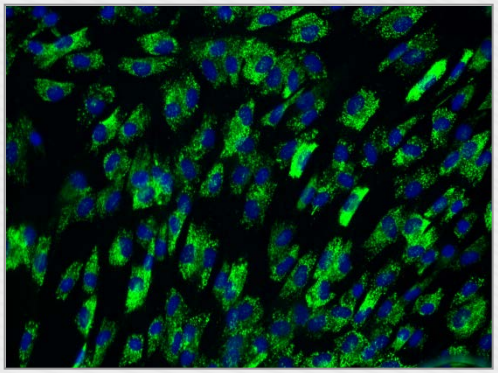
Control

Collagen I: +



1 ppm PRO-COLL-ONE+®

Collagen I: +++



1 ppm Palmitoyl Pentapeptide-3

Collagen I: +++

Immunocytology visualization of the effect of PRO-COLL-ONE+ ® on the synthesis of collagen I.
Comparison with Palmitoyl Pentapeptide-3

Principle

Objective:

1. quantify *in vivo vs.* placebo the anti-wrinkles efficacy of PRO-COLL-ONE+® formulated at 2% in an emulsion
2. compare this efficacy with two reference molecules

Panel:

- **Study of PRO-COLL-ONE+®:**
  - **placebo group: 25 volunteers, mean age 54 ± 8**
  - **PRO-COLL-ONE+® group: 25 volunteers, mean age 53 ± 8**

- **Study of reference molecules with random attribution to each half face, 43 volunteers distributed as follows:**
  - **31 half-faces for the placebo (mean age 55 ± 9)**
  - **27 half-faces for Palmitoyl Pentapeptide-3 (mean age 54 ± 10)**
  - **28 half-faces* for retinol (mean age 52 ± 9)**

*** 10% dropped out for reasons of product application**

II. Study of the anti-wrinkles properties of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

Method

Method:

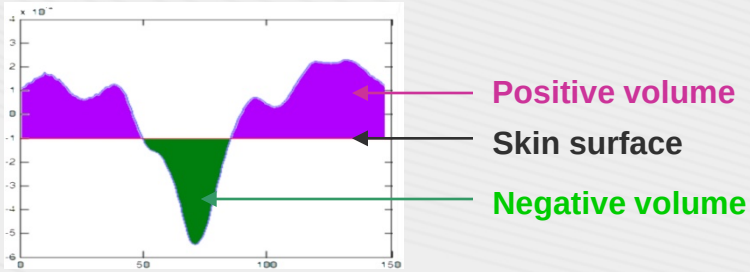
- acquisitions by fringe projection (Eotech, France)

- analysis of acquisitions with Optocat software (Eotech, France)

Studied parameters:

*** 3D roughness parameters: Sa and Sq**

*** volume parameters: positive and negative volumes**



Products: **PRO-COLL-ONE+® formulated at 2% *vs.* placebo**

3% Palmitoyl Pentapeptide-3 (synthetic peptide) and 0.075% retinol *vs.* placebo

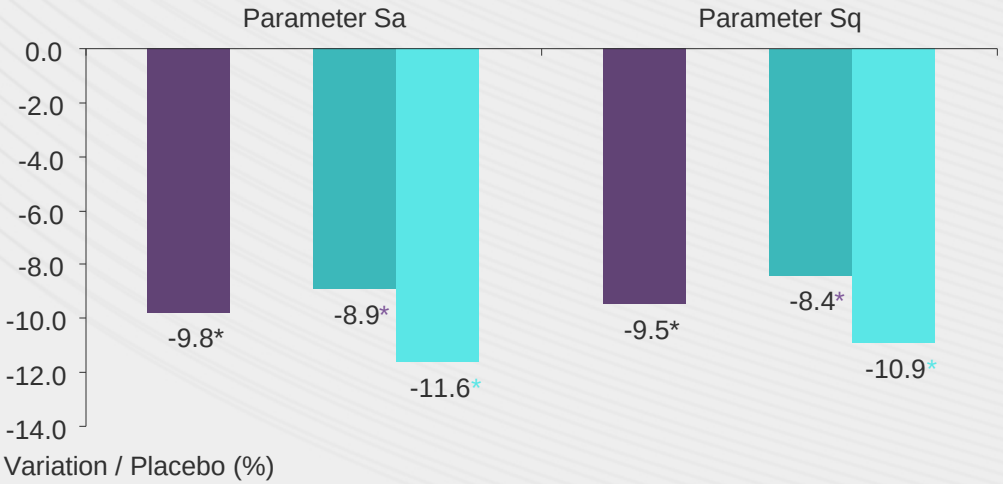
Zone treated: **crow's feet**

Time of treatment: **56 days of twice daily applications**

II. Study of the anti-wrinkles properties of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

Results



**: Significant differences according to modified Student's t test (P < 0.05)*

**: Significant differences according to Student's t test (P < 0.05)*

** : Différences significatives selon le test de Wilcoxon-Mann-Whitney (p<0,05)*

Effect of PRO-COLL-ONE+® formulated at 2% *vs.* placebo on 3D roughness parameters of crow's feet after 56 days of twice daily applications. Comparison with Palmitoyl Pentapeptide-3 and retinol.

In the conditions of this study, after 56 days of twice daily applications and compared to the placebo, PRO-COLL-ONE+® formulated at 2% in an emulsion smoothes skin relief of the crow's feet by significantly reducing:

- parameter Sa by 9.8% (P = 0.0014)

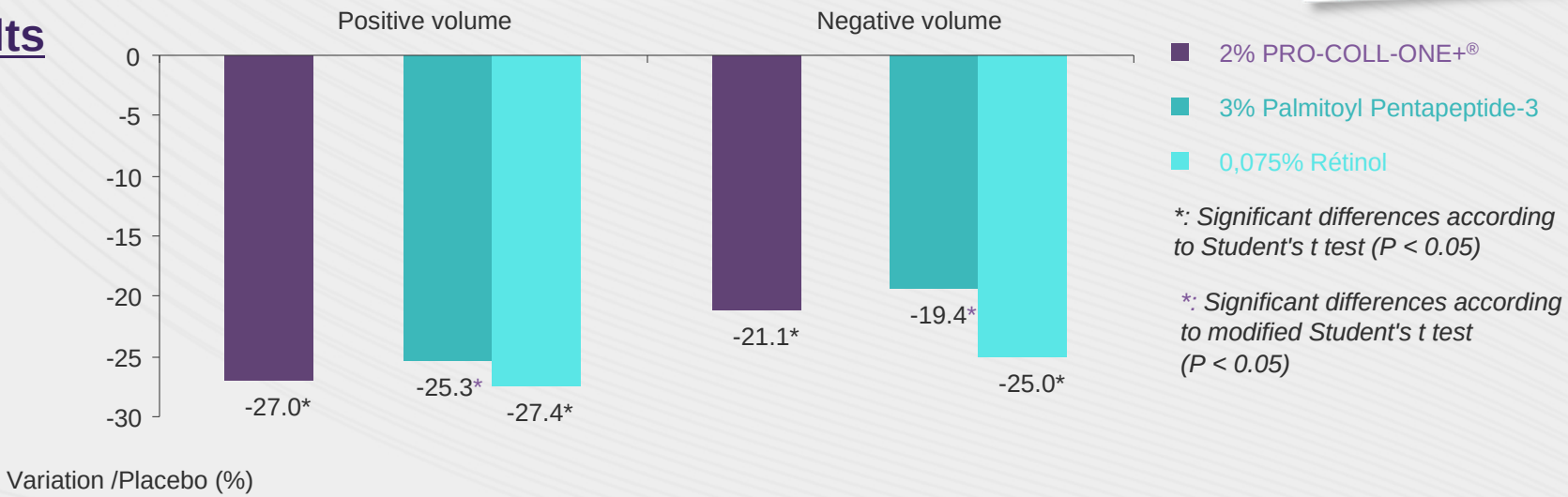
- parameter Sq by 9.5% (P = 0.0026)

The decrease of parameters Sa and Sq was observed in 72% and 76% of volunteers, respectively.

II. Study of the anti-wrinkles properties of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

Results



Effect of PRO-COLL-ONE+® formulated at 2% *vs.* placebo on volume parameters of wrinkles after 56 days of twice daily applications.
Comparison with Palmitoyl Pentapeptide-3 and retinol.

