Active Beauty
PrimalHyal™ Ultrafiller
The dermal fillers challenger

Gold
PrimalHyal™
Ultrafiller
in-cosmetics Latin America
Innovation Zone
Best Ingredient
Award 2018

Crafted by white technology

Givaudan

engage your senses

Focus on the product

Hyaluronic Acid and its key role in organism

Our body naturally contains an average of 15 grams of **hyaluronic acid** (HA), found in every part of our organism: skin, joints, scalp, eyes, soft tissues, and synovial fluid[1]. Due to its chemical nature, HA can hold up a great quantity of water, but its turnover time is very short, with approximately a third of our body HA renewed everyday[2]. In each body location, HA serves different functions, correlated to its molecular weight (MW): high MW HA has more of a water retention function, while lower MW HA enhances biological activities, and plays a role of signal molecule. These physiological roles of HA in skin include:

- **moisturising** and holding water[3],
- maintaining **skin viscoelasticity and tonicity** by creating a hydrogel in the extracellular matrix[4],
- improving cell multiplication and communication through CD44 receptors[5],
- facilitating cellular mobility and viability by transportation of nutrients[6].

Ageing processes and dermal fillers

Upon **chronological ageing**, the metabolic efficiency of skin cells decreases, lowering their capability to produce major constituents of the extracellular matrix. Due to its high degradation rate, HA is among the first matrix polymer to disappear, leading to the first signs of ageing (fine lines, wrinkles). Meanwhile, antioxidant defences of ageing cells are strongly reduced, making them more sensitive to free radicals.
In parallel, **extrinsic ageing** is driven by daily expositions to a set of environmental aggressions: UV, blue light, pollution, chemicals or repeated mechanical stresses. All of these factors result in the production of Reactive Oxygen Species (ROS), damaging cells components and inducing an overexpression of matrix metalloproteinases (MMP), fostering the degradation of extracellular matrix fibres (such as collagen).

A majority of aesthetic dermal fillers is based on HA, as it is a native component of skin with high volume filling properties (water binding capacities). However the use of these dermal fillers requires local injections with possible complications, and despite their cost, they cannot restore skin natural protection.
It was therefore crucial to find an alternative to protect the skin and its constituents along our lifetime, but also to restore some of its key properties when possible, and get rid of our wrinkles.

PrimalHyal™ Ultrafiller, the cosmetic alternative to dermal fillers

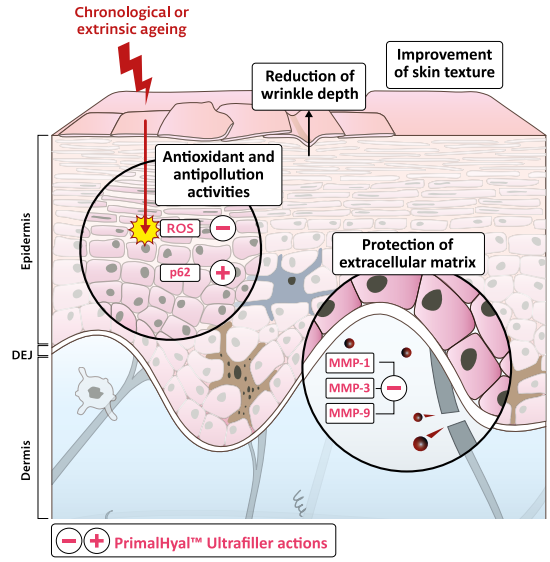
To answer this unmet need, Active Beauty experts have designed PrimalHyal™ Ultrafiller, a new generation of topical HA, by optimising the bioavailability of a specific MW of HA, thanks to its full acetylation.

PrimalHyal™ Ultrafiller behaves as a filler by penetrating deeply into the skin, quickly reducing wrinkles. It also stimulates skin epidermis and dermis metabolisms to:

- Protect the extracellular matrix by decreasing collagenases production and collagen degradation, even under photo-pollution,
- Restore the antioxidant defences through the sequestosome pathway[7], helping skin to fight against free radicals.

Four clinical tests have highlighted the unique consumers benefits of PrimalHyal™ Ultrafiller:

- Flash filler effect: decrease of wrinkles in only 1 to 6 hours,
- Anti-ageing effect: restore skin antioxidant potential, reduce nasogenian wrinkles and improve skin texture.



