CLARIANT

ACTIVES & NATURAL ORIGINS

CycloRetin™
Activating ageless beauty

Thanks to its unique cyclic peptide, CycloRetin delivers retinol's benefits without its irritating effects, by ensuring retinol homeostasis and optimal use by the skin, to relaunch skin matrix production and improve epidermal structure. It is clinically proven to outperform bakuchiol on skin sagging and demonstrates comparable performance on skin firmness and radiance increase over 28 days. Outstandingly, it is as effective as retinol at reducing wrinkles in only 14 days.



CycloRetin™

TECHNICAL INFORMATION

INCI Name	Glycerin, Water, Pseudostellaria Heterophylla Extract
Origin	From prince ginseng, an adaptogenic plant grown in China's scenic valleys
Use level	0.3%-2.5%
Active compound	Heterophyllin B (cyclic peptide)
Specification	Appearance: light yellow to orange yellow liquid Odor: characteristic Solubility: water soluble Shelf life: 3 years
Compliance	China NMPA registered; ECOCERT COSMOS APPROVED; ISO 16128 100% NATURAL ORIGIN; PALM-FREE; 100% Renewable Carbon Index; NATRUE Approved

KEY RESULTS

In-vitro & ex-vivo	**Retinoid pathway:** stimulates 7 targets to ensure retinol homeostasis		
	Collagen level: equivalent to retinol 3 times more efficient than bakuchiol	**+133%**	
	Matrix 3D architecture:		
	SPARC (collagen assembly):	**+99%**	
	Fibrilin I (elastic fibers organization):	**+393%**	
Clinical	**Firmness:**		
	after 28 days:	**+12.6%**	at 0.3% vs. placebo, or vs. 0.5% bakuchiol
	after 56 days:	**+19%**	
	as efficient as bakuchiol		
	Sagging:		
	after 28 days:	**-10.7%**	
	x 1.4 time higher than bakuchiol		
	Radiance:		
	x 9.5 times better than placebo after 28 days		
	as efficient as bakuchiol		
	Wrinkles:		at 1% vs. 0.3% retinol
	after 14 days:	**-5.1%**	
	after 28 days:	**-7.1%**	
	similar efficacy to retinol		

KEY PROPERTIES

— Firms and lifts the skin up

— Defies the effects of gravity by reducing age-related sagging

— Improves complexion radiance

TESTS

— *In-vitro*

— *Ex-vivo*

— Clinical

DISTINCTIVE SIGNS

— A natural, efficient and non-irritating alternative to retinol

— Same efficacy as retinol at reducing wrinkles after 2 weeks, surpasses bakuchiol at improving sagging after 4 weeks

— Cyclic peptides offer modern solutions - better bioavailability, formulation stability, and overall performance

— Traceable supply chain from a time-tested traditional Chinese medicine plant

— Easy to formulate, water soluble, stable, China-registered, efficient at 0.3%

APPLICATIONS

— Age-defying creams for day and night

— Anti-gravity solutions

— Rejuvenating serums

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03.2024

CLARIANT

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CycloRetin™
Activating ageless beauty



CYCLORETIN™

Fighting skin sagging without compromise

SKIN-FRIENDLY BEAUTY: PERFORMANCE WITHOUT COMPROMISE

The landscape of skincare is ever evolving, shaped by new expectations and changing narratives. Gone are the days when it was acceptable for beauty to require sacrifices, when performance was more important than naturalness and gentleness. Influenced by lifestyle, stress and environmental factors, skin needs have also changed, and skin sensitivity has undeniably become a bigger issue. All this has led to a significant shift in today's mindsets, particularly regarding the approach to minimizing the signs of aging. While retinol has been a favorite with skincare consumers since the 1980s, its popularity is now facing a major challenge – unwanted irritation.



DEFYING TIME

As we age, our skin undergoes changes in both the dermal and epidermal layers. In the dermis, fibroblasts play a key role in producing the components of the extracellular matrix, including collagen for firmness and elastin for elasticity. Other compounds support dermal structures and prevent their degradation, influencing the mechanical properties of the skin. Meanwhile, within the epidermis, mechanisms that hinder self-renewal can lead to thinning.

To counter these natural but visible effects, like fine lines, loss of firmness and sagging, the skin needs extra care. This is where retinoids come in – such as retinol, retinaldehyde, and retinyl palmitate. These compounds activate specific receptors, such as the retinoic acid receptor (RAR), thereby initiating the synthesis of collagen for example and dermal matrix. This also triggers biological mechanisms that enhance epidermal cell proliferation, strengthening the protective function of the skin.

Encouraging the natural cycles of the skin, i.e. the recurring processes that contribute to the skin's renewal and structural maintenance, is essential. Hence, compounds that activate retinoid pathways, without causing side effects, offer a promising approach to addressing the effects of aging in modern society. The result? Revitalized, youthful-looking skin that stands the test of time, paving the way to enduring beauty.

PRINCE GINSENG: A MILD CYCLIC PEPTIDE

While contrary to its name prince ginseng, or *Pseudostellaria heterophylla*, is not a ginseng plant, its milder nature make it a popular substitute. Used as an adaptogen in herbal medicine, its roots naturally contain exceptional molecules: cyclic peptides. Compared to their linear counterparts, these compounds exhibit better stability thanks to their three-dimensional cyclic structure, and greater selectivity for cell receptors, increasing their performance[1]. Highly effective even at low concentrations, peptides play a crucial role in supporting youthful skin appearance. This is because they are short chains amino acids, the building blocks of skin proteins, and help stimulate the production of collagen and elastin.

In traditional Chinese medicine, this valuable plant has been prized for over 3,000 years for its many medicinal virtues, ranging from treatment of fatigue to invigorating the spleen and anti-oxidant benefits. While exploring the benefits of cyclic peptides in our lab, we discovered prince ginseng's outstanding potential to restart the skin matrix production cycle.

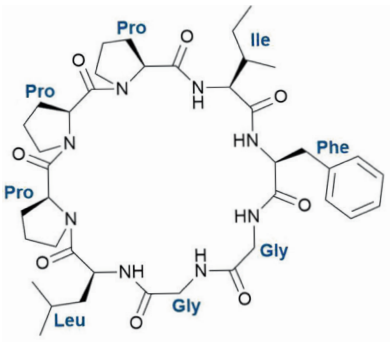
WELL-AGING FEATURES THANKS TO SAG REDUCTION AND TIGHTENING EFFECTS

CycloRetin harnesses prince ginseng's powers through heterophyllin B, a natural cyclic peptide, and activates the age-defying benefits inspired by retinol while being naturally gentle. This unique biomarker is a versatile compound that allows CycloRetin to provide a broad range of skin benefits. These range from extracellular matrix synthesis and the quality of skin structures to fortifying effects on fibroblast metabolism. Additional benefits include bolstered barrier properties and skin hydration pathways, which are essential for maintaining a strong barrier function.

CycloRetin is better received by the skin than retinol, and delivers a fast efficacy in reducing wrinkles in just 14 days. When used for 4 weeks, CycloRetin reshapes facial features by reducing skin sagging more effectively than bakuchiol, and boosts skin firmness as well as improves complexion radiance. Potent yet natural, the active suits modern mindsets: By opening up a gentler approach to skincare, it offers a smoother journey to ageless beauty without compromise.



Heterophyllin B

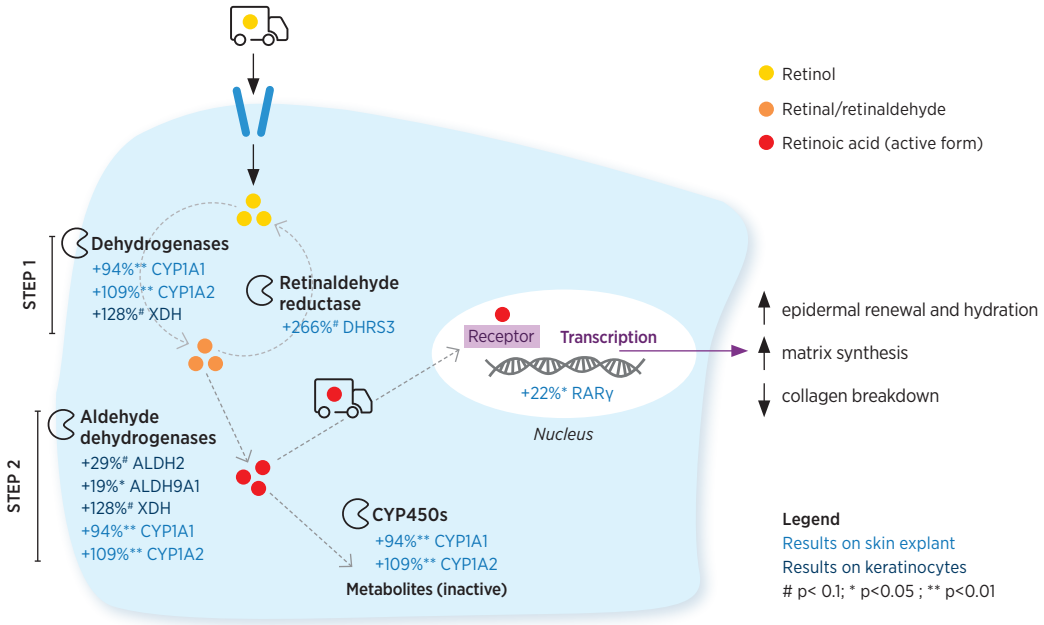


[1] Structure-activity analysis of histatin, a potent wound healing peptide from human saliva: cyclization of histatin potentiates molar activity 1000-fold, *The FASEB Journal* (2009)

Experimental Data

EFFECT ON RETINOID PATHWAY

Retinol alternative approach is increasingly appealing, with the aim to stimulate the retinoid pathway, ensuring a balanced level within skin cells and optimal use. Two complementary models were used: CycloRetin at 0.3% on a skin explant or CycloRetin at 0.15% on keratinocytes, using transcriptomic analysis.



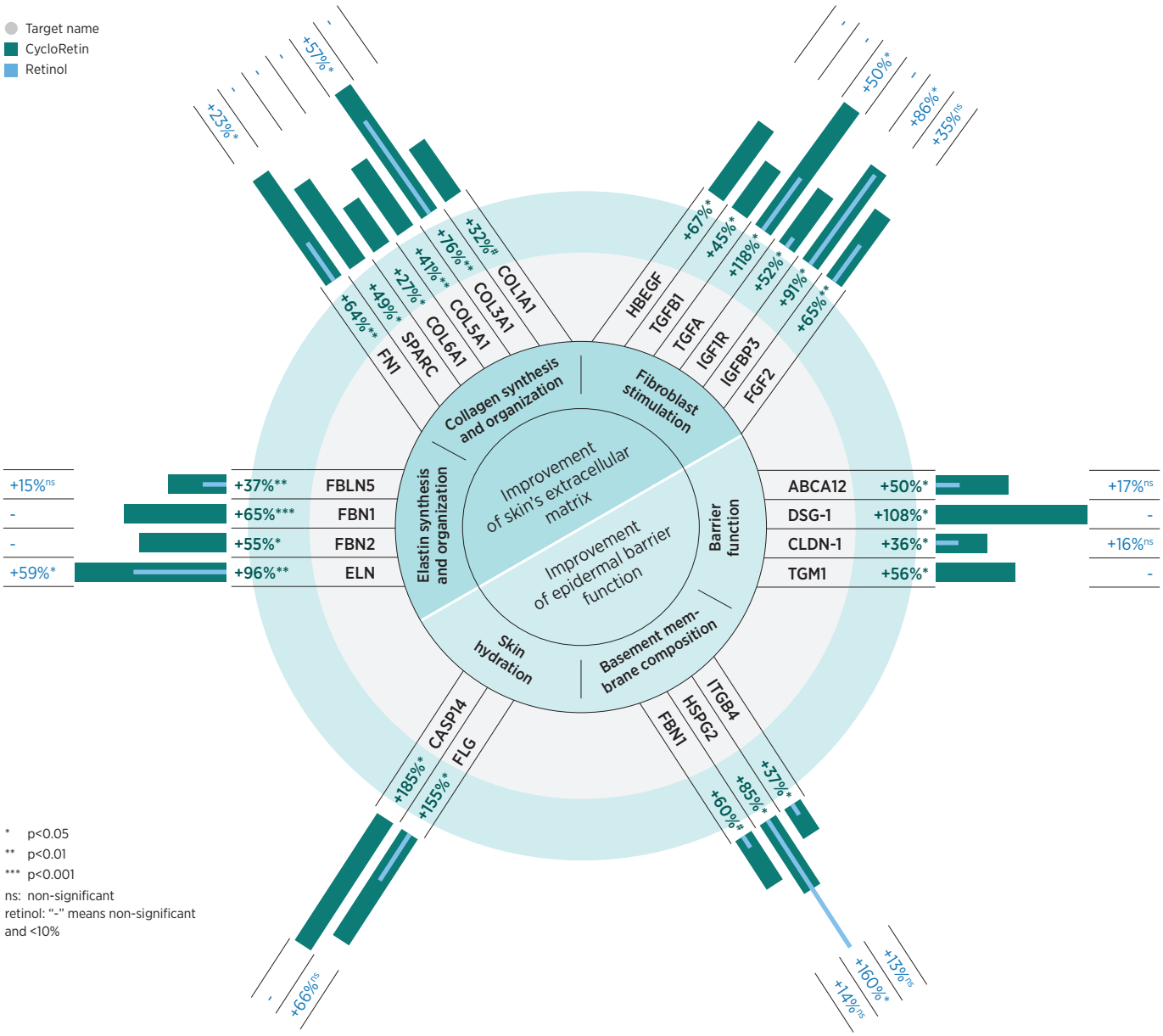
- **CYP1A1, CYP1A2, XDH, DHRS3 and ALDH(s)**: the level of retinol conversion enzymes is increased to optimize retinoid pathway homeostasis for a better use of intra-cellular retinol.
- **RARγ**: the most abundant epidermal receptor for retinoic acid in the epidermis

CycloRetin increases the expression of important enzymes involved in retinol conversion, and of the most dominant retinoid receptor in the epidermis. This suggests it can trigger similar benefits to retinol without overloading the skin, reducing the risk of adverse effects.

Experimental Data

IMPROVEMENT OF EPIDERMIS AND DERMIS STRUCTURE

– CycloRetin at 0.15% or retinol at 10 ppm (0.001%) on keratinocytes
– CycloRetin at 0.01% or retinol at 10 ppm (0.001%) on fibroblasts
– Positive regulation of epidermis and dermis through transcriptomic analysis



* p<0.05
** p<0.01
*** p<0.001
ns: non-significant
retinol: "-" means non-significant and <10%

CycloRetin acts on multiple aspects of dermis and epidermis organization:
- Stimulation of fibroblasts ensures cell renewal, matrix production and dermal homeostasis. The growth factors play an important role in harnessing skin-regenerating properties.
- Activation of collagen and elastin helps to maintain skin structure over time.
- Barrier function protects the skin from external threats while maintaining skin health.
- Basement membrane supports dynamic processes of cell renewal and barrier integrity.
- Boosting skin hydration elements is crucial for maintaining skin health, as well as elasticity.

Compared to retinol, CycloRetin has superior effects on almost all of the skin's natural building blocks, helping to achieve skin that looks younger for longer.

BOOST OF COLLAGEN CONTENT

By stimulating fibroblast metabolism, CycloRetin should promote type I collagen synthesis, the most abundant protein in the body. This was evaluated on fibroblasts, after 3 days of incubation with or without CycloRetin and its biomarker heterophyllin B at equidose. Two benchmarks were included: retinol and its most famous plant-based alternative, bakuchiol; their concentrations were chosen in accordance with their described and documented efficacy. TGF-β1 at 10 ng/mL was used as positive control.

Increase in pro-collagen I (% of control)

TGF-β1	Retinol (10 ppm = 0.001%)	Bakuchiol (10 ppm = 0.001%)	CycloRetin (0.1%)	Heterophyllin B (0.10 ppm = 0.00001%)
+74%*	+158%***	+44%^{ns}	+133%***	+95%**



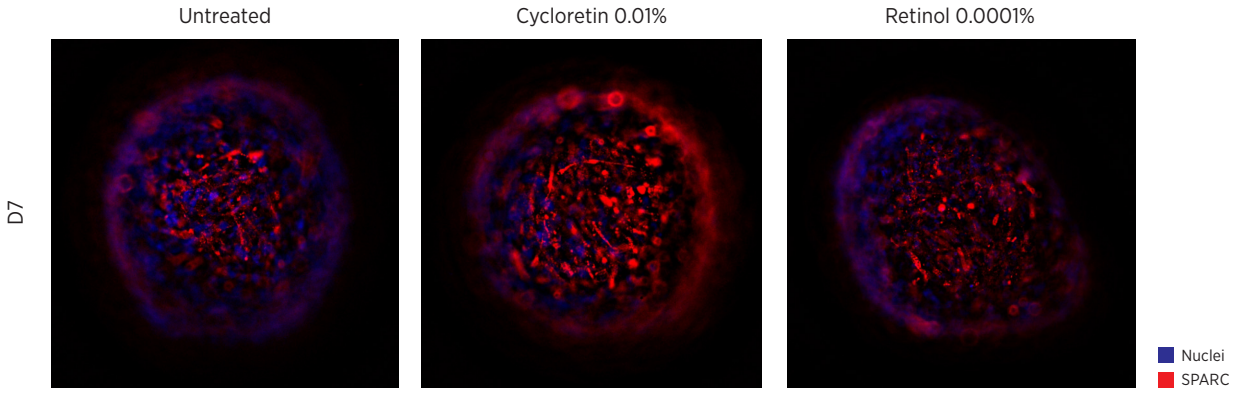
* p<0.05
** p<0.01;
*** p<0.001;
ns: non-significant
$$ p<0.01 vs. bakuchiol

Thanks to its content in unique cyclic peptide, CycloRetin boosts the collagen I level in the skin by 133%. It is as efficient as retinol and 3 times more efficient than bakuchiol.

Experimental Data

ENHANCEMENT OF EXTRACELLULAR MATRIX ARCHITECTURE

We performed a unique spheroid assay to examine the matrix architecture regulation via SPARC, that modulates collagen assembly. This model uses only cells, without surrounding extracellular elements, cultivated in a 3D environment, and allows exploring the fibers' organizational properties without exogenous interference.



p<0.05; ns: non-significant vs. untreated

CycloRetin clearly increases SPARC expression in the dermis, helping maintain skin shape over time.

CYCLORETIN PROVIDES THE BENEFITS OF RETINOL WITHOUT ITS CONCERNS

CycloRetinol activates the retinoid metabolism and has superior effects on the production and organization of the cellular matrix compared to retinol. To assess the skin irritation feature, a patch test assay on human skin was performed with either a placebo formula, CycloRetin at 0.3%, 0.5% and 1%, or retinol at usual dosage ranging from 0.1% to 0.3%.

Placebo	Non-irritating
CycloRetin 0.3%	**Non-irritating**
CycloRetin 0.5%	**Non-irritating**
CycloRetin 1.0%	**Non-irritating**
Retinol 0.1%	Slightly irritating
Retinol 0.3%	Slightly irritating

The results confirm the safety of CycloRetin: While retinol demonstrated potential for irritation even at the minimal dose of 0.1%, the active ingredient does not cause irritation even at 1%, the highest tested dose. Compared to traditional retinol, CycloRetin offers a gentler yet equally effective approach to skin care.

Clinical Evaluation

In our efforts to support a youthful skin appearance, we harnessed the power of retinoid metabolism to enhance skin framework. CycloRetin showed the ability to reactivate the dermal extracellular matrix, surpassing bakuchiol and equivalent to retinol in term of collagen production, delivering optimal performance without causing irritation.

STUDY #1 VERSUS BAKUCHIOL

22 women (45-65 yo) showing wrinkles, reduced firmness and dull complexion

0.3% CycloRetin vs. placebo

56 days, 2x/day

12 women (45-64 yo) showing wrinkles, reduced firmness and dull complexion

0.3% CycloRetin vs. 0.5% bakuchiol

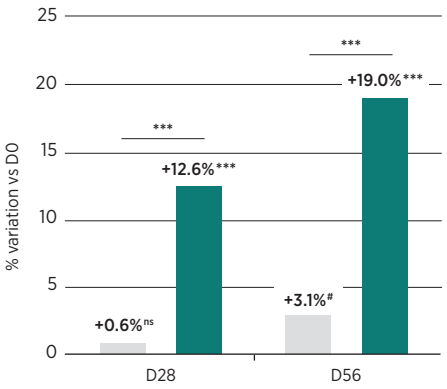
56 days, 2x/day

> Skin firmness (R0 parameter), measured with a Cutometer
> Sagging, scored by a trained assessor on a 5-point scale
> Luminosity L* lightness value, via colorimetry

FIGHTING LOSS OF FIRMNESS

Firm skin easily returns to its original shape when pulled or strained. Skin firmness is determined by the structural protein collagen, as well as proper 3D organization.

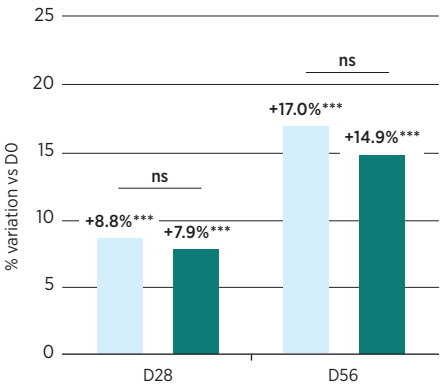
Firmness improvement vs. placebo



x6 vs. placebo after D56

Placebo
CycloRetin 0.3%

Firmness improvement vs. bakuchiol



Same effect as bakuchiol

Bakuchiol 0.5%
CycloRetin 0.3%

\# p< 0.1
*** p<0.001
ns: non-significant

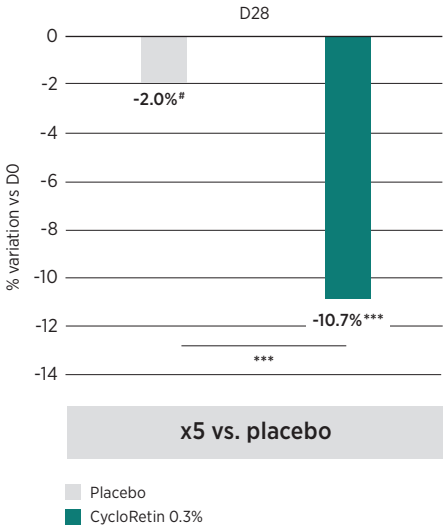
Thanks to its effects on skin matrix synthesis and in particular on collagen I, CycloRetin significantly improves skin firmness by 13% after 28 days, with further intensified effects after 56 days of use. This is observed in 100% of the volunteers. The ingredient performs 6 times better than the placebo and is similarly effective as the bakuchiol benchmark.

Clinical Evaluation

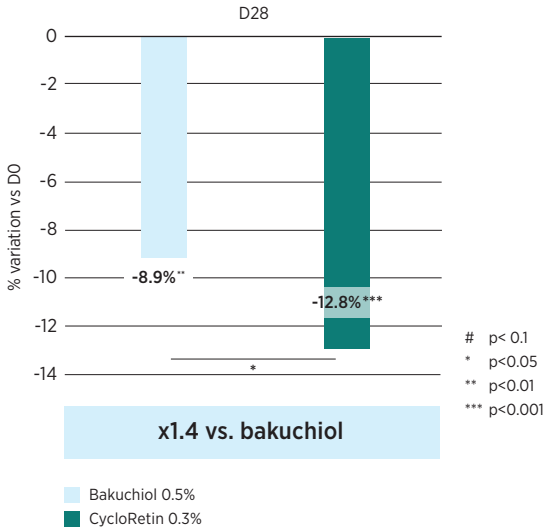
ANTI-SAGGING ACTION

Over time, gravity takes its toll on the skin, causing it to stretch downward. For many women, visible sagging is a big concern. Improving the structural properties of the underlying skin layers should help to mitigate this phenomenon.

Improved skin sagging vs. placebo



Improved skin sagging vs. bakuchiol

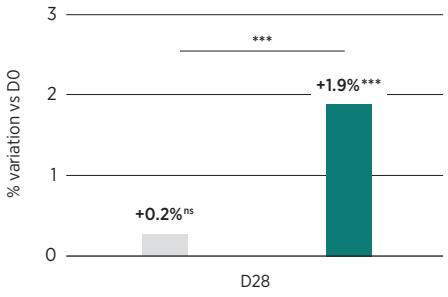


CycloRetin defies the effects of gravity by reducing age-related sagging, in an even more effective way than bakuchiol. This helps to keep facial features sculpted and defined.

SKIN RADIANCE

The rising demand for skin brightening ingredients reflects consumers' desire for a radiant complexion. Since retinol is known for its ability to even out complexion, we also assessed skin brightening in our study. The L* parameter is a measure for skin luminosity.

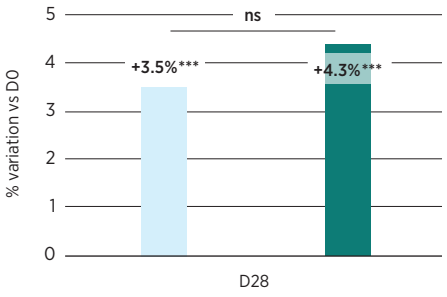
Enhanced skin luminosity vs. placebo



x9.5 vs. placebo

Placebo
CycloRetin 0.3%

Enhanced skin luminosity vs. bakuchiol



Same effect as bakuchiol

Bakuchiol 0.5%
CycloRetin 0.3%

*** p<0.001
ns: non-significant

After 28 days, an improvement in skin brightness was observed, promoting greater clarity of complexion. The ingredient is as effective in improving luminosity as bakuchiol.

Clinical Evaluation

STUDY #2 VERSUS RETINOL

20 women* (45-65) showing wrinkles

* *7 volunteers withdrew from the study due to irritation reactions from retinol exposure*

1% CycloRetin vs. 0.3% retinol

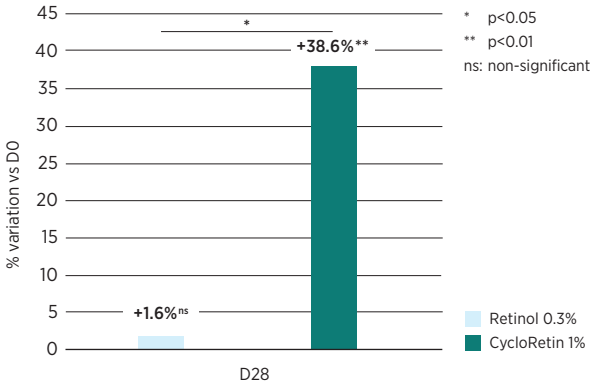
28 days, 2x/day

> Dermis density, via echography
> Global wrinkles and crow's feet wrinkles, measured with AEVA
> Pictures, via AEVA

DERMIS DENSITY

The density of the fibers in the dermis is closely linked to the quantity of extracellular matrix elements. Using an echography device based on ultra-sounds, changes in dermis density were assessed. An increase contributes to better skin firmness and reduction in wrinkles.

Density increase



x24 vs. retinol

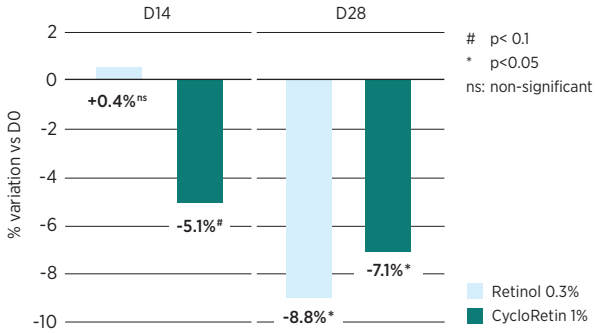
By stimulating the production and organization of collagen and elastin in the dermis, CycloRetin plumps the skin from the inside through a strong increase in skin density, surpassing by far retinol's effect.



