LUCASMEYER COSMETICS

ACTIVE INGREDIENTS

TECHNICAL FILE

SWT-7™

SELF-REGENERATIVE STEM CELL TECHNOLOGY

Stimulates stem cells and growth factor production

-

Improves epidermis regeneration and thickness

-

Resurfaces skin and blurs vertical wrinkles after only 7 days!

SUMMARY

SWT-7™ L	
INCI NAME	Isopropyl Palmitate (1°) (and) Lecithin (2°) (and) Water (3°) (and) Swertia Chirata Extract (4°)
CAS	142-91-6 (1°) 8002-43-5 (2°) 7732-18-5 (3°) 97766-44-4 (4°)
EINECS	205-571-1 (1°) 232-307-2 (2°) 231-791-2 (3°) 307-906-8 (4°)
SWT-7™ H	
INCI NAME	Maltodextrin (1°) (and) Swertia Chirata Extract (2°)
CAS	9050-36-6 (1°) 97766-44-4 (2°)
EINECS	232-940-4 (1°) 307-906-8 (2°)
ORIGIN	Titrated swertiamarin extracted from Indian gentian leaves under 2 versions: - hydrosoluble powder: SWT-7™ H - liposoluble liquid (microdispersion): SWT-7™ L
COSMETIC PROPERTIES	• Stimulates epidermal growth factor production by adipose-derived stem cells • Improves hypodermis-epidermis cell communication • Increases keratinocyte proliferation • Improves epidermis regeneration and thickness
SKIN BENEFITS / POTENTIAL CLAIMS	• Blurs vertical wrinkles • Improves skin texture • Decreases lip contour wrinkles • Reduces lipstick migration for a more confident look • Gives a younger-looking skin and more peaceful facial expression
APPLICATIONS	• Anti-aging care • Anti-wrinkle care • Lip contour care • Lipistick • Eye contour care • Regenerating care • Wound healing care • Anti-stretch mark • Cosmeceuticals • Men's care
RECOMMENDED DOSAGE	• 0.5-1%: Preventive action • 1-2%: Treating action
USAGE PH RANGE	5-8
INCORPORATION	**For emulsion:** to be added in the appropriate phase before emulsification step **For water free (SWT-7™ L)/oil free (SWT-7™ H) product**: to be added to other ingredients (hot or cold process) **For lipstick:** to be added before pouring
INCOMPATIBILITIES	In progress

INTRODUCTION

Advanced medical research is an unlimited source of inspiration for the identification of new biological mechanisms of action applicable to the cosmetic industry. Influenced by the latest discoveries in regenerative medicine (tissue engineering) for the treatment of burn injuries, Lucas Meyer Cosmetics designed SWT-7™, capable of stimulating keratinocyte proliferation to regenerate thin epidermis and improve the look of aged and wrinkled skin. This never-seen before mechanism of action is based on stem cell therapy with a cell-to-cell communication between adipose-derived stem cells (ADSC) and keratinocytes through growth factors action.

Titrated in swertiamarin, an iridoid extracted from Indian gentian leaves, SWT-7™ is a patented natural high tech anti-aging ingredient acting through an innovative biomimetic pathway on the reduction of the appearance of wrinkles, especially vertical wrinkles.

In order to widen its applications, Lucas Meyer Cosmetics developed 2 grades:

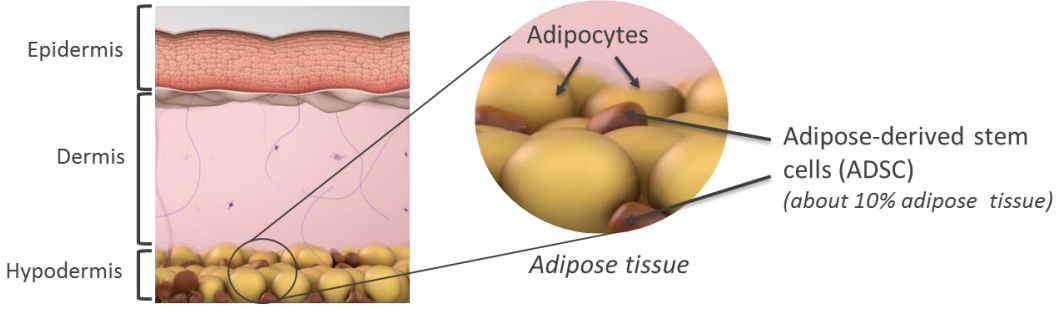
- **SWT-7™ H**, hydrosoluble version to be introduced in any type of O/W and W/O emulsions; and oil free formulas.
- **SWT-7™ L**, liposoluble version to be introduced in any type of O/W and W/O, water free formulas (oily serum, balm, etc.), even lipstick.

EPIDERMIS REGENERATION FROM ADIPOSE-DERIVED STEM CELLS: A BREAKTHROUGH MECHANISM FOR SKIN ANTI-AGING ACTION

Adipose-derived stem cells & skin regeneration

Using the self-renewal properties of stem cells, stem cell therapy emerged as a promising new approach for regenerative and reconstructive medicine, an advanced science aimed to replace, repair or regenerate tissues and organs affected by the aging process, injury or disease. Since then, many studies have been conducted to understand all the characteristics and properties of stem cells.

After many years of research in skin tissue engineering, adipocyte-derived stem cells (ADSC) have been identified as a key player in the skin regenerative process with a cell-to-cell communication pathway. ADSC produce high content of growth factors, substances acting as a messenger, to communicate with keratinocytes in order to stimulate their proliferation, indispensable to regenerate epidermis and heal wound.



Therefore, the use of ADSC and their growth factors have become a hot topic to create *in vitro* reconstructed skin faster in order to treat severely burned patients better, in terms of time and results.
Skin auto-grafts are widely used as an effective and rapid treatment of burns but this technique is very challenging for large zones. In this case, skin obtained by *in vitro* culture of keratinocytes taken on the patient is more adapted but requires longer treatment. The revolutionary discovery of adipose-derived stem cells (ADSC) and the understanding of their properties permitted to envisage new therapeutic methods, faster and more qualitative.

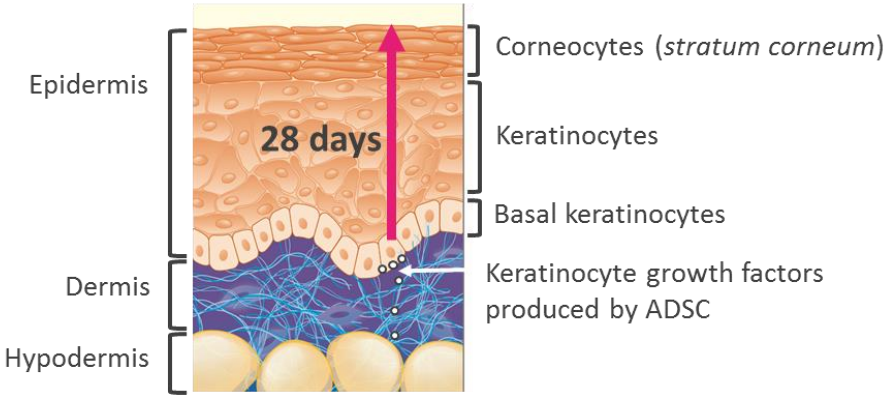
In the aesthetic surgery area, this new knowledge already led to the creation of a new method, Lipo-seeding®, consisting in the auto-injection of ADSC in order to improve the quality and the aspect of skin from the inside.

Inspired by advanced research in skin tissue engineering, SWT-7™ has been designed to activate skin ADSC to promote the release of growth factors in order to auto-regenerate skin.

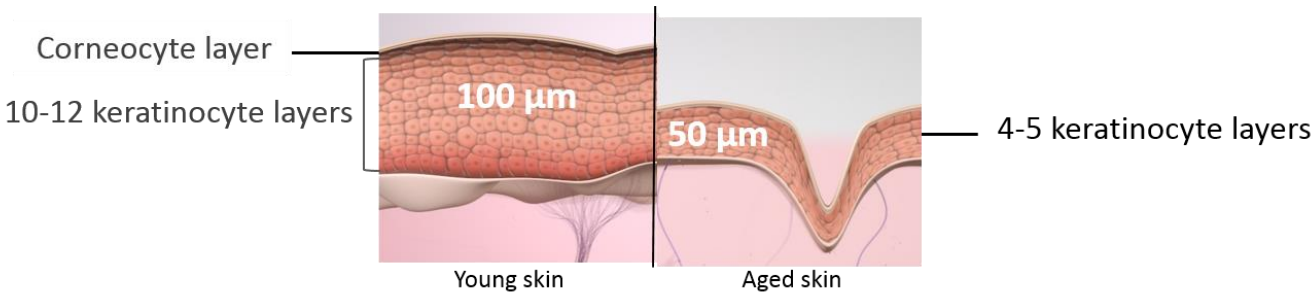
Epidermis aging & wrinkles

Human epidermis, skin's upper layer, is a multi-layered epithelium composed of 80% of keratinocytes at different stages of the keratinization process. Epidermis is continually renewed due to a permanent production of new keratinocytes.

Regulated by growth factors, keratinocyte production starts in the stratum basal layer where basal keratinocytes divide into two identical daughter cells (=proliferation). One remain as a basal keratinocyte while the other one migrates in approximately 28 days to the upper layer of the epidermis, *stratum corneum*, where it becomes a corneocyte which flakes away during the desquamationprocess. This natural proliferation process also allows skin regeneration during the wound healing process. A higher production of growth factors is released to rapidly produce new keratinocytes able to migrate into the wound in order to fill in the gap created by the injury and to recover a normal skin aspect.



Epidermis aging is characterized by a loss of 10% keratinocytes per decade due to the decrease of their proliferation capacity linked to a diminished production of growth factors over time. This physiological change leads to a thinner epidermis, which highly accelerates wrinkle formation since the natural skin renewing/repairing process is reduced.



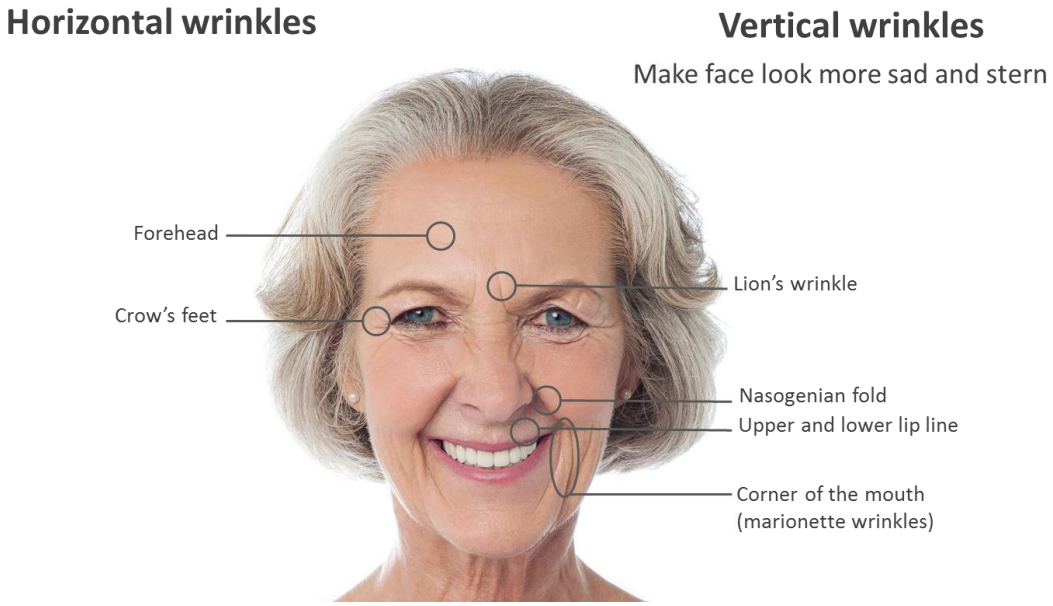
SWT-7™ demonstrated its ability to induce a strong production of growth factors from adipose-derived stem cells. This release of growth factors is also proven to promote keratinocyte proliferation resulting in a thicker epidermis with higher number of keratinocytes layers.

