

We make
beauty natural

Colla-Gain™

Anti-Wrinkle Active Derived from Pomegranate Flowers

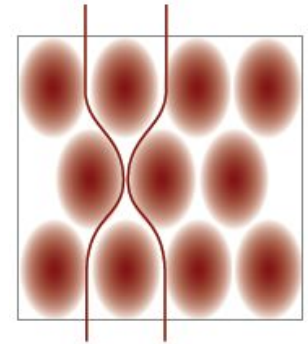
Comprehensive Information

Get an Idea...

Colla-Gain™

Gaining collagen – with a solution inspired by nature

- Wrinkle reduction
- Skin regeneration
- Improvement of skin density and smoothness
- Protection against the impact of UV-A exposure
- Increase of cell viability
- Skin lightening



Colla-Gain™

Combination of pomegranate flower extract and a vitamin C derivative on a maltodextrin carrier



INCI

EU/US: Maltodextrin, Alcohol, Punica Granatum Flower Extract, Sodium Ascorbyl Phosphate

Colla-Gain™

- water soluble powder
- pale yellowish to beige colour

Benefits

Colla-Gain™ generates visible cosmetic effects

- **Promotes rejuvenated and firm skin**

- stimulation of collagen synthesis

- inhibition of collagen breakdown

- improvement of skin density and smoothness

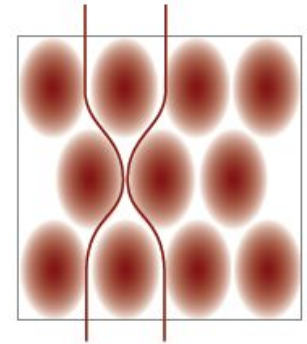
- **Supports healthy and fresh looking skin**

- increase of cell viability

- protection from UV-A impact

- **Provides skin lightening effects**

- reduction of melanin production



Why Using Pomegranate?

- The pomegranate is one of the oldest fruit known to man.
- Appeared in Persia around 4,000 BC.
- Its abundance of seed has always been a symbol of fertility, wealth and good luck.
- Ancient Egyptians believed the pomegranate to be an aphrodisiac.
- Pomegranate has been represented in art since ancient times.
- Pomegranate peel extract stimulated type I procollagen synthesis and inhibits matrix metalloproteinase-1 (MMP-1; interstitial collagenase) production by dermal fibroblasts
(M.N. Aslam et al. / Journal of Ethnopharmacology 103 (2006) 311 – 318)



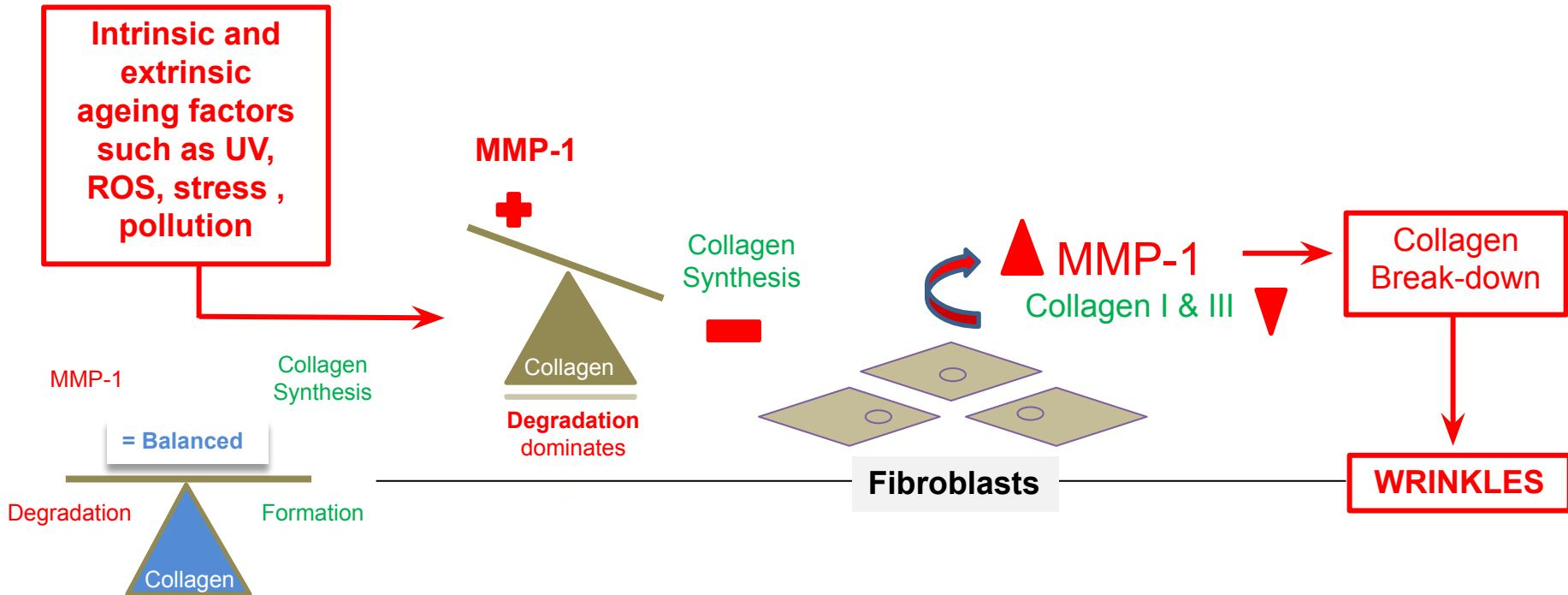
Why Using Pomegranate Flower?