GLOBAL WRINKLES REDUCTION

The improvement of dermis density should result in wrinkles softening, therefore a global analysis of wrinkles was conducted. The face is prone to many types of wrinkles that form vertical and horizontal lines. These include expression wrinkles as well as aging wrinkles, which slowly develop.

Rapid Wrinkles Reduction



Faster action after D14, and same effect as retinol after D28

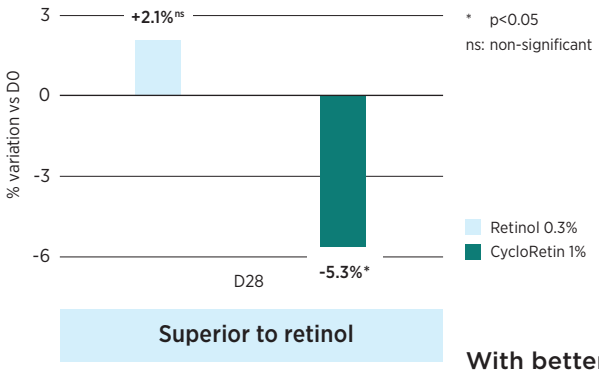
In only 2 weeks, CycloRetin reduces all type of deep facial wrinkles, much faster than retinol, with a building effect overtime to help prevent the formation of future lines.

Clinical Evaluation

CROW'S FEET WRINKLES IMPROVEMENT

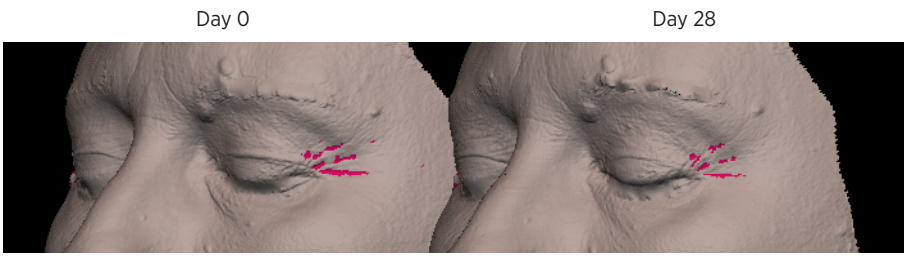
Crow's feet wrinkles readily show signs of aging, especially when smiling. To preserve a joyful, youthful appearance around the delicate eye area, focus was placed on this region.

Fewer crow's feet wrinkles



With better efficacy than retinol, CycloRetin visibly smooths and plumps the eye area wrinkles, providing a youthful appearance.

Visible crow's feet wrinkles reduction



KEY MARKETING FEATURES

- Ingredient capitalizes on modern consumer expectations and the demand for effective skincare that does not compromise on skin friendliness

- Allows developing a product that builds on the popularity of retinol but doesn't have its side effects, while also including trendy peptides

- Supports the natural cycles of the skin with cyclic peptides, found exclusively in *Pseudostellaria heterophylla*

TECHNICAL INFORMATION		
INCI Name	Glycerin (and) Water (and) *Pseudostellaria Heterophylla* Extract	
Use level	Can be used at 0.3-2.5% in various formulation types (clinical studies: 0.3% and 1%)	
Specification	Appearance	> light to dark orange liquid
	Odor	> Characteristic
	Solubility	> Water soluble
	pH (25°)	> 4.00~7.00
	Specific gravity (25°)	> 1.15~1.25
	Heterophyllin B	> 50 ppm
Benefits	Improves skin firmness Boosts skin brightness Limits skin sagging Enhances collagen production Reduces wrinkles	
Application	Anti-aging line for day and night Skin firmness cream Well-aging serum	

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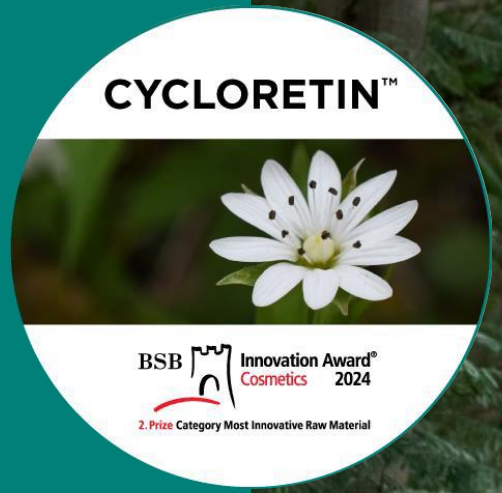
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CycloRetin™

Activating Ageless Beauty



CLARIANT

Clariant Actives & Natural Origins



PAST

Beauty sacrifices to stay young – standing still in time

More simple skin care routine – basic skin care practices

Increased awareness about protecting the skin from the sun and first cosmetics containing retinol debut in the market

Usage of synthetic ingredients are higher



Consumers are looking for safe and natural ingredients, with high performance

PRESENT

A lot of options in the market and a more complete routine – mix of ingredients

Awareness that not only age impacts the skin, but also the lifestyle and the environment – now more consumers indicate their skin is sensitive

Demand for potent ingredients without drawbacks – damaging the skin



Embracing inherent beauty and encouraging the natural cycles of the skin

FUTURE

Gentle Beauty: rise in skin sensitivity led to increased need for safe and quality products

Sophisticated Beauty: changes in societal attitudes, growing awareness of overall skin health, greater emphasis on appearance

Effective Beauty: deeper understanding of skin’s unique needs

The long-standing hero retinol faces modern challenge

- Retinol is a true anti-aging hero
- Recognized to reduce wrinkles and fine lines, improve firmness and skin tone

- Causes unwanted irritation, lacks stability
- Mostly synthetic
- Best used at nighttime
- On-going discussion to limit its maximum concentration



Trendy stars in Beauty & Personal Care

Peptides are starring ingredients supporting end-products performance

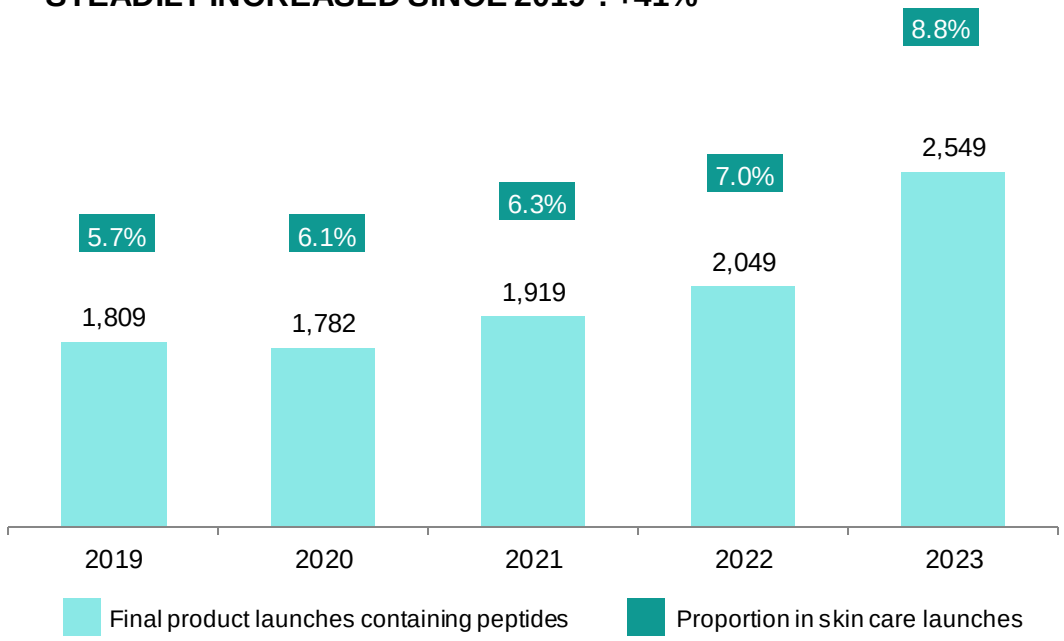
Stable molecules, they demonstrate unwavering efficacy in reducing aging signs and inflammation, even at low doses

Short chains of amino acids, building blocks of skin proteins (collagen, elastin)

Around 20,000

peptide-related patents for cosmetics application (until 2023)

SKINCARE LAUNCHES CONTAINING PEPTIDES HAVE STEADILY INCREASED SINCE 2019[1]: +41%



Sources: [1] Mintel GNPD

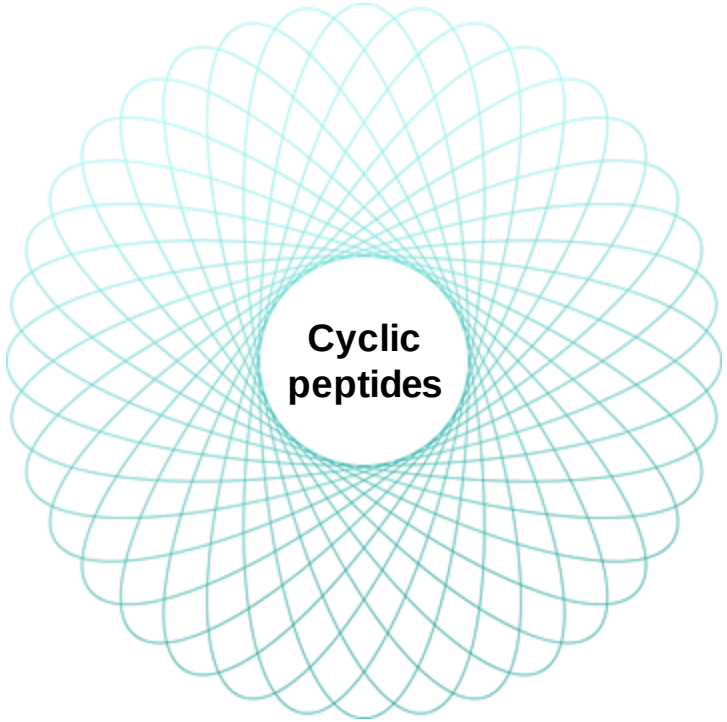
Groundbreaking evolution: cyclic peptides providing superior performance

Unlike linear peptides, which have a start and end point, cyclic peptides form a closed loop which gives them specific characteristics:

Prized in pharmaceutical industry
One of the most alluring prospects in modern drug discovery: **46%** of approved peptides are cyclic[1]

Targeted effects
Precise and effective outcomes

Bioavailability
Better cell permeability for significant impact[2]

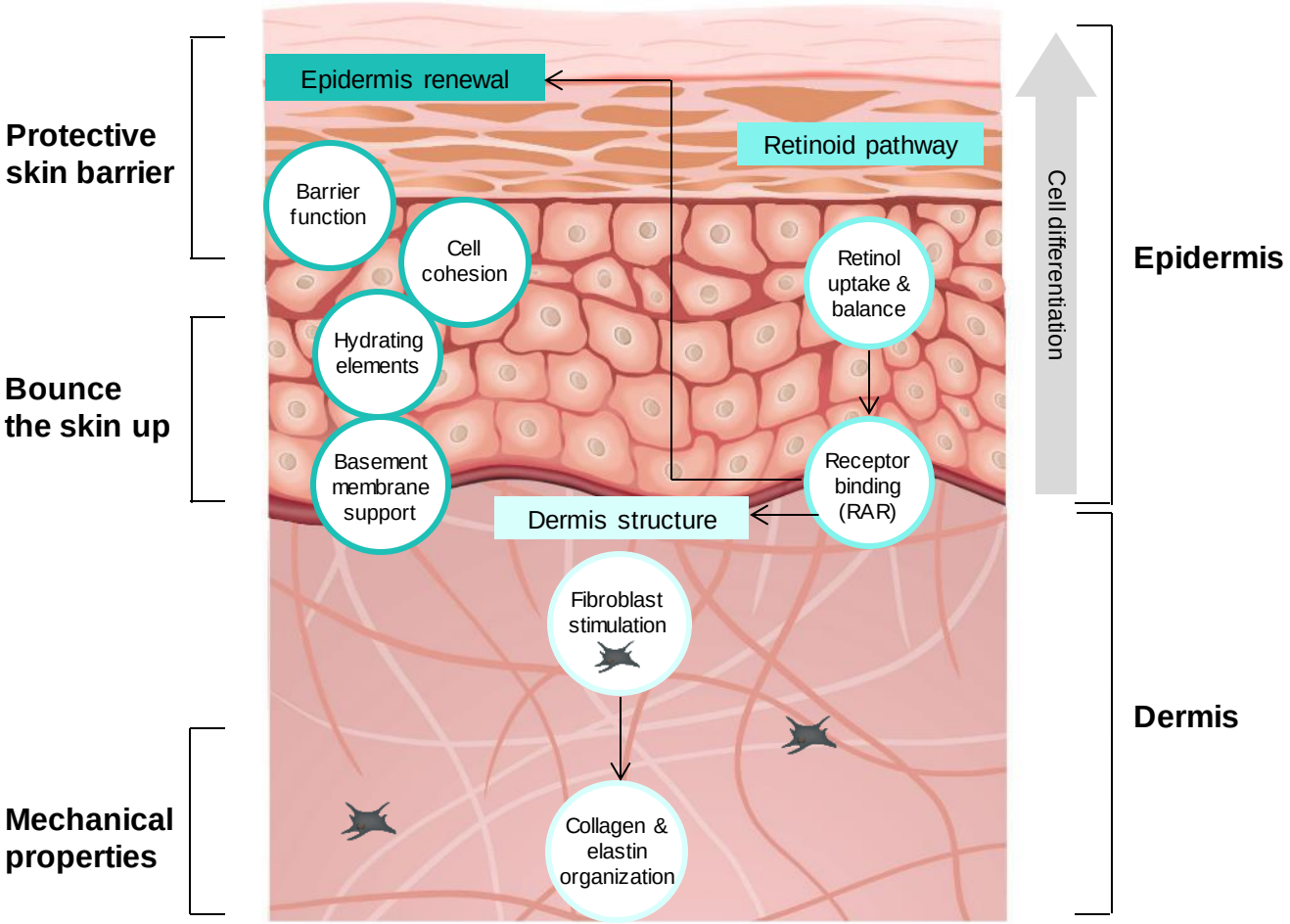


Excellent stability
- 30 times more stable than linear peptides[3]
- Privileged structure resistant to chemical and enzymatic degradation[4]

Bioactivity
Up to 1000-fold more potent when compared with uncyclized[5]

Sources: [1] Cyclic Peptides in Pipeline: What Future for These Great Molecules?; [2] Natural Cyclic Peptides as an Attractive Modality for Therapeutics: A Mini Review; [3] Solution stability of linear vs. cyclic RGD peptides; [4] Cyclic Peptides as Potential Therapeutic Agents for Skin Disorder; [5] Structure-activity analysis of histatin, a potent wound healing peptide from human saliva: cyclization of histatin potentiates m olar activity 1000-fold

Supporting extracellular matrix and skin framework to maintain the skin over time



Aging impacts dermal and epidermal layers:

- Impairment of cell renewal process
- Degradation of extracellular matrix
- Reduction in epidermis thickness
- Weaken of skin barrier function

The solution: relaunching production of skin matrix and maintaining epidermis structure, to encourage the natural cycles of skin

CycloRetin™

Activating ageless beauty



Market opportunity: Harnessing the benefits of two superstars to optimize skin and reduce aging signs appearance

Inspired by the iconic ingredient **RETINOL** without its side effects

Retinol has been adopted in more than **4,700** cosmetics products[1]

PEPTIDES are efficient at low dose and well-known molecules

50% of women in China associate oligopeptides as having anti-aging benefits[2]



Skin-friendly approach serving the modern mindset:

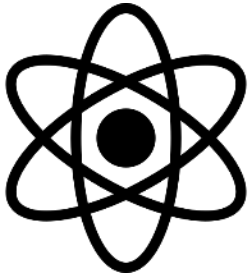
The only natural cyclic peptide, rivaling retinol and bakuchiol, developed for daytime application, addressing ageless beauty without compromise

Sources: [1] Mintel GNPD, [2] Mintel report, 2022

Powers of Nature: Plant-derived cyclic peptides

OCCURRENCE

- Around **500 cyclopeptides**[1] were discovered from plants
- Identified in 26 families and 120 species (mostly found in Caryophyllaceae and Rhamnaceae families)
- Size from 2 to 80 amino acids



BENEFITS

- Highly targeted solutions (affinity with cell protein surfaces)
- Cyclic form enhances the potential of peptides in skin-related disorders[2]
- Important roles as defense molecules for the host, against insects and other pathogens[1]

EXPLORATION

- Not produced by humans
- Three technologies for production: **isolation from host**, chemical synthesis (high environmental impacts), biological synthesis (genetic modifications)[3]

Sources: [1] Plant Cyclopeptides; [2] Cyclic Peptides as Potential Therapeutic Agents for Skin Disorders; [3] Discovery and applications of naturally occurring cyclic peptides

The science at the rescue to found out natural cyclic peptides

Plant-derived peptides



CYCLIC PEPTIDE for enhanced activity meeting natural requirements

Plant selection



Extensive literature analysis led to **PRINCE GINSENG** plant identification

CycloRetin™



- From *Pseudostellaria heterophylla* (prince ginseng) root extract
- Use of fractionation to target the biomarker **heterophyllin B** (cyclic peptide): **66 times more concentrated** than standard plant extract
- Well-characterized and native botanical peptide
- **Only green solvents** are used in the process

Natural Peptides: from the plant to the active

Plant
naturally producing peptides that could be beneficial for the skin

Raw extract
obtained from dried plant to extract soluble molecules

Fractionation
to separate the different groups of molecules present in the raw extract and get an extract concentrated in plant peptides

Solubilization
in glycerin to get the final product



Prince ginseng is grounded in ancestral knowledge



100 years

of use in clinics in China[1]



Adaptogenic plant

recognized to strengthen the body against stress



1 of 721

kinds of herbs[1] recorded in traditional Chinese medicine (TCM) in 1757 **Time-tested practices**



Not a ginseng

but used for children as a substitute for its milder effects

Source: [1] Chemistry, pharmacology and analysis of Pseudostellaria heterophylla: a mini-review

Zoom on…



Botanical name: *Pseudostellaria heterophylla*
Vernacular name: Prince Ginseng, Tai zi shen
Family: Caryophyllaceae
Origin: native to East Asia, found in China, Japan and Korea



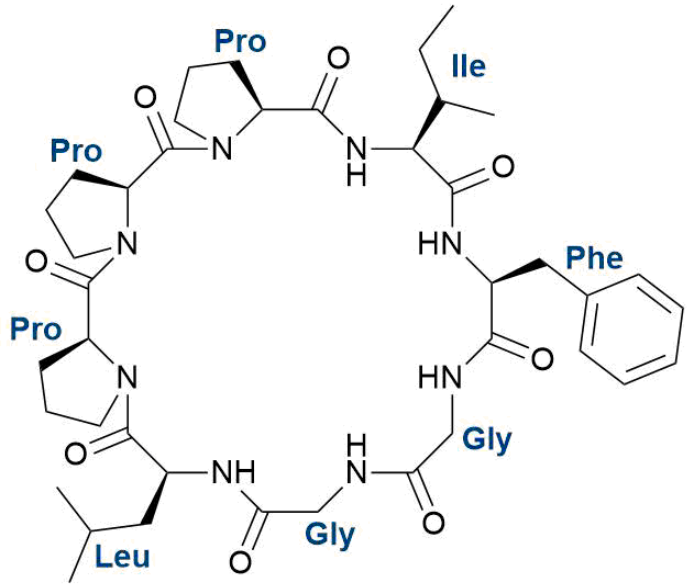
Ancestral uses: support immune system, enhance vitality, treatment of fatigue and lack of appetite

- Commonly used TCMs, demand for this herb is rising annually
- Particularly appreciated by children to overcome weaknesses

Heterophyllin B: a remarkable plant-derived cyclic peptide

HETEROPHYLLIN B

- A cyclic octapeptide with a single ring formed with peptide bonds and eight L-amino acids
- Specific bioactive peptide from *Pseudostellaria heterophylla*
- Promising circular molecule reducing irritation and oxidative stress



Heterophyllin B

Glycine and proline are two most prevalent amino acids in skin collagen

Chinese valleys exploration to discover the age-defying powers of prince ginseng

SOURCING

- Seek the best harvests in **geo-authentic area** ensuring authentic botanicals grown in indigenous region, in Jurong, Jiangsu Province, China
- Cultivated in shady and wet place under the forest in the valley at 800-2700 meters above sea level
- Reasoned approach: no pesticide, no fertilizer

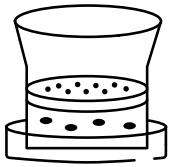
Evaluations: experimental data

In-vitro and *ex-vivo* studies

STEP 1: Retinol alternative through retinol homeostasis

Gene level

Skin explant (34 yo)

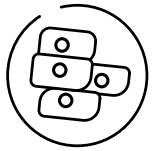


+/- CycloRetin at 0.3%

D0 D1

Transcriptomic analysis

NHEK



+/- CycloRetin at 0.15%

D0 D1

Transcriptomic analysis

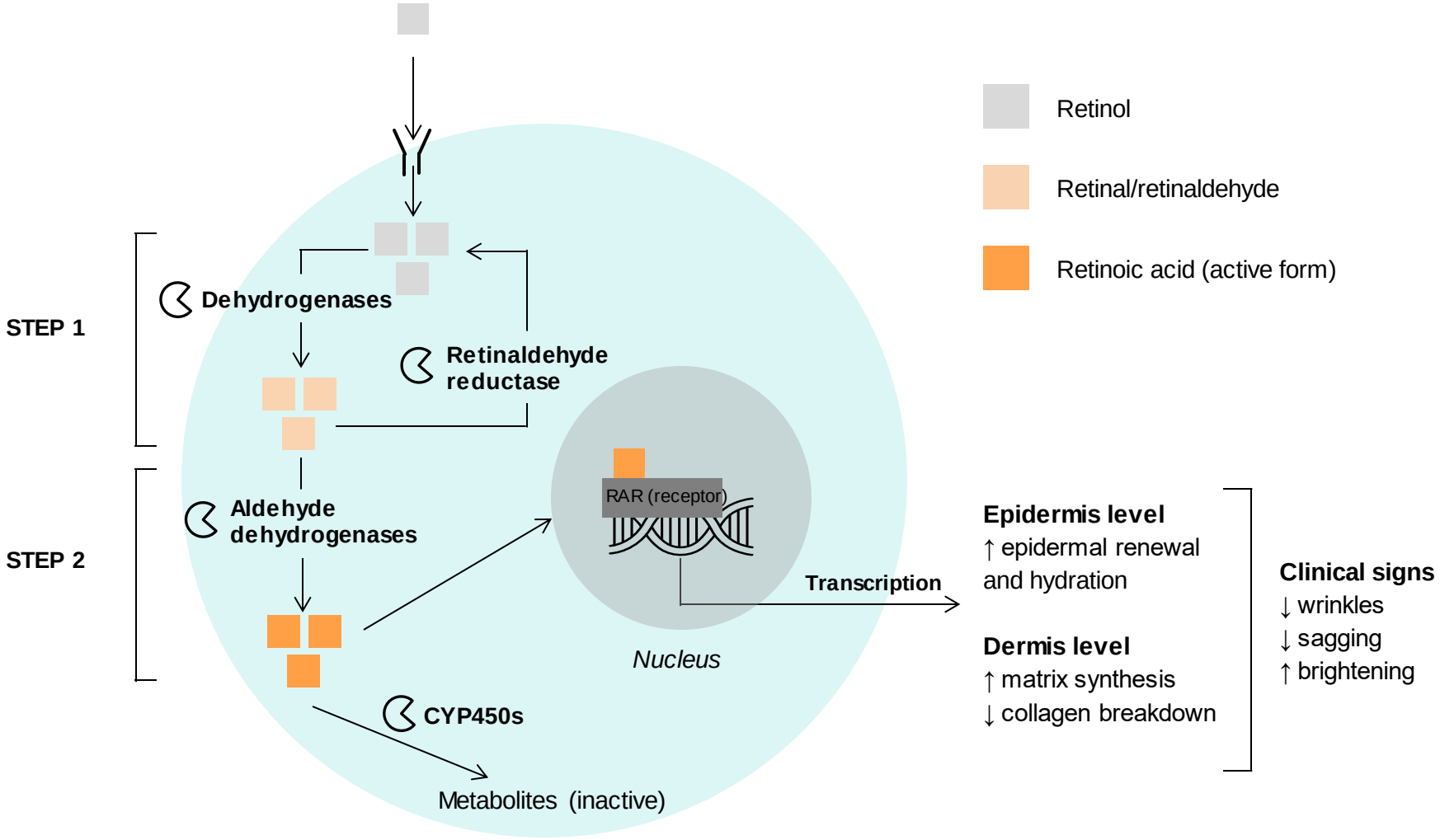
The retinoid pathway to boost skin structure

WHY?