[1] E. Papakonstantinou et al, 2012 [2] R. Stern, 2004 [3] F. Masson, 2010 [4] C. Chun et al, 2016
[5] B. P. Toole, 2004 & L. Li et al, 2006 & J. A. P. Gomes et al, 2004 [6] M. Gallorini et al, 2017 [7] I. Riz et al, 2016 & Y. Katsuragi et al, 2015

Biological activity

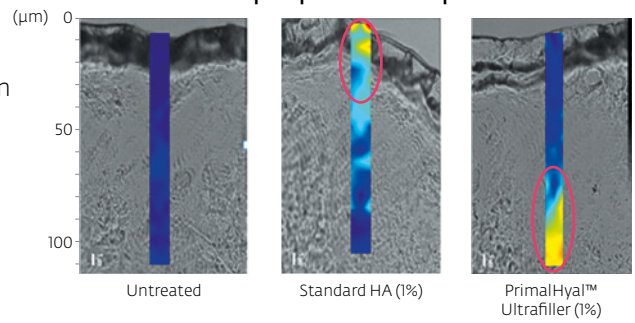
Deeper skin penetration and long-lastingness

1. Deeper skin penetration

Skin explants from a Caucasian 37 years old donor were topically treated for 8 hours with 1% of standard HA (same range of MW than PrimalHyal™ Ultrafiller, but non-acetylated) or PrimalHyal™ Ultrafiller, and analysed by Raman spectroscopy to assess products penetration.

Results: The standard HA shows an average skin penetration from 20 to 50µm. PrimalHyal™ Ultrafiller **penetrates much deeper, with most of the signal around 100µm in the skin.**

Skin depth penetration profile

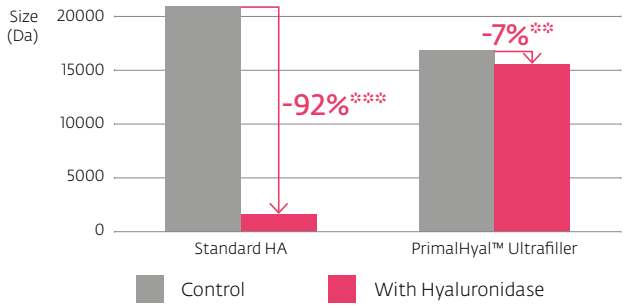


2. Biological long-lastingness

The resistance of standard HA and PrimalHyal™ Ultrafiller to biological degradation was analysed by incubating them 16 hours with hyaluronidase, and HPLC analysis was used to estimate the level of degradation (reduction of MW).

Results: PrimalHyal™ Ultrafiller **shows a high resistance to biological degradation with only 7% of degradation**. Standard HA is almost entirely degraded in these conditions (92%). PrimalHyal™ Ultrafiller **half life is estimated to be 13 times longer than standard HA.**

Resistance to hyaluronidase degradation



**p<0.01 Student's t-test ***p<0.001 Student's t-test

Multifunctional: antioxidant, antipollutant & anti-ageing

A transcriptomic analysis was performed on fibroblasts and keratinocytes pre-treated with PrimalHyal™ Ultrafiller, respectively at 0.01% and 0.1%.

Cellular Function	Gene names		(fold expression)
Antioxidant	Thioredoxin reductase 1	TXNRD1	(+1.7) *
	Aldo-keto reductase	AKR1C3	(+2.1) **
Anti-Pollution	NAD(P)H quinone dehydrogenase	NQO1	(+2.5) ***
	Heme oxygenase	HMOX1	(+3.0)*
Anti-Ageing	Matrix Metallo Proteinases	MMP-1	(-2.0) ***
		MMP-3	(-1.9) ***
		MMP-9	(-1.3) ***

*p<0.05 Student's t-test **p<0.01 Student's t-test ***p<0.001 Student's t-test

Results: PrimalHyal™ Ultrafiller **influences the expression of a whole set of genes** involved in multiple pathways targeting **cellular antioxidant defences, anti-pollution and anti-ageing.**

Biological activity

Epidermis defence activation: antioxidant & antipollution

1. Antioxidant activity (*in vitro*)

▸ **Thioredoxin reductase expression**: Normal human keratinocytes (NHEKs) were pre-incubated with PrimalHyal™ Ultrafiller or standard HA at 0.1% for 2 hours. An oxidative stress was then performed by adding H_2O_2 (500 µM) for 15 minutes, and stopped by a change of culture medium containing PrimalHyal™ Ultrafiller or standard HA at 0.1%. TRXR1 expression was analysed by immunofluorescence after 24 hours of post-stress incubation.