- The flower, seed and fruit have been used in food, medicines, cosmetics and in sacred rituals for nearly 6,000 years.
- The Romans used pomegranate flowers to make a red dye.
- Pomegranate flowers have been used in both Unani and Ayurvedic medicines for the treatment of diabetes.

□ The numerous beneficial effects of the plant in general and the broad utility of the flower in particular prompted us to **explore the utility of the pomegranate flower for cosmetic applications.**



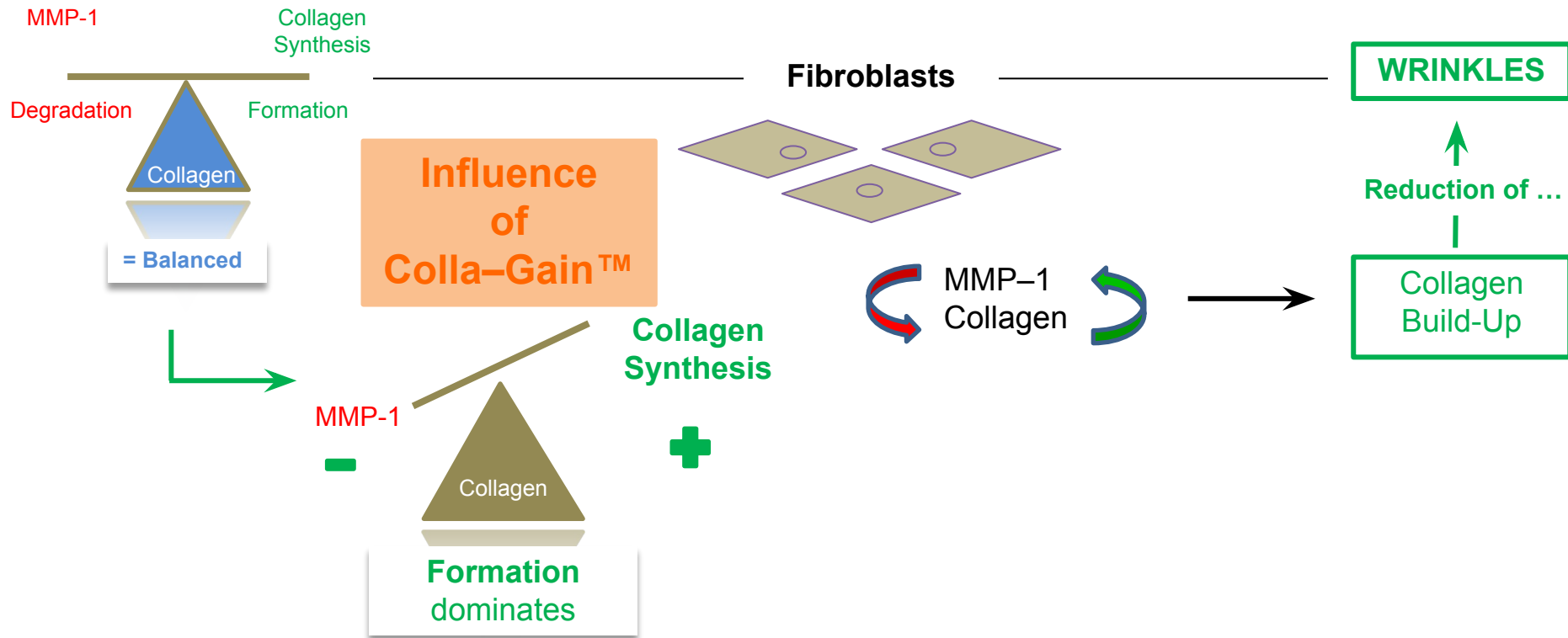
Collagen Breakdown in Fibroblasts



Ageing factors such as UV and Reactive Oxygen Species (ROS) are triggers for

- **the change from a balanced situation**
(equilibrium of MMP-1 activity vs. collagen synthesis)
- **to a state where the collagen decomposition dominates**
(owed to enhanced activity of MMP-1)

Influence of Colla-Gain™ on Collagen Breakdown



Colla-Gain™ supports the balance in a positive way

- it reduces the formation of MMP-1 and hence the collagen decomposing activity
- it boosts the synthesis of collagen and eventually supports wrinkle reduction

Efficacy Assessment of Colla-Gain™



in vitro studies

I MMP-1 gene expression analysis

testing the gene expression-inhibiting properties of pomegranate flower extract (PGFE) in dermal fibroblasts

II MMP-1 synthesis

testing the anti-ageing potential of Colla-Gain™ by measuring the production of MMP-1 in aged dermal fibroblasts

III UV-A cytotoxicity assay / cell viability

testing the influence of PGFE on the viability of dermal fibroblasts after UV-A irradiation

IV Skin lightening assay

testing the inhibition potential of Colla-Gain™ on melanin synthesis in melanocytes/keratinocytes

Efficacy Assessment of Colla-Gain™

I MMP-1 gene expression analysis

Examination of MMP-1 gene expression-inhibiting properties in a real-time Reverse Transcription Polymerase Chain Reaction (real-time RT-PCR) screening