To counter aging weaknesses, our skin needs extra care

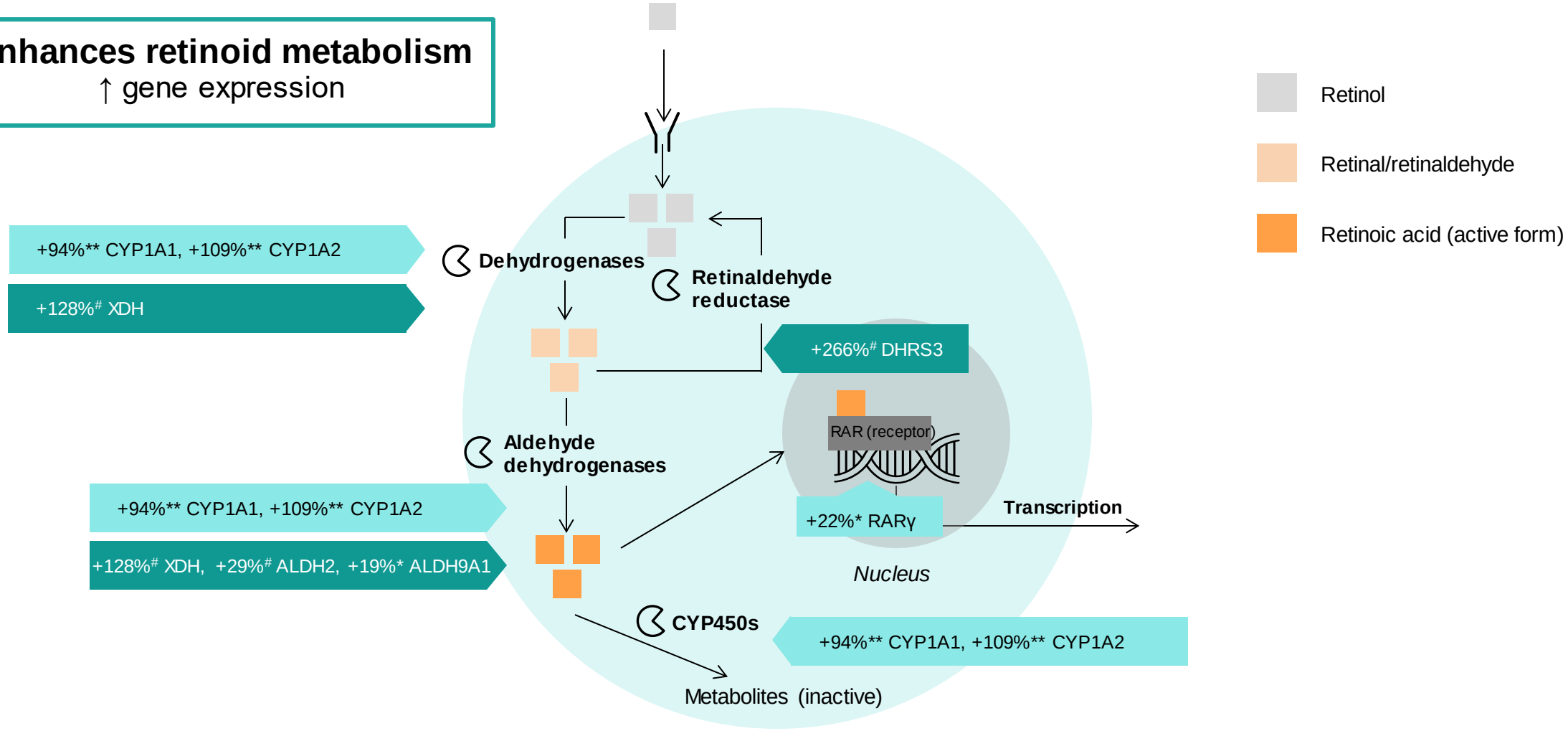
STRATEGY

Stimulating cells, like retinol would do without the need of its topical application, to promote similar activities while preventing from deleterious effects



CycloRetin ensures efficient use of intra-cellular retinol

Enhances retinoid metabolism
↑ gene expression

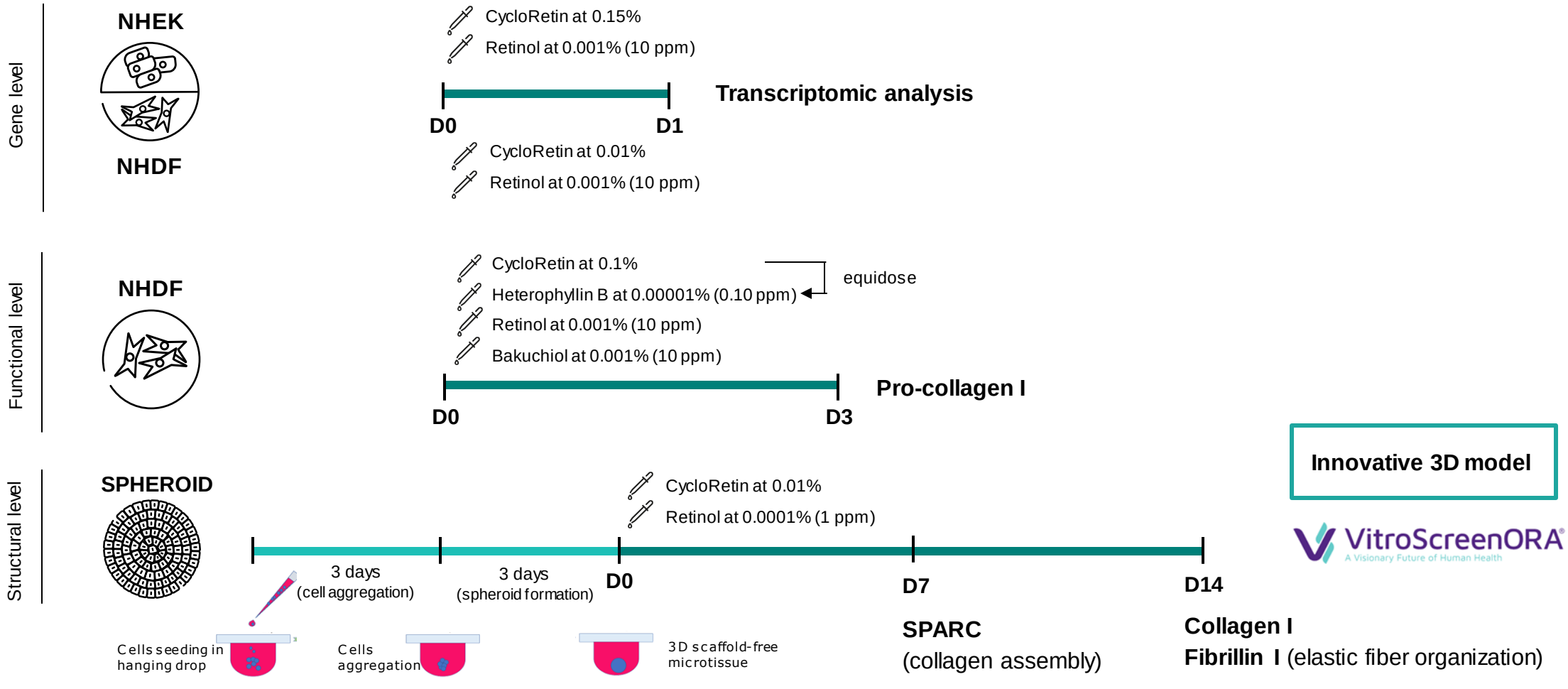


CycloRetin at 0.15%, *in-vitro* CycloRetin at 0.3%, *ex-vivo*

# p< 0.1; * p<0.05; ** p<0.01

In-vitro studies

STEP 2: Exclusive signature through epidermis and dermis structures improvement



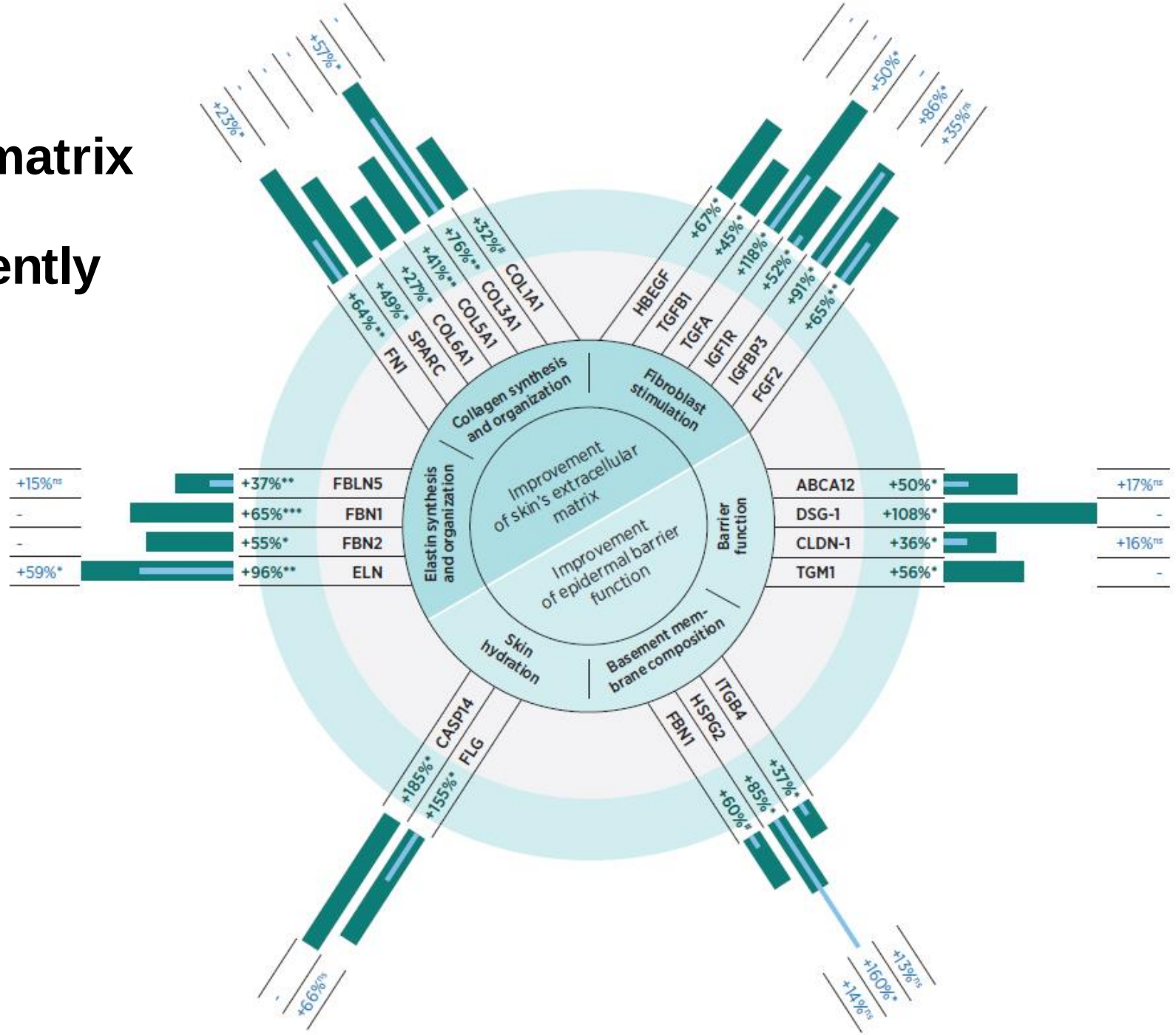
CycloRetin boosts skin matrix and regulates epidermal homeostasis more efficiently than retinol

↑ gene expression

Target name

CycloRetin

Retinol

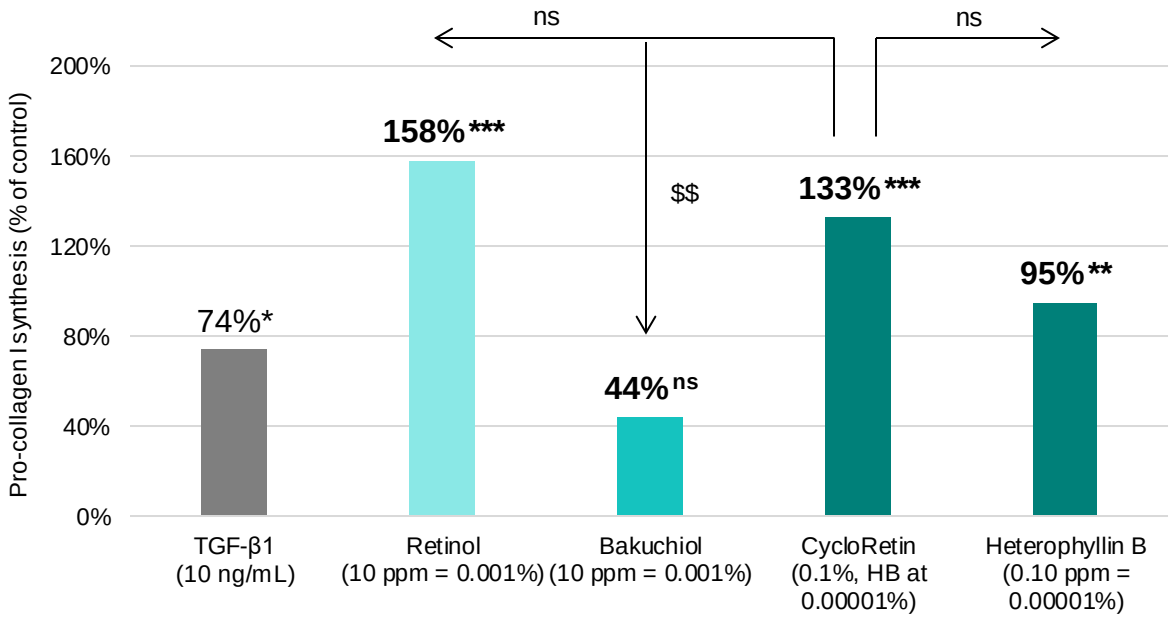


Legend:
* p<0.05; ** p<0.01; *** p<0.001
ns: non-significant
Retinol: "-" means no-regulation and non-significant

Increase of collagen synthesis leading to dermis structure improvement

3x more efficient than **Bakuchiol**

- **Endpoint:** pro-collagen, by ELISA
- **Benchmarks:** retinol or bakuchiol (concentrations chosen in accordance with their documented efficacy)
- **Positive control:** TGF-β1

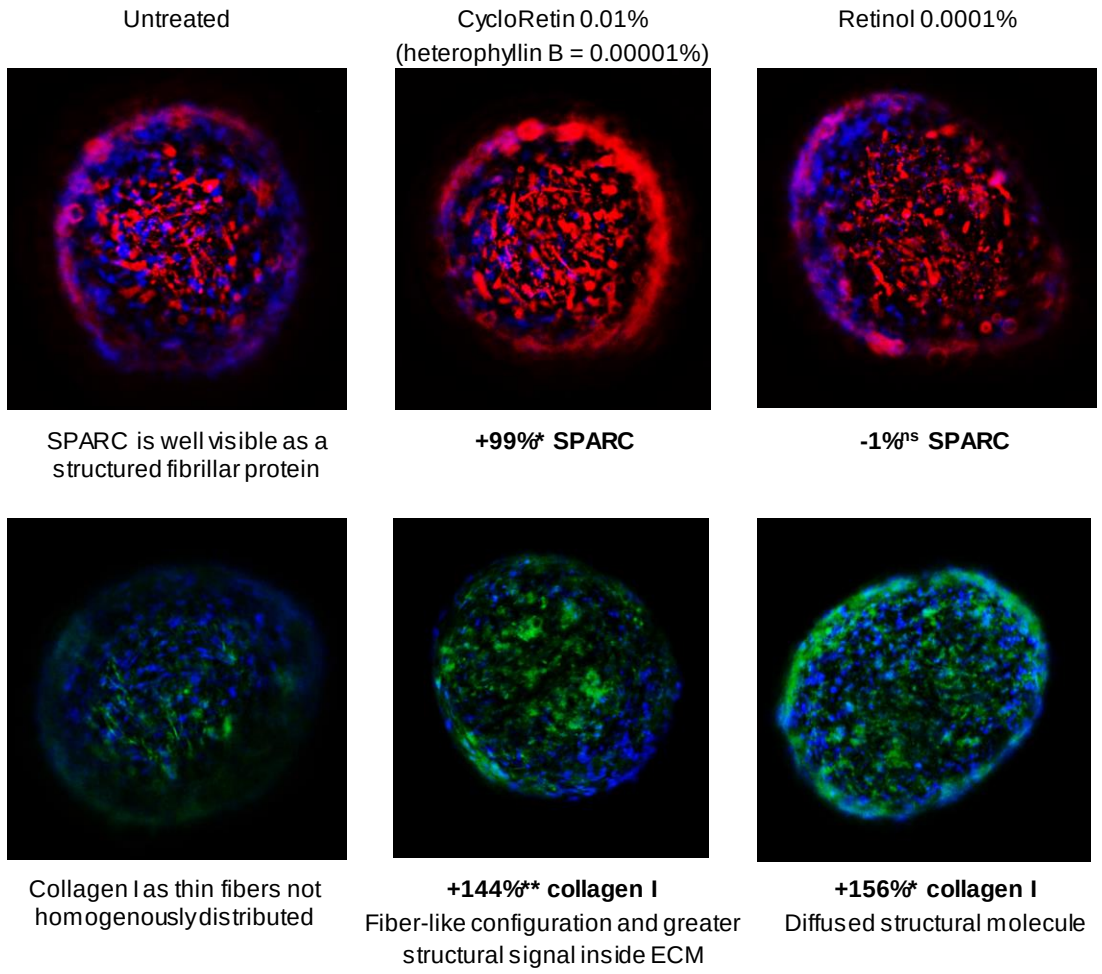


* p<0.05 ; ** p<0.01; *** p<0.001 ns: non-significant
$$ p<0.01 vs. Bakuchiol

Thanks to its content in unique cyclic peptide, Cycloretin **boosts collagen I level** in the skin **by 133%**, and its biomarker by 95%. It is **as efficient as retinol** and **3 times more efficient than bakuchiol**.

Enhancement of skin matrix maturation and architecture

- **Model:** innovative 3D spheroid assay of dermis. Fibroblasts are cultivated in a 3D environment without ECM elements
- **Endpoints:** protein SPARC (7 days), that modulates collagen assembly, and protein Collagen I (14 days)
- **Benchmark:** retinol

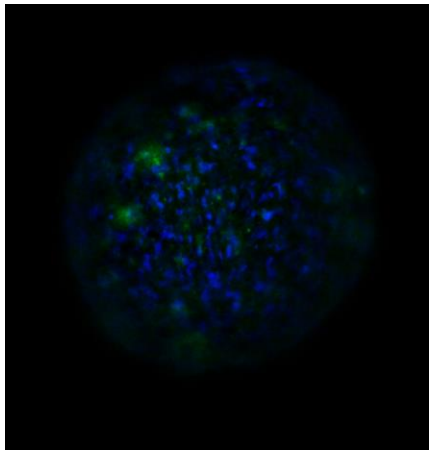


CycloRetin increases SPARC and collagen I levels in the dermis, helping maintain skin shape over time.

Enhancement of skin matrix maturation and architecture

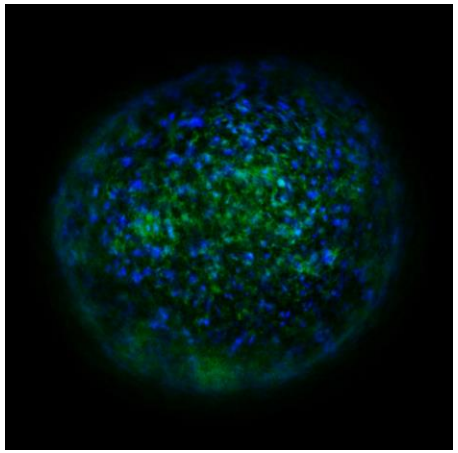
- **Model:** innovative 3D spheroid assay of dermis. Fibroblasts are cultivated in a 3D environment without ECM elements
- **Endpoints:** protein fibrillin I (14 days) to support elastic fibers organization
- **Benchmark:** retinol

Untreated



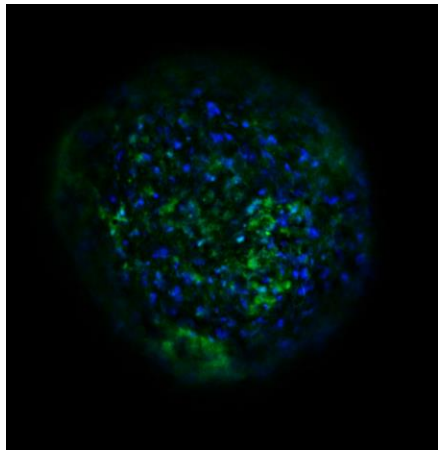
Fibrillin I as a diffused signal with a very thin filamentous structure

CycloRetin 0.01%
(heterophyllin B = 0.00001%)



+393%* fibrillin I**
Thin fibers are visible as dense network embedding cells.

Retinol 0.0001%



+234%* fibrillin I**
More visible thin fibers

Nuclei

Fibrillin I

*** p<0.001 versus untreated

CycloRetin increases fibrillin I level in the dermis, helping maintain skin shape over time.

CycloRetin: extended biological efficacy

1. Stimulation of retinoid metabolism, like retinol would do

Retinol homeostasis

6 targets

CYP1A1/A2, XDH, ALDH2/9A1, DHRS3

Retinol receptor

+22%

RARγ (the main RAR receptor in epidermis)

2. Reactivation of skin matrix production and protection of skin mechanical properties

Fibroblast stimulation: **6 targets**

Collagen synthesis & organization: **6 targets**

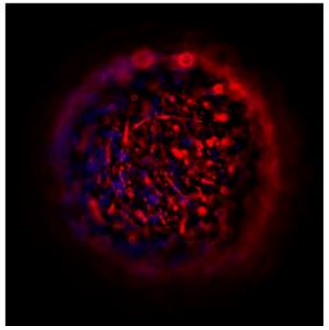
Elastin synthesis & organization: **4 targets**

Transcriptomic analysis

+133% collagen I

— **x3 better** than Bakuchiol

— Same as retinol



+99% SPARC protein

3. Improvement of epidermis structure

Barrier function

up to +108%

ABCA-12, DSG-1, CLDN-1,TGM1

Hydration

up to +185%

CASP14, FLG

Basement membrane composition

up to +85%

ITGB4, HSPG2, FBN1

Transcriptomic analysis

Clinical data

A gentle peptide-based solution compatible with sensitive skin

- **Context:** Non-protected retinol causes irritation
- **Protocol:** occlusive patch test assay on human, 48h
- **Investigated formulas:** placebo / CycloRetin at 0.3%, 0.5% and 1% / retinol at 0.1% and 0.3% (usual doses)

Placebo	Non-irritating
CycloRetin 0.3%	Non-irritating
CycloRetin 0.5%	Non-irritating
CycloRetin 1.0%	Non-irritating
Retinol 0.1%	Slightly irritating
Retinol 0.3%	Slightly irritating

Low doses

Retinol, even at the lowest recommended dose, induces slight irritation

On the contrary, CycloRetin is a mild ingredient, delivering similar performance to retinol, without the unpleasant side effects.

Clinical protocol #1 vs. Bakuchiol

Placebo	Bakuchiol
22 women (45-65 yo) showing wrinkles, reduced firmness and dull complexion	12 women (45-64 yo) showing wrinkles, reduced firmness and dull complexion
0.3% CycloRetin vs. placebo	0.3% CycloRetin vs. 0.5% Bakuchiol
56 days, 2x/day on half of the face	56 days, 2x/day on half of the face

BAKUCHIOL®

Famous plant-based retinol alternative (*Psoralea corylifolia)* China compliance only in progress

- Skin firmness (R0 parameter), measured with a Cutometer
- Sagging, scored by a trained assessor on a 5-point scale
- Luminosity L* lightness value, via colorimetry
- Pictures, via Visia

Formula:

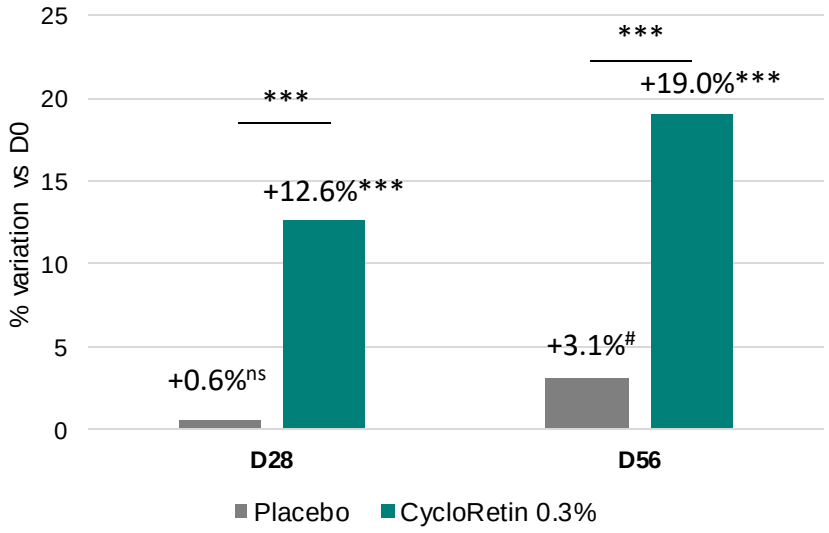
Ingredients	Quantity (%) Cycloretin formula	Quantity (%) Placebo formula	Quantity (%) Bakuchiol formula
Water	q.s	q.s	q.s
Ammonium Acryloyldimethyltaurate/VP Crosspolymer	0.80	0.80	0.80
Dicaprylyl Ether	1.00	1.00	1.00
Citric Acid (and) Sodium Citrate	1.00	1.00	1.00
CycloRetin	**0.30**	**-**	**-**
Bakuchiol	**-**	**-**	**0.50**
Phenoxyethanol (and) Methylparaben (and) Ethylparaben	1.10	1.10	1.10
Fragrance	0.003	0.003	0.003

Improved skin's strength and smoothness, for a lifted and tightened appearance

CycloRetin fights loss of firmness

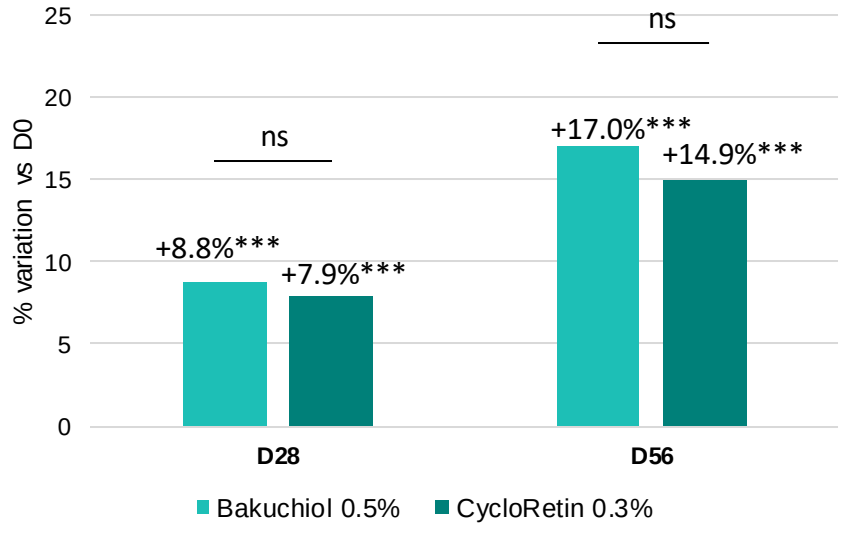
Skin firmness depends on the amount and quality of collagen fibers.

FIRMNESS MAINTENANCE VERSUS PLACEBO



PLACEBO
x6 after D56

FIRMNESS MAINTENANCE VERSUS BAKUCHIOL



BAKUCHIOL
Same effect

Legend: # p< 0.1 *** p<0.001 ns: non-significant

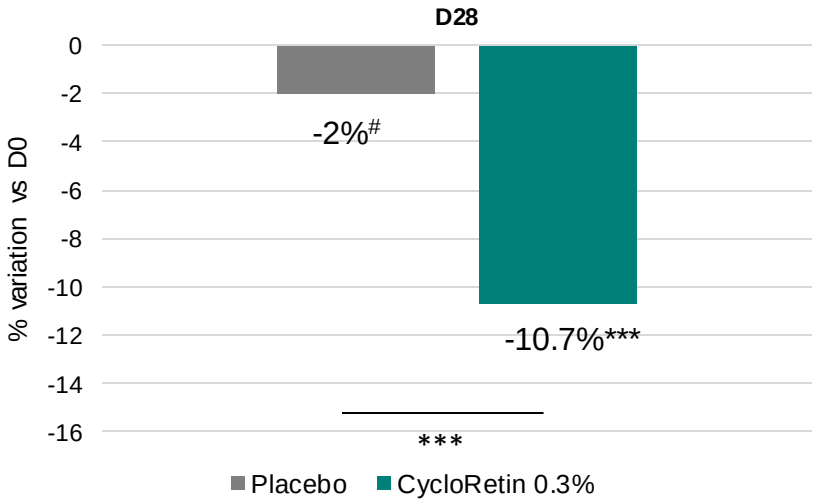
CycloRetin improves skin firmness after 28 days and more intensely after 56 days, by 6 times more than placebo and similarly to bakuchiol.

CycloRetin lifts sagging skin

Redefined facial contours and toned skin

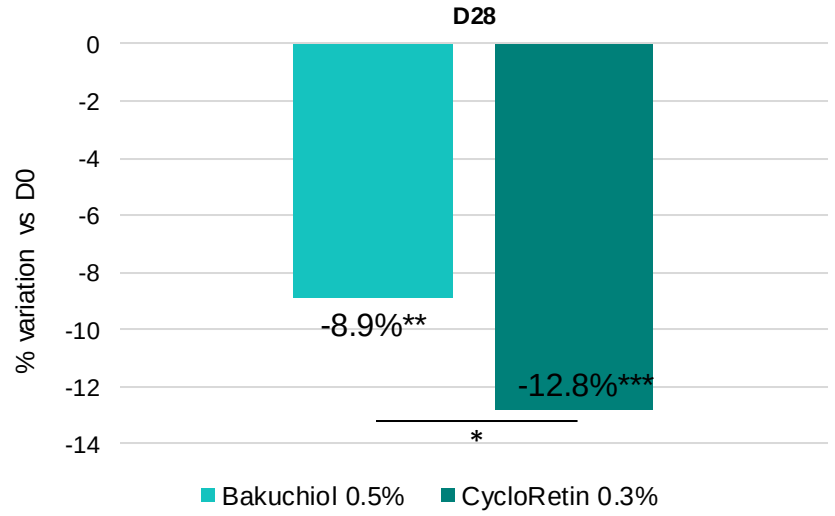
Due to the constant effects of gravity, time tends to stretch the skin downward.