By targeting epidermis, SWT-7™ improves the epidermis thickness and fills in wrinkles for a resurfacing effect

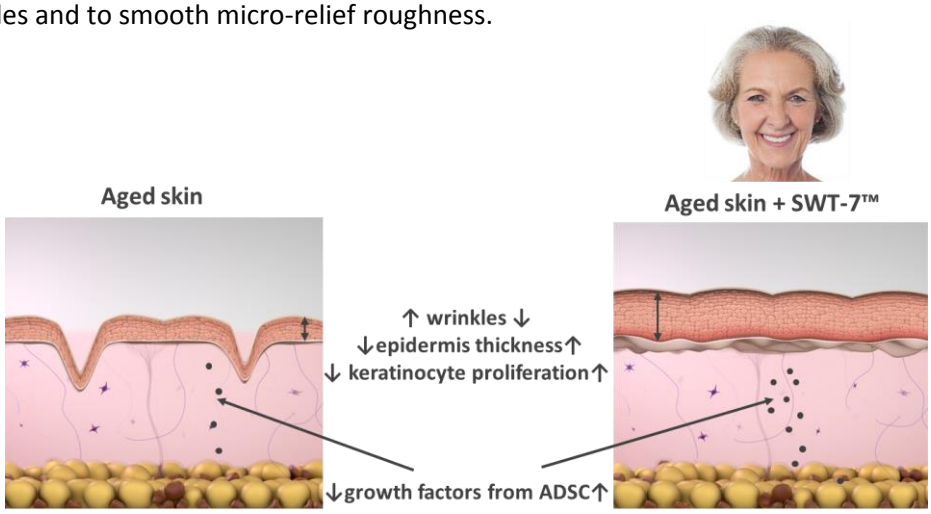
SWT-7™ L & H, 2 VERSIONS TO BLUR VERTICAL WRINKLES

By mimicking the wound healing process, the appearance of wrinkles can be reduced by stimulating the natural skin regeneration process.

The clinical study focused on vertical wrinkles as they are known to make faces look stern and sad. Besides the lion's wrinkle located between eyebrows, they are mainly located in the lower part of the face: nasogenian fold, corner of the mouth, upper and lower wrinkles of lip contour.



The stimulation of the skin regeneration process and the increase in epidermis thickness is a fast way to fill in the gap of the wrinkles and to smooth micro-relief roughness.



4 CLINICAL STUDIES!
Anti-vertical wrinkle & skin texture smoothing effect
Reduction of vertical wrinkles volume
Anti-wrinkle action on lip contour (smokers)
Reduction in lipstick migration

By stimulating the skin to self-regenerate, SWT-7™ rapidly improves epidermis thickness to shade off and blur the appearance of wrinkles. This resurfacing effect on vertical wrinkles results in a younger look and a more peaceful facial expression.

Swertiamarin, a pure molecule from Indian gentian

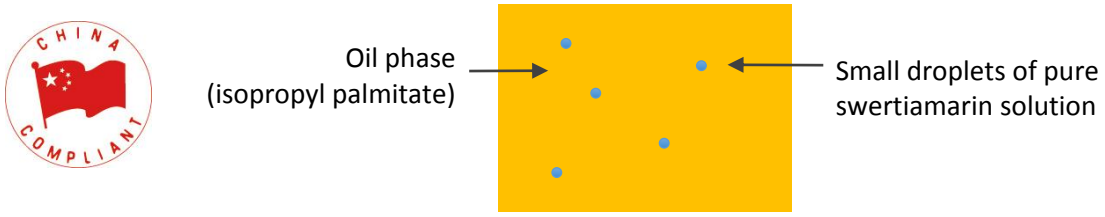
SWT-7™ specific action is due to its titrated content in swertiamarin (1.25%) extracted from Indian gentian leaves. Swertiamarin chemically belongs to iridoid family, a group of molecules typically found in medicinal plants.

Native to temperate Himalayas and found on high altitude hills (1200 to 3000 m), Indian gentian (*Swertia chirata* from the *Gentianaceae* family) is a traditional Ayurvedic herb with 4-angled flowers of greenish yellow colour tinged with purple.

The whole plant is an extremely bitter tonic digestive herb that lowers fevers, and also a stimulant. It has a beneficial effect on the liver, promoting the flow of bile; it is also useful for treating dyspepsia. Aqueous extracts were used for their anti-parasitic properties against malaria and fever, and more recently for their anti-microbial and wound healing properties.

In order to make this pure swertiamarin easy to formulate in any kind of product, two versions have been developed with a final concentration in swertiamarin of 1.25%.

SWT-7™ L: The liposoluble version is the fruit of a highly innovative technology using phospholipids as microdispersion provider. Small droplets of water solution of swertiamarin are dispersed in an oil phase easy to add to lipophilic compounds of final cosmetic products. Resistant to high temperature, it can be heated and introduced in a lispstick or oil balm composition.



SWT-7™ H: The hydrosoluble version form is a maltodextrin-based powder form.

EFFICACY STUDIES

IN VITRO EFFECT of SWT-7™ ON WOUND HEALING

Effect of adipocyte culture media treated by SWT-7™ on keratinocyte monolayer after injury

OBJECTIVE

The aim of this study was to evaluate the effect of SWT-7™ on adipose tissue capacity to produce essential components for epidermal layer development. In this way, the wound closure effect of the incubation media of normal human adipose tissue (naturally containing about 10% Adipose Derived Stem Cells) treated with SWT-7™ was tested on a model of normal human keratinocytes cultured in monolayers.

PROTOCOL

Tested product: Pure swertiamarin (batch: DK110622) was tested at the following concentrations: **0.0025% and 0.005%** (0.025 mg/mL and 0.05 mg/mL) respectively equivalent to 0.2% and 0.4% of SWT-7™.

Biological materials

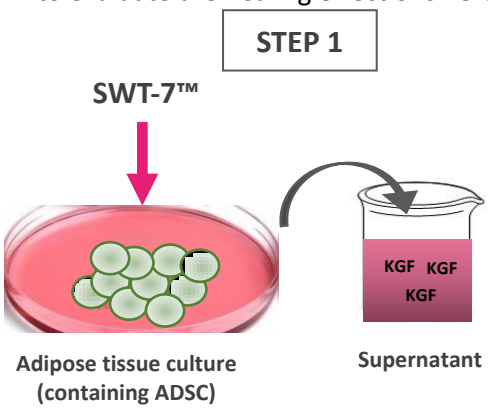
Human normal adipose tissue was obtained from a piece of surgical resection. The subject was 34 years old.
Human normal keratinocytes were obtained from abdominoplasty. The subject was 45 years old.

Culture protocol

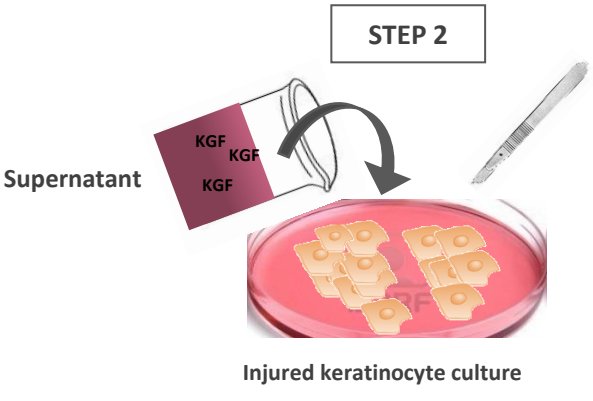
Suspensions of adipocytes were incubated at 37°C under gentle agitation for 15 hours in the absence (blank) or in the presence of increasing concentrations of swertiamarin.
Swertiamarin was dissolved at 1% (10 mg/mL) in DMSO before dilution in the incubation medium of adipose tissue, with a constant concentration of 0.15% (1.5 mg/mL) DMSO.

At the same time keratinocytes were incubated at 37°C in a humid atmosphere and 5% CO_2. Once confluence is reached, a mechanical scratch wounding of confluent monolayers was performed in each culture wells.

Keratinocytes monolayer was then incubated in the absence (blank) or presence of adipose tissue culture medium treated with increasing concentrations of swertiamarin. These media were diluted 1:10 in keratinocyte medium to evaluate the healing effect of swertiamarin after 72 hours.



SWT-7™ **stimulates** the production of **keratinocyte growth factors** by ADSC



The supernatant containing **kereatinocyte growth factors** is then **added to injured keratinocyte layer**

Evaluation protocol

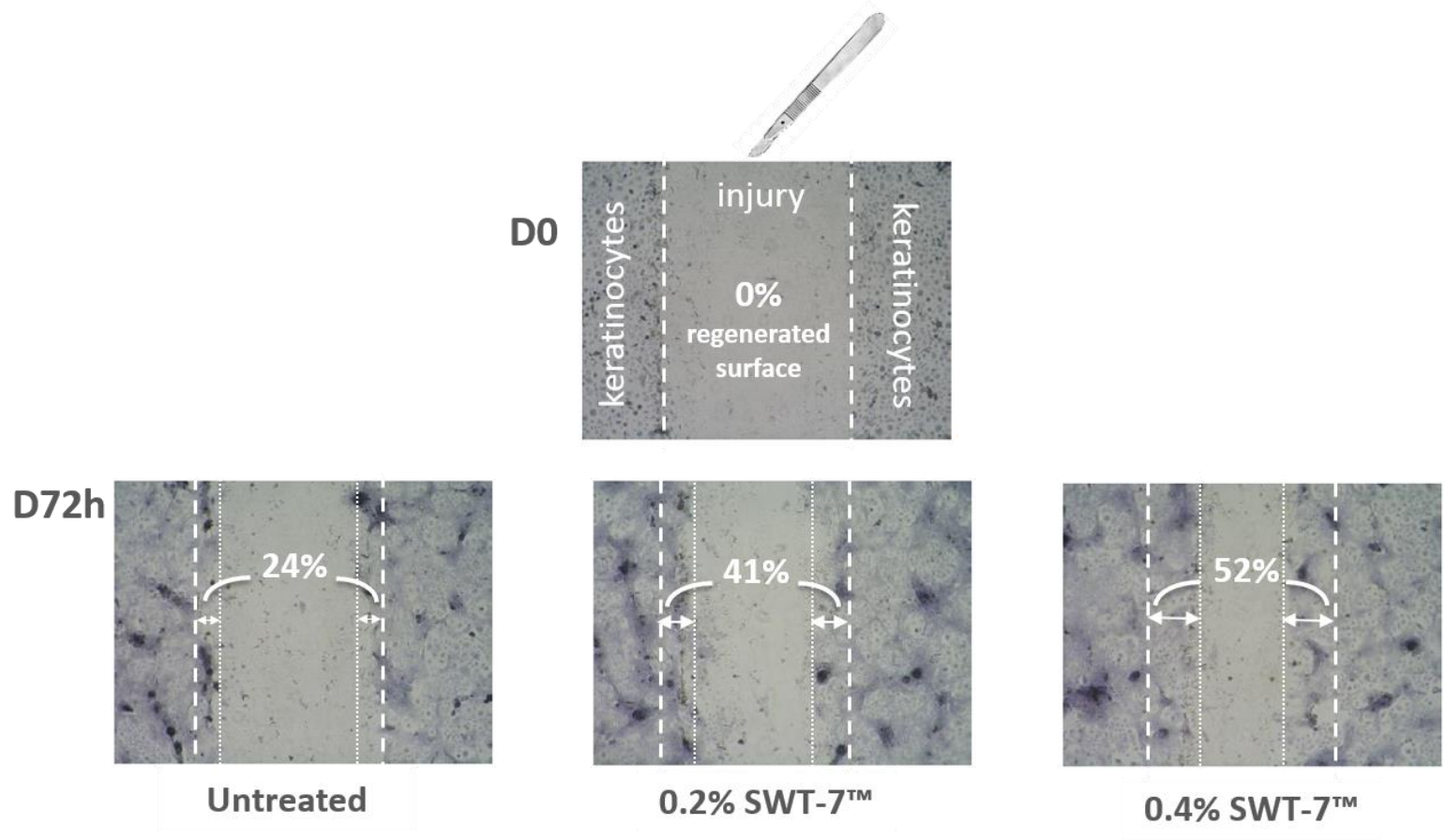
The surface of the wound which was not colonized by keratinocytes was measured using image analysis software (Image J) at D0 and at the end of the incubation period (72H).