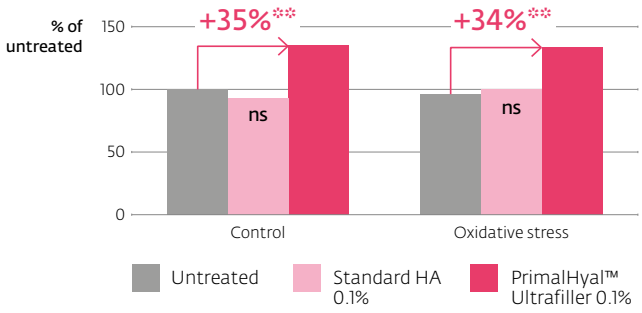
▸ **ROS production**: In a second time, NHEKs were pre-stimulated with PrimalHyal™ Ultrafiller or standard HA at 0.1% for 24 hours, and ROS production was then measured with or without an oxidative stress induced by Tert-Butyl Peroxide (5mM).

Results: PrimalHyal™ Ultrafiller significantly **increases the expression of thioredoxin reductase** both in basal and oxidative stress conditions, **up to +35%**. As a consequence, it significantly **decreases ROS production,** down to -30% under oxidative stress.

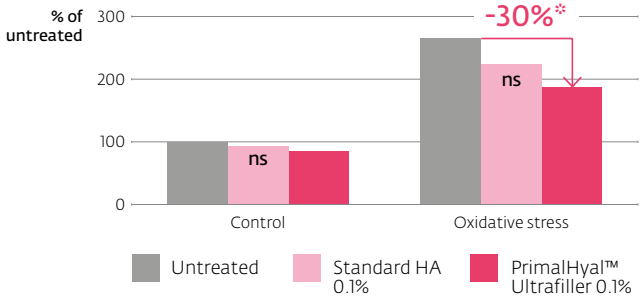
The standard HA shows no activity to protect keratinocytes from free radicals.

*p<0.05 Student's t-test **p<0.01 Student's t-test ns: non-significant

TRXR1 expression



Free radicals production



2. Antipollution activity (*ex vivo*)

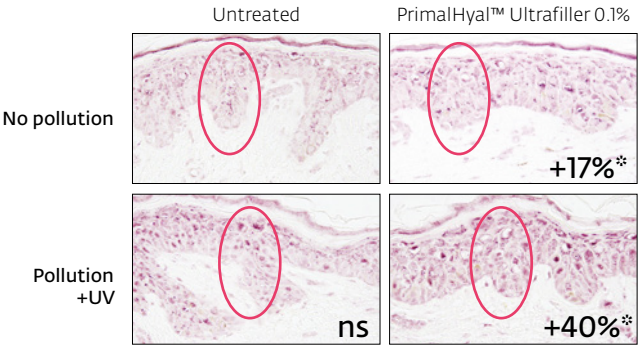
Human skin explants were left untreated or pre-treated with PrimalHyal™ Ultrafiller (0.1%) for 24 hours, then submitted to environmental photo-pollution (heavy metals and hydrocarbure) and UV exposure (13.5 J/cm²), using a Pollubox®, for 1.5 hours. Immunostaining was then performed to highlight sequestosome (p62) expression, a protein involved in skin antipollution defence mechanisms. A second characterisation was performed, quantifying malondialdehyde (MDA), a chemical typically present in the skin damaged by an oxidative stress.

Results: PrimalHyal™ Ultrafiller **stimulates the production of sequestosome**, from +17% without pollution, **up to +40%** under pollution and UV exposure. **It activates in advance skin defences against photo-pollution in a "vaccine like" concept.**

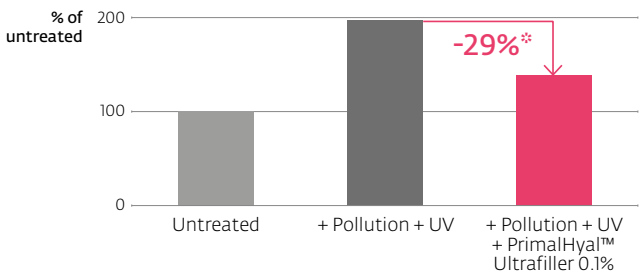
This sequestosome activation enables **a significant antipollution activity,** reducing the MDA production **down to -29%** under pollution and UV exposure.