IMPROVED SKIN SAGGING VERSUS PLACEBO



PLACEBO **x5**

IMPROVED SKIN SAGGING VERSUS BAKUCHIOL



BAKUCHIOL **x1.4**

Legend: # p< 0.1 * p<0.05 ** p<0.01 *** p<0.001

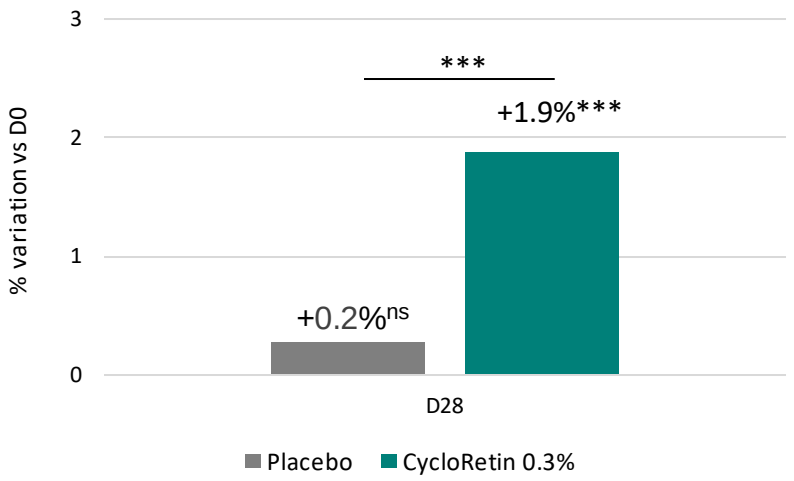
CycloRetin defies the impacts of gravity by visibly reducing sagging over time, surpassing bakuchiol.

CycloRetin improves skin luminosity

Illuminated complexion

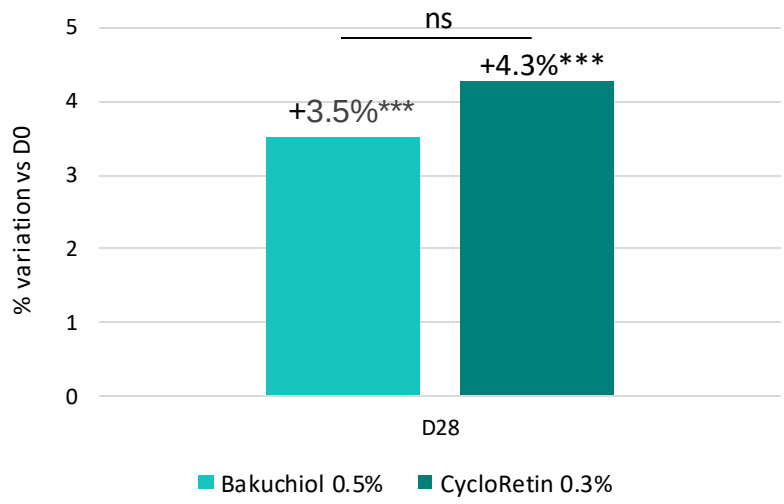
Retinol is known to even out complexion, the study also assessed skin brightening analysis.
The L* parameter represents skin luminosity.

ENHANCED SKIN LUMINOSITY VERSUS PLACEBO



PLACEBO
x9.5

ENHANCED SKIN LUMINOSITY VERSUS BAKUCHIOL

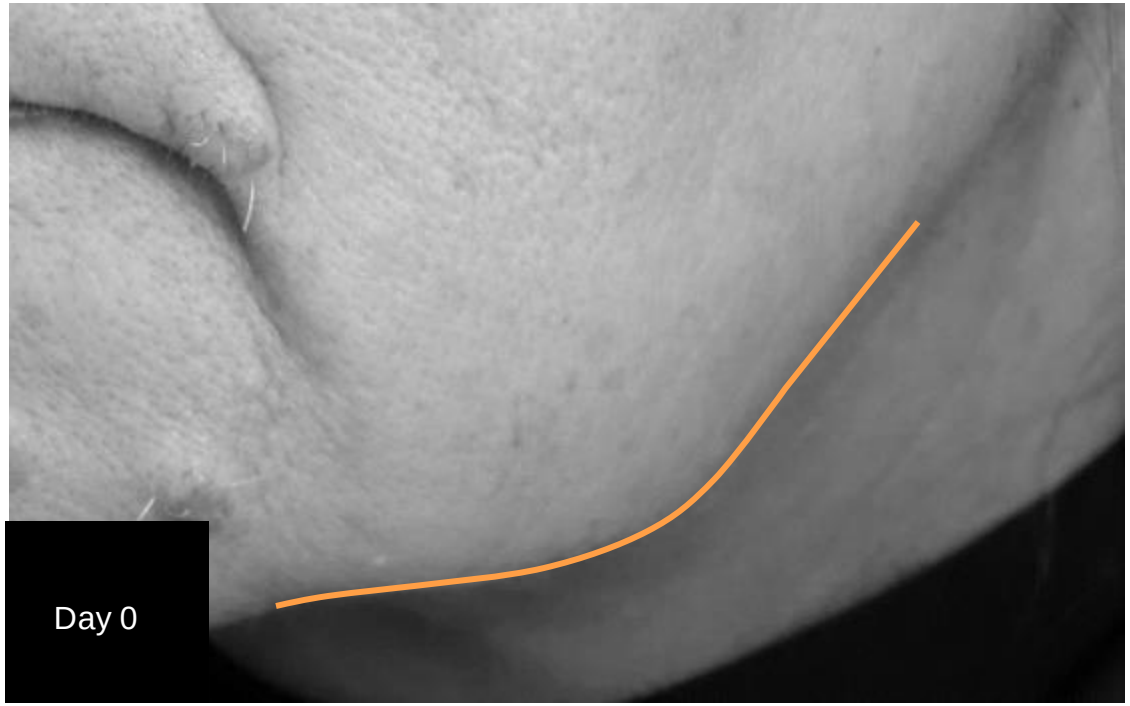


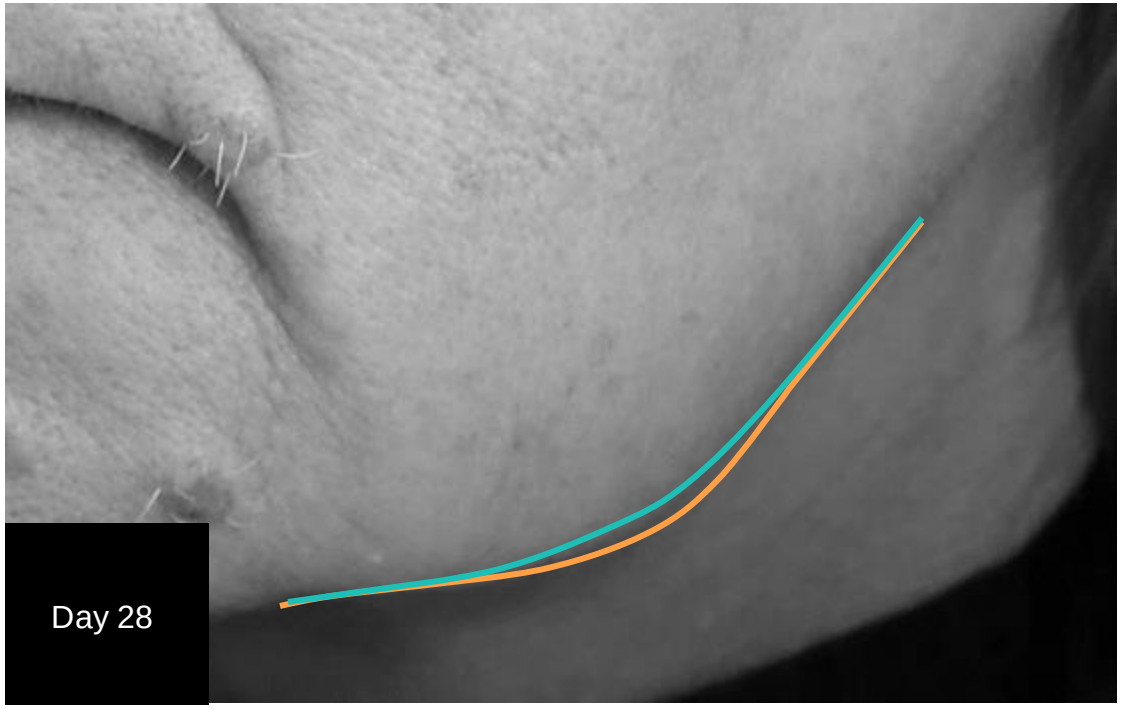
BAKUCHIOL
Same effect

Legend: *** p<0.001 ns: non-significant

After 28 days, skin brightening is improved, promoting skin clarity.

Significant improvement on facial contour - Volunteer #29 (60 yo)





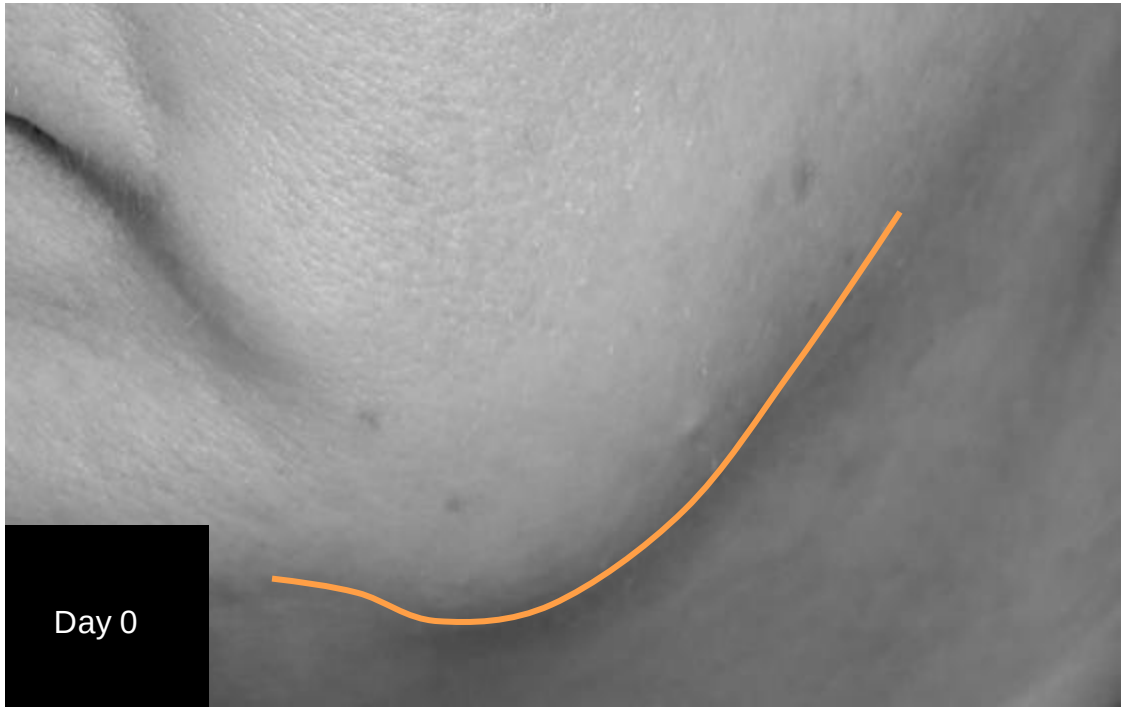
Sagging: -6.60%

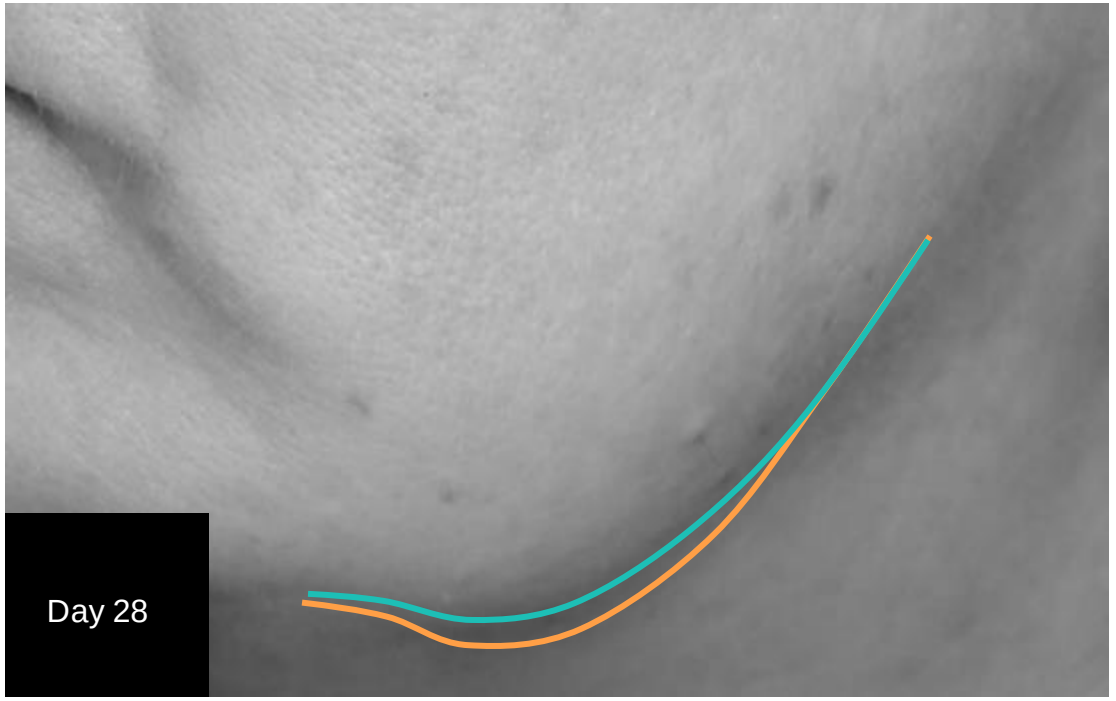
Basal sagging D0

Sagging reduction D28

CycloRetin captures demand for visible results by smoothing out sagging and double chin.

Significant improvement on facial contour - Volunteer #34 (46 yo)





Basal sagging D0

Sagging reduction D28

Sagging: -17.17%

CycloRetin captures demand for visible results by tightening drooping skin and illuminating the skin.

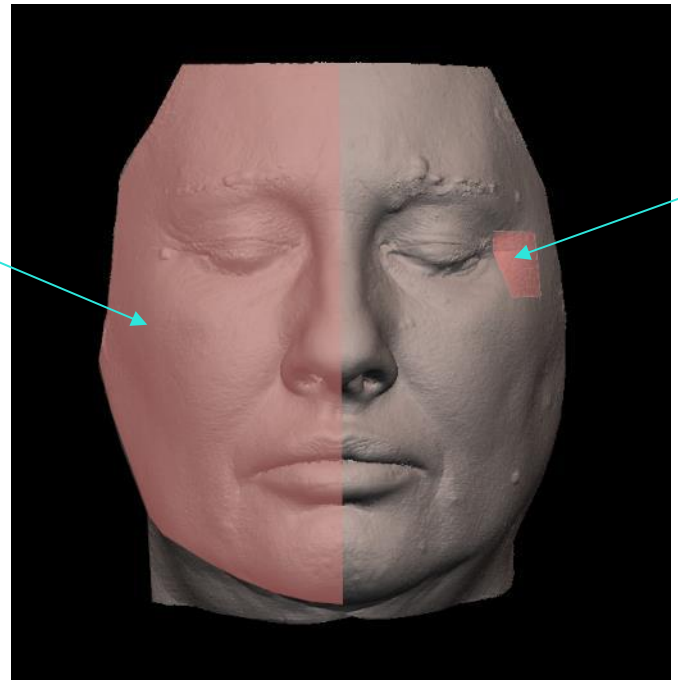
Clinical protocol #2 vs. Retinol

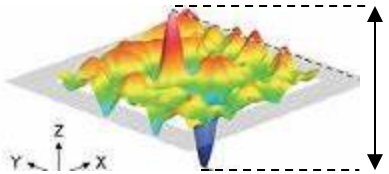
Retinol

20 women* (45-65 yo) showing wrinkles
** 7 volunteers withdrew from the study due to irritation reactions from retinol exposure*

1% CycloRetin vs. **0.3% retinol**

28 days, 2x/day on half of the face





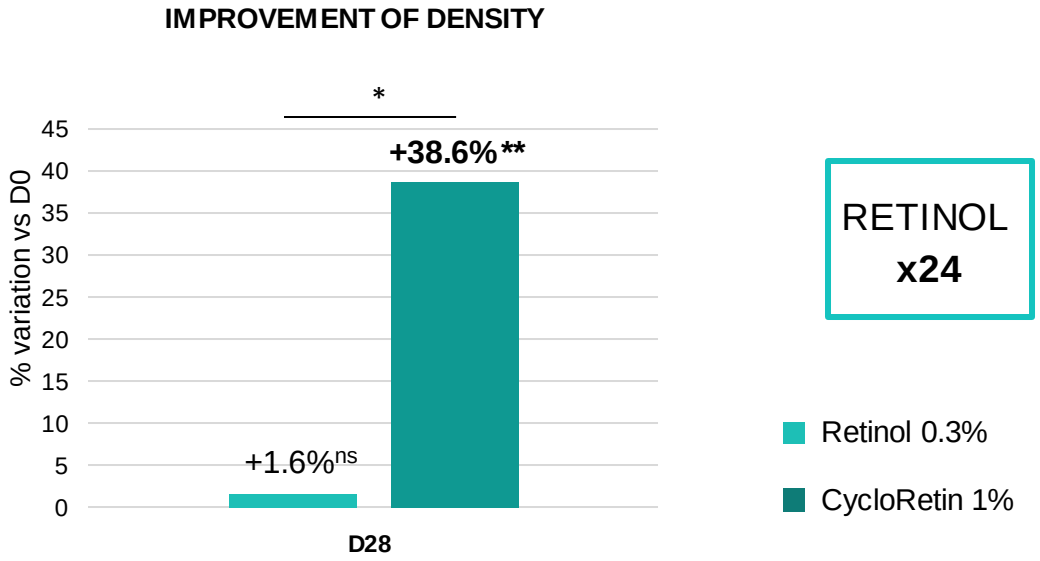
Rz = variance from highest to lowest points on the surface

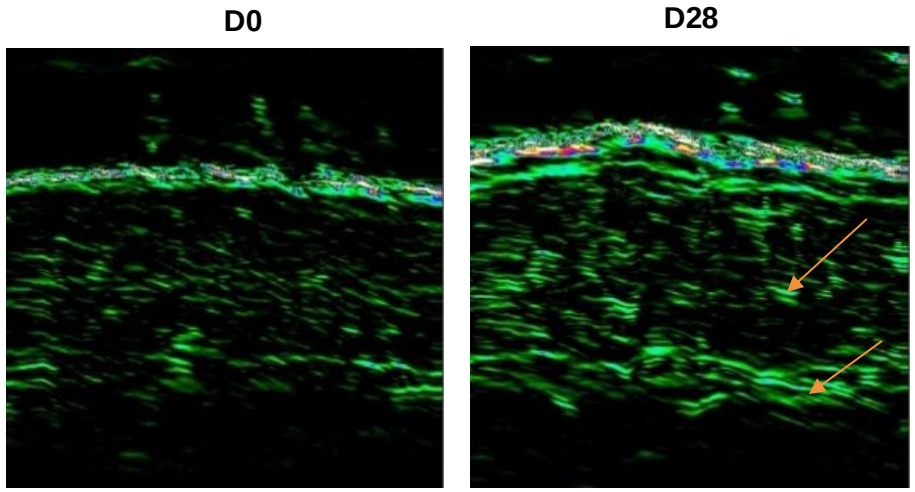
Global density = surface occupied by wrinkles

- Dermis density, via echography
- Global wrinkles (global density) and crow's feet wrinkles (Rz parameter), measured with AEVA
- Pictures, via AEVA

CycloRetin redensifies the dermis

The density of the fibers in the dermis is closely linked to the quantity of extracellular matrix elements.





CycloRetin enhances the density of skin matrix (more intense green fluorescence marking).

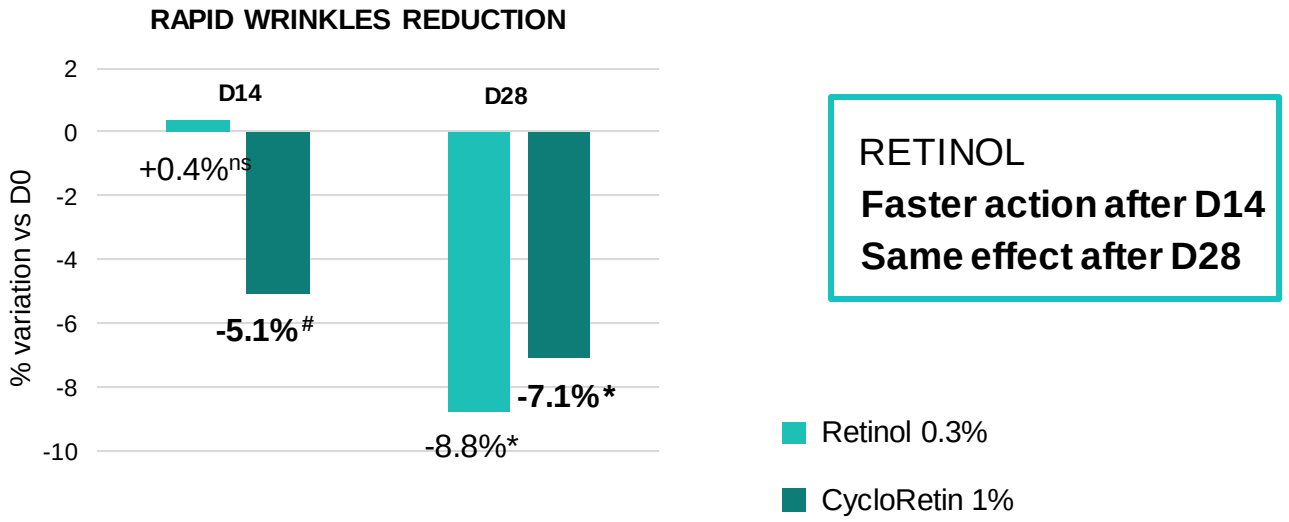
Legend: * p<0.05 ** p<0.01 ns: non-significant

By stimulating the production of collagen and skin architect compounds, CycloRetin plumps the skin from the inside through a strong increase in skin density.

Smoothed appearance

CycloRetin quickly minimizes the appearance of wrinkles

The face is prone to many types of wrinkles that form vertical and horizontal lines. These include expression wrinkles as well as aging wrinkles, which slowly develop.



RETINOL
Faster action after D14
Same effect after D28

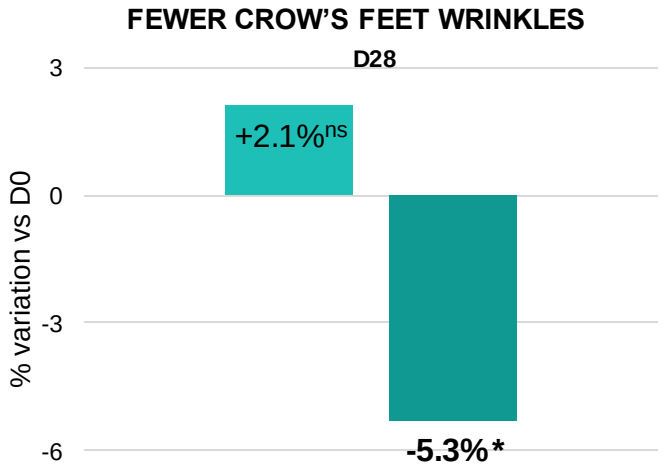
Legend: # p< 0.1 * p<0.05 ns: non-significant

In only 2 weeks, CycloRetin **reduces all type of deep facial wrinkles**, much faster than retinol, with a building effect overtime to help **prevent the formation of future lines**.

Joyful appearance and youthful look

Crow's feet wrinkles are softened

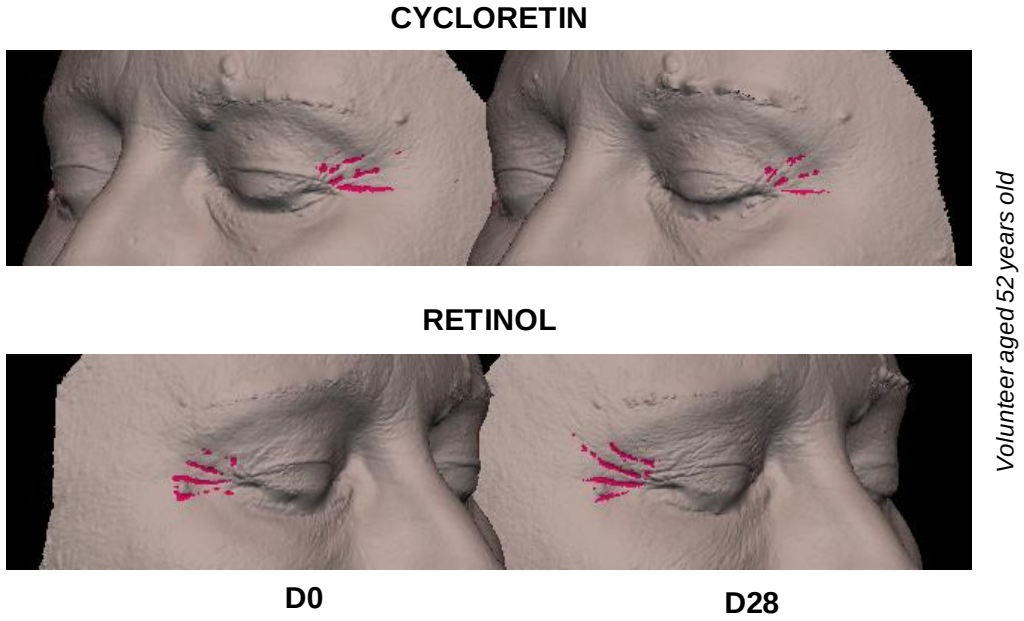
The crow's feet wrinkles at the outer corners of the eyes rapidly become more prominent with smiling over time and are exacerbated by frequent facial expressions.



Superior to RETINOL

Retinol 0.3%

CycloRetin 1%



Legend: * p<0.05 ns: non-significant

With better efficacy than retinol, CycloRetin visibly **smooths and plumps the eye area wrinkles**, providing a youthful appearance.