In the conditions of this study, after 56 days of twice daily applications and in comparison to the placebo, PRO-COLL-ONE+® formulated at 2% in an emulsion reduces crow's feet wrinkles by significantly decreasing:

- positive volume by 27.0% (P = 0.0014)

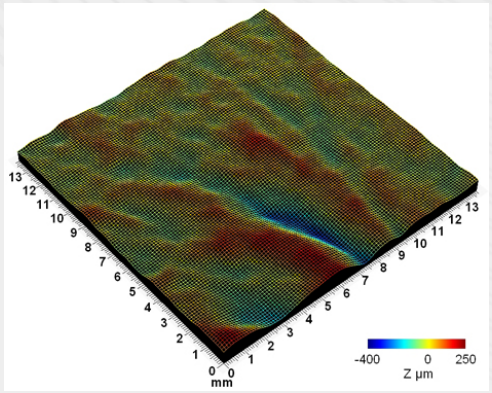
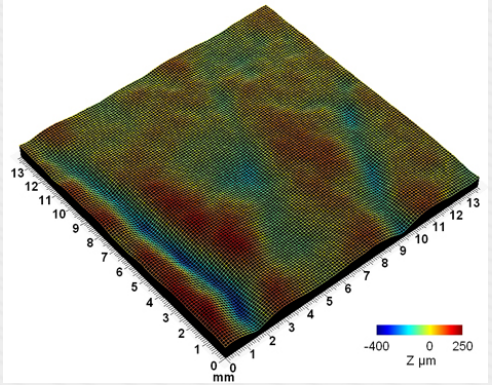
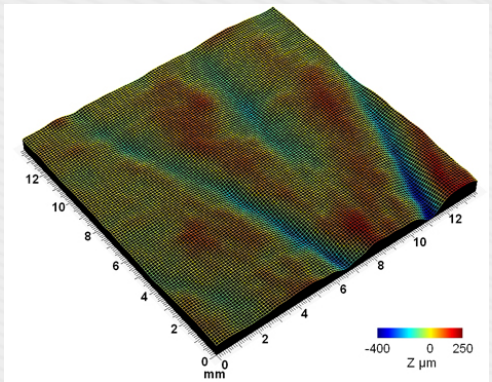
- negative volume by 21.1% (P = 0.0010)

A reduction in positive and negative volumes was observed in 76% and 72% of the volunteers, respectively.

II. Study of the anti-wrinkles properties of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

Results

2% PRO-COLL-ONE+®	3% Palmitoyl Pentapeptide-3	0.075% Retinol
		
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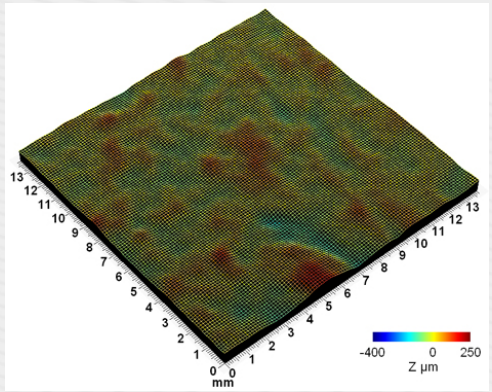
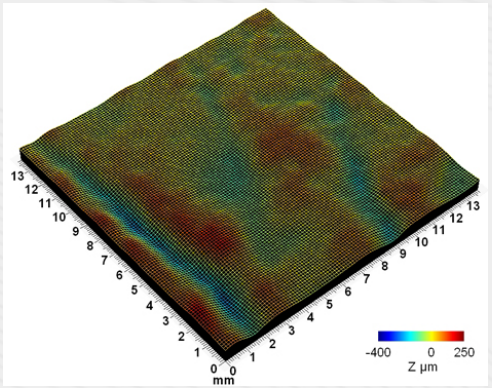
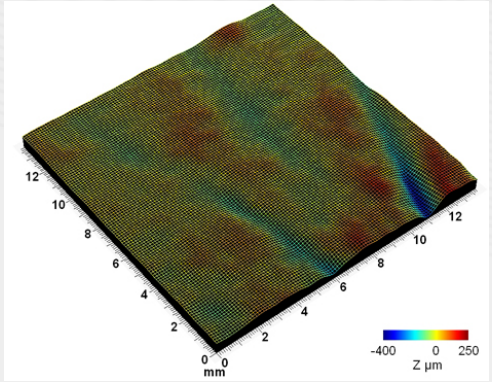
		