*p<0.05 Student's t-test ns: non-significant

Sequestosome activation



Pollution damages reduction (MDA)



Biological activity

Dermis active protection: anti-ageing activity

1. Dermal matrix protection (*in vitro*)

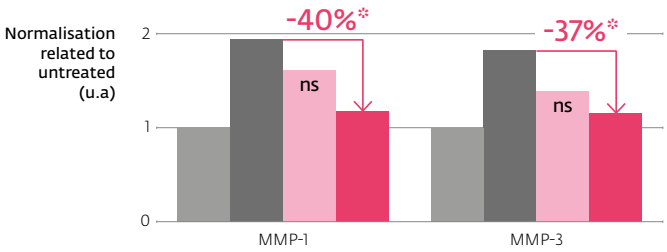
Fibroblasts from a 52 years old donor were pre-incubated with 0.1% of PrimalHyal™ Ultrafiller or standard HA for 2 hours and then exposed or not to an oxidative stress induced by H_2O_2 (200µM).
MMP-1 and MMP-3 production was assessed 48 hours after the oxidative stress by Luminex® and Collagen I degradation was measured by fluorescence analysis using DQ-collagen I.

Results: PrimalHyal™ Ultrafiller significantly **inhibits MMP-1 and MMP-3 collagenases production** in oxidative stress conditions, **down to -40%**.
It also significantly **decreases the degradation of collagen I, down to -42%** under oxidative stress.

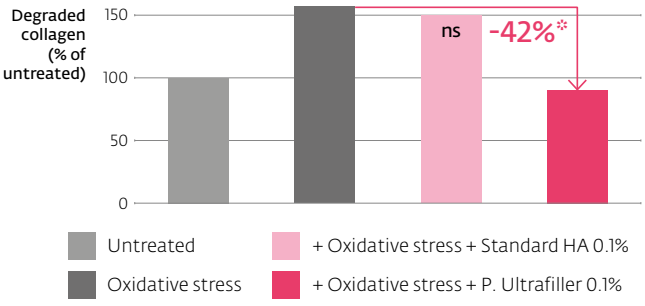
The standard HA shows no activity to reduce collagenases activity nor to protect collagen I.

*p<0.05 Student's t-test ns: non-significant

Decrease of MMP-1 and MMP-3 collagenases



Protection of collagen type I



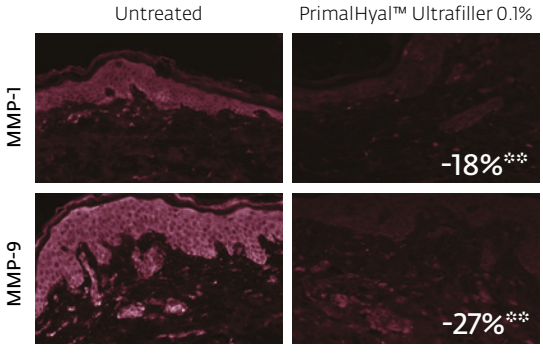
2. Protection against chronological ageing (*ex vivo*)

Skin explants from a 59 years old donor were treated with PrimalHyal™ Ultrafiller at 0.1% for 5 days. MMP-1 and MMP-9 were then quantified in the dermis, by using red fluorescence microscopy.

Results: PrimalHyal™ Ultrafiller **induces a significant decrease of MMP-1 and MMP-9 in the dermis, down to respectively -18% and -27%**, proving its efficacy against chronological ageing.

**p<0.01 Student's t-test

MMP inhibition (chrono. ageing)



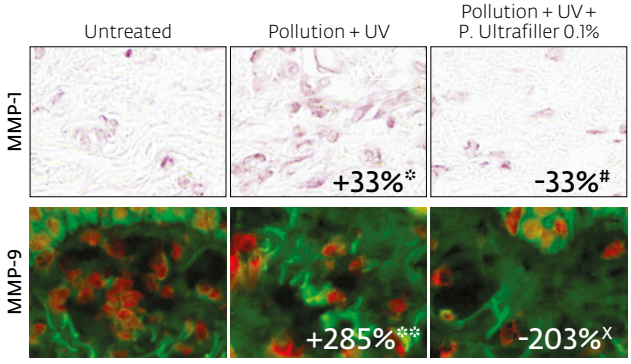
3. Protection against extrinsic (photo) pollution (*ex vivo*)

Photo-pollution effect on the MMP expression was measured on human skin explants in the same condition than for the Sequestosome activation test (see *Epidermis defence activation, 2. Antipollution activity*).