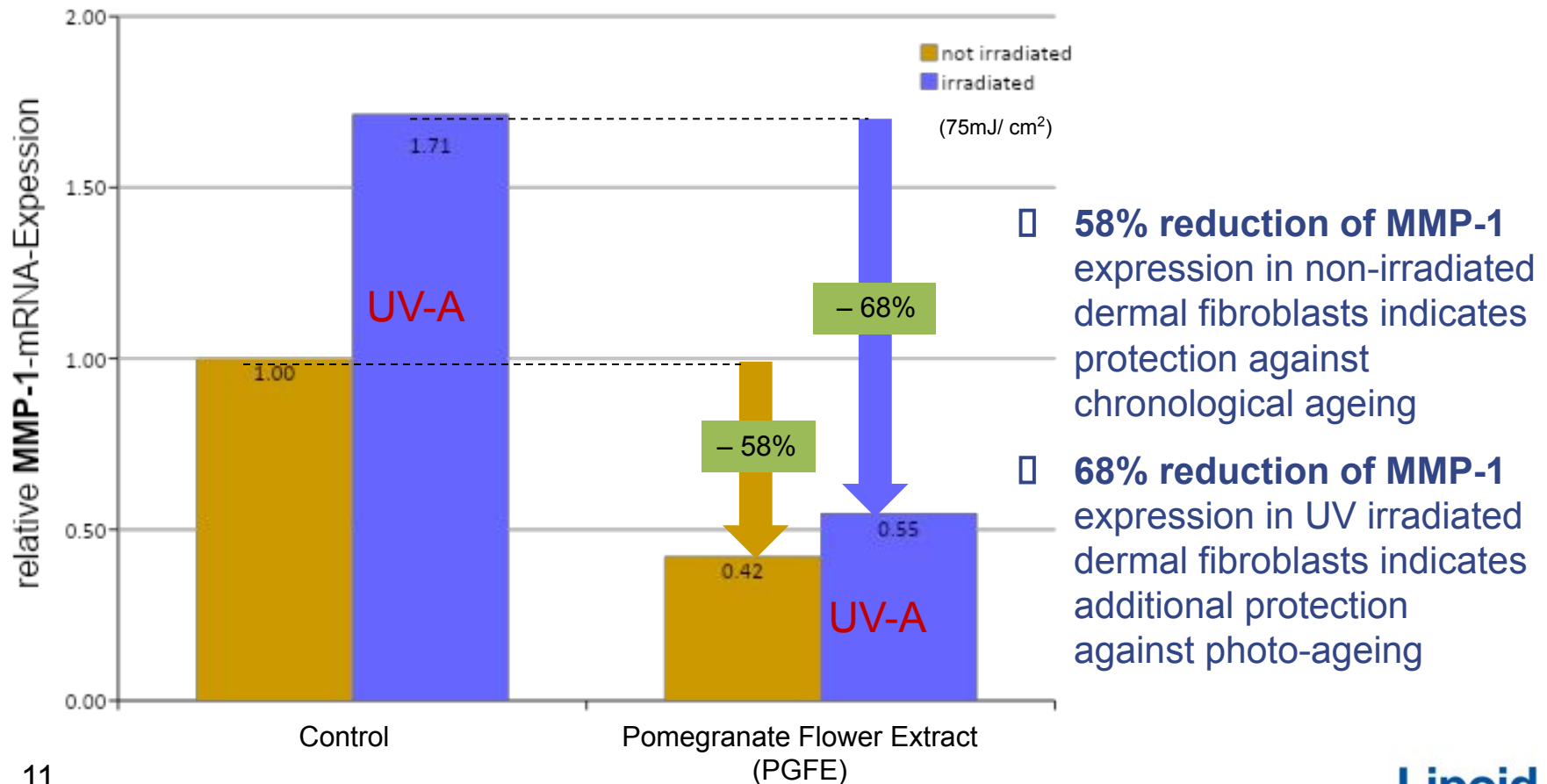
Test parameters:

- Cultivation of dermal fibroblasts in 6 well culture plates and 24 hours treatment with 0.1% DMSO (negative control) vs. PGFE at 0.711%
- Irradiation of 50% of both control and test group with 75 mJ/cm² UV-A light to induce further expression of MMP-1, i.e. to simulate of photo-ageing (UV-A penetrates deeper than UV-B and reaches the fibroblasts in the dermis)
- Extraction of mRNA from irradiated and non-irradiated cells 24 hours after irradiation
- Determination of the relative MMP-1 gene expression using real time RT-PCR

Efficacy Assessment of Colla-Gain™

I MMP-1 gene expression analysis

Examination of MMP-1 gene expression-inhibiting properties in a real-time Reverse Transcription Polymerase Chain Reaction (real-time RT-PCR) screening



Efficacy Assessment of Colla-Gain™

II MMP-1 inhibition

Examination the anti-ageing potential in an Enzyme-Linked Immuno Sorbent Assay (ELISA)