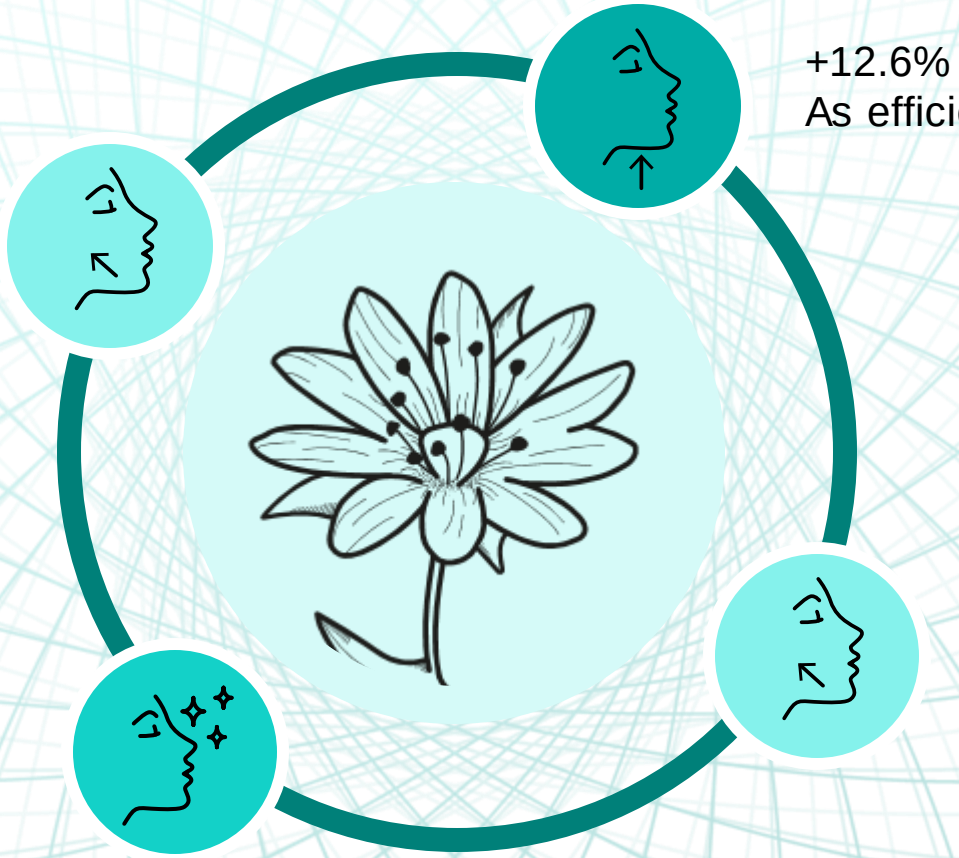
CycloRetin: outstanding performance

FIRMNESS

+12.6% after D28, +19% after D56
As efficient as bakuchiol

APPEARANCE OF WRINKLES

-5.1% after D14, -7.1% after D28
As effective and faster than retinol



SAGGING

-10.7% after D28
x 1.4 time higher than bakuchiol

RADIANCE

x 9.5 times better than placebo
As efficient as bakuchiol

Take-home messages

— With adaptogen prince ginseng from enchanting Chinese mountains, harnessing the skin-regenerating powers of unique cyclic peptide

— Operates through retinoid metabolism and a proper signature to relaunch collagen production and improves epidermal structure, outpassing retinol benefits

— Smooths wrinkles after just 2 weeks, and firms skin, sculpts the facial contour, brightens skin after 4 weeks

— Serves beauty without compromise: provides same efficacy as retinol on wrinkles, without irritation, and outperforms bakuchiol on skin sagging

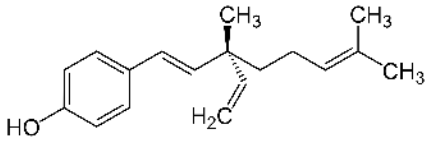


How CycloRetin stands out from the crowd



BAKUCHIOL

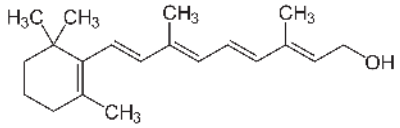
- Famous retinol-alternative
- One China-allowed source





RETINOL

- Iconic molecule with well recognized benefits
- Drawbacks: irritation, not adapted to day cream, difficult to formulate, unstable

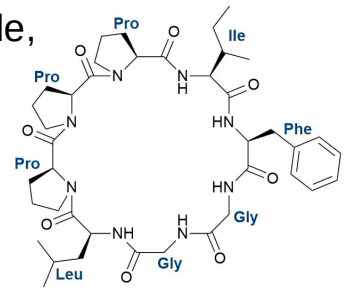


Ageless beauty



CYCLORETIN

- Trendy cyclic peptide-based structure: **native and plant-derived**
- Dual activity on retinoid metabolism and global skin structure
- **Easy to formulate**, water soluble, highly stable, China-approved
- Works fast, in only a few weeks
- **Suitable for daytime**
- Clinically proven at only 0.3%



Heterophyllin B

CLARIANT

Technical information and formulation tips



Claim and application suggestions

BENEFITS

- Gentle and natural retinol alternative empowered by natural cyclic peptides
- Collagen and elastin booster
- Facial contour line definition
- Provides skin firmness enhancement
- Limits skin sagging
- Improves the appearance of wrinkles
- Tightens and refines the skin
- Enhances skin brightening

APPLICATIONS

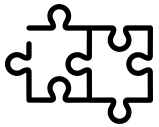
- Anti-aging line for day and night
- Age-defying creams
- Anti-gravity care
- Well-aging serums
- Plumping serums
- Rejuvenating night products
- Firming creams

Formulation tips for CycloRetin



TECHNICAL DATA

- pH tolerance: 3 to 8
- Heat tolerance: add below 50°C
- Light tolerance: store away from natural light



COMPATIBILITY

- Compatibility Ethanol: up to 100%
- Compatibility Oils: not soluble
- Compatibility Glycols: up to 100%

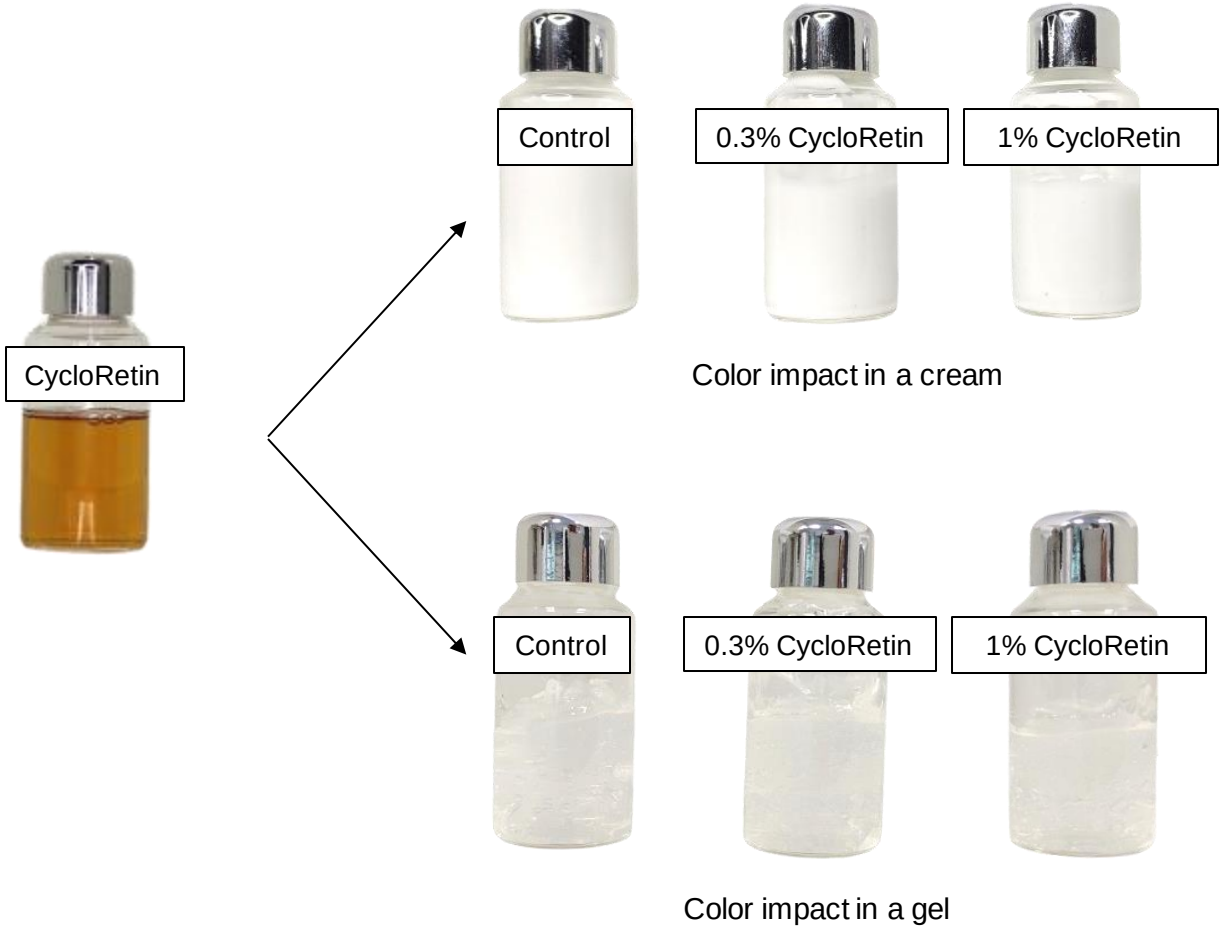


IMPACT ON CHASSIS

- cream chassis: full compatibility*
- acrylate gel chassis: full compatibility, creates slight yellow colour
- Surfactant chassis: full compatibility*

* Full compatibility: no precipitation, no change in colour and odour after 3 cycles (-5°C to 40°C) and 1 month at 50°C

Color impact when formulated



Formulation tips for CycloRetin

	Solvents	1% CycloRetin
Oil phase	Squalene	Insoluble
	Macadamia Oil	Insoluble
Water phase	Glycerin	Soluble
	Butylene Glycol	Soluble
	Propanediol	Soluble
	Alcohol (100%)	Soluble
	Water/Alcohol (10:90)	Soluble
	Water/Alcohol (50:50)	Soluble
	Water/Alcohol (80:20)	Soluble
	Water (100%)	Soluble

Protocol:

I. 1% (w/w) of CycloRetin were added to the quantity of solvent needed to reach 100%.

II. Blend was mixed with high shear for 1 min.

Technical information

INCI Name	Glycerin (and) Water (and) Pseudostellaria Heterophylla Extract
Use level	Can be used at 0.3–2.5% in various formulation types (clinical studies: 0.3% and 1%)
Patent number (pending)	CN202311386949.5
Specification	– Appearance: light to dark orange liquid – Odor: characteristic – Solubility: water soluble – pH (25°C): 4.00 ± 7.00 – Biomarker content: Heterophyllin B ≥ 50 ppm
Origin	China
Shelf life	3 years



China NMPA registered

Approved by ECOCERT RAW MATERIAL
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Thank you for your attention!

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*For sales to customers located within the United States and Canada the following applies in addition

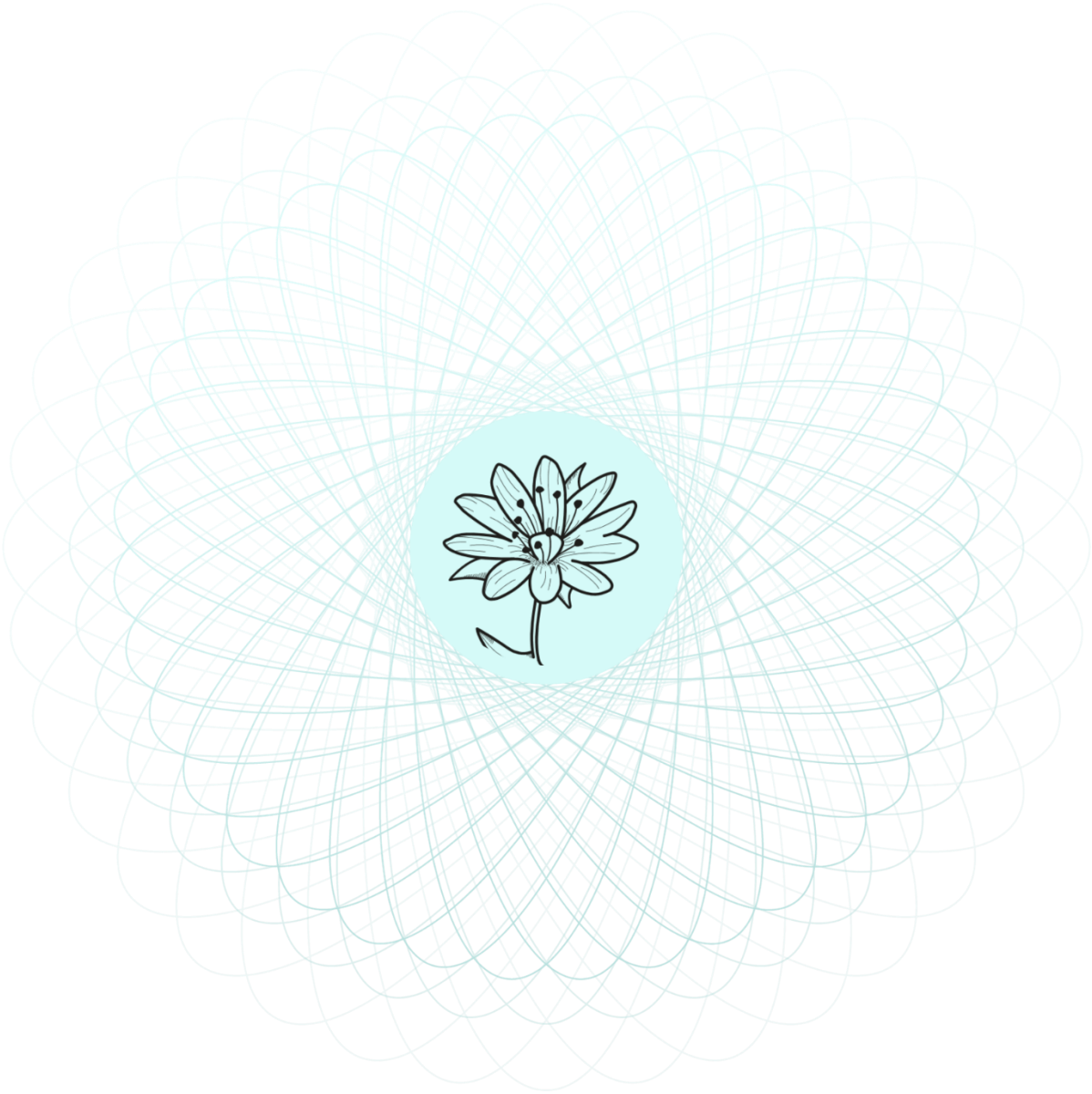
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LUCAS MEYER COSMETICS
by Clariant

CYCLORETIN™

Activating Ageless Beauty



LUCAS MEYER COSMETICS
by Clariant

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SCIENTIFIC BACKGROUND

Skin aging results from a confluence of many contributing factors

Both intrinsic (e.g.: time, genetic factors, hormones) and extrinsic (e.g., UV-exposure, pollution) factors drive skin aging. Characteristics of chronological aging include visible signs such as thin and dry skin, fine wrinkles, decreased elasticity and aberrant pigmentation. Among epidermal thinning causes, a decrease in proliferation and renewal capacity of basal keratinocytes is currently reported. Moreover, the deregulation of some important key proteins involved in barrier function is reported, such as filaggrin, that binds keratin fibers for skin structure but also aids in retaining skin moisture by promoting the formation of the natural moisturizing factor or NMF. The cornified envelope, a key structure for optimal skin barrier function, is also affected by aging. This cornified envelope is composed primarily from proteins such as involucrin or loricrin that are cross-linked together by transglutaminase enzymes. The expression of these proteins declines with aging as well as transglutaminases activity. This leads to defects in barrier performance that allow greater trans-epidermal water loss.

More than only epidermis defect's occurring in skin aging, the extracellular matrix of dermis undergoes also structural alterations and degradations, leading to the increase in wrinkles, loss of elasticity and firmness of the skin. At molecular level, a reduced synthesis of collagen types I and III and a disorganization of existing fibers is characteristic of chronologically aged skin and is exacerbated by photoaging. A shift of the ratio of collagen I to III (around 4:1 to 6:1) to get closer 1:1 is also reported [1,2]. In healthy human skin, type I and III collagen have relatively substantial roles, comprising of 80-85% and 10-15% of collagen, respectively [3]. Mechanistically, collagen I is a stiff fibrillar protein that gives tensile strength, whereas collagen III produces an elastic network that stores kinetic energy as an elastic rebound [4] and forms thinner, weaker fibers. So this age-related shift in collagen I : collagen III ratio leads to more fragile skin that is more prone to damages.

More than collagen fibers production, some important players for matrix organization have been reported. For example, SPARC (Secreted Protein Acidic and Rich in Cysteine), also known as osteonectine, is a matricellular protein that plays a role in regulating cell-matrix interactions and collagen fibers assembly. SPARC binds directly to collagen I and III in the dermis [5] [6] and regulates collagen deposition and fibrillogenesis. Unfortunately, its expression decreases with intrinsic and photo-aging processes leading to an aberrant accumulation of disorganized collagen bundles [7] [8].

Additionally, other protein modulations promote the well-known dermis loss of elasticity and firmness in skin aging context. Levels of matrix metalloproteinases (MMPs), that degrade collagen and elastin, are increased with age, while elastin fibers synthesis and microfibril proteins expression, such as fibrillin, that ensure skin flexibility, are decreased.

Growth factors are other players that are critical signalling proteins controlling many cellular processes vital for maintaining dermal structure, function, and healing after injury. They help to regulate fibroblast functions, extracellular matrix synthesis, angiogenesis, and tissue repair in the dermis. Unfortunately, several alterations of dermal growth factors signalling are described in a skin aging context, among them being the following examples:

- Transforming Growth Factor-beta (TGF-β): essential growth factor regulating proliferation and differentiation of skin cells and stimulating extracellular matrix protein production including collagen and fibronectin [9],
- Fibroblast Growth Factors (FGF) that promote growth and migration of keratinocytes during re-epithelialization in wound healing [10] [11],
- Insulin-like Growth Factor 1 (IGF-1) is produced by human fibroblasts and its receptor (IGF-1R) is expressed by keratinocytes for the ultraviolet-B (UVB) response. The IGF-1/IGF-1R signaling was demonstrated to play a key role in maintaining human epidermal homeostasis (UVB response, collagen, elastin and glycosaminoglycan stimulated production) but is dramatically impaired in aged skin, contributing to thinning of the epidermis and dermal extracellular matrix leading to skin atrophy [12] [13],
- Platelet-derived growth factor (PDGF) and Epidermal growth factor (EGF) are also important growth factors for fibroblast migration, proliferation, collagen synthesis, and angiogenesis. Declining of both growth factors disrupts signalling between the epidermis and dermis and affects fibroblast activation and extracellular matrix synthesis.

In addition to epidermis and dermis alteration through aging, another skin compartment is highly modulated, the dermal–epidermal junction (DEJ). This area is composed by complex network of interconnecting proteins and the finger-like projections of rete ridges help to provide skin with both structural integrity, mechanical stability and to ensure optimal exchanges between epidermis and dermis. DEJ is also impacted by skin aging and some major structural proteins are downregulated (collagen IV, collagen VII, collagen XVII, integrin β4, and laminin-332) [14]participating to the flattened appearance with a reduced surface area due to loss of rete ridges [15].

In a nutshell, skin aging is a complex phenomenon encompasses epidermal and dermal remodelling, with deep decreases of skin barrier function, DEJ composition and structure integrity, as well as dermal matrix production. All these modifications strongly induce the skin's progressive functional decline over time.

Retinol: the gold standard solution for anti-aging skin care

Among the many solutions offered by cosmetics to counteract the effects of skin aging, retinoids are molecules of choice.

Retinoids (like retinol or ROL, retinaldehyde or RAL, all-trans retinoic acid or atRA or RA) have potent effects on cellular growth, differentiation, and proliferation, which underlie their efficacy as anti-aging skin treatments. They exert their effects through the binding of the active metabolite atRA to nuclear receptors called RARs and RXRs, which act as ligand-activated transcription factors that regulate gene expression by interacting with retinoid response elements (RAREs) on target genes. In skin cells like keratinocytes, fibroblasts and melanocytes, retinoids stimulate collagen production, accelerate cell turnover to improve skin tone and texture, improve barrier function and epidermal thickness, inhibit matrix metalloproteinases that degrade collagen, and decrease pigmentation.

Mechanistically, when the body's requirement for vitamin A increases, retinol is excreted from the liver to the circulation. Retinol then combines with RBP4 to form a complex (holo-RBP4). When this complex reaches a target tissue, it interacts with a specific membrane protein called STimulated by Retinoic Acid 6 (STRA6), and the RBP4 then comes back into the circulation as apo-RBP4. After retinol is transported into target cells, it undergoes a series of oxidative reactions to transform into the active metabolite, all-trans retinoic acid (figure 1).

These reactions are mediated by enzymes called retinol dehydrogenases (RDHs) such as Retinaldehyde Reductase 9 (DHRS9) or Xanthine dehydrogenase (XDH) which catalyse the oxidation of retinol to retinaldehyde. Retinaldehyde dehydrogenases (RALDHs) further oxidize retinaldehyde into all-trans-retinoic acid. The cytochrome P450 enzymes such as CYP1A1, CYP1B1 also play major roles in retinoid metabolism.

CYP1A subfamily contains only two functional genes, CYP1A1 and CYP1A2. CYP1A could metabolizes several substrates and the reaction types of CYP1A include hydroxylation, demethylation, epoxidation, and oxidation notably for retinol and retinaldehyde [16]. Indeed, as mentioned above, the oxidation of ROL to RA is mainly achieved by two dehydrogenation reactions. Compared with other CYP isoforms, CYP1A1 and CYP1A2 exhibited retinol dehydrogenase activity and at the same time, CYP1A1 and CYP1A2 also showed high activity of retinal dehydrogenase, forming the functional metabolite retinoic acid [17]. CYP1A subfamily could oxidize retinol while some others cytochrome P450s enzymes as CYP26s only break down retinoic acid as part of catabolism by forming polar metabolites of RA (4-OH-RA and 4-oxo-RA).

All this cell signalling is only possible thanks to some binding proteins which are essential intracellular carriers that regulate the availability of retinoids/retinoic acid. More than RBP mentioned above for delivery of retinol from liver stores to target tissues like skin, Cellular retinol Binding Protein (CRBP) facilitates retinol uptake and stimulates its enzymatic conversion to active metabolites or storage form conversion into lipid droplets. Cellular Retinoic Acid Binding Protein (CRABP) either shuttles RA to the nucleus (CRABP II) for interaction with retinoic acid receptors (RARs) or to the catabolism by cytochrome P450 enzymes (CRABP I).

In summary, retinoid intracellular metabolism involves a network of binding proteins, oxidoreductases and cytochrome P450s for conversion of retinol into the active metabolite retinoic acid which then exert its anti-aging effects via nuclear receptor signalling (figure 1).

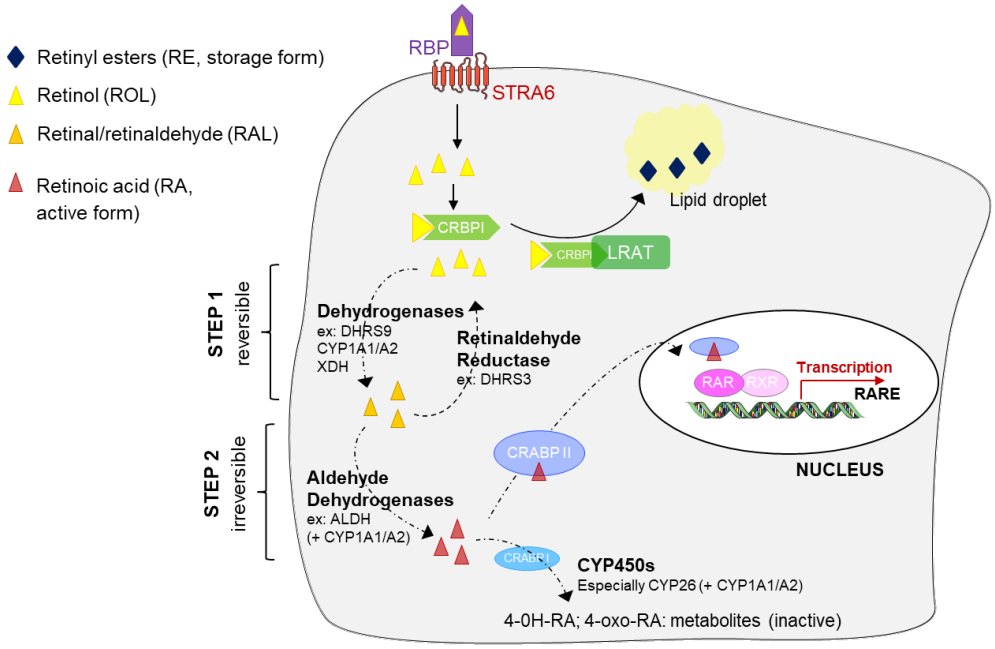


Figure 1: schematic representation of retinoid metabolism

RAR are not equivalently expressed in the human skin

As mentioned above, retinoid effects are mediated by nucleic acid receptors activation. The nuclear retinoic acid receptor family called RARs was the first to be described and consists of three main subtypes: RAR-α, RAR-β, RAR-γ. Each RAR subtype has different ligand binding affinities, expression patterns and target genes. They are activated by the all-trans-retinoic acid (at-RA) and have distinct DNA binding domains and functions depending on the formation of homo or heterodimers. In the human skin, RARs partner with retinoid X receptors (RXRs) to form heterodimers.

Both RARs and RXRs are present in the normal skin. The RAR-γ subtype accounts for nearly 90% of RARs in the human epidermis, whereas the RXR-α subtype accounts for nearly 90% of the RXRs. Therefore, the normal human skin is regulated by paired heterodimers composed of RAR-γ and RXR-α (figure 2). Moreover RXR-α can also form heterodimers with other receptors leading to other benefits than the one mediated by RAR.

The heterodimer complex binds to specific elements in the DNA known as Retinoic Acid Response Elements (RARE) in the promoter region of retinoid-responsive genes, leading to the transcription of the targets of retinoic acid.

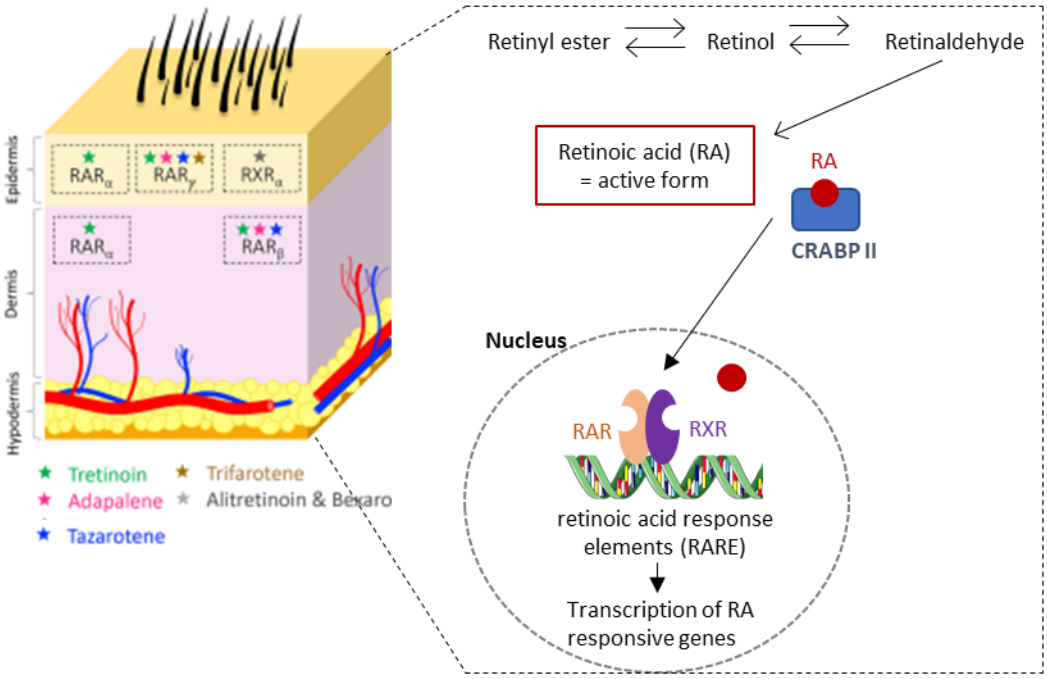


Figure 2: schematic representation of the expression and localization of RAR in the skin. Adapted from [18]

Retinol drawbacks

Although retinol is one of the most popular skin anti-aging agent in the cosmetic field, it is also known to provoke side effects such as irritations, erythema, pruritus, peeling, stinging, or burning, and to be difficult to formulate and stabilize. Therefore, to avoid or limit such drawbacks, retinol is usually stabilized and/or encapsulated in delivery systems such as liposomes for example.

Another strategy to minimize the side effects of retinoids in skin consists in selectively target specific retinoic acid receptor (RAR) subtypes. In line with this, topical retinoids that selectively bind to and activate RARγ were developed such as Tazarotene and more recently Trifarotene, a novel fourth-generation retinoid acting selectively on RAR-γ receptor with best efficacy and safety profile [19] [20,21].