Statistics

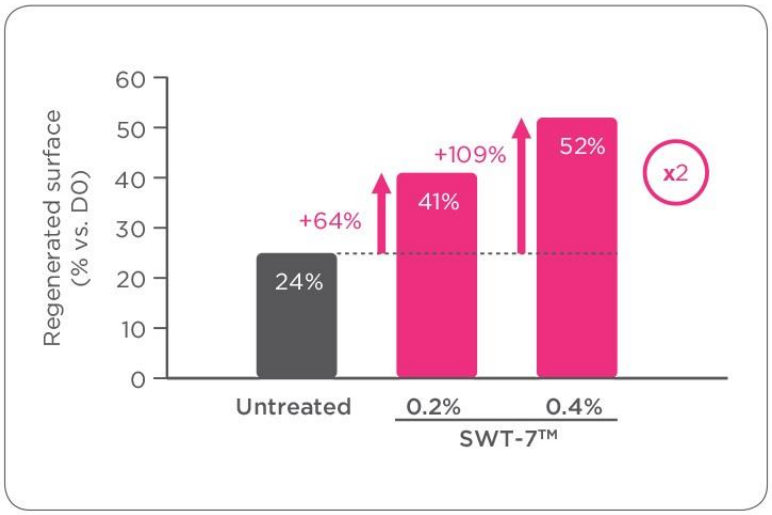
Results are given as a percentage of the surface recolonized from the blank condition, by photographic fields observed (mean + / - standard deviation, SD) in SWT-7™ equivalent.

The statistical significance of differences between the conditions DMSO (Blank) and SWT-7™ was assessed by analysis of variance (One Way ANOVA) followed by Holm Sidak test (*: p <0.05).

RESULTS



REGENERATION OF INJURED KERATINOCYTE CULTURE



The incubation media of normal human adipose tissue treated with SWT-7™ on keratinocyte monolayer, had a visible, dose-dependent and significant regenerative effect at 0.025 mg/mL (eq. 0.2% of SWT-7™) (+64.6%) and 0.05 mg/mL (eq. 0.4% of SWT-7™) (+109.6%) compared to the untreated control.

CONCLUSION

The results confirmed the capacity of SWT-7™ to stimulate adipose-derived stem cells to produce keratinocyte growth factors permitting epidermal regeneration.

SWT-7™ accelerates keratinocyte proliferation for a strong regenerative effect

EX VIVO EVALUATION OF SWT-7™ EFFECT ON EPIDERMAL REGENERATION

INTRODUCTION

Adipose tissue has been newly identified as very important for its paracrine activity. Recently, reconstructive surgery researches have highlighted the importance of hypodermis and more specifically of Adipose Derived Stem Cells (ADSC) for skin regeneration [Mizuno H. *et al.*, 2011]. To confirm the mechanism of action of SWT-7™, an *ex vivo* study has been performed using SWT-7™ in formula.

OBJECTIVE

The aim of this study was to confirm the relation between adipose tissue stem cells activation and epidermal proliferation to assess the regenerative effect of SWT-7™on human living skin explants. For this, SWT-7™ effect was evaluated on human explants with hypodermis (containing naturally adipose derived stem cells (ADSC)) or without hypodermis (no ADSC).

PROTOCOL

Tested formula

- Active formula (batch: 13 350 02 C145) containing 2% of SWT-7™.

Phase	Ingredient	%
A	Heliogel™	3
	Caprylic/Capric Triglyceride	15
B	Water	79.2
	SWT-7™	2.00
	Dekaben	0.8

- Placebo formula (batch: 13 350 01 C145)

Phase	Ingredient	%
A	Heliogel™	3
	Caprylic/Capric Triglyceride	15
B	Water	81.2
	Dekaben	0.8

Biological materials

Explants were obtained from abdominoplasty. The subject was 47 years-old.

From this surgical resection two kinds of explants were prepared.

- Skin explants: Epidermis, dermis without hypodermis (no ADSC)
- Full skin explants: Epidermis, dermis, hypodermis (with ADSC)

Incubation protocol

Explants were kept in survival in BEM medium (BIO-EC's Explants Medium) at 37°C in a humid, 5% CO_2 atmosphere.

Tested formulas were applied (1µl morning and 1µl evening) on the surface of the explants at days 0, 1, 2, 3, 6 and 7.

Sampling

On D0 and after 9 days, the 3 explants were collected and cut in 2 parts. One part was fixed in buffered formol solution and the second frozen at -80°C.

The culture medium was collected on D9 to perform the dosage of KGF (FGF-7).

Histological protocol

Explants were fixed, dehydrated and impregnated in paraffin. 5-µm-thick sections were made using a Leica RM 2125 Minot-type microtome. The microscope observations were realized using a Leica DMLB or Olympus BX43 microscope. Pictures were digitized with an Olympus DP72 camera and the Cell^{D} data storing software.

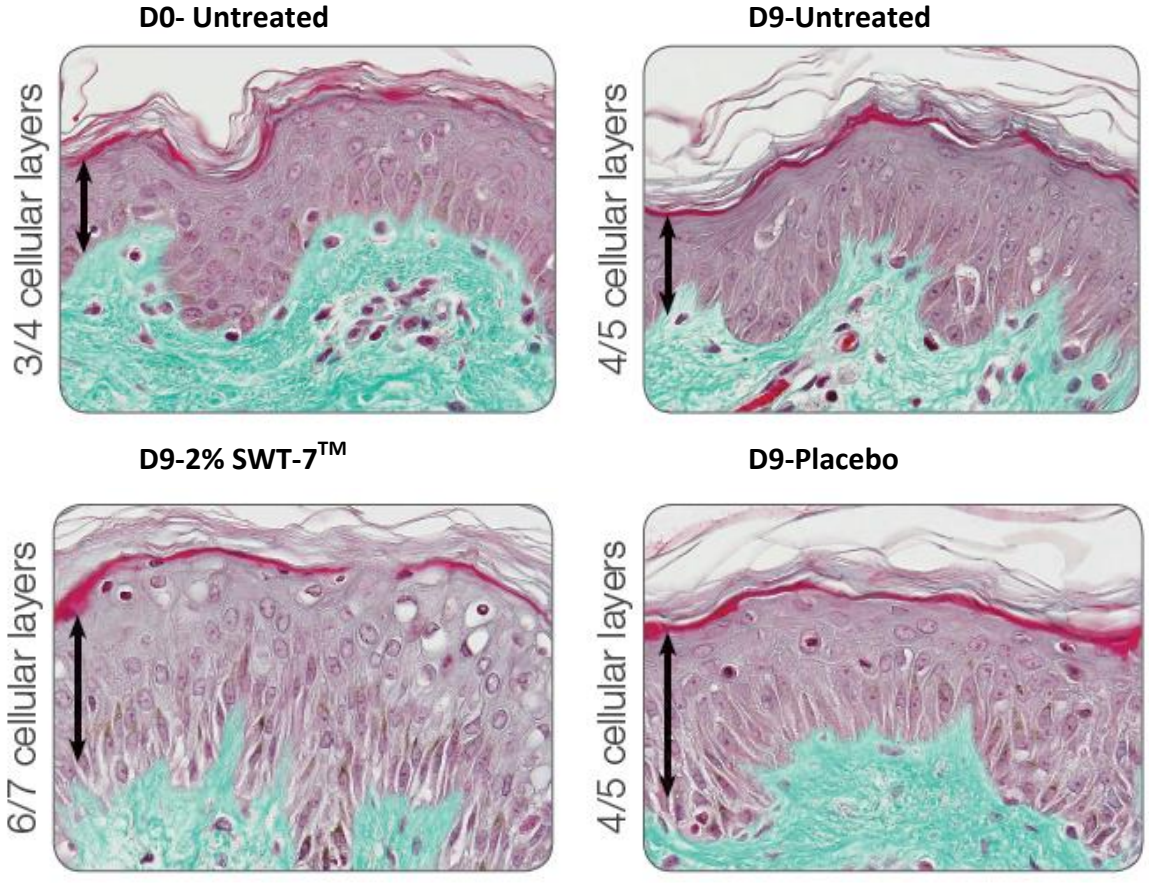
KGF dosage

KGF (FGF-7) dosage was performed with the RayBiotech human FGF-7 Elisa kit (ref. ELH-FGF-7-001). Absorbance was measured at 450 nm using infinite M200Pro Tecan microplate reader and Magellan7 software.

The concentrations of KGF (FGF-7) in the culture media are indicated in pg/mL.

RESULTS

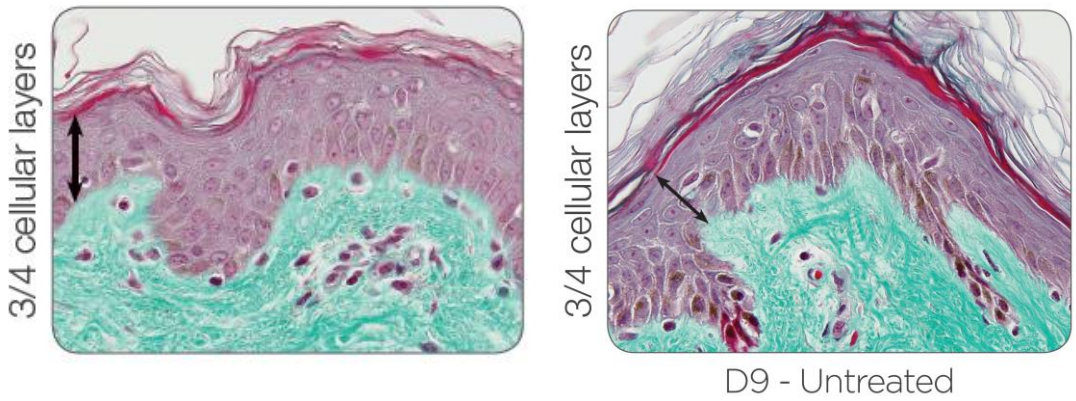
General morphology on skin explants without hypodermis (without ADSC)

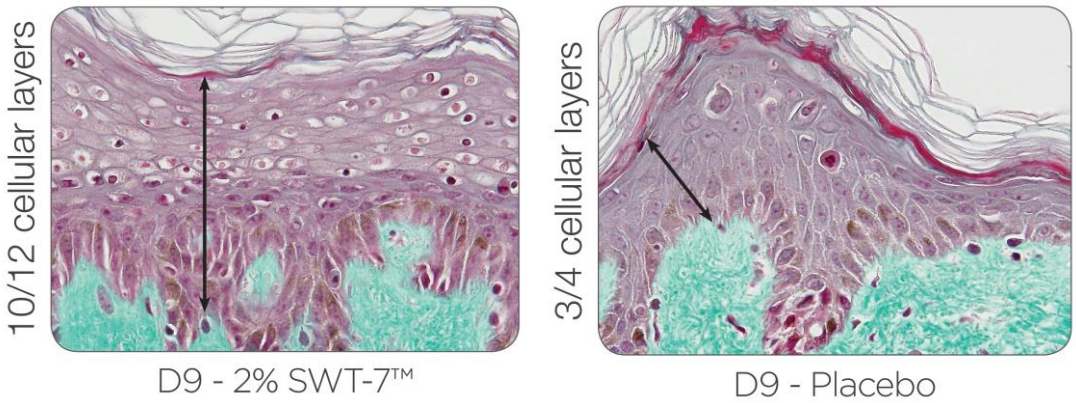


On the skin explants without hypodermis (no ADSC), after 9 days in culture, the morphology of the untreated skin explant is close to the one observed at D0. The treatment of skin explants with SWT-7™ increases the number of cellular layers to 6/7 (vs 4/5 for the control), whereas the skin explants treated with the placebo shows no difference in comparison with the untreated explants.