D56	D56	D56

Visualization of the anti-wrinkles effect of PRO-COLL-ONE+® formulated at 2% on crow's feet. Comparison with Palmitoyl Pentapeptide-3 and retinol.

Zone of interest: 15 x 15 mm

III. Subjective evaluation of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

Principle

Objective: **quantify *in vivo vs.* placebo the sensations felt during the twice daily use of 2% PRO-COLL-ONE+®, 3% Palmitoyl Pentapeptide-3 or 0.075% retinol**

Panel: **same panel as before**

Method

Method: **self-evaluation questionnaires containing closed questions**

Products: **PRO-COLL-ONE+® formulated at 2% vs. placebo**
Synthetic peptide: 3% Palmitoyl Pentapeptide-3, and 0.075% retinol *vs.* placebo

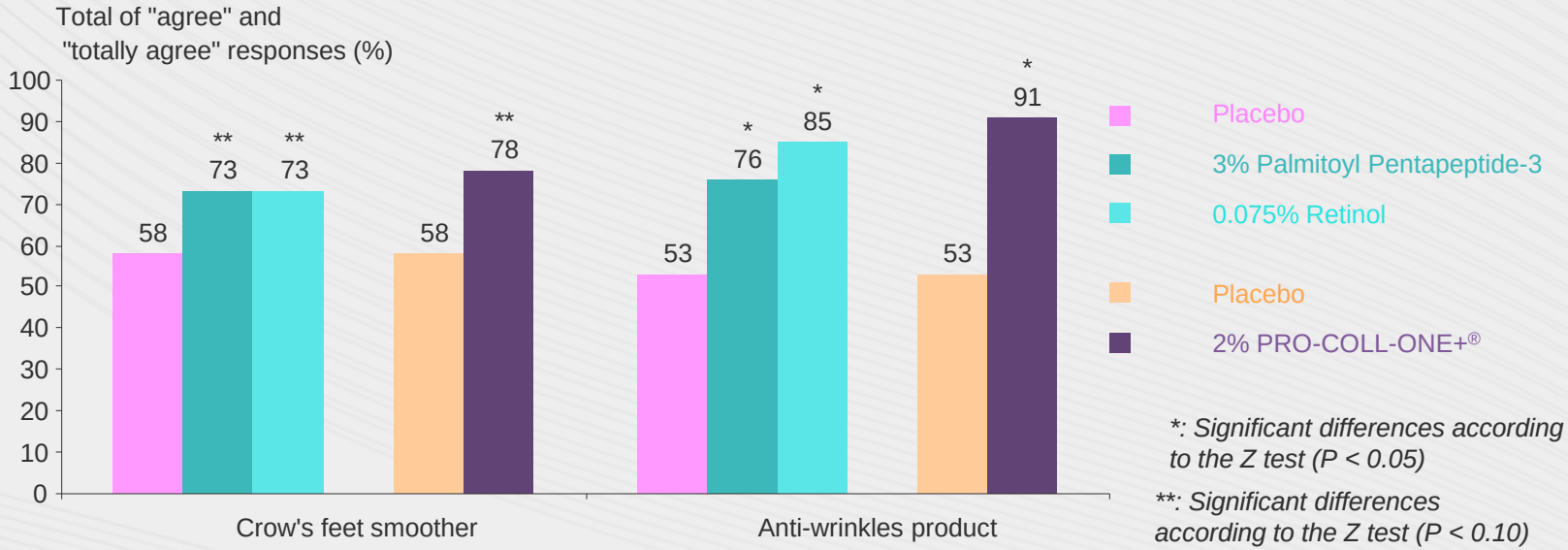
Zones treated: **Half-face**

Treatment time: **56 days of twice daily applications**

III. Subjective evaluation of PRO-COLL-ONE+®

Comparison with Palmitoyl Pentapeptide-3 and retinol

Results



**: Significant differences according to the Z test (P < 0.05)*

***: Significant differences according to the Z test (P < 0.10)*

Subjective evaluation of PRO-COLL-ONE+® formulated at 2% in an emulsion after 56 days of twice daily applications. Comparison with Palmitoyl Pentapeptide-3 and retinol.