In summary, while retinoids can activate all three RAR subtypes, RARγ plays a predominant role in mediating most anti-aging effects in skin cells. The specific targeting of receptors like RARγ allows to maximize benefits while minimizing adverse effects.

Bakuchiol: the first natural retinol alternative

Pure bakuchiol was the first active molecule alternative to retinol to be developed. Bakuchiol is a meroterpene found mainly in the seeds of *Psoralea corylifolia*. It has been demonstrated to exhibit anti-inflammatory, antioxidant, anti-aging and anti-acne activities. Mechanistically, bakuchiol is reported to target several cellular pathways similar to those targeted by retinoids, including numerous retinoid-responsive genes and upregulation of collagen and extracellular matrix synthesis enzymes, without effect on genes encoding the

retinoic acid receptors (RAR-β and RAR-γ) [22]. All these regulations lead to visible effects on skin, namely improvement in wrinkles, pigmentation, and firmness.

Despite the increasing use of Bakuchiol as a natural alternative to retinoids, some studies have reported cases of contact dermatitis and skin irritation associated with its topical application [23,24] [24]. Therefore, there is still value in identifying natural alternatives of retinoids from plant sources that provide comparable anti-aging activity with improved dermatological safety and tolerance.

Pseudostellaria heterophylla: a plant species with ancestral uses in Traditional Chinese Medicine

Pseudostellaria heterophylla is an herbaceous perennial belonging to the Caryophyllaceae family. This plant species has tubers and solitary erect stems up to 20 centimeters tall. The flower has 5 white petals, but some flowers are cleistogamous and lack petals (Pseudostellaria heterophylla in Flora of China, efloras.org)



The plant species, known commonly as *hai er shen* (Chinese: 孩兒參, kid ginseng, child ginseng) or *tai zi shen* (Chinese: 太子參, crown prince ginseng), is native to East Asia (China, Japan, Korea). It grows in humid mountain forests at altitudes of between 1,000 and 3,000 meters.

Pseudostellaria heterophylla is a mild adaptogen and, in Traditional Chinese Medicine, the tuberous rhizomes are used in formulas that enrich qi, nourish yin, and generate fluids in cases of deficiency. Symptoms treated include fatigue, weakness, shortness of breath, dry mouth/throat, spontaneous sweating, etc. It is often used in cases of chronic illness, where its more long-term mild effects can be more beneficial to the patient. It is also used as a nutritional supplement for children with a weak physique [25].

Modern research has confirmed some of the plant's pharmacological activities, including immunostimulant, anti-inflammatory, antioxidant and liver cell-protective effects.

An authentic harvesting and sourcing

Pseudostellaria heterophylla tuberous rhizomes are harvested in autumn and used fresh or dried. They contain triterpenic saponins, polysaccharides and various other bioactive compounds such as cyclic peptides [26].

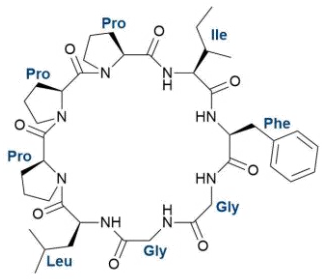
The plants used to produce our active ingredient Cycloretin™ are sourced from a village located in a geo-authentic region (Jurong, Jiangsu Province, China) of *Pseudostellaria heterophylla* [27], guaranteeing exceptional quality and an optimized phytochemical composition. Furthermore, no pesticides or fertilizers are used for their cultivation

An original and active plant-specific biomarker: Heterophyllin B, a natural cyclopeptide

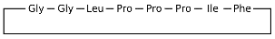
Cyclic peptides are bioactive molecules naturally present in certain plants and involved in their defense against pathogens. Unlike linear peptides, they have a closed cyclic structure, making them more stable and

resistant to degradation enzymes [28]. Some cyclic peptides have interesting therapeutic properties, and they are attracting a great deal of interest in the development of new drugs. Until May 2023, one hundred and fourteen peptides have been approved by the regulatory authorities as therapeutic agents, which included pharmaceuticals and diagnostic tools. Among the approved peptides, the cyclic peptides represent 46% of the total approvals [29]. Their unique, stable structure and diversity of biological functions make them highly attractive molecules for a wide range of therapeutic applications, notably in oncology or against resistant infections. Cyclic peptides may have greater potential as therapeutic agents than their linear counterparts due to their superior cell membrane permeability (example Cyclosporin A by passive diffusion) [30], better stability thanks to their three-dimensional cyclic structure, and greater selectivity for cell receptors, increasing their performance [31]. For example, scientific papers mention that peptide cyclization could increase either peptide stability by 30 times either molar acitivity by 1000 fold for histatin, a potent wound healing peptide from human saliva.

Surprisingly, to date, very few cosmetic applications were developed based on their interesting properties.



Heterophyllin B



Heterophyllin B is a natural cyclic octapeptide with a single ring formed with peptide bonds and eight L-amino acids, which belongs to the Caryophyllaceae-like cyclopeptides and is abundant in the tuberous roots of *P. heterophylla*. Heterophyllin B is one of the characteristic chemical components of *P. heterophylla* and is used as the quality control index for evaluating Pseudostellariae radix in the Chinese pharmacopoeia [32].

Cycloretin™ production process has been specially designed with a fractionation step to enrich the product in Heterophyllin B (and 7 other cyclopeptides). Only natural-based solvents are used during the process.

PRODUCT CHARACTERISTICS

INCI Name	Glycerin (and) Water (and) Pseudostellaria Heterophylla Extract
Use level	Can be used at 0.3–2.5% in various formulation types (clinical studies: 0.3% and 1%)
Patent number (pending)	CN202311386949.5
Specification	Appearance: light to dark orange liquid Odor: characteristic Solubility: water soluble pH (25°C): 4.00 ± 7.00 Biomarker content: Hetorophyllin B ≥ 50 ppm
Origin	China
Shelf life	3 years



China NMPA registered

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BIOLOGICAL EVALUATION

1. Effect of CycloRetin™ on retinoid metabolism

Aim and biological rational

The objective of this study was to determine, using different experimental models, the ability of CycloRetin™ to modulate the transcription of retinoid metabolism players.

1.1 Improved expression of retinol-retinoic acid conversion players and retinoid nuclear receptor

Model protocol

This test was performed on human skin explants. Upon receiving of the abdominal biopsy, the adipose tissue was removed, and skin explants were then cut into pieces (2 cm x 2 cm). CycloRetin™ containing cream or placebo (composition in table 1) were then topically applied (5 mg/cm²) on the surface of the skin explants

before its incubation for **24 hours** in a humidified incubator at 37°C and 5% CO_2 (figure 3). All experimental conditions were performed in n=3.

Ingredient	INCI	Placebo	CycloRetin™ 0.3%
Water	Water	96.10	95.80
Aristoflex AVC	Ammonium Acryloyldimethyltaurate/VP Crosspolymer	0.80	0.80
Cetiol OE	Dicaprylyl Ether	1.00	1.00
Buffer solution pH 6	Citric Acid (and) Sodium Citrate	1.00	1.00
Phenonip ME	Phenoxyethanol (and) Methylparaben (and) Ethylparaben	1.10	1.10
Fragrance	Fragrance	0.003	0.003
Bakuchiol	Bakuchiol	-	-
CycloRetin™	Pseudostellaria heterophylla extract (and) Glycerin (and) Water	-	**0.30**

Table 1: composition of formulations used for *ex vivo* experiment

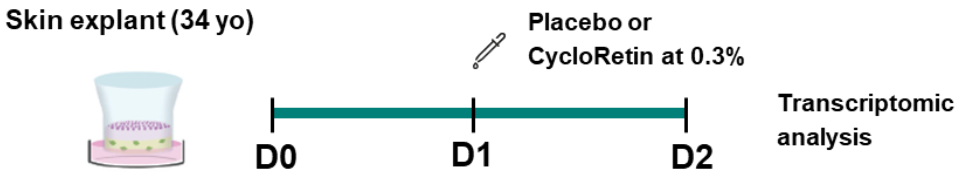


Figure 3: schematic representation of experimental model for transcriptomic analysis on human skin

At the end of the incubation, samples were mechanically homogenized using a Precellys® Evolution homogenizer (Bertin Instrument) and total RNA was extracted from each sample using TriPure Isolation Reagent® according to the supplier's instructions. The amount and quality of RNA were evaluated using capillary electrophoresis (Bioanalyzer 2100, Agilent technologies). The complementary DNA (cDNA) was synthetized by reverse transcription of total RNA or mRNA in presence of oligo(dT) and « Transcriptor Reverse Transcriptase » (Roche). The cDNA quantities were then adjusted before PCR step. PCR (Polymerase Chain Reaction) were performed using the « LightCycler® » system (Roche Molecular System Inc.) according to the supplier's instructions.

The relative expression value was calculated with the formula: $(1/2^{\text{number of cycles}}) \times 10^6$. The PCR array used in the present study included 3 reference genes (GAPDH, RPS28 and ACTB). These housekeeping genes

were used for data normalization since their expression is constitutive and theoretically stable. Consequently, the level of expression of the target markers was compared to the mean expression level of these 3 markers for all test conditions. The data were expressed in percentage of increase in comparison to the placebo condition. Statistical comparisons were performed by an unpaired Student's t-test

Results

Improved expression of retinol metabolism markers

Genes regulated by CycloRetin™ at 0.3%	Relative gene expression ratio between the placebo group and the treated group	
	% of increase versus placebo (0%)	p-value
CYP1A1	+94% **	0.007
CYP1A2	+109% **	0.003
RAR gamma	+22% *	0.022

Table 2: transcriptomic regulation of retinoid signalling pathway related genes

This experiment highlights that CycloRetin™ improves the transcription of two major cytochromes involved in the metabolization of retinoids (retinoic acid synthesis or degradation) and the expression of the most represented nuclear retinoic acid receptor in the epidermis (subtype RAR-γ).

1.2 Effect on retinoid metabolism pathway

Model protocol

This test was performed on primary human keratinocytes (NHEK), coming from a donor aged of 47 years old. 24 hours after seeding in 6 wells plate, primary NHEK at passage 3 were treated 24 hours by CycloRetin™ at 0.15% (figure 4).

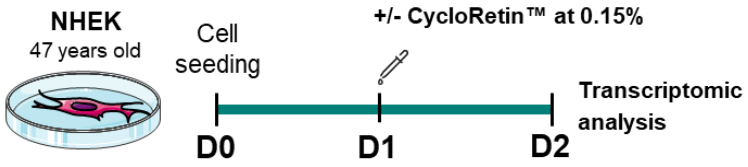


Figure 4: schematic representation of experimental model for retinoid metabolism transcriptomic analysis on primary human keratinocytes

After treatment, cells were harvested, and mRNA extraction was performed thanks to RNeasy Plus Mini Kit (Qiagen). mRNA quality evaluation was performed using a fragment analyzer (Advanced Analytical) and an

Agilent kit (reference DNF472-0500 High Sensitivity RNA Analysis Kit). Reverse transcription step allowing the obtention of cDNA template was performed on 0.5µg of RNA with the CFX96 thermal cycler (Bio-Rad Laboratories, Hercules, CA). Then, cDNA templates were diluted with nuclease free H2O and mixed with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). 20µL of diluted cDNA were added on each well of predesigned 96-well panel for retinoid metabolism evaluation (retinol metabolism H96, Bio-Rad Laboratories, Hercules, CA). PCR was run on a CFX96 thermal cycler. Raw data and statistic (Student's t-test) were analyzed on Bio-Rad CFX manager. The PCR array used in the present study included 3 reference genes (GAPDH, TBP and HPRT1). These housekeeping genes were used for data normalization since their expression is constitutive and theoretically stable. Consequently, the level of expression of the target markers was compared to the mean expression level of these 3 markers for all test conditions. The data were expressed in percentage of increase in comparison to the untreated condition. Statistical comparisons were performed by an unpaired Student's t-test and consider a p-value < 0.05 as significant versus control group

Results

Up-regulation of several Retinoid-related genes

Genes regulated by CycloRetin™ 0.15% in NHEK	Relative gene expression ratio between the control group and the treated group	
	% of expression versus control (0%)	p-value
XDH Dehydrogenase for retinoid metabolization: increase the pool of retinoic acid (active form of retinol)	+ 128% #	0.062
ALDH2 **ALDH9A1** Aldehyde Dehydrogenases: increase the pool of retinoic acid	+ 29% # + 19% *	0.056 0.014
DHRS3 Retinaldehyde reductase reduces back retinal to retinol to avoid excessive irritation	+ 266% #	0.086

Table 3: transcriptional effect of CycloRetin™ on retinoid metabolism key players in keratinocytes.

Statistical significance: # 0.05<p<0.1; *p<0.05

This experiment demonstrated that CycloRetin™ improves the transcription of essential dehydrogenases (ALDHs and XDH) that convert either retinol to retinal, or retinal to retinoic acid, or both steps. The result is an increase of the intracellular pool of retinoic acid, the active form of retinol. Moreover, we observed an increase of the DHRS3, an important retinaldehyde reductase for the control of intracellular retinoic acid content.

1.3 Conclusion

Taking together, all these transcriptional data on retinoid metabolism showed the capacity of CycloRetin™ to improve the global retinoid homeostasis by increasing RARγ expression for an optimal effect of

endogenous retinol and activation of several enzymes involved in the retinoic acid production and the control of intracellular content (figure 5).

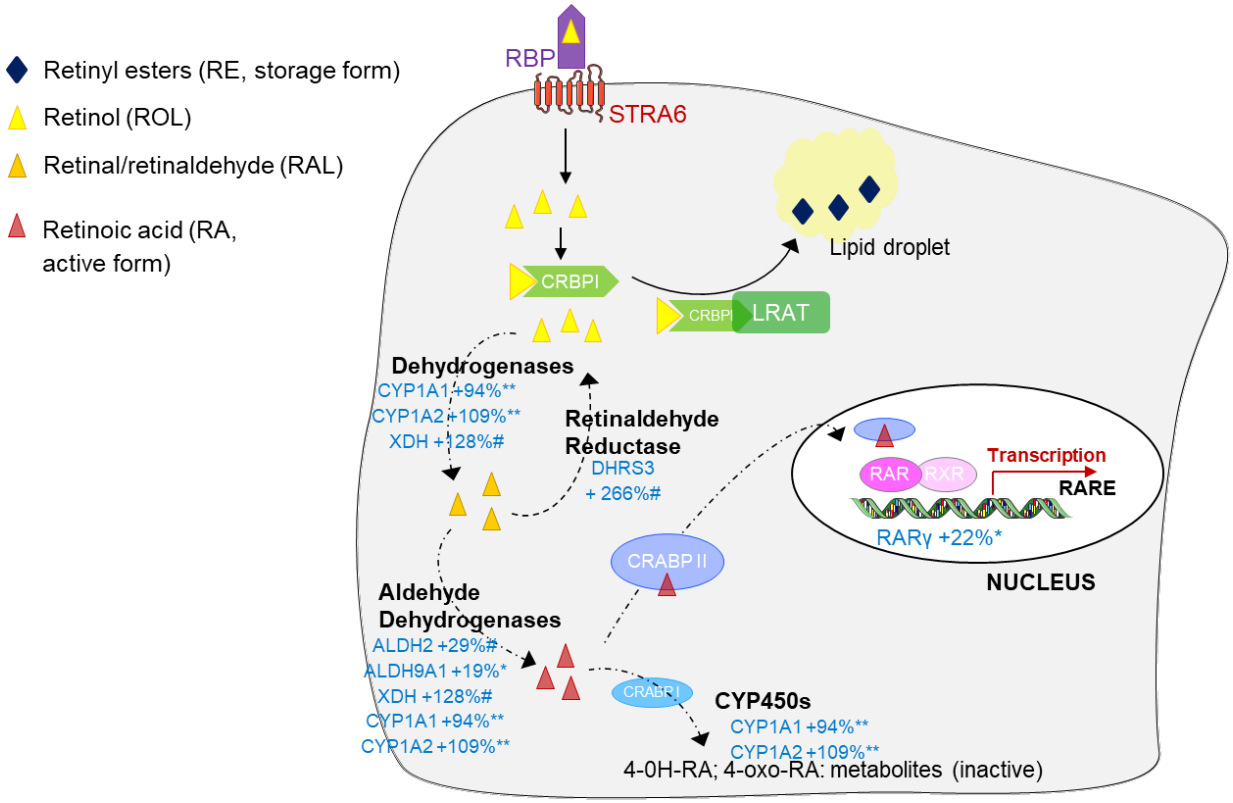


Figure 5: summary of CycloRetin™ 's effect on retinoid metabolism

2. CycloRetin™ anti-aging signature

Aim and biological rational

The objective of the following experiments was to study the effect of CycloRetin™ on epidermis and dermis key components by transcriptomic analysis. We compared our active ingredient to retinol to determine both its own anti-aging signature and the similarities of action with retinol, on normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF).

Model protocol

These tests were performed on NHEK at passage 3 and NHDF at passage 6, coming from a donor aged of 47 years old. NHEK were exposed to CycloRetin™ at 0.15% or Retinol 0.001%. NHDF were exposed to CycloRetin™ at 0.01% or Retinol 0.001% (figure 6). Each treatment was performed for 24h.

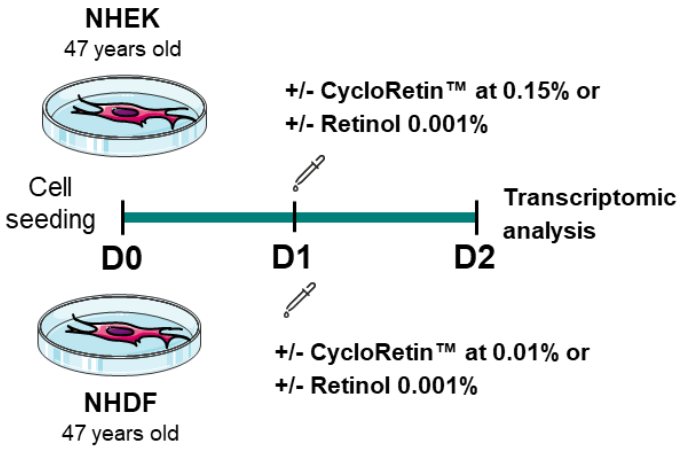


Figure 6: schematic representation of the experimental model for the anti-aging signature of CycloRetin™ analysis on primary human keratinocytes and fibroblasts

After treatment, cells were harvested, and mRNA extraction was performed thanks to RNeasy Plus Mini Kit (Qiagen). mRNA quality evaluation was realized using a fragment analyzer (Advanced Analytical) and an Agilent kit (reference DNF472-0500 High Sensitivity RNA Analysis Kit). Reverse transcription step allowing the obtention of cDNA template was performed on 0.5µg of RNA with the CFX96 thermal cycler (Bio-Rad Laboratories, Hercules, CA). Then, cDNA templates were diluted with nuclease free H2O and mixed with Taqman® Fast Advanced Master Mix. 20µL of diluted cDNA were added on each well of customized TaqMan Arrays (home designed 96 genes plates focused on dermal or epidermal compartment) (Life technology Waltham MA). PCR was run on a CFX96 thermal cycler. The PCR array used in the present study included 3 reference genes (GAPDH, 18S and HPRT1). These housekeeping genes were used for data normalization since their expression is constitutive and theoretically stable. Consequently, the level of expression of the target markers was compared to the mean expression level of these 3 markers for all test conditions. The data were expressed in percentage of increase in comparison to the untreated condition. Statistical comparisons were performed by an unpaired Student's t-test and consider a p-value < 0.05 as significant versus control group

Results

Results are given in the following tables for NHEK and NHDF:

Genes		CycloRetin™ 0.15%		Retinol 0.001%	
		% versus control (0%)	p-value	% versus control (0%)	p-value
Barrier function					
ATP binding cassette subfamily A member 12 ABCA12 is a keratinocyte transmembrane lipid transporter protein associated with the transport of lipids in lamellar granules to the apical surface of granular layer keratinocytes	**ABCA12**	+50% *	0.036	+17% ns	0.411
Desmoglein 1 Component of intercellular desmosome junctions. Involved in the interaction of plaque proteins and intermediate filaments mediating cell-cell adhesion	**DSG-1**	+108% *	0.018	Down-regulation *	0.004
Transglutaminase 1 Involved in the formation of the cornified cell envelope, which is a structure that surrounds skin cells and helps form a protective barrier between the body and its environment	**TGM1**	+56% *	0.032	Down-regulation	0.228
Claudin-1 The transmembrane protein claudin-1 is a major component of epidermal tight junctions (TJs), which create a dynamic paracellular barrier in the epidermis	**CLDN-1**	+36% *	0.048	+16% ns	0.464
Hydration					
Caspase 14 Non-apoptotic caspase involved in epidermal differentiation; role in keratinocyte differentiation and is required for cornification. Regulates maturation of the epidermis by proteolytically processing filaggrin	**CASP14**	+185% *	0.033	Down-regulation *	0.001
Fillagrin Aggregates keratin intermediate filaments and promotes disulfide-bond formation among the intermediate filaments during terminal differentiation of mammalian epidermis	**FLG**	+155% *	0.001	+66% ns	0.145
Basement membrane composition					
Heparan sulfate proteoglycan 2 It interacts with basement membrane components such as laminin-1 and collagen IV; Perlecan deposition in the basement membrane zone correlated with formation of multilayered epidermis	**HSPG2**	+85% *	0.011	+160% *	0.048
Integrin B4 Integrin alpha-6/beta-4 is a receptor for laminin. Is required for the regulation of keratinocyte polarity and motility. ITGA6:ITGB4 binds to IGF-1 and this binding is essential for IGF-1 signaling	**ITGB4**	+37% *	0.044	+13% ns	0.526
Fibrillin 1 In tissues such as skin, microfibrils form the periphery of the elastic fiber, acting as a scaffold for the deposition of elastin	**FBN1**	+60% #	0.079	+14% ns	0.616

Table 4: transcriptional effect of CycloRetin™ 0.15% or Retinol 0.001% on NHEK. Percentage of regulation and p-value were calculated compared to control condition. Statistical significance: # 0.1<p>0.05; * p<0.05; ** p<0.01

Genes		CycloRetin™ 0.01%		Retinol 0.001%	
		% versus control (0%)	p-value	% versus control (0%)	p-value
Collagen network					
Collagen type I alpha 1 chain Type I collagen is by far the most abundant protein in human skin. The collagen network is organized and maintained by dynamic mechanical tension provided by the fibroblasts responsible for its production	**COL1A1**	+32% #	0.072	0%	0.976
Collagen type III alpha 1 chain Collagen type III occurs in most soft connective tissues along with type I collagen	**COL3A1**	+76% **	0.002	+57% *	0.016
Collagen type V alpha 1 chain Type V collagen is a member of group I collagen (fibrillar forming collagen)	**COL5A1**	+41% **	0.002	Down-regulation ns	0.506
Collagen type VI alpha 1 chain Collagen VI acts as a cell-binding protein	**COL6A1**	+27% *	0.033	+7% ns	0.600
Secreted protein acidic and cysteine rich SPARC is a matricellular protein that plays a role in regulating collagen assembly and organization of the extracellular matrix of the skin	**SPARC**	+49% *	0.018	Down-regulation ns	0.980
Fibronectin 1 Fibronectins bind cell surfaces and various compounds including collagen; they stabilize collagen fibers during deposition, can add elasticity to guarantee flexibility or enhance rigidity	**FN1**	+64% **	0.002	+23% *	0.046
Elastin network					
Elastin Elastin is the protein responsible for the elasticity of vertebrate tissues. It is elastin which gives the elastic fibers of the dermis their elasticity and resilience and allows the skin to return to its original position when it is pinched or stretched	**ELN**	+96% **	0.007	+59% *	0.032
Fibulin 5 Essential for elastic fiber formation, is involved in the assembly of continuous elastin (ELN) polymer and promotes the interaction of microfibrils and ELN	**FBLN5**	+37% **	0.006	+15% ns	0.134
Fibrillin 1 Fibrillin-1 also plays a key role in tissue homeostasis through specific interactions with growth factors. Moreover, fibrillins are a major component of elastic fibers and include fibrillins-1, -2 and -3. Fibrillins show tissue-specific expression, and fibrillin-1 is expressed in the dermis	**FBN1**	+65% ***	0.001	Down-regulation ns	0.814
Fibrillin 2 In normal skin, fibrillin-2 is detected at the dermal-epidermal-junction and in vessel walls and has increased expression in wound healing	**FBN2**	+55% *	0.010	Down-regulation ns	0.992
Fibroblasts stimulation					
Fibroblast growth factor 2 Acts as a ligand for FGFRs. Plays an important role in the regulation of cell survival, cell division, cell differentiation and cell migration	**FGF2**	+65% **	0.006	+35% ns	0.121
Heparin binding EGF like growth factor Members of the epidermal growth factor (EGF) family are the most important growth factors involved in epithelialization during cutaneous wound healing	**HBEGF**	+67% *	0.027	+6% ns	0.488
Insulin like growth factor 1 receptor Receptor tyrosine kinase which mediates actions of insulin-like growth factor 1 (IGF1)	**IGF1R**	+52% *	0.032	+9% ns	0.289
Insulin like growth factor binding protein 3 IGFBP-3 ensures epidermal homeostasis	**IGFBP3**	+91% *	0.037	+86% *	0.015
Transforming growth factor alpha TGFA acts synergistically with TGF beta to promote anchorage-independent cell proliferation	**TGFA**	+118% *	0.034	+50% *	0.049
Transforming growth factor beta 1 TGF-β is a prototypic regulator of extracellular matrix (ECM) deposition, positively regulating the expression of components of the ECM	**TGFB1**	+45% *	0.024	+5% ns	0.582

Table 5: transcriptional effect of CycloRetin™ 0.01% or Retinol 0.001% on NHDF. Percentage of regulation and p-value were calculated compared to control condition. Statistical significance: # 0.1<p>0.05; * p<0.05; ** p<0.01; *** p<0.001

Conclusion

Taking together, all these data shed lights on the ability of CycloRetin™ to strongly modulate genes involved in barrier function, basal membrane composition, hydration, collagen and elastin synthesis and organization, as well as in fibroblast stimulation by essential growth factor expression. Moreover, CycloRetin™ outperforms retinol on some gene expression.

This confirms that CycloRetin™ combines its effects on retinoid metabolism to strong epidermis and dermis positive regulations. This dual mode of action ensures CycloRetin™ 's strong anti-aging signature by improving epidermis function as well as ECM composition in the dermis (figure 7).

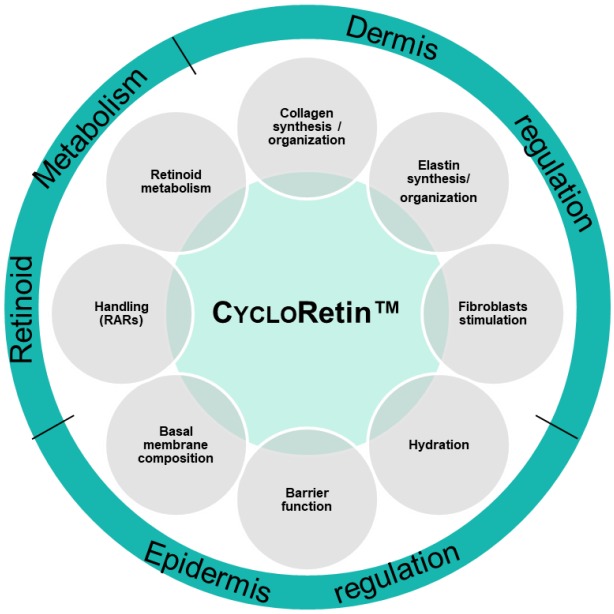


Figure 7: Summary of CycloRetin™ anti-aging signature

3. Matrix improvement benefit of CycloRetin™

Aim and biological rational

The objective of this study was to confirm, using different experimental models, the ability of CycloRetin™ to improve the dermal matrix. The first model aims to determine by ELISA assay the synthesis of type I pro-collagen on human dermal fibroblasts. In parallel, an innovative model of 3D scaffold-free dermal spheroids, developed by the Vitroscreen company, was used to observe the ability of CycloRetin™ to improve the collagen and elastin synthesis and organization.