General morphology on full skin explants with hypodermis and ADSC

D0- Untreated **D9-Untreated**

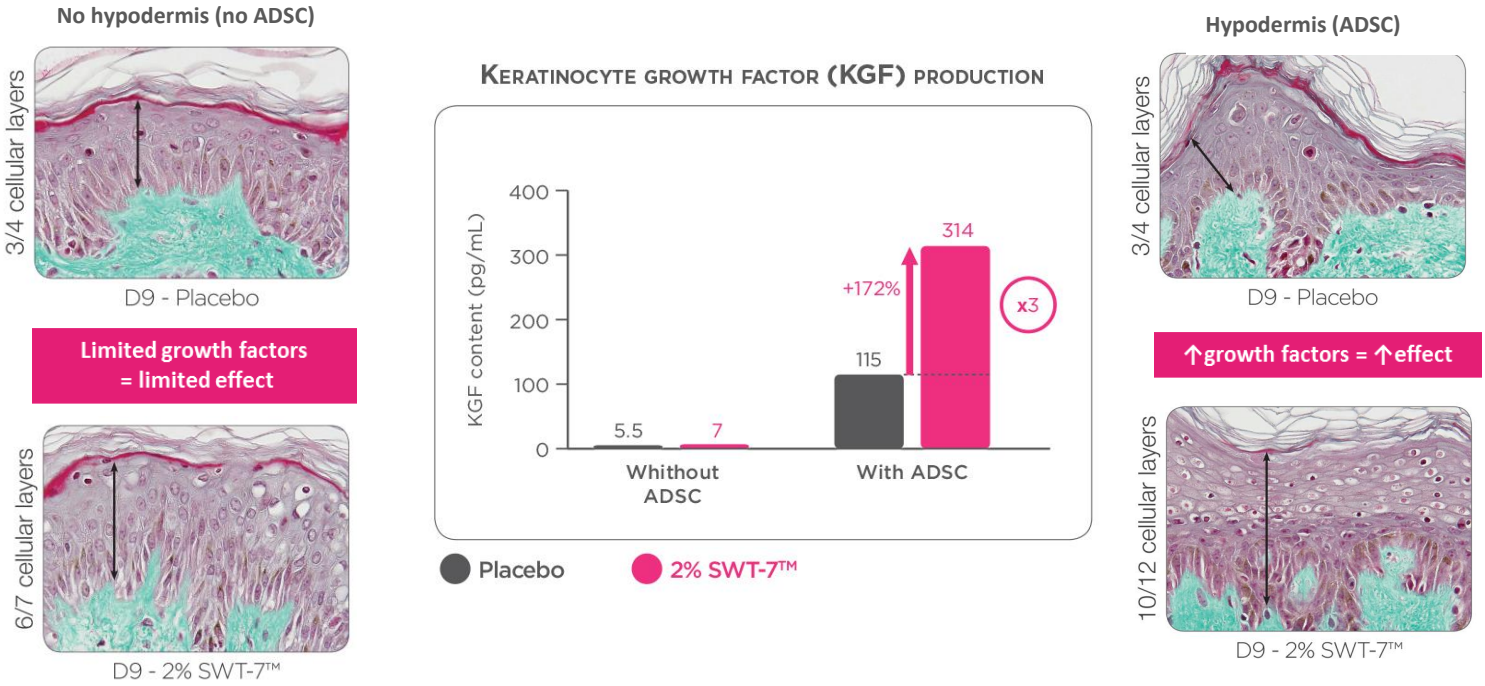




On full thickness skin explants with hypodermis (with ADSC), after 9 days in culture, the morphology of the untreated skin explants is similar to the one observed at D0. The treatment of the full thickness skin explants with SWT-7™ induces a strong hyperplasic acanthosis (increase in the number of cellular layers to 10/12 vs 4/5) whereas the skin explants treated with the placebo show no difference in comparison with the untreated explants.

KGF dosage

The amount of KGF (FGF-7) was evaluated in the culture media collected from the different explants treated with or without SWT-7™.



Only few amount of KGF was measured in the skin explant without hypodermis (no ADSC) treated with SWT-7™ whereas we observed a strong increase in KGF production on explants with hypodermis treated with SWT-7™. These results confirmed that this growth factor is produced by ADSC from hypodermis.

CONCLUSION

Epidermis thickness increase is due to a higher keratinocyte growth factor production by ADSC stimulated by SWT-7™

CLINICAL STUDY

- **Anti-vertical wrinkle & skin texture smoothing effect**
- **Reduction in vertical wrinkles volume**
- **Anti-wrinkle action on lip contour (smokers)**
- **Reduction in lipstick migration**

EVALUATION OF ANTI-AGING EFFECT ON VERTICAL WRINKLES AND SKIN TEXTURE

Picture analysis with VISIA CR filters

OBJECTIVE

The aim of the study was to evaluate the **anti-wrinkles and smoothing effect** on vertical wrinkles of SWT-7™ formulated at 2% in a finish product during **28 days**.

PROTOCOL

Subjects

17 healthy female volunteers aged between 45 and 60 years with vertical wrinkles in the lower part of the face were recruited for this clinical study. For the anti-wrinkles study, only 16 persons gave interpretable results.

Test conditions

For 28 days the volunteers applied an emulsion containing either 2% of SWT-7™ or a placebo. Creams were applied randomized split-face twice a day in the morning and evening.

Tested creams

Placebo

Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	Micro-dispersion without swertiamarin	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Active formula : 2% SWT-7™

Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	SWT-7™L	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Clinical evaluation

At days 0, 7 and 28, an evaluation of the anti-wrinkle effect on both half-lower-faces was realized by the use of the VISIA-CR to take photographies and apply specific filter for texture of the skin and wrinkles.

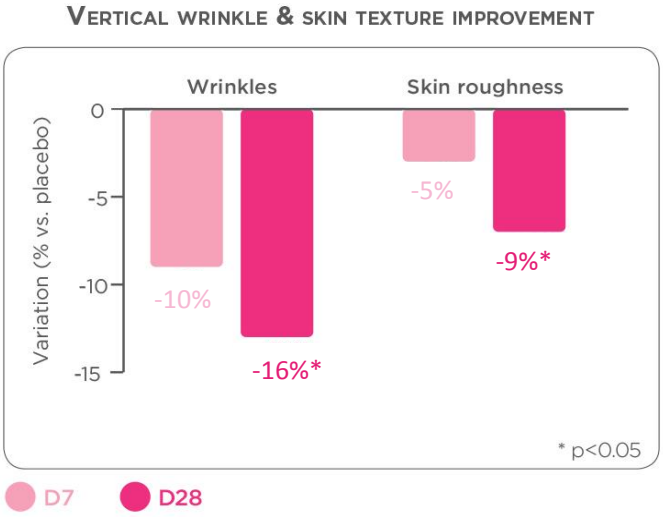
Visia-CR is a facial imaging system for clinical research. It permits to deliver excellent standardized image capture with multiple lighting modalities ideal for capturing specific skin conditions. In our study we have focused on skin wrinkles and texture.

Statistical methods

The statistical analysis consists in the comparison on the values obtained on treated zones by each product at the different times of kinetics, compared to before application.

Data were analyzed with a paired student t-test. This method tests whether the mean of sample differences between pairs of data is significantly different from the hypothetical mean, zero under the null hypothesis (H0). The alternative hypothesis (H1) was that the average difference was either greater or less than 0 (two-tailed test).

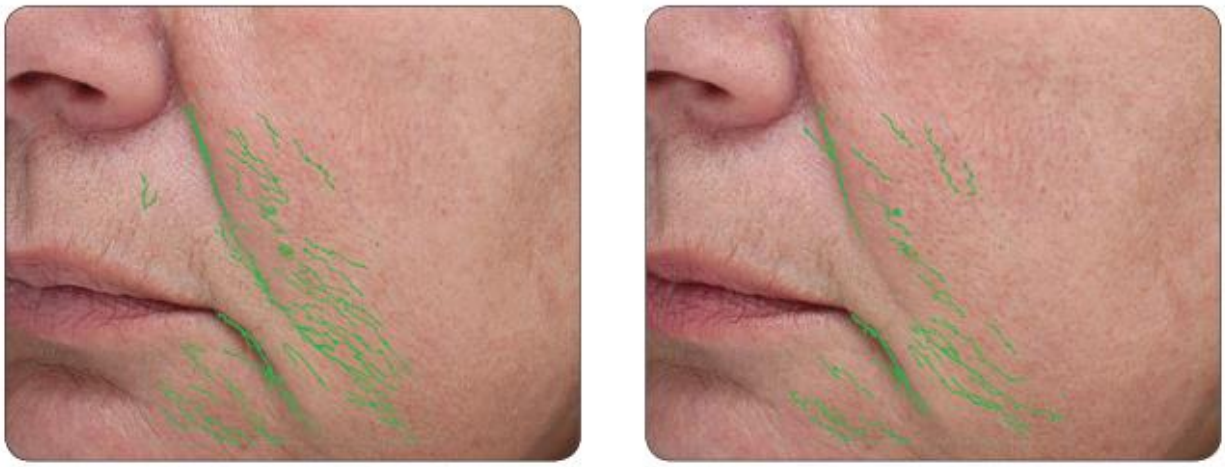
RESULTS



At D7, maximum wrinkle reduction up to -53%
At D7, maximum skin roughness reduction up to -42%

87% positive response after 28 days

D0 versus D7 (wrinkle filter) - Volunteer 7 (51 years)



D0 versus D7 and D28 (texture filter) - Volunteer 7 (51 years)

At 7 days, the active formula permitted an improvement of wrinkles and skin texture compared to placebo. This effect was significantly confirmed at 28 days with a result of -16% ($p<0.05$) for the wrinkles and -9 % ($p<0.05$) for the roughness.

SWT-7™ blurs the appearance of vertical wrinkles after only 7 days!

EVALUATION OF ANTI-AGING EFFECT ON VERTICAL WRINKLE VOLUME

Print analysis by fringe projection

OBJECTIVE

The aim of the study was to evaluate the **anti-wrinkles and smoothing effect** on vertical wrinkles of SWT-7™ formulated at 2% in a finish product during **28 days**.