Results: PrimalHyal™ Ultrafiller induces a significant **decrease of MMP-1 and MMP-9 in the dermis** even under pollution and UV exposure, **down to respectively -33% and -203%,** proving its efficacy against extrinsic ageing.

*p<0.05 Student's t-test *vs* Untreated **p<0.01 Student's t-test *vs* Untreated,
#p<0.1 Student's t-test *vs* Pollution + UV X p<0.05 Student's t-test vs Pollution + UV

MMP inhibition (extrinsic ageing)



Clinical efficacy

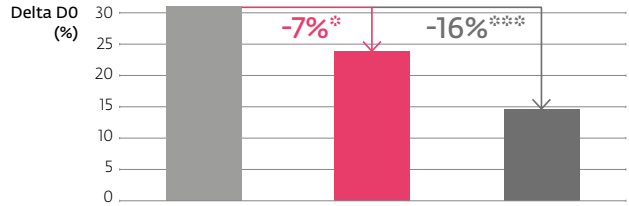
Skin antioxidant potential restoration (Clinical test #1)

A double blind clinical evaluation was carried out on 20 volunteers (women from 18 to 65, 47 years old in average). Volunteers applied twice a day either no product, a cream containing PirmalHyal™ Ultrafiller (0.1%) or the same cream containing 2% of Vitamin E Acetate on their thighs for 56 days. Lipid peroxidation (LPO) was measured just after UV exposure (5 J/cm²) at D0 and D28, and Ferric Reducing Antioxidant Parameter (FRAP, skin antioxidant capacity) at D0 and D56 in basal conditions (no UV exposure).

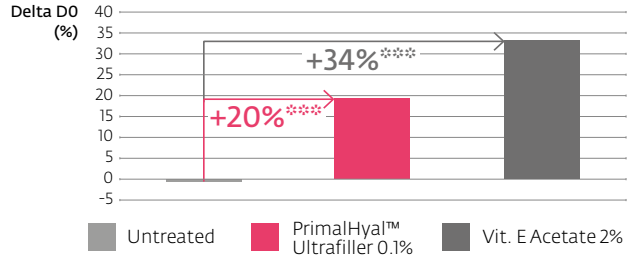
Results: Even at a dosage 20 times lower than Vitamin E acetate, PrimalHyal™ Ultrafiller significantly **decreases UV-induced lipid peroxidation** in 28 days.
PrimalHyal™ Ultrafiller **significantly restores up to +20% the skin antioxidant power (FRAP)** in 56 days.

*p<0.05 Student's t-test, *** p<0.001 Student's t-test

Decrease of skin oxidised lipids (LPO - 28 days)



Boosting skin antioxidant capacities (FRAP - 56 days)



Untreated | PrimalHyal™ Ultrafiller 0.1% | Vit. E Acetate 2%

Flash filler effect in 1 to 6 hours (Clinical test #2)

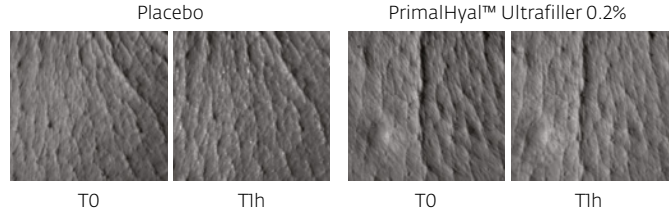
1. Crow's feet wrinkles filling

A double blind clinical evaluation was carried out on 21 volunteers (women from 42 to 70, 63 years old in average) having crow's feet wrinkles. Volunteers applied once either a cream containing 0.2% of PrimalHyal™ Ultrafiller or the same cream without the active (placebo) on each side of their face. Profilometery measurements were performed on crow's feet wrinkles 1 and 6 hours after application, and compared to T0.