In the conditions of this study and in comparison to the placebo, after 56 days of twice daily use, volunteers using PRO-COLL-ONE+® formulated at 2% reported that the product smoothed crow's feet (P = 0.0761) and was anti-wrinkles (P = 0.0016).

PRO-COLL-ONE+® was judged to be comparable to 3% Palmitoyl Pentapeptide-3 and 0.075% retinol.

C) Complementary information

Raw material

- **Latin name:** *Glycine soja*
- **English name:** soybean
- **French name:** soja
- **Countries of origin:** France, South America
- **Applications:** food, medicine
- **Part of the plant:** fiber

Complementary information
Description

Aspect

Form:	aqueous solution
Aspect:	limpid liquid
Odor:	characteristic
Color:	amber
Bacteriology:	sterile product

Characterization

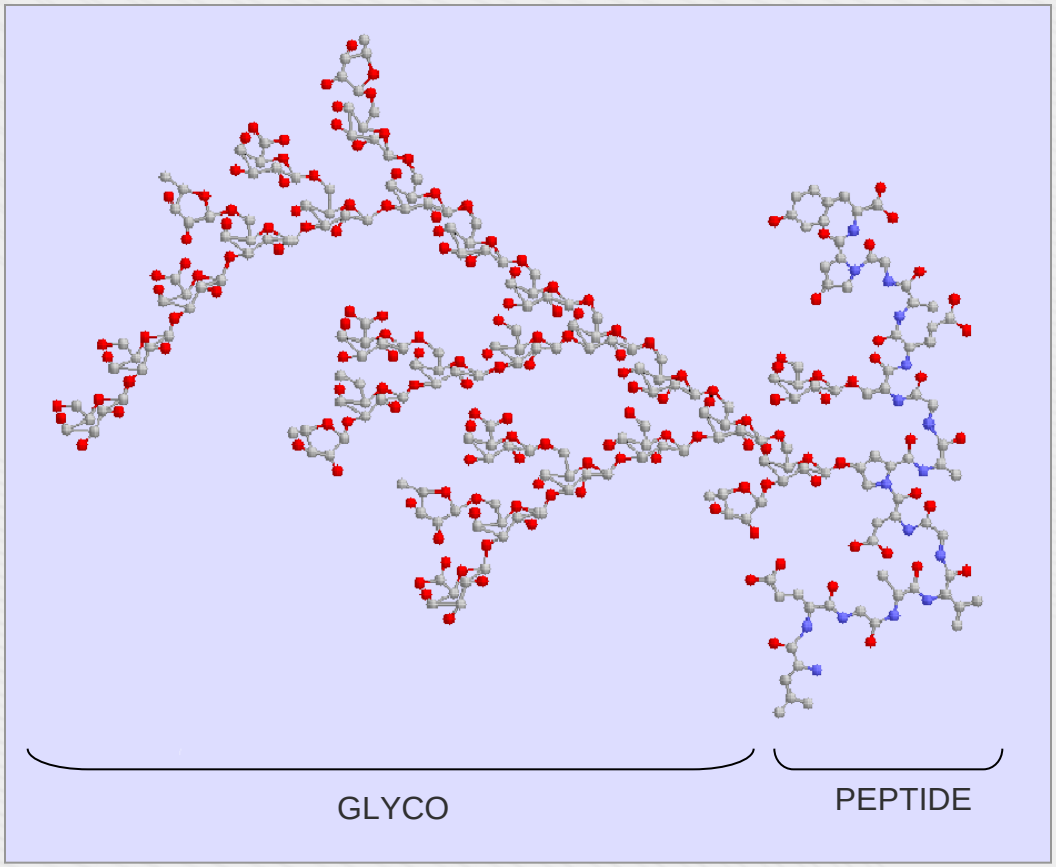
Dry matter:	22 - 32 g/l
Total sugars (Dubois method):	12 - 22 g/l
Hydroxyproline:	≥ 18 mg/g of proteins
pH:	5.0 – 6.0
Stabilizer:	Ethylhexylglycerin 0.20%
Preservative:	Phenoxyethanol 0.50%