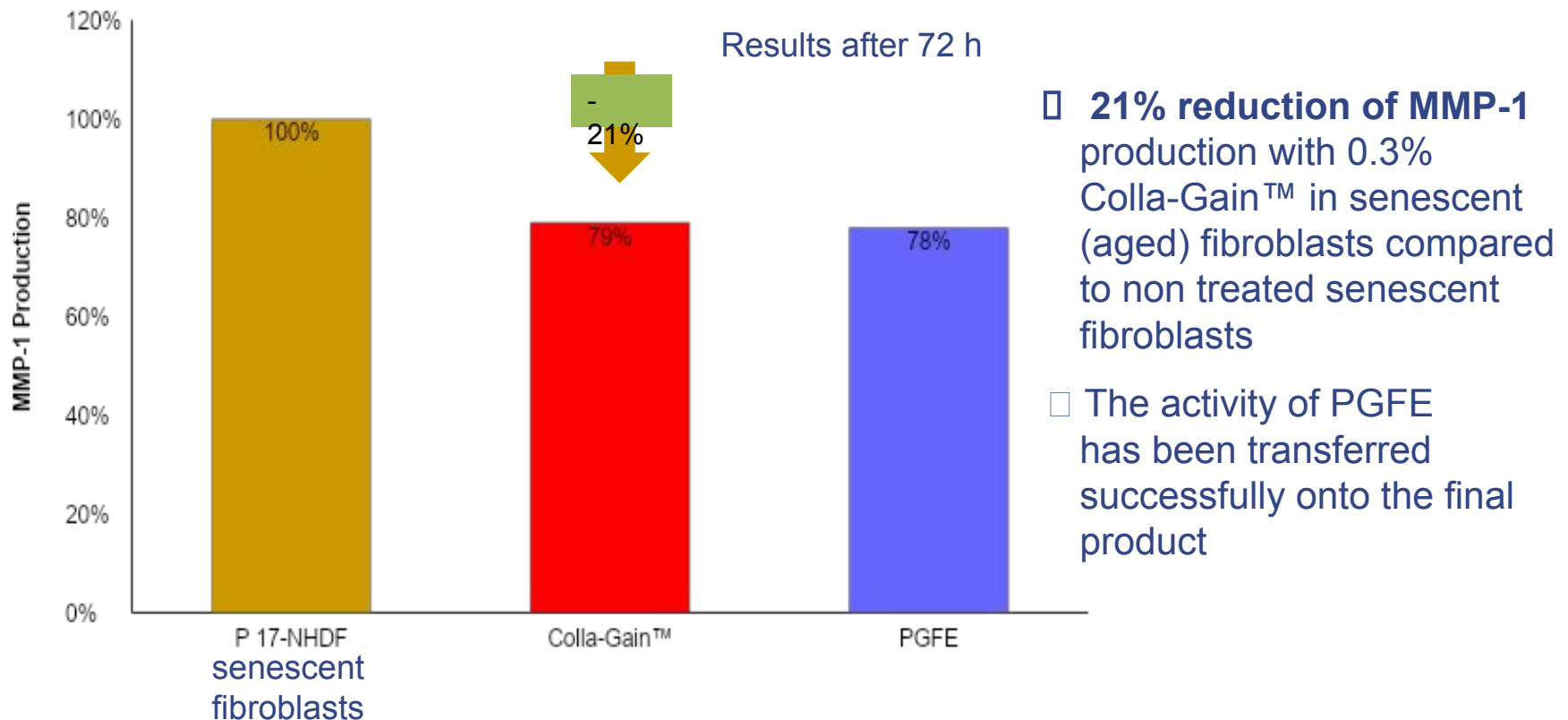
Test parameters:

- Cultivation of Normal Human Dermal Fibroblasts (NHDF) up to senescence (passage 17 (P-17)).
- Cultivation of the senescent (= aged) dermal fibroblasts (“P17-NHDF”) in 96 well culture plates, incubation for 72 hours with 0.3% Colla-Gain™, and comparison to untreated control cells.
- Quantification of the secreted MMP-1 after 72 hours using *ELISA*.

Efficacy Assessment of Colla-Gain™

II MMP-1 inhibition

Examination the anti-ageing potential with the Enzyme-Linked Immuno Sorbent Assay (ELISA)



Efficacy Assessment of Colla-Gain™

III UV-A cytotoxicity assay / Cell viability

Examination of the cell protection potential under UV-A-irradiation

Test parameters:

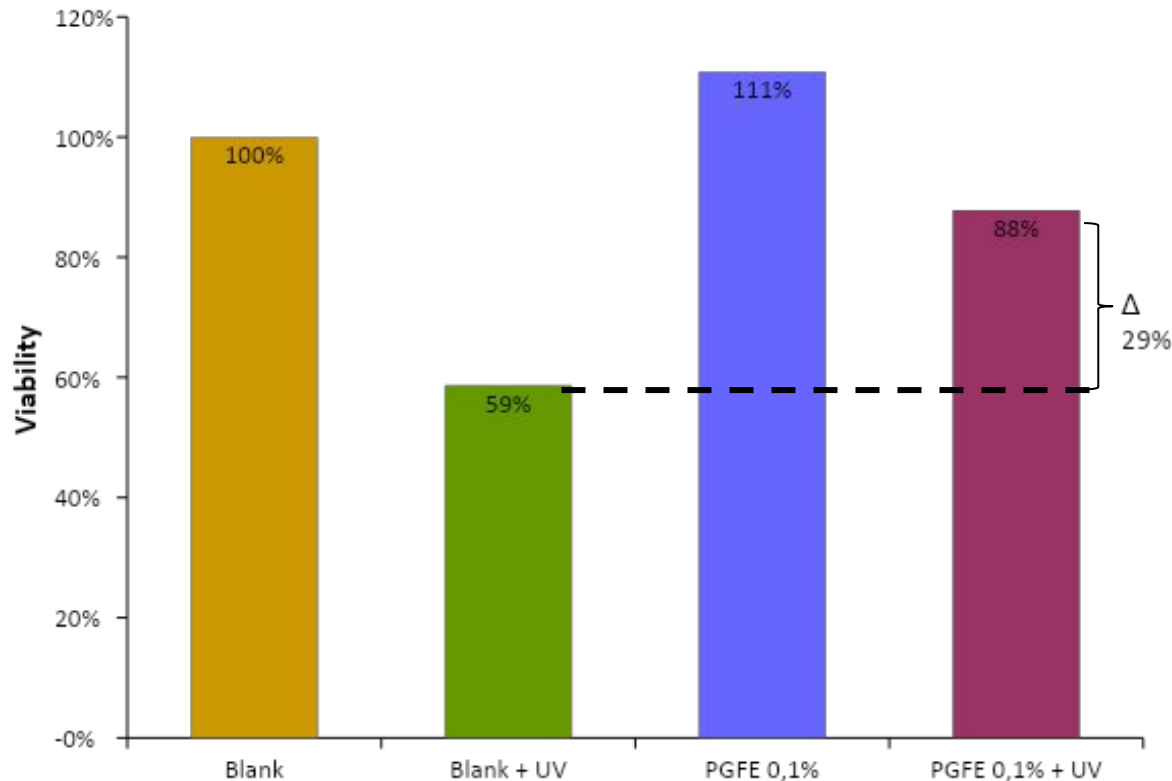
- Treatment of one group of NHDF with 0.1% PGFE. At the same time a control group was left untreated.
Triple-fold irradiation of half of the cultures of each group with UV-A light (12.5 mJ/cm²; every 24 hours).
- Determination of cell viability by means of an a XTT cytotoxicity test 24 hours after the last irradiation.

XTT = (2,3-bis[2-methoxy-4-nitro-5-sulfopheny]-2H-tetrazolium-5-carboxyanilide inner salt)

Efficacy Assessment of Colla-Gain™

III UV-A cytotoxicity assay / Cell viability

Examination of the cell protection potential under UV-A-irradiation



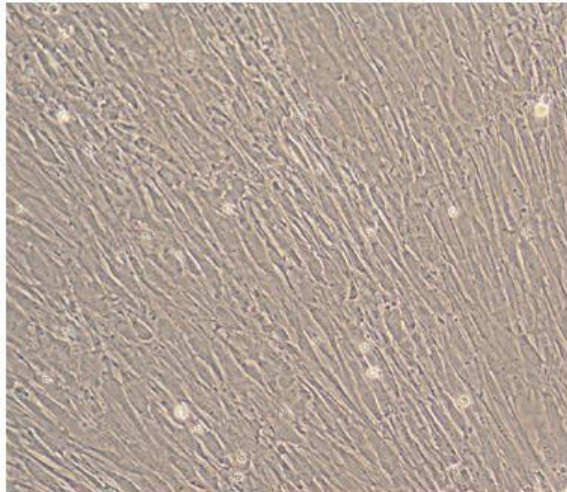
□ **12% damage** of UV-irradiated cells at 0.1% PGFE compared to 41% damage of the fibroblasts without PGFE treatment