PROTOCOL

Subjects

17 healthy female volunteers aged between 45 and 60 years with vertical wrinkles in the lower part of the face were recruited for this clinical study.

Test conditions

For 28 days the volunteers applied an emulsion containing either 2% of SWT-7™ or a placebo. Creams were applied randomized split-face twice a day in the morning and evening.

Tested creams

Placebo

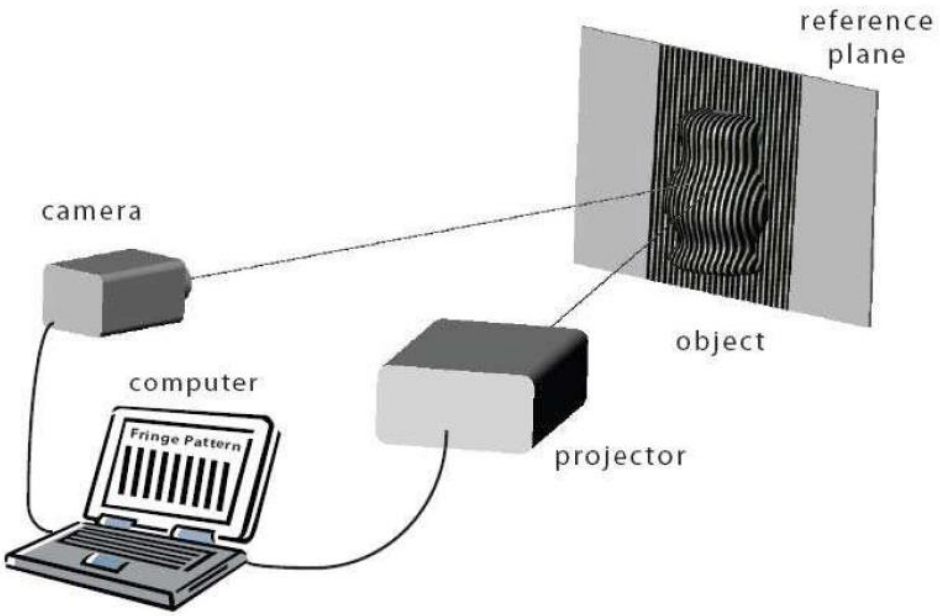
Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	Micro-dispersion without swertiamarin	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Active formula : 2% SWT-7™

Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	SWT-7™L	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Clinical evaluation

At days 0, 7, 14 or 28, an evaluation of the anti-wrinkle effect on both half-lower-faces was realized by 3D analysis (fringe projection) of skin print to measure the volume of the wrinkles.

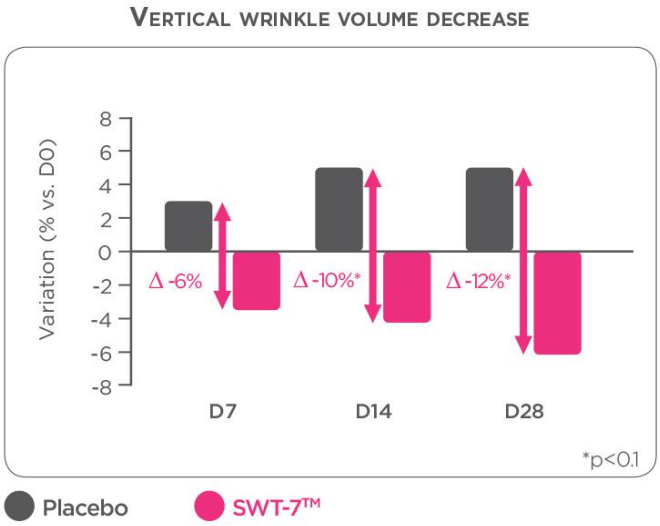


Statistical methods

The statistical analysis consists in the comparison on the values obtained on treated zones by each product at the different times of kinetics, compared to before application.

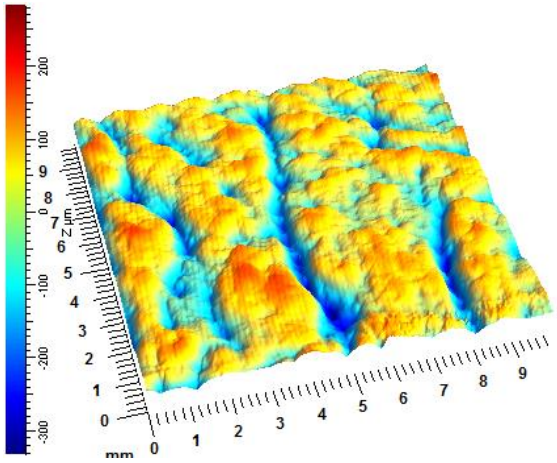
Data were analyzed with a paired student t-test. This method tests whether the mean of sample differences between pairs of data is significantly different from the hypothetical mean, zero under the null hypothesis (H0). The alternative hypothesis (H1) was that the average difference was either greater or less than 0 (two-tailed test).

RESULTS

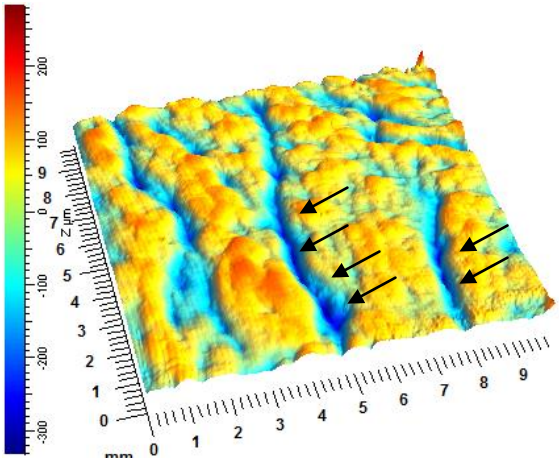


At D7, maximum wrinkle volume reduction up to -49%

D0



D14



At 7 days, the active formula permitted to obtain a 6% variation of the wrinkle volume compared to placebo. This trend was significantly confirmed at 14 and 28 days with respectively 10% and 12% ($p<0.1$).

SWT-7™ blurs the appearance of vertical wrinkles after only 7 days!

EVALUATION OF ANTI-WRINKLE EFFECT ON LIP CONTOUR
In vivo profilometry and evaluation by a dermatologist

OBJECTIVE

The aim of the study was to evaluate the **anti-wrinkle** effect on lip contour vertical wrinkles of SWT-7™ at 2% in a finished product formula during a 28 days clinical trial. The study has been carried out by a dermatologist.

PROTOCOL

Subjects

10 healthy female volunteers with smoking habits (marked wrinkles around the lips), aged between 45 and 65 years with deep wrinkles, especially around lips.

Test conditions

For 28 days the volunteers applied an emulsion cream containing either 2% of SWT-7™ or a placebo. The trial cream and the placebo cream were applied randomized split-face twice a day in the morning and evening.

Tested creams

Placebo (batch: 14.090.01/02.C138)

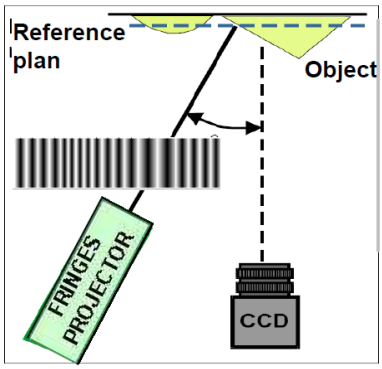
Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	Micro-dispersion without swertiamarin	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Active formula (batch: 14.091.01/02.C138)

Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	SWT-7™L	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Clinical evaluation

At days 0, 7 or 28, a hemi-face analysis of cutaneous relief variations was done using Primos® 3D Pico.

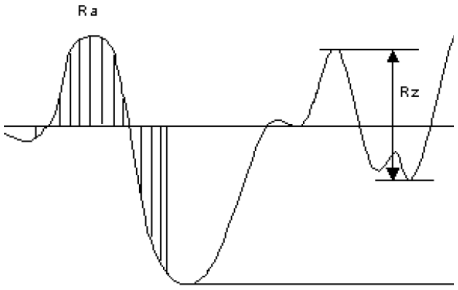


Primos 3D Pico technique consists in calculating a phase image from images with interference fringe projection.

This analysis was focused on wrinkles of lip contour of each volunteer. This technique allow to:

1. Measure average roughness (Ra) and mean roughness depth (Rz). Indeed, the image obtained by fringe projection allows determining the height of each point.

   Ra: the average roughness (in µm): defined like the ratio between the surface integrated around the mean value on the profile length. A decrease in Ra characterizes a smoothing effect.
   Rz: the average relief (in µm): average value of all the maxima (between peaks and hollow) on the profile length. A decrease of Rz characterizes an anti-wrinkle effect after a long use.

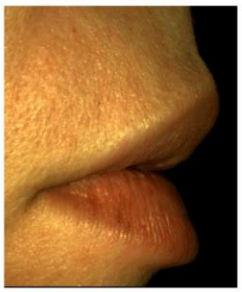


2. Illustrate the expected visual effects using macrophotographs

The acquisition software allows to obtain 2D and 3D measurements and to determine parameters of the cutaneous relief on 32 radiuses distributed like a star on the zone of interest. An automatic system of repositioning allows the precise re-identification of the zone measurement.

Statistical methods

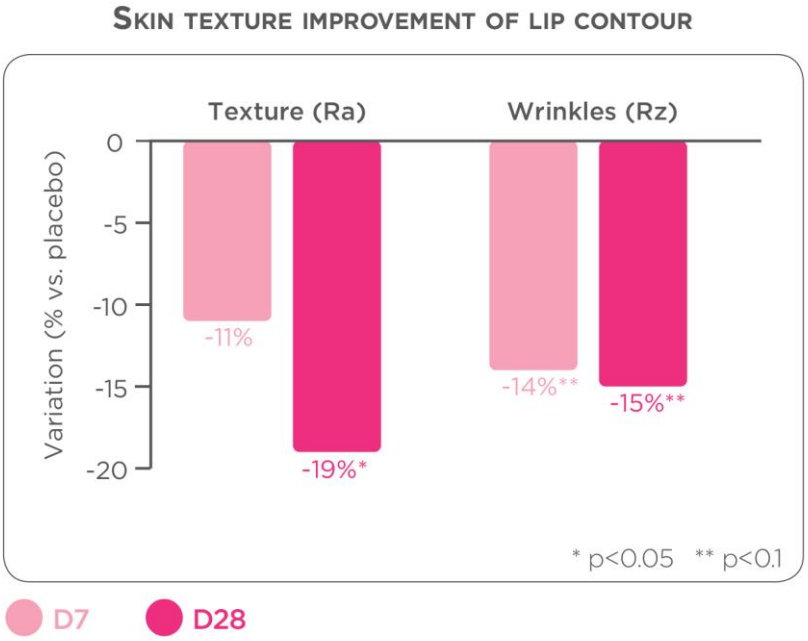
Example of 3D photo



The statistical analysis consists in the comparison on the values obtained on treated zones by each product at the different times of kinetics, compared to before application.