Identification and quantification in formula: active **from 1%**

Complementary information

Active fraction

Fraction containing hydroxyproline rich glycopeptides (HRGPs) whose glycosylated chains contain primarily galactose, galacturonic acid and mannose



Modelling of an unitary pattern of the structure of PRO-COLL-ONE+®

Stability

pH:	stable between pH 5 and 10
Temperature:	can withstand temperatures up to 80°C for at least 2 hours
Ethanol:	Ethanol / Water up to 20/80 (v/v)

Compatibility in formulation

Cosmetic formulas	Stability
Aqueous solution	+
Clear gel	+
Opaque gel	+
Emulsified gel	+
Non-ionic emulsion	+
Anionic emulsion	+
Cationic emulsion	+

Complementary information

Cosmetic applications

Compatibility towards cosmetic starting materials

- Thickeners: total compatibility
- Emulsifiers: total compatibility
- Solvent: alcohol max 20%

Proposed cosmetic formulas

- Anti-wrinkles day cream
- Night cream
- Serum

Complementary information

Regulatory data

Latin name (LINNE):	*Glycine soya*
EEC name:	Hydrolyzed Soy Fiber
CAS n°:	68607-88-5
EC n°:	271-770-5
I.N.C.I. name (USA):	Hydrolyzed Soybean Fiber
Japanese name:	Hydrolyzed Soybean Fiber
JCIA n°:	562 593

PRO-COLL-ONE+®

Les incontournables SILAB:
SILAB actives you can't perform without®

**Identified and characterized natural molecules:
soybean HRGPs**

↓

Boosts collagen I synthesis

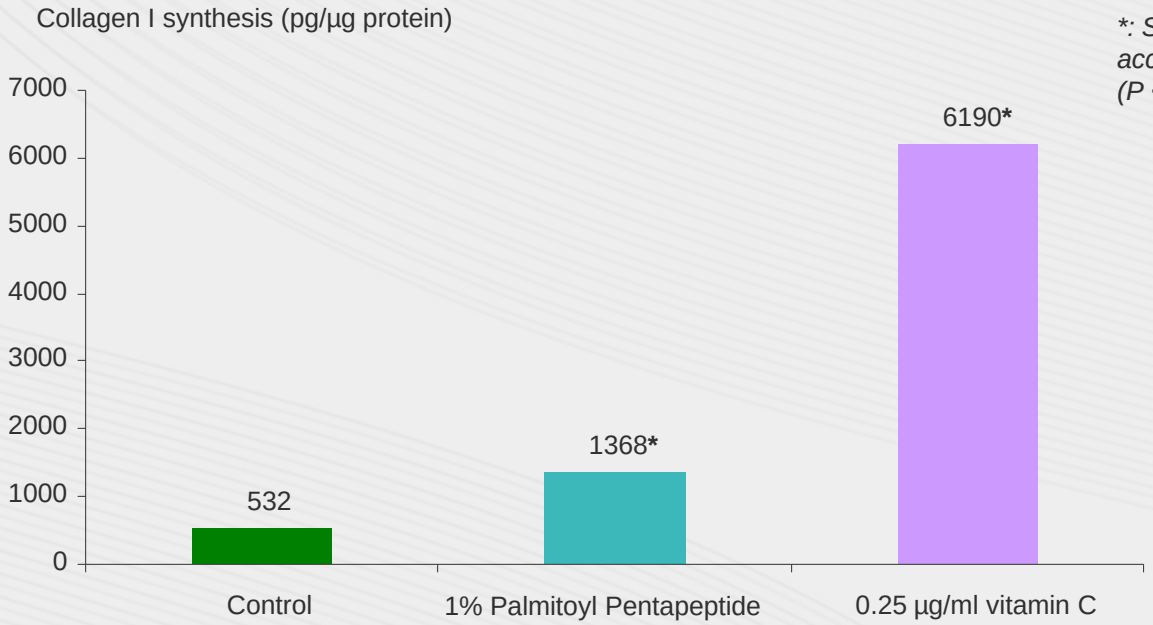
↓

**Smoothes the microrelief and reduces
crow's feet wrinkles**

Study of the efficacy of reference molecules

✓ *In vitro:* synthesis of collagen I by ELISA assay

- Synthetic peptide: 1% (1 ppm) Palmitoyl Pentapeptide-3
- Positive control: 0.25 µg/ml (0.25 ppm) vitamin C