Efficacy Assessment of Colla-Gain™

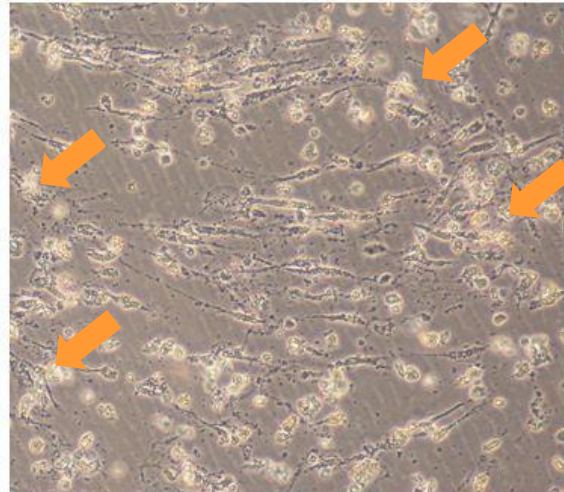
III UV-A cytotoxicity assay / Cell viability

Examination of the cell protection potential under UV-A-irradiation

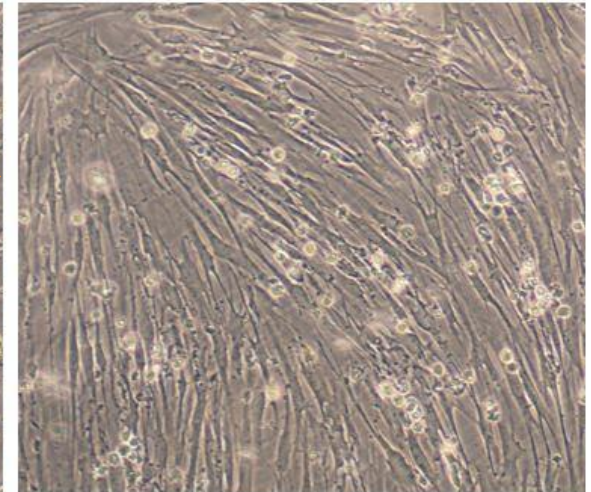
Microscopic imaging of the treated fibroblasts



Not irradiated
(regular appearance
of the fibroblasts;
the lines indicate a healthy
structure; few clumps only)



Irradiated
(yellow arrows =
clumps of damaged cells)



Irradiated + 0.1% PGFE
(reduced number of damaged
cell clumps; better visibility of
fibroblast structure and lines)