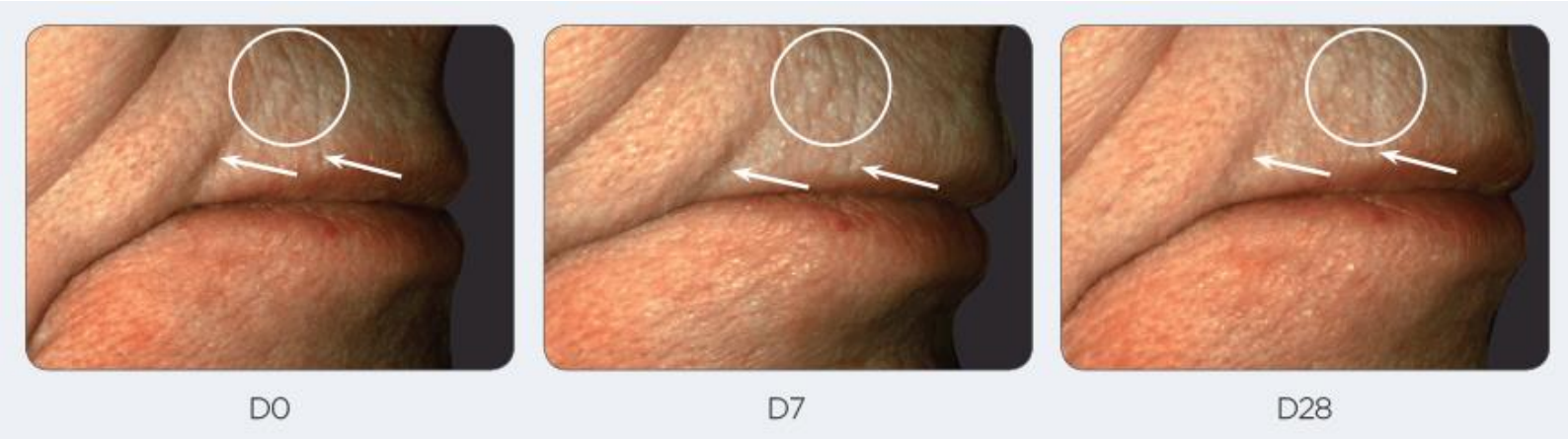
Data were analyzed with a paired student t-test. This method tests whether the mean of sample differences between pairs of data is significantly different from the hypothetical mean, zero under the null hypothesis (H0). The alternative hypothesis (H1) was that the average difference was either greater or less than 0 (two-tailed test).

RESULTS



Texture:
Up to -16% D7
Wrinkles:
Up to -68% D7

Volunteer 34 (64 years) smoker



A smoothing effect (Ra) was observed from 7 days and even confirmed at 28 days with -19% on the average roughness ($p<0.05$).

An optimal effect was reached by SWT-7™ on the wrinkles (Rz) from 7 days with -14% ($p<0.1$).

SWT-7™ reduces lip contour wrinkles after only 7 days!

EVALUATION OF ANTI-WRINKLE EFFECT ON LIP CONTOUR *Lipstick migration study*

OBJECTIVE

The aim of the study was to evaluate the capacity of SWT-7™ at 2% to treat the vertical wrinkles around the mouth and to limit lipstick migration after a 28 days application. The study has been carried out by a dermatologist.

PROTOCOL

Subjects

10 healthy female volunteers, aged between 46 and 63 years with wrinkles especially around lips.

Test conditions

For 28 days the volunteers applied an emulsion cream containing 2% of SWT-7™. The trial cream was applied twice a day in the morning and evening.

Tested cream

Active formula (batch: 14.091.01/02.C138)

Phase	Ingredient	%
A	Deionized Water	69.95
	Dermofeel™ PA3	0.10
B	Glycerin	2.00
	Satiaxane CX911	0.25
C	Heliofeel	4.00
	SWT-7™L	2.00
	LIPEX® 102	7.00
	Sweet Almond Oil	8.00
	Lanette® 22	2.50
	Vitapherole E1000	0.20
D	Dermosoft™ 1388	4.00
		100.00

Clinical evaluation

- Migration of a lipstick

Photographs of the lips were taken with a crossed polarized light and Prolite® flashs, using a D7100 Nikon camera with a 60mm Nikon.
Views were realized with a shutter speed at 1/125 of second and diaphragm on f22.
The positioning of the subjects were standardized in order to respect the 1/3 proportion.

The pictures were visualized on a computer screen. The analysis was realized by comparing the migration of the lipstick 2 h after product application at D0 and D28.
Migration:
The grading was realized by the technician in charge of the study and an independent technician trained to score "migration" was done by comparing placebo to treated area according to a five points' scale:

0	No migration
1	Light migration
2	Moderate migration
3	Important migration
4	Very important migration

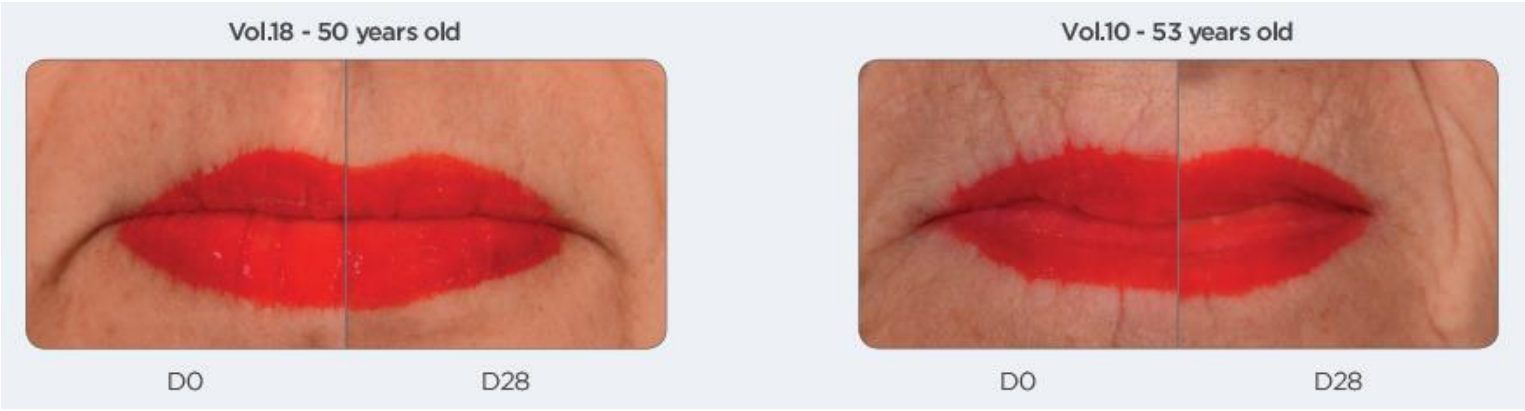
Below, an example of lipstick migration:

After 2 hours

The reference of lipstick used was: Miss Europe, Rouge Divin, Ref 149.

RESULTS

A two time decrease in lipstick migration was observed after 28 days of treatment with SWT-7™ ($p<0.05$) (The average scorage of the migration at the beginning of the study was 1.5 and after the 28 days of treatment we obtained 0.75).



Up to 7 time less lipstick migration

Lipstick bleeds can quickly downgrade a women's look, but with SWT-7™ we noticed a decrease in migration of lipstick giving women a more confident look

CONCLUSION

Inspired from one of the most advanced medical research in regenerative surgery, SWT-7™ provides a breakthrough mechanism against skin anti-aging process through cell-to-cell communication. Titrated in swertiamarin, SWT-7™ activates adipose-derived stem cells to promote the release of growth factors able to stimulate keratinocyte proliferation. The increase in the number of keratinocyte layers in epidermis leads to a thicker and regenerated skin which refills and blur fine lines and wrinkles, particularly vertical ones in order to recover a younger and more peaceful facial expression.
The two available forms, liposoluble or hydrosoluble, offer a solution for the formulation of any type of product, even lipstick.

COSMETIC APPLICATIONS

- Anti-aging care
- Anti-wrinkle care
- Lip care
- Eye contour care
- Regenerating care
- Wound healing care
- Anti-stretch mark
- Men' care

RECOMMENDATION FOR USE

Process

For emulsion: to be added in the appropriate phase before emulsification step
For water free product/oil free product: to be added to other ingredients (hot or cold process)
For lipstick: to be added before pouring

RECOMMENDED DOSAGE:

0.5 - 1%: preventive anti-aging care
1 - 2%: intensive treatment

COMPLEMENTARY STUDIES

ASSESSMENT OF THE FIBRONECTIN SYNTHESIS INDUCED BY SWT-7™ IN NORMAL HUMAN DERMIS FIBROBLASTS

INTRODUCTION

Fibronectin is a major adhesive protein whose role is to anchor cells to extracellular materials. Through its interaction with integrins, fibronectin can also modulate cell functions. In normal skin, fibronectin is located at the DEJ and in connective tissue of dermis.

Fibronectin is sensitive to proteolytic cleavage, a phenomenon that tends to increase with aging, contributing to the accumulation of denatured protein within the dermis and a decrease in the amount of fibronectin in dermis.

OBJECTIVES

The aim of this study was to determine the effects of SWT-7™ on the stimulation of fibronectin synthesis in human fibroblasts.

PROTOCOL

Tested product

The pure Swertiamarin was tested at following concentrations: 0.005 and 0.01% respectively (0.05 and 0.1 mg/mL) correspond to level of SWT-7™ between 0.2 to 0.4%.

Reference product

TGF-β was used as a positive control at 10 ng/mL (Sigma T7039).

Biological material

Normal human dermal fibroblasts (NHDF) were isolated from normal human dermis.

Incubation protocol

The culture was realized with DMEM (Eurobio, CMODME70-08) containing 10% fetal calf serum (Eurobio, CVFSV00-0U), 1% antibiotics (penicillin/streptomycin, Eurobio, CABPES01-OU) and 1% L-glutamine (Eurobio, CSTGLUOO-OU) at 37°C under 5% CO_2 and 95% humidity.

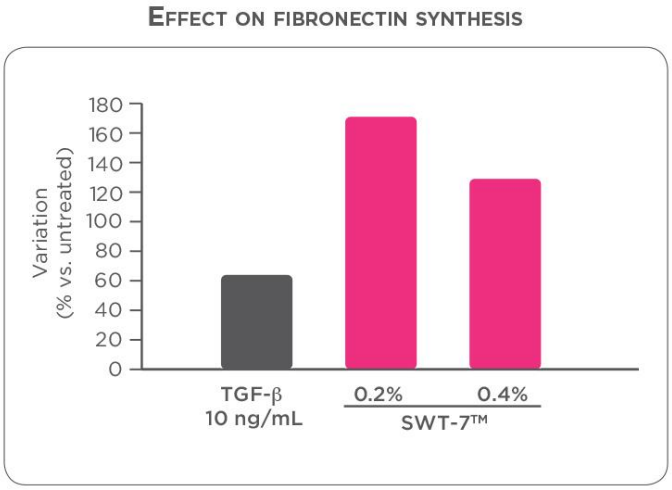
$2.5x10^4$ cells were seeded in micro plates in DMEM complete for 24h. The culture medium is then replaced with serum-free DMEM for an additional 24 hours to promote a quiescence state of cells.

TGF-β or swertiamarin was then added for an additional 24 hours. At the end of the incubation period, supernatants were collected for ELISA (BMS028, Ebiosciences) and cell viability assays (MTS test).

Evaluation protocol

The results are indicated according to the concentration of fibronectin contained in culture supernatants reported to the concentration in SWT-7™ equivalent

RESULTS



SWT-7™ had a positive effect on stimulation of fibronectin synthesis compared to the control. The positive control TGF-β increase fibronectin by + 64% whereas 0.4% of SWT-7™ increase by + 171%.
SWT-7™ at only 0.4% directly increase the synthesis of fibronectin by + 171% compared to the control and even compared to the positive control TGF-β (+ 64%) which is already known as enhancer of synthesis of fibronectin. SWT-7™ is able to directly improve the cohesion between epidermis and dermis.

CONCLUSION

SWT-7™ stimulates fibronectin synthesis with a direct action on fibroblasts.