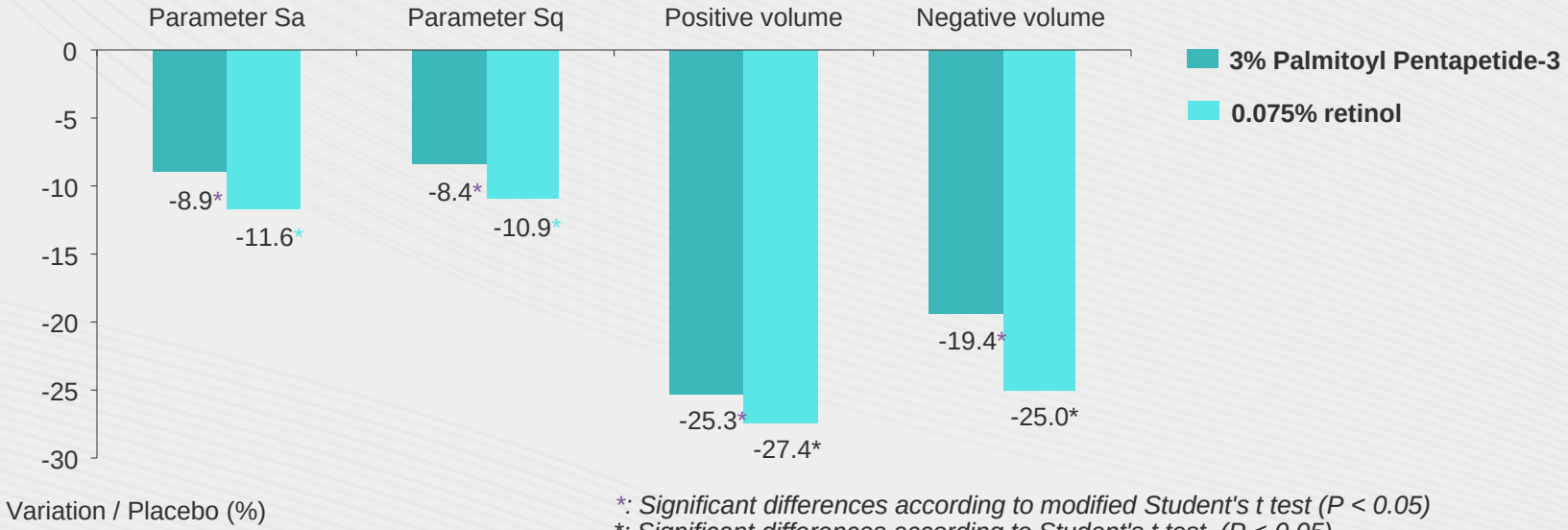
**: Significant differences according to Student's t test ($P < 0.05$)*

Tested on normal human fibroblasts, 1% Palmitoyl Pentapeptide-3 and vitamin C at 0.25 µg/ml significantly stimulate the synthesis of collagen I by 157% and 1064%, respectively.

Study of the efficacy of reference molecules

✓ *In vivo:* anti-wrinkles effect by fringe projection

- Synthetic peptide: 3% Palmitoyl Pentapeptide-3
- 0.075% retinol



*: Significant differences according to modified Student's t test ($P < 0.05$)
*: Significant differences according to Student's t test ($P < 0.05$)
*: Significant differences according to the Wilcoxon-Mann-Whitney test ($P < 0.05$)

After 56 days of twice daily applications and compared to the placebo, 3% Palmitoyl Pentapeptide-3 and 0.075% retinol significantly smooth skin relief and attenuate wrinkles.