3.1 Effect on pro-collagen 1 synthesis

Model protocol

Tests were performed on NHDF coming from a donor aged of 35 years old in a first experiment and of 50 years old in a second experiment. NHDF were exposed 72h to CycloRetin™ at 0.1% or Heterophyllin B at 0.0001% or Retinol at 0.001% or Bakuchiol at 0.001% (figure 8). TGF-β1 at 10 ng/mL was used as a positive control.

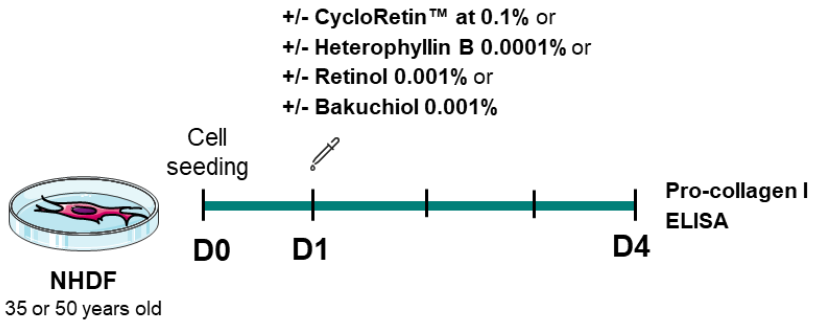


Figure 8: schematic representation of experimental model to analyse the ability of CycloRetin to improve the pro-collagen I synthesis, in comparison to retinol and Bakuchiol

After treatment, supernatants were collected. Type I pro-collagen was measured by ELISA according to the instructions of manufacturer (#DPCA00, Bio-techne, Minneapolis, MN 55413, USA) and compared to placebo. Total amount of proteins was measure for each condition by a Bradford assay for pro-collagen I content normalization (pg of pro-collagen I/µg proteins) All values were expressed as mean +/- standard deviation (SD). Results for both fibroblasts (35 and 50 years old) were combined. Statistical analysis was performed using Dunnett test and consider a p-value < 0.05 as significant compared to control condition or Cycloretin.

Results

Type I pro-collagen synthesis

Condition	Pro-collagen I content (pg of pro-col I/ µg of protein) +/- SD	%	p-value versus Control	p-value versus CycloRetin
Control	2.75 +/- 0.32	100		
TGF-β1	4.78 * +/- 0.58	174	0.01	* 0.02
Retinol (0.001%)	7.10 *** +/- 0.70	258	0.0001	ns 0.74
Bakuchiol (0.001%)	3.96 ns +/- 0.43	144	0.27	** 0.002
CycloRetin (0.1%)	6.39 *** +/- 0.48	233	0.0001	1
Heterophyllin B (0.00001%)	5.37 ** +/-0.33	195	0.001	ns 0.4

Table 6: pro-collagen I synthesis following retinol or Bakuchiol or CycloRetin™ or heterophyllin B treatment versus untreated condition. Statistical significance: * p<0.05; ** p<0.01; *** p<0.001; ns: non-significant

As expected and described in the literature, both TGFβ1 and retinol are able to induce a strong increase in the pro-collagen I synthesis by dermal fibroblasts (+74% and +158% compared to untreated condition, respectively). More surprisingly, Bakuchiol, the famous retinol natural alternative, induces a slight and non-significant increase in pro-collagen I. CycloRetin™ significantly boosts collagen I level in the skin by 133%, and its biomarker heterophyllin B by 95%. This data highlights that CycloRetin™ efficiency is supported by its unique content in cyclopeptides.

Thanks to these data, we confirmed that CycloRetin™, our natural retinol alternative, is as efficient as retinol and almost 4 times more efficient than bakuchiol.

3.2. Matrix improvement in an innovative 3D-scaffold-free dermal spheroids

Model protocol

Briefly, human dermal fibroblasts from a 50-year-old female donor were amplified for 7 passages, then put 3 days in Akura® plate to begin the cell aggregation. Aggregates are then transfered in a Akura Plate V2 (TRAP) for spheroid formation for 3 days. After this period, treatments with retinol at 0.0001% or Cycloretin at 0.01% started (figure 9). Samples were collected at D7 or D14 and fixed in formalin for histological analysis.

After fixation in formalin buffered solution 10%, samples were incubated with SPARC (anti-SPARC rabbit polyclonal, Abcam, ab225716) - Pro-collagen I (anti-Pro-col I mouse monoclonal, CliniScience, abx132015) and Fibrillin I (anti-FBN1 mouse monoclonal, LaboSpace, LS-B5512) primary antibodies overnight at 4 ◦C. Alexa 555

donkey anti-rabbit and Alexa 488 goat anti-mouse were used as a secondary antibodies while nuclei were stained with DAPI.

Microscopy analyses were performed using a Leica THUNDER Imager, allowing the acquisition in fluorescence of multiple focal planes. Staining quantification were done on the most representative focal plane (stack) for at minimum 8 spheroids per condition. Marked area were normalized to the number of nuclei for each spheroid. Statistical analysis was performed using t-test and consider a p-value < 0.05 as significant versus control condition.

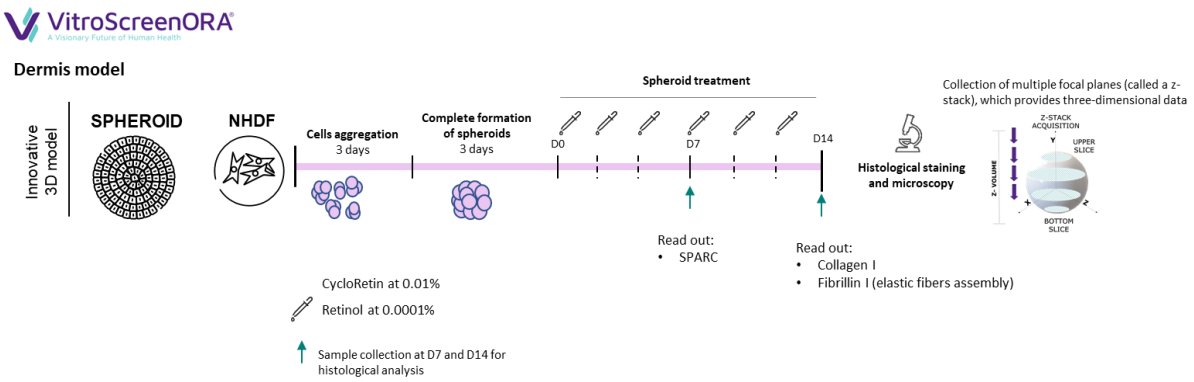
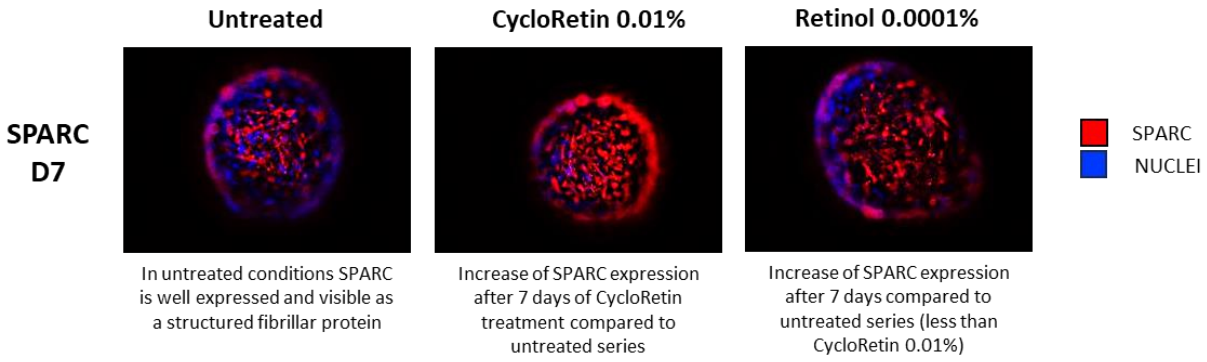


Figure 9: schematic representation of innovative 3D model of dermis to analyse the effect of CycloRetin on the ECM synthesis and network organization

Results

Representative images of the immunolabelling and quantification of the expression of the targets are shown hereafter (figure 10 and table 7).



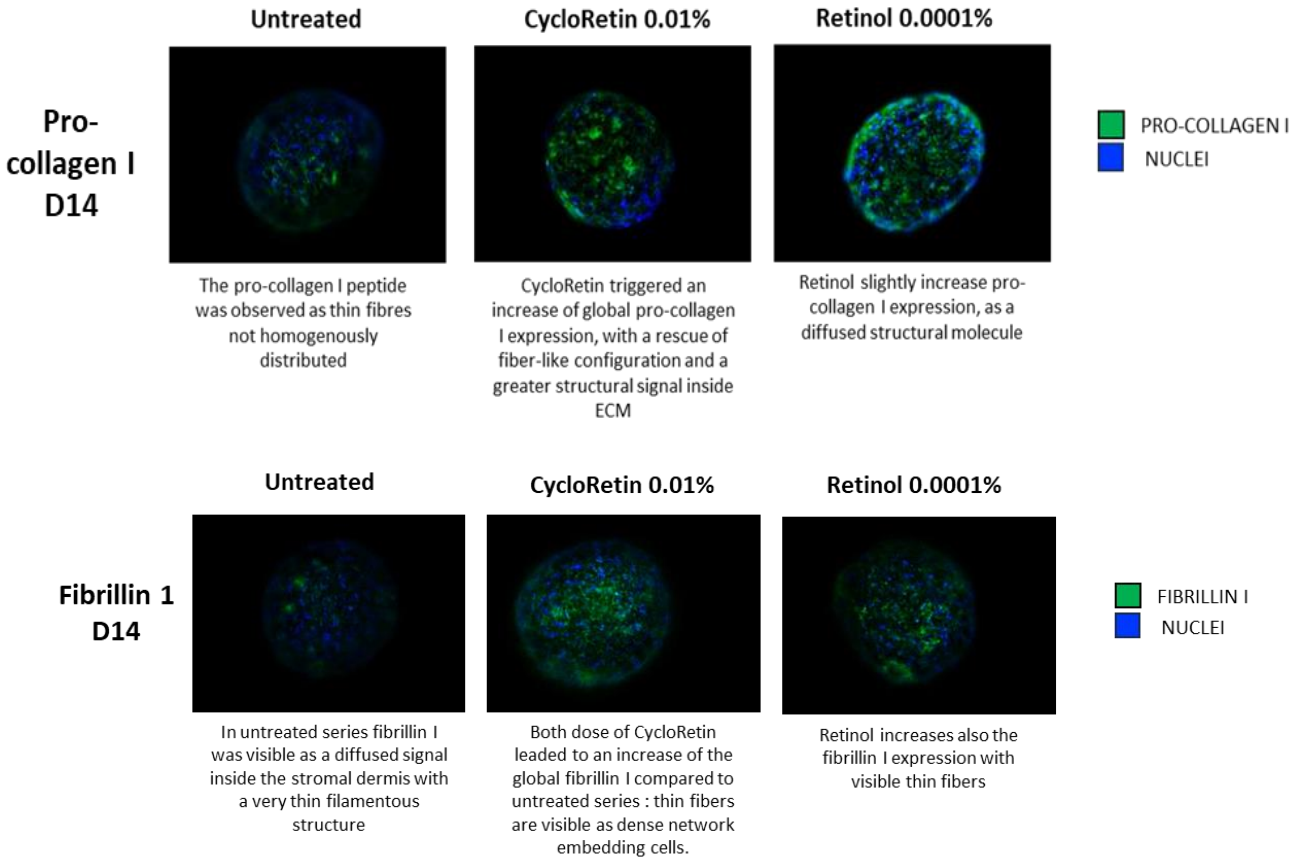


Figure 10: immunostaining for SPARC, pro-collagen I and Fibrillin 1 (FBN1) on dermis spheroids.

	Timepoint		Untreated	Cycloretin 0.01%	Retinol 0.0001%
SPARC Collagen organization	D7	Marked Area/Number of nucleus (a.u) +/- SD	78 +/- 38	155 +/- 73	77 +/- 45
		%	**100%**	**199% ***	**99%** ns
		p-value vs NT	1	0.0322	0.9854
		p-value vs Retinol	ns 0.9854	*# 0,051*	1

	Timepoint		Untreated	Cycloretin 0.01%	Retinol 0.0001%
PRO-COLL I	D14	Marked Area/Number of nucleus (a.u) +/- SD	50 +/- 38	122 +/- 44	128 +/- 75
		%	**100%**	**244% ****	**256% ***
		p-value vs NT	1	0.0017	0.0228
		p-value vs Retinol	* 0.0228	ns 0,837	1

	Timepoint		Untreated	Cycloretin 0.01%	Retinol 0.0001%
FBN I Elastic Fibers organization	D14	Marked Area/Number of nucleus (a.u) +/- SD	24 +/- 12	117 +/- 83	79 +/- 41
		%	**100%**	**493% *****	**334% *****
		p-value vs NT	1	6.37×10^{-6}	8.96×10^{-7}
		p-value vs Retinol	*** 8.96×10^{-7}	ns 0,184	1

Table 7: Quantification of immunostaining performed on dermis spheroids. Percentage of regulation and p-value were calculated compared to control condition or retinol. Statistical significance: # 0.1<p>0.05; * p<0.05; ** p<0.01; *** p<0.001

This innovative model of dermis allows to determine the ability of CycloRetin™ to lead fibroblasts to produce their own matrix (collagen type I) but also the associated protein for the collagen and elastin network organization (SPARC and FBN1 respectively). In this way, we observed that CycloRetin™ significantly increases the SPARC expression after 7 days (+99%) compared to the untreated control, with no efficiency of retinol. This interesting result is combined with a significant increase of pro-collagen I protein expression in the spheroids after 14 days. This result highlights and confirms that Cycloretin improves the collagen content (cf transcriptomic analysis and ELISA assays) but also the collagen network organization, demonstrating its superiority compared to retinol for a more efficient dermal matrix. Moreover, we demonstrated that CycloRetin is also able to potentialize the elastic network organization by improving significantly the protein expression of fibrillin 1 by ~400% (confirmation of the transcriptomic results (tables 4 and 5)).

3.3 Conclusion

Taking together, all these functional data highlighted the capacity of CycloRetin™ to enhance skin matrix composition, maturation, and architecture with a better efficiency than retinol and backuchiol.

CLINICAL PERFORMANCE OF CYCLORETIN™

1. Effect of CycloRetin™ on firmness, luminosity and sagging compared to Bakuchiol

Aim and scientific rational

Based on the encouraging results on retinol equivalent activities, and as bakuchiol was described as efficient as retinol [33], we tested CycloRetin™ versus a placebo to highlight its proper efficacy, but also versus Bakuchiol at the recommended concentration of 0.5% to challenge our active compared to this well-known retinol alternative.

Tested products

CycloRetin™ was formulated at 0.3% and was tested vs placebo for group 1 (22 volunteers) and vs bakuchiol 0.5% for group 2 (12 volunteers). Formulations details were described in table 8.

Ingredient	INCI	Placebo	Bakuchiol 0.5%	CycloRetin™ 0.3%
Water	Water	96.10	95.60	95.80
Aristoflex AVC	Ammonium Acryloyldimethyltaurate/VP Crosspolymer	0.80	0.80	0.80
Cetiol OE	Dicaprylyl Ether	1.00	1.00	1.00
Buffer solution pH 6	Citric Acid (and) Sodium Citrate	1.00	1.00	1.00
Phenonip ME	Phenoxyethanol (and) Methylparaben (and) Ethylparaben	1.10	1.10	1.10
Fragrance	Fragrance	0.003	0.003	0.003
Bakuchiol	Bakuchiol	-	**0.50**	-
CycloRetin™	Pseudostellaria heterophylla extract (and) Glycerin (and) Water	-	-	**0.30**

Table 8: Formulation details of the tested products (cream-gel)

Panel characteristics

34 Caucasian women of 45-65 years old, with no sensitive skin, presenting wrinkles/ fine lines on crow's feet, a lack of firmness on face and a dull complexion, were enrolled. They were randomly dispatched in the 2 groups. In both groups, CycloRetin™/Placebo (22 volunteers) and CycloRetin™/Bakuchiol (13 volunteers),

products were used twice a day in randomized half-face. The age range was kept: 45 - 65 years old for both groups. 1 volunteer was removed from group CycloRetin™/Bakuchiol due to missing values at day 0.

The recommendations of the Declaration of Helsinki and the guidelines of the International Conference on Harmonization Good Clinical Practice were observed.

Study design

The study lasted 56 days, with an intermediate timepoint at D28. Brightness on cheek and sagging were evaluated after 28 days of use, whereas cutometry on temples and pictures taken with VISIA® were performed at day 28 and 56 (Figure 11).

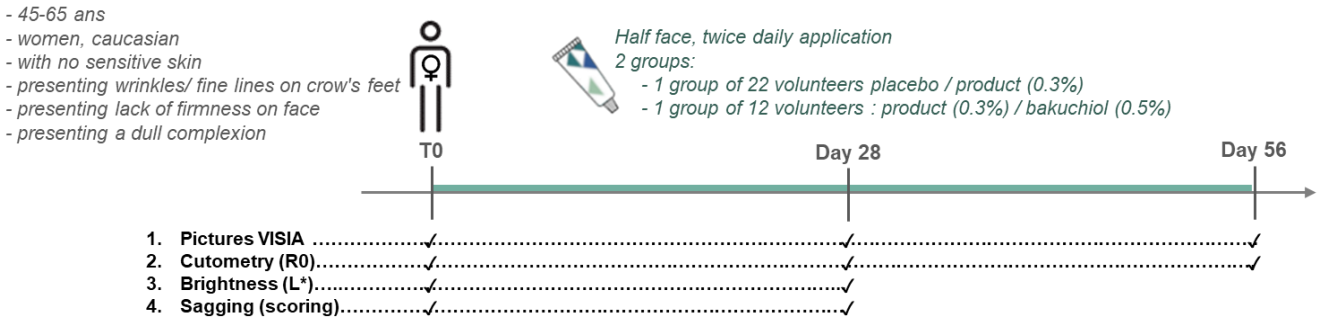


Figure 11: Clinical study protocol

Methods of analysis

Cutometry

The skin has two rheological properties:

• a visco-elastic behavior with high elastic component,

• a natural tension which varies depending on the age and the studied zone.

This tightness is bound to a directional network line of the skin (Langer network).

The rheological properties of the connective tissue are linked to its structure: collagen and elastin fibers arranged in three-dimensional network play an important role.

MPA 580 Cutometer® (Courage & Khazaka) is an *in vivo* non-invasive method to evaluate skin rheological properties: measures of biological extensibility and elasticity variations. The technique consists of the suction of the skin in the orifice of a probe by a constant vacuum pressure and for a constant duration. The depth of penetration of the skin into the probe is measured, without friction and mechanical effects, by using two optical prisms located at the opening of this probe. Measurements are performed with a 2mm probe with a 450mbar constant pressure and one cycle of measurement. Suction and relaxation times are of 3 seconds. Each measurement is an average of two acquisitions, performed on the temples, at both D0, D28 and D56.

Depending on the software used, the measured parameters are expressed in Ue, Uf,... or R0, R1,... (Figure 12). In this study, cutaneous firmness parameter R0 is studied. As R0 decrease represents an increase of firmness, the absolute value of R0 was used to calculate the % variation of firmness over time.

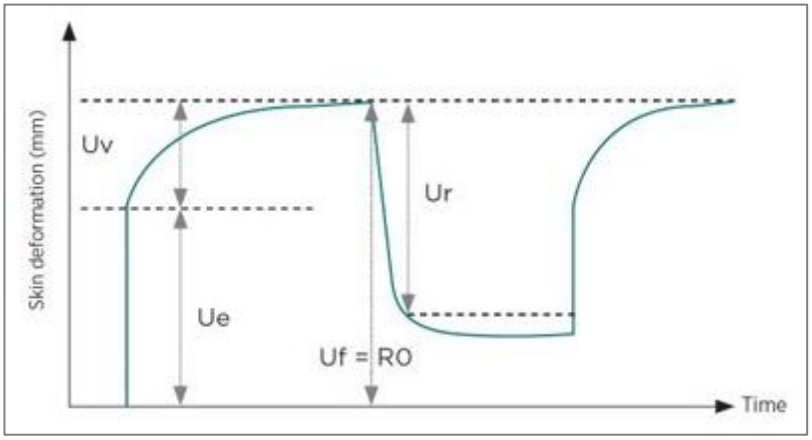


Figure 12: Skin deformation curve obtained with a Cutometer®.

Brightness measurement

Skin colorimetric measurements are done with a Spectrophotometer® CM700-d (Minolta) equipped with an 8 mm diameter head. The Spectrophotometer® converts colors perceived by man to a digital code composed of three parameters:

- L*: for clarity (from dark to light)
- a*: for the green-to-red spectrum
- b*: for the blue-to-yellow spectrum

a* and b* are chrominance parameters, L* is a luminance parameter.
In this study, brightness parameter L* is studied, after 28 days of cream application.

Sagging measurement

The analysis of skin firmness (anti-sagging) is done after visit D28 via scoring photographs by 3 trained technician experts, based on a specific scoring scale (figure 13). The pictures are visualized on a computer screen in a black room. The analysis is performed comparing the pictures taken on D0 and after 28 days of products use on hemi-face.

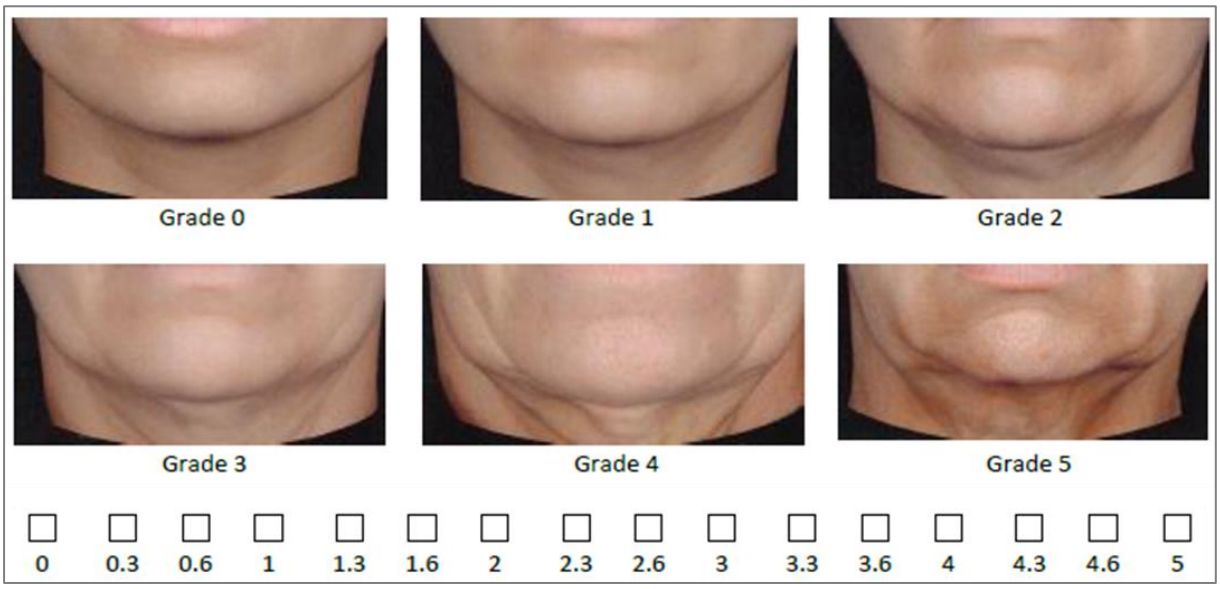


Figure 13: Ptosis (Volume 1, Caucasian type - Skin aging atlas)

Pictures acquisition

The device used is the VISIA® from CANFIELD imaging systems. It enables to take pictures with different types of lightening in a very rapid way. The control of the repositioning takes place directly on data-processing screen using an overlay visualization of the images at each time of acquisition.

In this study, we focused on standard light pictures.

Calculation

Results are expressed as % variation vs Day 0 for each product according to the following formula:

$$\% \text{ variation} = (Dx - D0) / D0 * 100$$

For the R0 parameter of cutometry, as a decrease of the parameter represents an increase in firmness, we expressed the results as absolute values

To compare the effects of products between them, we used this formula:

$$\text{Comparison factor} = \% \text{ variation product 1} / \% \text{ variation product 2}$$

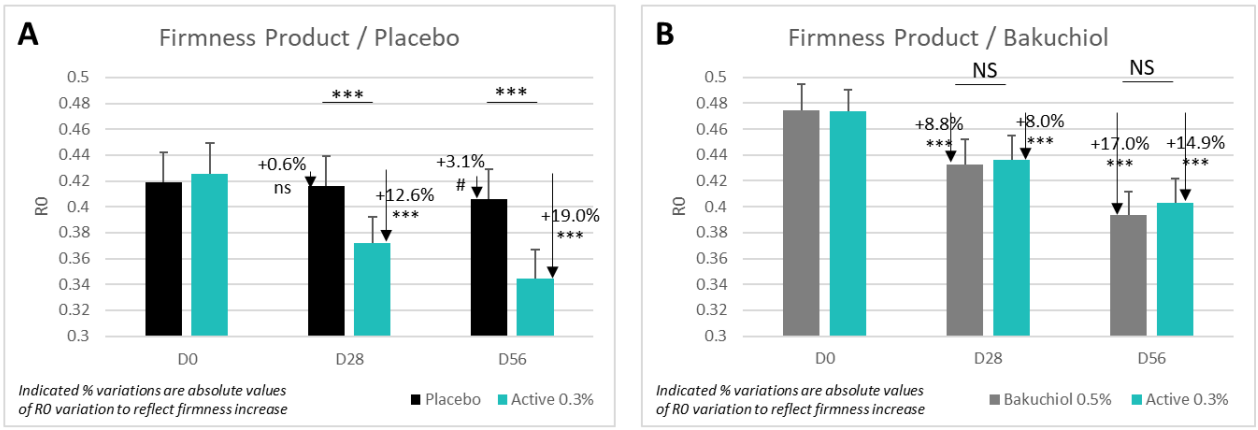
Product 1: formula with CycloRetin™ - Product 2: placebo formula or bakuchiol formula

Statistical analysis

In the clinical study, as the tested products (chassis formulation alone or cosmetic active ingredient) were applied on half-faces of subjects, samples are paired. To compare the two groups (CycloRetin™ vs Placebo and CycloRetin™ vs Bakuchiol), as well as the evolution over time (vs D0), data were analyzed with the paired Student's t-test (if the normality of the distributions is confirmed) or Wilcoxon tests (if the normality of the distributions is rejected), considering a p value < 0.05 as statistically significant. The normality of the distributions was checked using the Shapiro-Wilk test with a level of significance set at 1%.