ASSESSMENT OF THE COLLAGEN XVII SYNTHESIS INDUCED BY SWT-7™ IN NORMAL HUMAN KERATINOCYTES

INTRODUCTION

Collagen is a protein which has mechanical properties. It confers to the skin her resistance thanks to his role in junctions systems. Collagen XVII is located particularly in hemi-desmosomes. In this way, it permits adhesion between dermis and dermo-epidermal junction (DEJ).

OBJECTIVE

The aim of this study was to determine the effects of SWT-7™ on the stimulation of collagen XVII synthesis in normal human keratinocytes in culture.

PROTOCOL

Tested product

The pure Swertiamarin was tested at following concentrations: 0.01 and 0.0075% (respectively 0.1 and 0.075 mg/mL) corresponding to level of SWT-7™ between 0.6 to 0.8%.

Reference product

TGF-β was used as a positive control at 10 ng/mL (Sigma T7039).

Biological material

The keratinocytes NCTC 2544 are isolated from normal human epithelial cell line. The culture was realized with DMEM (Eurobio, CMODME70-08) containing 10% fetal calf serum (Eurobio, CVFSV00-0U), 1% antibiotics (penicillin / streptomycin, Eurobio, CABPES01-OU) and 1% L-glutamine (Eurobio, CSTGLUOO-OU) at 37°C under 5% CO_2 and 95% moisture.

Incubation protocol

For the test, $5x10^4$ cells were seeded in micro plates in DMEM complete for 24 h at 37°C under 5% CO_2 and 95% humidity. The culture medium is then replaced with DMEM containing 1 % fetal calf serum, 1% antibiotics (penicillin/streptomycin) and 1% L-glutamine at 37°C under 5% CO_2 and 95% moisture for 24 hours more.

After the incubation period, TGF-β or swertiamarin was added for 24 hours of treatment before immunolabelling.

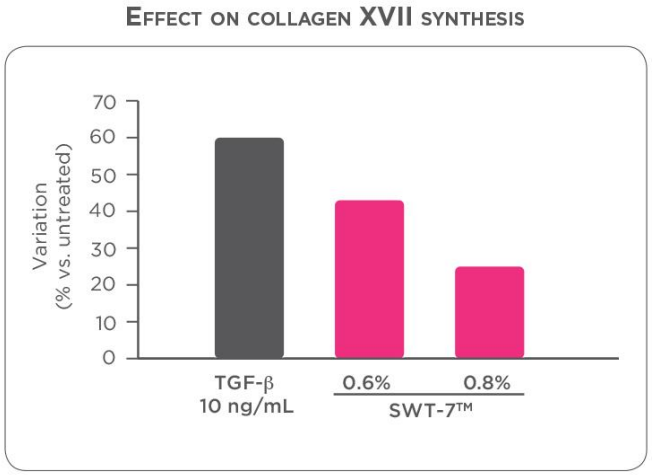
Collagen XVII immunolabelling

Collagen XVII was specifically labeled with a collagen XVII antibody (S.CRUZ, ref. SC-26395) diluted at 1% and revealed with a second antibody (anti-goat IgG-FITC, CRUZ, ref. SC-2024) coupled to the fluorescein (green fluorescence).

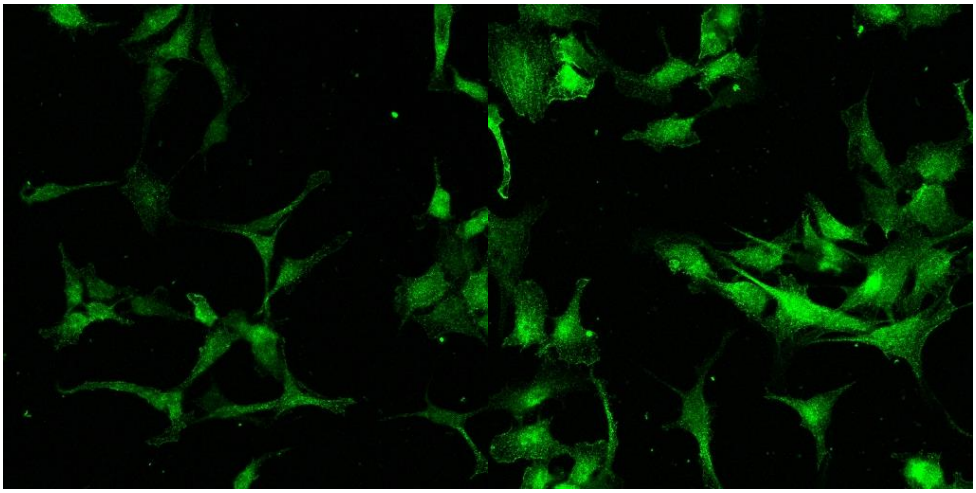
Histograms obtained by Image J indicated the average of the green fluorescence intensity for a given surface. A percentage of fluorescence can be calculated and a comparison can be realized between the control and the treated assay.

The results are indicated according to the concentration of collagen XVII containing in culture supernatants reported to the concentration in SWT-7™ equivalent.

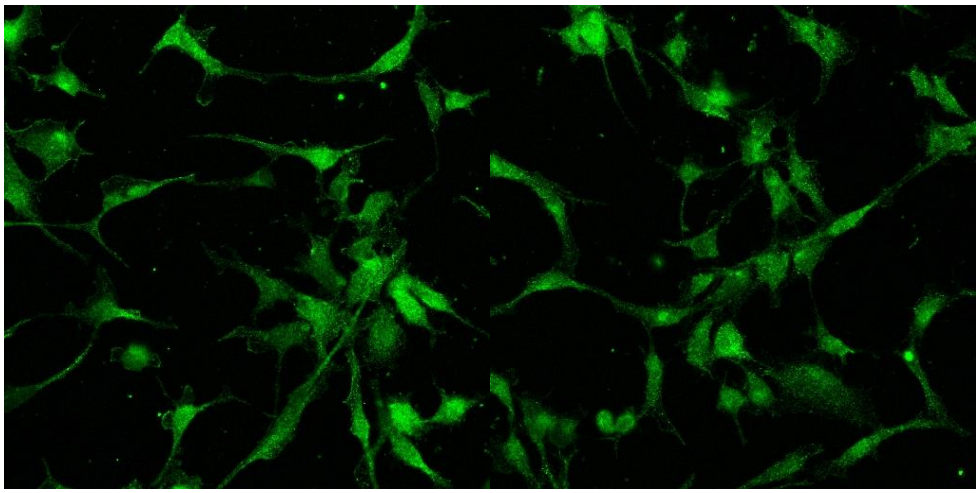
RESULTS



SWT-7™ at 0.6 and 0.8% stimulates the collagen XVII synthesis compared to the control (respectively + 25 and + 43%).



Control TGF β 10 ng/mL



SWT 0.0075% SWT 0.01%

SWT-7™ stimulated the collagen XVII synthesis at 0.6% (+ 43%) compared to the control in a direct way (no need of adipose tissue).

CONCLUSION

SWT-7™ stimulates collagen XVII synthesis improving the cohesion between epidermis and dermis.

ASSESSMENT OF THE LAMININS SYNTHESIS INDUCED BY SWT-7™ IN KERATINOCYTES

INTRODUCTION

The basement membrane is involved in the repair and regeneration of skin damaged by UV exposure or aging, and provides anchoring. Laminins are major proteins in the basement membrane, a protein network foundation for most cells and organs. The laminins are an important and biologically active part of the basal lamina, influencing cell differentiation, migration, and adhesion between cells and basal lamina.

OBJECTIVE

The aim of this study was to determine the direct effect of SWT-7™ on the stimulation of laminins synthesis in normal human keratinocytes in culture by ELISA dosage.

PROTOCOL

Tested product

Pure Swertiamarin was tested at following concentrations: 0.005 - 0.0075 and 0.01% (respectively 0.05 – 0.075 and 0.1mg/mL) corresponding to level of SWT-7™ of 0.4%, 0.6% and 0.8%.

Reference product

TGF-β was used as a positive control at 10 ng/mL (Sigma T7039).

Biological material

The keratinocytes NCTC 2544 are isolated from normal human epithelial cell line. The culture was realized on monolayer with DMEM (Eurobio, CMODME70-08) containing 10% fetal calf serum (Eurobio, CVFSV00-0U), 1% antibiotics (penicillin/streptomycin, Eurobio, CABPES01-OU) and 1% L-glutamine (Eurobio, CSTGLUOO-OU) at 37°C under 5% CO_2 and 95 % moisture.

Incubation protocol

7500 cells/well in 96-well plates are seeded in microplates in enriched DMEM for 24h. The culture medium is then replaced with DMEM medium containing 5% of FCS for an additional 24 h to promote a quiescence state of cells.

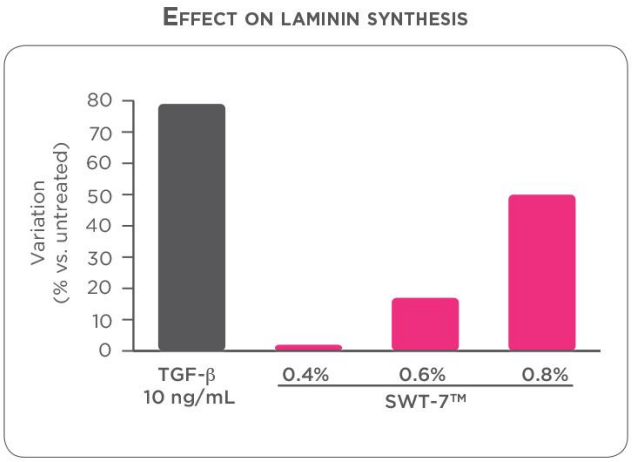
TGF-β or swertiamarin was then added for an additional 24 hours.

Evaluation protocol

At the end of the incubation period, supernatants were collected for ELISA (ref TAKMK107Z) and cell viability assays (MTS test).

Results are indicated according to the percentage activation of the laminins synthesis reported to the concentration in SWT-7™ equivalent and to cellular viability.

RESULTS



SWT-7™ had a dose-dependent direct effect on laminins production with a significant effect at 0.01% (+50%)

SWT-7™ stimulated the laminins synthesis at 0.8% (+50%) compared to the control thus reinforcing the skin basement membrane functions in the epidermis without the help of hypodermis.

CONCLUSION

SWT-7™ stimulates laminin synthesis for a better anchoring of the DEJ (dermo-epidermal junction).

ANNEX

GENE EXPRESSION PROFILING ANALYSIS OF SWT-7™-TREATED ADIPOCYTES

-

Functional network identification with the analytical research tool Predict Search™

INTRODUCTION

Adipocyte genic expression coupled with Predict Search software allow to identify new functions or pathway through emerging keyword, discovered activities triggered by new molecules and so to determine contextual biological processes and activities within functional networks. By determining the main signaling pathway induced by a molecule, this software can predict the global physiological impact and biochemical cascades as the result of the modulation gene expression.

OBJECTIVE

The objective of the study was to determine the transcriptional effects of SWT-7™ in human differentiated subcutaneous adipocytes.

PROTOCOL

Tested molecule:

Pure swertiamarin (batch #STK4417) was tested at 0.005% (0.05mg/ml) equivalent to 0.4% of SWT-7™.