Efficacy Assessment of Colla-Gain™

IV Skin lightening assay

Examination of the inhibition potential on melanin synthesis

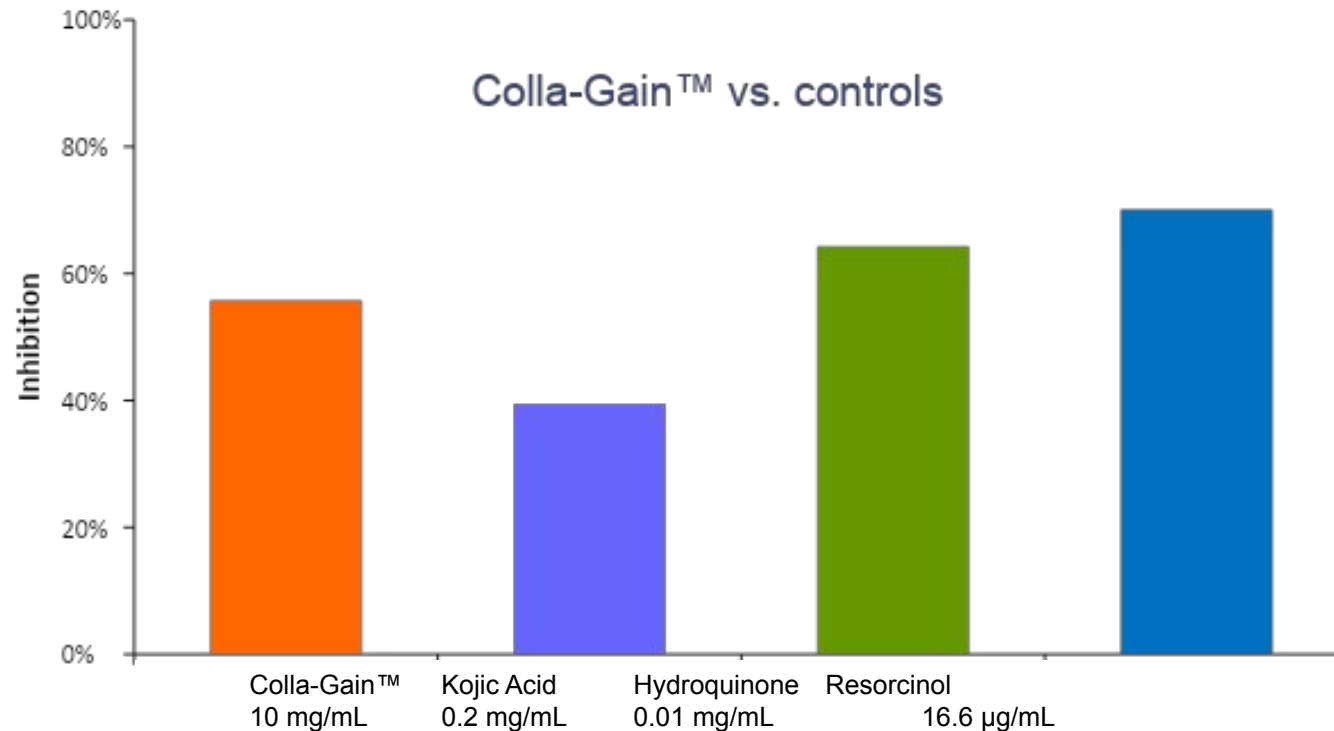
Test parameters:

- Determination of the whitening effect of Colla-Gain™ in co-cultures of primary human melanocytes and immortalized human keratinocytes.
- Cultivation of primary human melanocytes on a confluent monolayer of keratinocytes for 24 hours.
- Treatment of the co-cultivated cells with test substances for 6 days (replacement of culture media with fresh media every 2 days).
- Washing of the cells, lysis and determination of the melanin content in the lysate using photometry at 405 nm.
- Calculation of the inhibition by comparison to an untreated control group.

Efficacy Assessment of Colla-Gain™

IV Skin lightening assay

Examination of the inhibition potential on melanin synthesis



□ 55% of melanin inhibition at 1% Colla-Gain™

Control substances used at their highest non-cytotoxic concentrations

Summary in vitro Studies of Colla-Gain™



I **MMP-1 gene expression analysis (transition level)**

The key ingredient of Colla-Gain™ (i.e. PGFE) provides