Results: PrimalHyal™ Ultrafiller has **a flash reduction effect in 1 hour on the length of fine crow's feet wrinkles, down to -8.7%**. PrimalHyal Ultrafiller also significantly **reduces down to -26.5% the number of deeper crow's feet wrinkles in 6 hours.**

*p<0.05 Student's t-test

Crow's feet reduction



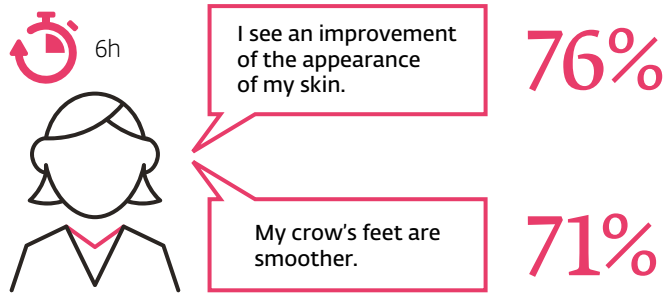
Flash effect in 1 hour	P. Ultrafiller 0.2% (*vs* placebo)
Crow's feet (length of furrows 0-0.5 mm)	-8.7%*

Flash effect in 6 hours	P. Ultrafiller 0.2% (*vs* placebo)
Crow's feet (number of wrinkles 0.5-2 mm)	-26.5%*

2. Auto-evaluation after 6 hours

All the volunteers were asked to assess the efficacy of the product on their crow's feet and the general appearance of their skin 6 hours after application.

76% of the volunteers could see an improvement of their skin's appearance, and 71% could feel their crow's feet becoming smoother.



Clinical efficacy

Mid and long-term anti-ageing activity (Clinical tests #3 and #4)

1. Nasogenian wrinkles reduction (1 month)

A double blind clinical evaluation was carried out on 20 volunteers (women from 35 to 70, 56 years old in average) having wrinkles in the nasolabial area.

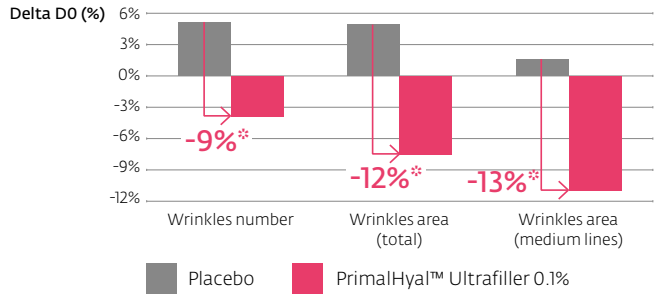
Volunteers applied either a cream containing 0.1% of PrimalHyal™ Ultrafiller or the same cream without the active (placebo) on each side of their face for 28 days, twice a day (morning and evening).

VISIA® analysis was performed after D0 and D28 to assess wrinkles number and area.

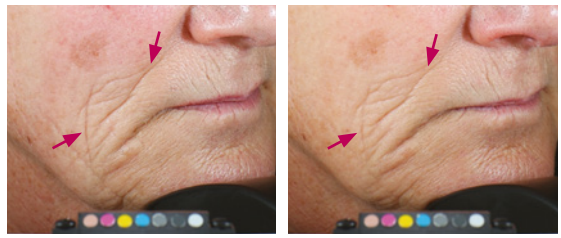
Results: In 1 month, PrimalHyal™ Ultrafiller significantly **decreases down to -9% the number of nasogenian wrinkles, and down to -13% the nasogenian wrinkles area.**

*p<0.05 Student's t-test

Evolution of nasogenian wrinkles (1 month)



Nasogenian wrinkles reduction



D0 D28

2. Improvement of skin texture (2 months)

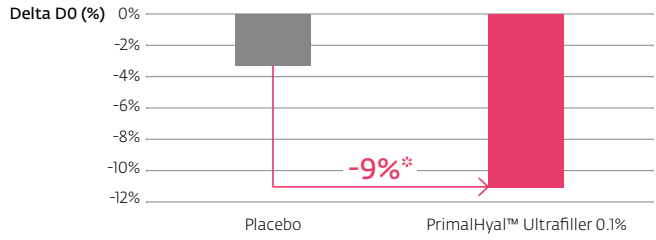
A double blind clinical evaluation was carried out on 30 volunteers (women from 50 to 70, 57 years old in average) having wrinkles on the face.
Volunteers applied either a cream containing 0.1% of PrimalHyal™ Ultrafiller or the same cream without the active (placebo) on each side of their face for 56 days, twice a day (morning and evening).