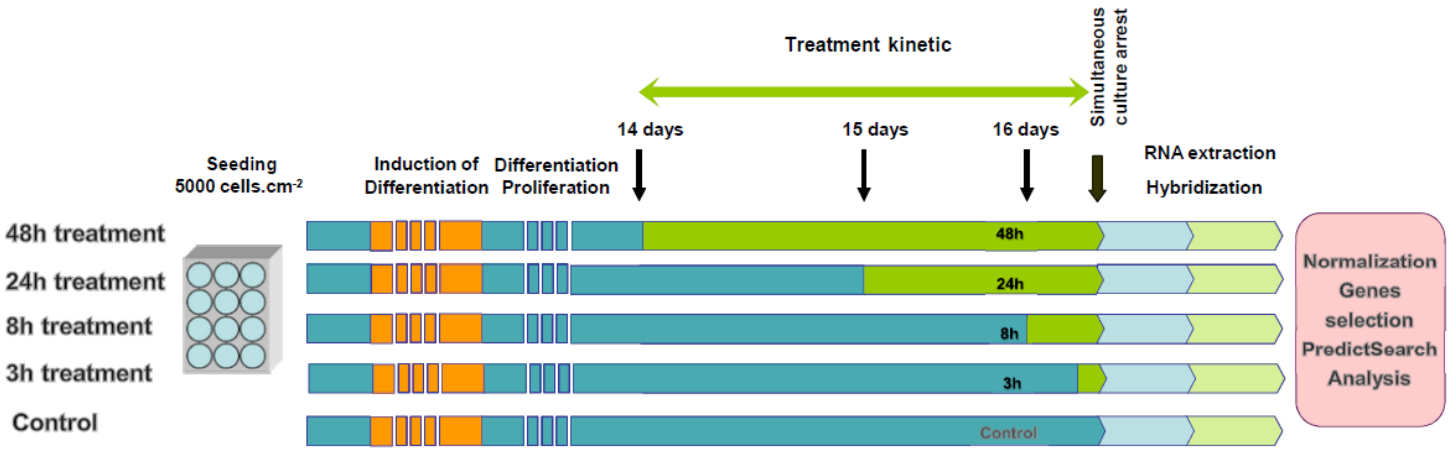
Biological material

Human subcutaneous pre-adipocytes (Ref C-12731) were isolated from skin biopsies processed during surgical intervention of healthy non-diabetic 18-60 years old patients with normal Body Mass Index (20 to 25).

Cells were amplified in appropriate culture medium (Ref C-27417), and then seeded in 9.6cm^2 wells (COSTAR cell culture plates 12 wells, Corning, Ref. CLS3513, Sigma-Aldrich) at a density of 5,000 cells per cm^2.

Three days after seeding, adipogenic differentiation was induced with specific medium (Ref C- 27437), maintained for 72 hours and replaced with adipocyte-specific medium (Ref C-27439). These conditions were maintained until full differentiation, i.e. 14 days. Each culture was duplicated in order to perform both RNA and protein extractions.

Experimental design:



RNA extraction:

Total RNAs were extracted using the RNEasy Mini kit (QiaGen), according to the manufacturer's recommendations. A DNAse I treatment has been performed to avoid genomic DNA contamination. Purified RNAs were quantified using the Nanodrop 1000 (ThermoFisher) spectrometer and qualitatively evaluated by capillary electrophoresis on Agilent BioAnalyzer.

- **Sample processing and microarrays hybridization**

500 ng RNA of each sample have been processed for retro-transcription, amplification and Cy3 labeling using the Quick Amp Labeling Kit, one-color (Agilent Technologies). Three Whole Human Genome Arrays (4x44K, Agilent Technologies) were hybridized as recommended by the manufacturer.

- **Data processing and normalization**

The microarray data were quantified according to the protocol of the quantification software (GeneExtractionFeature V10.5.5.1). The dataset was normalized by Quantile Method with the R statistical software (inter- and intra-array normalization).

- **Genes selection and main results**

Gene modulation was evaluated by determining a ratio calculated between fluorophore intensity value of treated condition and intensity value of untreated condition. Initially, only genes expressed a fluorophore intensity value ≥ 60 have been taken into account. Then, genes submitted to PredictSearch™ analysis were selected when the ratio was superior or equal to 1.45 or activated and maintained at three consecutive time points.

DETAILED RESULTS

Swertiamarin-induced class II histone deacetylase **HDAC4** (1.7; 1.5; 1.5; 1.3) and partners reinforces the hypothesis of a negative retro-control of adipocyte differentiation. The histone deacetylase (HDAC) enzymatic complex is required for specific transcriptional repression, promoting DNA condensation when tethered to a promoter. It is especially active in stem cells to inhibit differentiation. Indeed, selective class II HDACs inhibition also impairs PPARγ signalling in adipose tissue, suggesting their important role in the repression of adipogenesis [Nebbioso A. *et al.*, 2010].

SKI (2.4; 2.5; 1.6; 1.1) [Nomura T. *et al.*, 1999], **NIPBL** (1.5; 1.8; 1.6; 2.0) [Jahnke P. *et al.*, 2008] and **ANKRD11/ANCO1** (1.5; 1.9; 1.3; 2.7) [Zhang A. *et al.*, 2004] recruit and/or are part of this complex. Moreover, the coordinated regulation of genes involved in such complex with other transcriptional repressors such as histone demethylases **JMJD1C** (1.9; 1.8; 1.6; 1.3) and **JARID2** (3.0; 3.0; 1.5; 0.9), known as repressors of differentiation, may also lead to the inhibition of adipocyte differentiation. This hypothesis is reinforced by the up-regulation of **RBAK** (1.7; 2.1; 1.4; 1.5), which interacts with the retinoblastoma protein to repress **E2F1** (1.0; 1.6; 2.2; 0.9) - dependent transcription [Skapek SX. *et al.*, 2000].

RBAK (1.7; 2.1; 1.4; 1.5) is specifically expressed in hematopoietic stem cells, suggesting its role in the maintenance of an undifferentiated state [Gomes I. *et al.*, 2002]. Furthermore, the histone demethylase **KDM5B** (1.7; 1.5; 1.5; 1.1) also represses gene transcription to maintain uncommitted progenitors and to block terminal differentiation [Dey BK. *et al.*, 2008].

Counterbalancing these transcriptional repressions associated to the inhibition of differentiation, **SUPT16H/FACT** (1.7; 2.2; 2.0; 1.7) promotes RNA pol II-driven transcription by interacting with specific histones to destabilize nucleosomal structure [Belotserkovskaya R. *et al.*, 2003]. **SUPT16H/FACT** plays a key role in the development and differentiation of smooth muscle cells [Kihara T. *et al.*, 2008].

On the other hand, intracellular cAMP levels are increased by the membrane protein **CRCP** (1.9; 2.3; 2.2; 1.6), and cAMP by itself can partially activate adipogenesis. This nucleotide can also specifically induce Krüppel-like factor 4, encoded by **KLF4** (1.9; 1.6; 1.5; 0.7). While **KLF4** functions as an immediate early regulator of adipogenesis via direct transcriptional induction of **CEBPB** (1.2; 0.8; 0.8; 0.8) [Birsoy K. *et al.*, 2008], it is also able to maintain cells in an undifferentiated state in repressing transcription [Yet SF. *et al.*, 1998], [Saulnier N. *et al.*, 2011]. Since expression of **CEBPB** and **PPARG** (1.1; 0.9; 1.1; 1.2) remains to basal levels, it can be suggested that induction of **KLF4** upon swertiamarin treatment limits the differentiation process.

Accordingly to the role of **KLF4** in self-renewal [Xu N. *et al.*, 2009], swertiamarin also induces stem cell-specific genes such as **RIF1**(2.3; 2.5; 2.1; 1.4), which is involved in the maintenance of telomere length and pluripotency in the germline; it is highly expressed in toti- and pluri- potent cells during early mouse development, and in adult germ cells [Adams IR and McLaren A., 2004]. In the same line, the stem cell-specific transcription factor **ETV5** (1.5; 1.7; 2.1; 1.3) is also required for spermatogonial stem cell self-renewal and for the regulation of the hematopoietic stem cell niche [Chen C. *et al.*, 2005].

Stem cell-specific genes often are involved in other development-associated processes such as morphogenesis or epithelial remodeling. For instance, in mesenchymal stem cells derived from bone marrow, **RORA** (1.8; 2.3; 1.9; 1.3) interacts with NM23-2 kinase, involved in organogenesis and differentiation [Meyer T. *et al.*, 200]. **FGF7/KGF** (1.6; 1.1; 1.6; 1.5) also stimulates morphogenesis in cooperation with

metalloproteinases, especially with **MMP3/Stromelysin** (0.9; 1.5; 2.1; 1.7) in mammary epithelium [Simian M. et al., 2001].

Organogenesis, growth and differentiation of the kidney require **PDK2/Polycystin-2** (1.7; 1.8; 1.6; 1.3), **EXOC5/SEC10** (2.1; 2.6; 1.7; 1.3) as well as iron as a cofactor for development. Iron can be delivered to cells by ferritin via its binding to the cell surface through **SCARA5** (1.5; 0.9; 1.9; 1.5). This mechanism allows kidney organogenesis, suggesting an important role for this scavenger receptors family, including **COLEC12/SCARA4** (1.6; 1.6; 1.9; 1.7), in early development [Li JY. *et al.*, 2009]. Such morphogens expression by subcutaneous adipose tissue suggests possible architectural skin rearrangements upon swertiamarin-treatment.

On the other hand, **FGF7/KGF** (1.6; 1.1; 1.6; 1.5), encoding Keratinocyte Growth Factor is a paracrine growth factor secreted by mesenchymal cells to exert positive effects on various epithelial cells. Recent work indicates that FGF7/KGF is expressed in subcutaneous adipose tissue [Gabrielsson BG. *et al.*, 2002]. The significant expression and the slight up- regulation of **FGFR1** (1.6; 1.9; 1.6; 0.8), the receptor of FGF7, suggest that this secreted GF may have a slight autocrine effect on adipocytes. Consistent to our data, FGF7/KGF stimulates pre-adipocyte proliferation without promoting their differentiation to mature adipocytes in 3T3-L1 model [Zhang T. *et al.*, 2010]. *In vitro*, this GF stimulates epidermis formation and keratinocytes proliferation onto acellular human dermis. They also stimulate keratinocytes migration, suggesting a role in wound healing [Erdag G. *et al.*, 2004], [She T. *et al.*, 2009]. The secretion of this GF by the skin micro-environment, especially subcutaneous adipocytes, could help epidermal regeneration upon swertiamarin stimulation.

SUMMARIZED RESULTS

Main genes expression variation indicating the maintenance of the adipocyte stem cells pool

- Induction class II histone deacetylase (**HDAC4**), histone demethylase (**JMJD1C**) : Inhibition adipocyte differentiation especially stem cells.
- Up regulation of RBAK, KDM5B, KLF4: Maintenance of uncommitted progenitor or undifferentiated state
- Up regulation RIF1 (stem cell-specific gene): Maintenance of telomere length and pluripotency in the germline
- Up regulation ETV5 (stem cell-specific transcription factor): Stimulation adipose derived stem cell (ADSC) self-renewal.
-

Main genes expression variation indicating hypodermic-induced regeneration of the epidermis

- Induction of **FGF7/KGF**, keratinocyte growth factor and also of its receptor **FGFR2b**
  - Autocrine effect: proliferation of preadipocytes without differentiation
  - Paracrine effect: stimulation of epidermis formation and keratinocytes proliferation

For more detailed results cf. to the Annex.

CONCLUSION

SWT-7™ regulates transcription of gene mainly implied in repression of differentiation. SWT-7™ would stop the differentiation process of stem cells, favoring their self-renewal and therefore limiting their transformation in differentiated adipocytes.

On the other hand, subcutaneous adipocytes stimulated by SWT-7™ could also play a key role in skin microenvironment signaling, sending epidermal regeneration signal through FGF7/KGF expression.

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