Results

Firmness increase



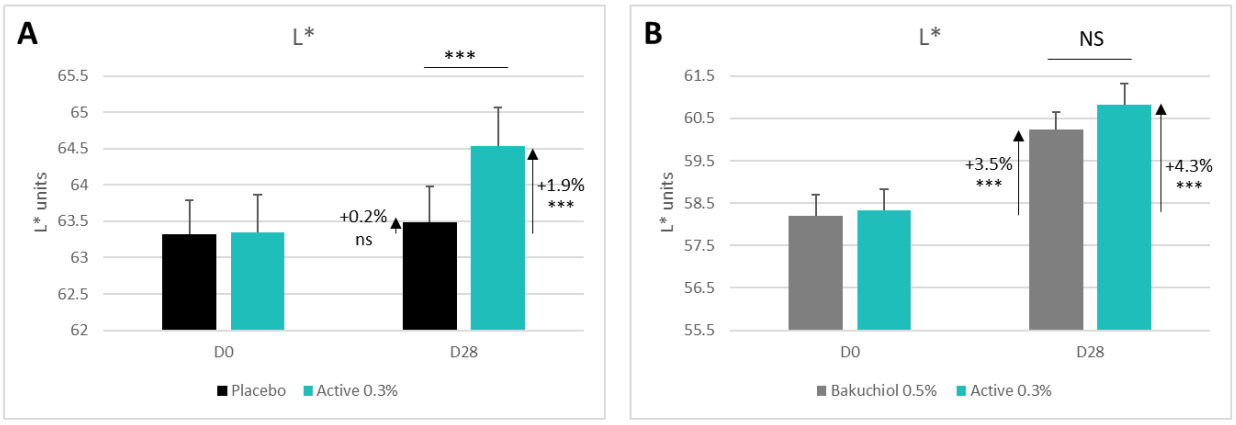
% of subjects with improvement
Placebo : D28: 60% - D56 : 65%
Active: D28: 100% - D56 : 100%

% of subjects with improvement
Bakuchiol: D28: 100% - D56 : 100%
Active: D28: 100% - D56 : 100%

Figure 14: Results obtained for firmness measurement by cutometry in both groups: CycloRetin™ /Placebo (A) and CycloRetin™ /Bakuchiol (B). All are expressed as R0 values mean and absolute % R0 variation vs Day 0. Statistics: ***: p<0.001; #: p<0.1; ns: p>0.1.

CycloRetin™ improves firmness with a cumulative effect over time (figure 14A). It increases firmness by decreasing R0 from 0.43 mm at day 0 to 0.37 mm at day 28 (corresponding to +12.6% firmness, p<0.001) then 0.34 mm at day 56 (+19.0% firmness, p<0.001) These effects are 6 times better than placebo, with highly significant differences at all timepoints (p<0.001). This positive effect on firmness is equivalent to the one of Bakuchiol at both timepoints, as the difference between the effects of the 2 products are non-significant, whereas they present the same significance over time (p<0.001 for both timepoints, figure 14B).

Brightness enhancement



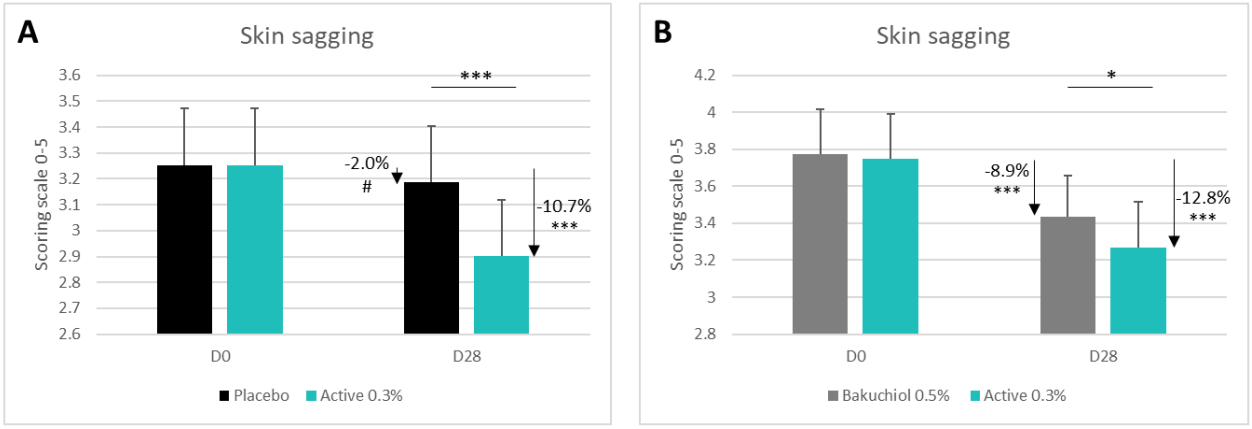
% of subjects with improvement
Placebo : D28: 57%
Active: D28: 90%

% of subjects with improvement
Bakuchiol: D28: 100%
Active: D28: 100%

Figure 15: Results obtained for brightness measurement by colorimetry (L*) in both groups: CycloRetin™/Placebo (A) and CycloRetin™/Bakuchiol (B). All are expressed as L units mean and % variation vs Day 0. Statistics: ***: p<0.001, ns: p>0.1.

CycloRetin™ is able to improve skin brightness (Figure 15A), as L* is significantly increased by +1.2 units (+1.9%, p<0.001) after 28 days of use, which is 9.5 times better than with placebo (p<0.001). Moreover, the efficacy of CycloRetin™ is comparable to the one of Bakuchiol (figure 15B), as both present similar variation and are non-significantly different from each other (+2.0 units for Bakuchiol corresponding to +3.5% (p<0.001) and +2.5 units for CycloRetin™ corresponding to +4.3% (p<0.001)).

Sagging improvement



% of subjects with improvement
Placebo : D28: 38%
Active: D28: 95%

% of subjects with improvement
Bakuchiol: D28: 58%
Active: D28: 100%

Figure 16: Sagging evaluation by scoring for CycloRetin™ /Placebo (A) and CycloRetin™ /Bakuchiol (B). All are expressed as scoring scale value mean and % variation vs Day 0. Statistics: ***: p<0.001; *: p<0.05; #: p<0.1; ns: p>0.1.

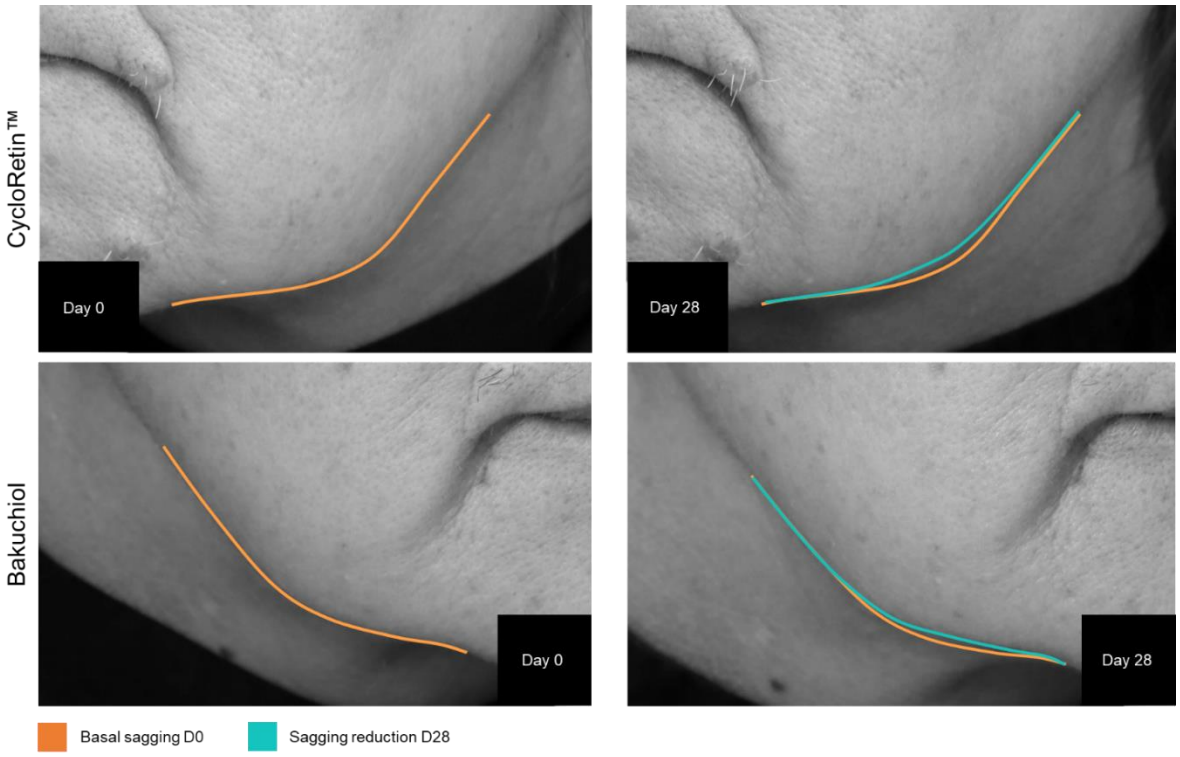


Figure 17: pictures from volunteer 29 (60 years old).

CycloRetin™ decreases sagging by -0.3 points (-10.7%, $p<0.001$) as soon as day 28, being 5 times better than placebo ($p<0.001$). The most interesting part is that it presents a better efficiency than the well-known retinol alternative: Bakuchiol. While Bakuchiol shows a decrease of only -0.3 points (-8.9%, $p<0.01$), our CycloRetin™ is able to decrease the sagging on this panel by -0.5 points (-12.8%, $p<0.001$), being 1.4 times significantly better than Bakuchiol ($p<0.05$). Moreover, CycloRetin™ shows a positive effect on all panelists (100%) when Bakuchiol acts only on 58% of volunteers.
Figure 17 shows volunteer 29, a 60-year-old woman, as an example of visual results on sagging.

Conclusion

To summarize, CycloRetin™ is able to:

- increase skin luminosity after 28 days of application
- show an anti-sagging effect after 28 days of application
- improve skin firmness after 28 days, that continues until 56 days

Thus, CycloRetin™ presents interesting effects on parameters known to be improved by retinol: brightness and firmness. Moreover, CycloRetin™ is as efficient as Bakuchiol, the well-known alternative to retinol, on these 2 parameters and outperforms Bakuchiol on sagging. Being more efficient than Bakuchiol, CycloRetin™ can be considered as a new retinol alternative.

2. Effect of CycloRetin™ on wrinkles and dermis density compared to Retinol

Aim and scientific rational

As CycloRetin™ 0.3% was shown as efficient as Bakuchiol on luminosity and firmness and even superior on sagging, we aimed to compare CycloRetin™ to Retinol, the well-known anti-aging molecule. For Retinol, we used the new maximum recommended concentration for cosmetic use in Europe: 0.3%. CycloRetin™ was used here at 1%.

Tested products

CycloRetin™ and Retinol were formulated as follow (table 9):

Ingredient	INCI	Retinol 0.3%	CycloRetin™ 1%
Water	Water	95.50	95.10
Aristoflex AVC	Ammonium Acryloyldimethyltaurate/VP Crosspolymer	0.80	0.80
Cetiol OE	Dicaprylyl Ether	1.00	1.00
Buffer solution pH 6	Citric Acid (and) Sodium Citrate	1.00	1.00
Phenonip ME	Phenoxyethanol (and) Methylparaben (and) Ethylparaben	1.10	1.10
Fragrance	Fragrance	0.003	0.003
Retinol GS50	Retinol (and) Polysorbate 20 (and) BHA (and) BHT	**0.60**	-
CycloRetin™	Pseudostellaria heterophylla extract (and) Glycerin (and) Water	-	**1.00**

Table 9: Formulation details of the tested products (cream-gel)

Panel characteristics

20 Caucasian women between 45 and 65 of age, presenting moderate dry skin (corneometry between 30 and 45 A.U.) and moderate to very severe signs of ageing (grades 2-4 Eiben-Nielson photonumeric scale for wrinkles and loss of elasticity) were enrolled. They were asked to apply CycloRetin™/Retinol products were used twice a day in randomized half-face.

The recommendations of the Declaration of Helsinki and the guidelines of the International Conference on Harmonization Good Clinical Practice were observed.

7 volunteers were removed from the study due to undesirable effect that prevents continuing with the application protocol. These effects were observed on the side of the face with the use of retinol formulation, volunteer reported skin redness with peeling and dryness.

Study design

The study lasted 28 days, with an intermediate timepoint at D14. The 3D skin topography parameters were recorded from full face 3D reconstructions, using AEVA-HE V4 (FoV, L) system (Eotech) at day 0, 14 and 28. Dermis density was measured in the cheek using echography (Ultrascan UC22 Echograph, Courage + Khazaka Electronic GmbH) at day 0 and 28 (Figure 18).

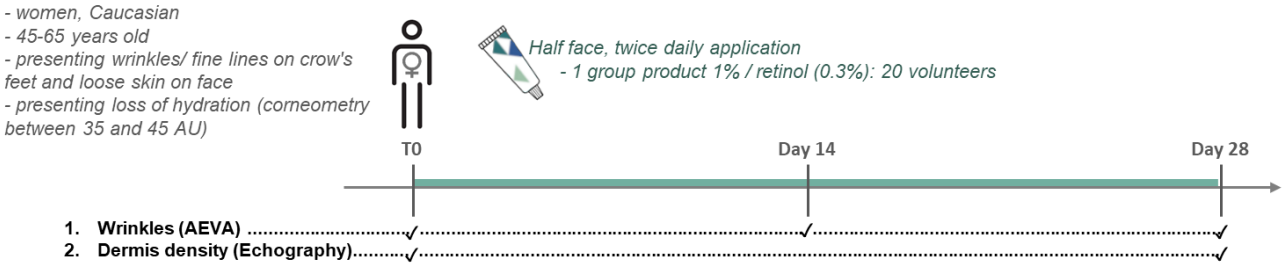


Figure 18: Clinical study protocol

Methods of analysis

Wrinkles measurement with AEVA

The volunteers were installed on the VisioTOP-500 bench with AEVA-HE system for accurate and stable positioning and re-positioning between the different measuring times. 3D acquisitions of full face were done at each timepoint. AEVA-HE system is a combination of known contrast lines (grey code) and sinusoidal fringes shifted on the object. The specific and unique algorithm reconstructs the face in 3D and is able to align all timepoints to calculate different parameters. We measured deep wrinkles on all hemi-face to have a global view of the effect on wrinkles even at early timepoint and we also focused on the crow's feet to see effect on this specific area at day 28.

For global wrinkles, faces were split in two and global wrinkles analysis was performed using AEVA software. Briefly, for deep wrinkles analysis, total wrinkles were detected and classified according to 3 levels: low (class 1), medium (class 2) and high (class 3) depth. A density of deep wrinkles (high, class 3) was calculated according to the following formula:

Deep wrinkles density = deep wrinkles area / total hemi-face area x 100.

Dermis density (echography)

The measurement of Ultrascan UC22 is based on ultrasound waves entering the skin and being reflected differently according to the different tissues. The reflected waves are transformed into electrical impulses and result in an image shown in 256 different colours in a B-scan.

With 22 MHz, we can look 6-8 mm deep into the skin in high resolution. It provides automatic measurement between set lines of dermis density (ultrasound density in % and as a color value between 0 and 256 a.u.).

Calculation and statistics

The % variation were calculated in the same way as described in the clinical study vs Bakuchiol and the same statistical analysis was performed also.

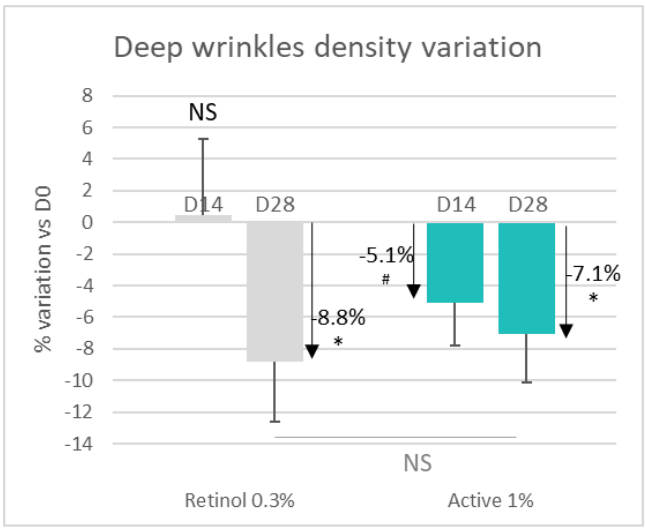
$$\% \text{ variation} = (Dx - D0) / D0 * 100$$

As for the first clinical study, as the tested products were applied on half-faces of subjects, samples are paired. To compare the two groups, as well as the evolution over time (vs D0), data were analyzed with the paired

Student's t-test (if the normality of the distributions is confirmed) or Wilcoxon tests (if the normality of the distributions is rejected), considering a p value < 0.05 as statistically significant. The normality of the distributions was checked using the Shapiro-Wilk test with a level of significance set at 1%.

Results

Global deep wrinkles reduction



% of subjects with improvement
Retinol: D14: 61.5% - D28: 58.3%
Active: D14: 53.8% - D28: 66.7%

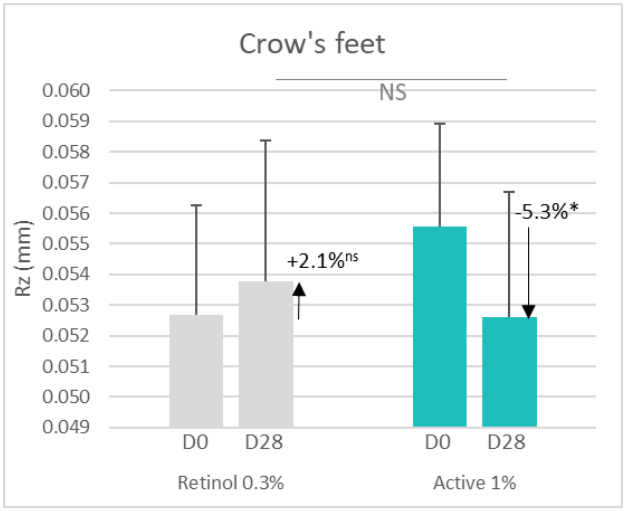
Figure 19: Global deep wrinkles variation by AEVA in all half-faces for Retinol and CycloRetin™. All are expressed as % variation of density related to face area. % variation are indicated in numbers (mean values vs Day 0). Statistics: *: p<0.05; #: p<0.1; ns: p>0.1.

CycloRetin™ decreases global deep wrinkles with a cumulative effect over time (figure 19). Wrinkles density variation is decreased of 0.23 at day 14 (corresponding to -5.1%, p<0.1) then 0.32 at day 28 (corresponding to -7.1%, p<0.05).

Retinol shows a significant decrease only after 28 days of use, of 0.39 at day 28 (corresponding to -8.8%, p<0.05).

Therefore, CycloRetin™ is able to decrease wrinkles faster than retinol does, and with more positive responses at day 28 (58.3% vs 66.7% of volunteers).

Crow's feet reduction

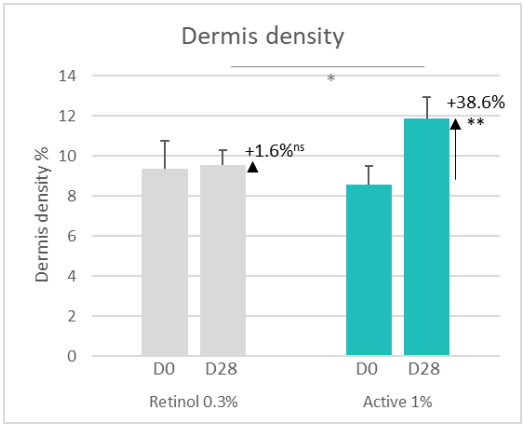


% of subjects with improvement
Retinol: 41.7%
Active: 66.7%

Figure 20: Measurement of crow's feet by AEVA for Retinol and CycloRetin™. All are expressed as Rz, the % variation are indicated in numbers (mean values vs Day 0). Statistics: *: p<0.05; ns: p>0.1.

In our experimental condition, Retinol failed to reduce crow's feet wrinkles (figure 20). CycloRetin™ is able to significantly decrease crow's feet wrinkles Rz from 0.056 mm at day 0 to 0.053 mm at day 28 (corresponding to -5.3%, p<0.05).

Improvement of dermis density



% of subjects with improvement
Retinol: 66.7%
Active: 83.3%

Figure 21: Dermis density measurement by echography for Retinol and CycloRetin™. All are expressed as % density. % variation are indicated in numbers (mean values vs Day 0). Statistics: *: p<0.05; **: p<0.01; ns: p>0.1.

CycloRetin™ improves dermis density in 28 days of use when Retinol does not (Figure 21). The density increases from 8.6 % at day 0 to 11.9% at day 28 with CycloRetin™ (corresponding to +38.6%, $p<0.01$). In our experimental condition, we cannot observe any significant improvement with Retinol.

Conclusion

To summarize these results vs retinol, CycloRetin™ is able to:

- decrease global deep wrinkles faster than retinol, as soon as day 14, and as efficiently as retinol after 28 days of use.
- decrease crow's feet wrinkles after 28 days, when retinol does not, in our experimental conditions
- increase dermis density after 28 days, when retinol does not, in our experimental conditions

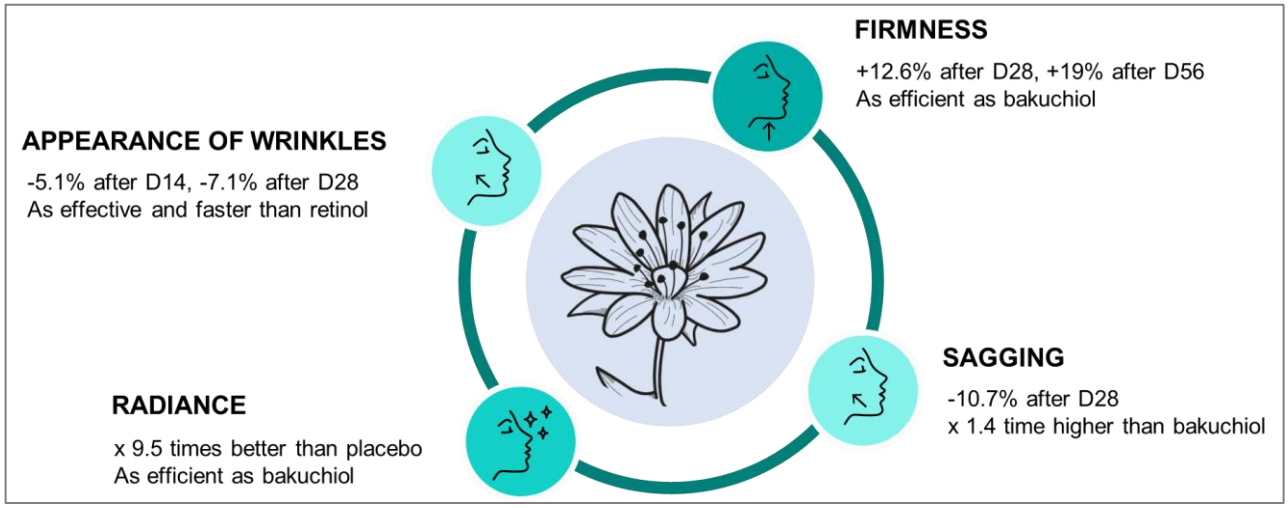


Figure 22: Summary of CycloRetin™ anti-aging benefits in comparison with Bakuchiol and Retinol.

3. CycloRetin™: a gentle peptide-based solution compatible with sensitive skin

Aim and scientific rational

Although retinol is the golden standard skin antiaging agent in cosmetic field, it is also known to provoke irritations, erythema, pruritus, peeling, stinging, or burning, and sensitivity on the skin. In this context, we performed patch test assay on humans to assess the non-irritative potential of CycloRetin™.

Model protocol

11 volunteers tested each product. The studied products were applied under the following conditions: occlusive patch tests (Finn Chambers® 8mm (50mm²)) charged with 20µL of pure products, applied on back, between hips and scapular part, for 48h.

The macroscopic skin examinations were carried out under standardized light, 15 30 minutes after removal of the patch tests and 24 hours later. If the subject had a cutaneous reaction >1, she/ he had to return to the centre and readings were done until complete reversibility of the cutaneous reactions.
The grading of the possible irritation reaction, on each zone that received the studied product and on the control zone was done according to the following scales:

- *Score 0*: No erythema, no oedema. Quotation: Absent
- *Score 0.5*: Fairly detectable erythema: discreet pinkness of one part of the tested area, Palpable, barely visible oedema. Quotation: Very mild
- *Score 1:* Discreet pinkness of the complete tested area or rather visible on one part of the tested area, Palpable and visible oedema. Quotation: Mild
- *Score 2:* clearly distinguishable, dull red erythema covering the whole tested area, obvious oedema thickness < 1 mm) with or without papule(s) or vesicle(s). Quotation: Moderate
- *Score 3:* deep dark or fiery bright red color covering all the tested area or moderate erythema diffusing outside the tested area. Severe oedema (thickness ≥ 1 mm or diffusing outside the tested area) with or without vesicle(s) or blister(s). Quotation: Severe.

A change in skin structure (dryness (D), roughness (R), thickness (T), reflectivity (Re)) that could be linked to the nature of the studied product or one of its components is clinically described and its intensity graded according to the following scale:

- *Score 0.5* = doubtful
- *Score 1* = mild
- *Score 2* = obvious
- *Score 3* = important.

From these readings, 2 indexes are calculated:

- cumulative irritation index (CII) according to the formula:
  CII = ∑ grades erythema oedema / total number of readings
- Mean Cumulative Irritation Index (MCII) according to the formula:
  MCII = ∑ CII / number of subjects

The obtained index (maximum 6) allows to arbitrarily classify the studied product according to the following scale:

- MCII < 0.25: Non irritating
- 0.25 < MCII < 0.50: Very slightly irritating
- 0.50 < MCII < 1: Slightly irritating
- 1 < MCII < 2: Moderately irritating
- MCII ≥ 2: Irritating

Tested products

Ingredient	INCI	Placebo	CycloRetin 0.3%	CycloRetin 0.5%	CycloRetin 1%	Retinol 0.1%	Retinol 0.3%
Water	Water	96.10	95.80	95.60	95.10	95.90	95.50
Aristoflex AVC	Ammonium Acryloyldimethyltaurate /VP Crosspolymer	0.80	0.80	0.80	0.80	0.80	0.80
Cetiol OE	Dicaprylyl Ether	1.00	1.00	1.00	1.00	1.00	1.00
Buffer solution pH 6	Citric Acid (and) Sodium Citrate	1.00	1.00	1.00	1.00	1.00	1.00
Phenonip ME	Phenoxyethanol (and) Methylparaben (and) Ethylparaben	1.10	1.10	1.10	1.10	1.10	1.10
Fragrance	Fragrance	0.003	0.003	0.003	0.003	0.003	0.003
Retinol 50C	Retinol (and) Polysorbate 20 (and) BHT (and) BHA (and) Heptane	-	-	-	-	**0.20**	**0.60**
CycloRetin™	Pseudostellaria heterophylla extract (and) Glycerin (and) Water	-	**0.30**	**0.50**	**1**	-	-

Table 10: Formulation details of the tested products (cream-gel)

Results

Product	MCII score	Class
Placebo	0.09	Non irritating
CycloRetin™ 0.3%	0.05	Non irritating
CycloRetin™ 0.5%	0.02	Non irritating
CycloRetin™ 1.0%	0.00	Non irritating
Retinol 0.1%	0.55	Slightly irritating
Retinol 0.3%	0.64	Slightly irritating

Table 11: classification of tested products according their M.C.I.I. score.

As expected, retinol, in our specific simple gel-cream formula, shows an irritating potential at both 0.1% and 0.3%. CycloRetin™, our new natural retinol alternative demonstrates a perfect skin compatibility, as from 0.3% to 1%, no skin irritation is seen.

Conclusion

CycloRetin™ is a safe alternative for retinol, ensuring no irritation issues.

GENERAL CONCLUSION

CycloRetin™, a specific extract of *Pseudostellaria heterophylla* root, enriched in a specific cyclopeptide heterophyllin B is a perfect natural and mild alternative to retinol. Indeed it

- ensures **retinol metabolism optimization** in the skin and its optimal use safely by the cells
- provides its **own anti-aging signature** with wide epidermis and dermis benefits, surpassing retinol & bakuchiol
- improves **collagen** and **elastic fibers** production and organization

for an optimal anti-aging effect, reducing skin sagging.



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