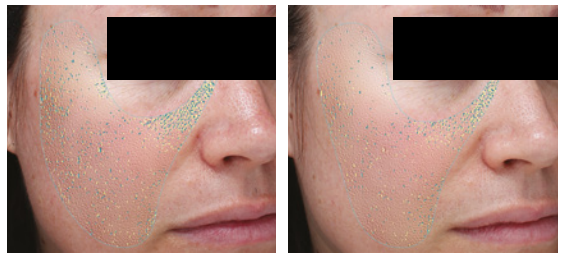
VISIA® analysis was performed after D0 and D56 to assess skin texture, and volunteers were asked to assess the product efficacy on their skin properties.

Results: PrimalHyal™ Ultrafiller enables a significant **9% improvement of skin texture in 2 months, while bringing noticeable skin benefits to volunteers.**

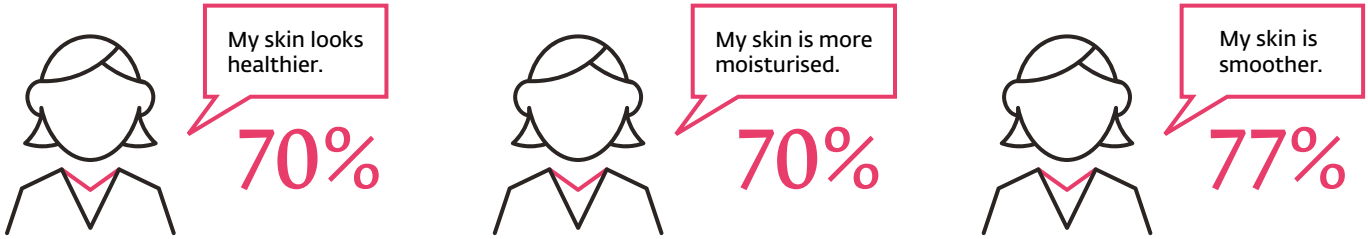
*p<0.05 Student's t-test

Evolution of skin texture (2 months)





Placebo PrimalHyal™ Ultrafiller 0.1%



Summary

Technical information

INCI:	Sodium Acetylated Hyaluronate
Origin:	Biotechnology
Preservation:	Preservative free
Appearance:	Light yellow powder
Solubility:	Water soluble
Dosage:	0.1-0.2%
Processing:	Can be added at the end of the formulation process in a premix 0.1% PrimalHyal™ Ultrafiller / 1.5% water, or in the water phase at the end of heating if in an emulsion. Formulate at temperature below 80°C, and pH between 4 and 12.

Claims

Claims:	Fast wrinkle reduction, skin texture improvement, nasogenian wrinkles reduction, crow's feet and nasogenian wrinkles filler, antioxidant defences booster, antipollution protection, UV-damages protection.
Applications:	Anti-ageing serum, flash anti-ageing cream, wrinkles reducing essence, cosmetic wrinkles filler, anti-ageing night cream, body anti-ageing lotion, dermocosmetic product, skin rejuvenating mist and spray.

Givaudan Active Beauty Sales Offices

Europe

Givaudan France SAS
19-23 rue de la Voie des Bans
FR-95100 Argenteuil (France)

Givaudan UK Ltd
Magna House
76-80 Church Street
Staines, TW18 4XR (United Kingdom)

Naturex SA
250 rue Pierre Bayle - BP 81218
84911 Avignon Cedex 9 (France)

Asia Pacific

Givaudan Singapore Pte Ltd
1 Pioneer Turn
627576 Singapore (Singapore)

Givaudan Shanghai Ltd
298 Li Shi Zhen Road
Pudong Zhang Jiang High Tech Park
201203 Shanghai (China)

Latin America

Givaudan do Brasil Ltda
Av. Engº Billings - 2185, Edificio 31, 1ºAndar - Jaguaré
05321-010 São Paulo - SP (Brazil)

North America

Givaudan Fragrances Corp.
40 W - 57th Street - Floor 17
NY 10019 - New York (United States)

global.cosmetic@givaudan.com

LEAFLET-PRIMALHYAL ULTRAFILLER-11.19