- 58% reduction of MMP-1 expression in non-irradiated dermal fibroblasts
- 68% reduction of MMP-1 expression in UV irradiated dermal fibroblasts

II **MMP-1 synthesis (protein level)**

- 21% reduction of MMP-1 production with 0.3% Colla-Gain™ in aged fibroblasts compared to non treated aged fibroblasts

III **UV-A cytotoxicity assay / Cell viability**

The key ingredient in Colla-Gain™ (i.e. PGFE) induces

- 12% damage of UV-irradiated cells at 0.1% PGFE compared to 41% damage of the fibroblasts without PGFE treatment

IV **Skin lightening assay**

1% Colla-Gain™ generates

- 55% of melanin inhibition

Efficacy Assessment of Colla-Gain™



no efficacy

Determination of a cream formulation with 1% Colla-Gain™ on female volunteers over a period of 56 days (application twice daily).

Influence of Colla-Gain™ on the density of dermis and epidermis

Application to volar forearms and face (n=20)

Methods: Ultrasonography, silicon imprints

- increase of skin density by an average of 32.5%

Influence of Colla-Gain™ on wrinkle reduction

Application to face (n=10)

Methods: PRIMOS, high resolution photography

- decrease of wrinkle depth by 15.8%

Efficacy Assessment of Colla-Gain™

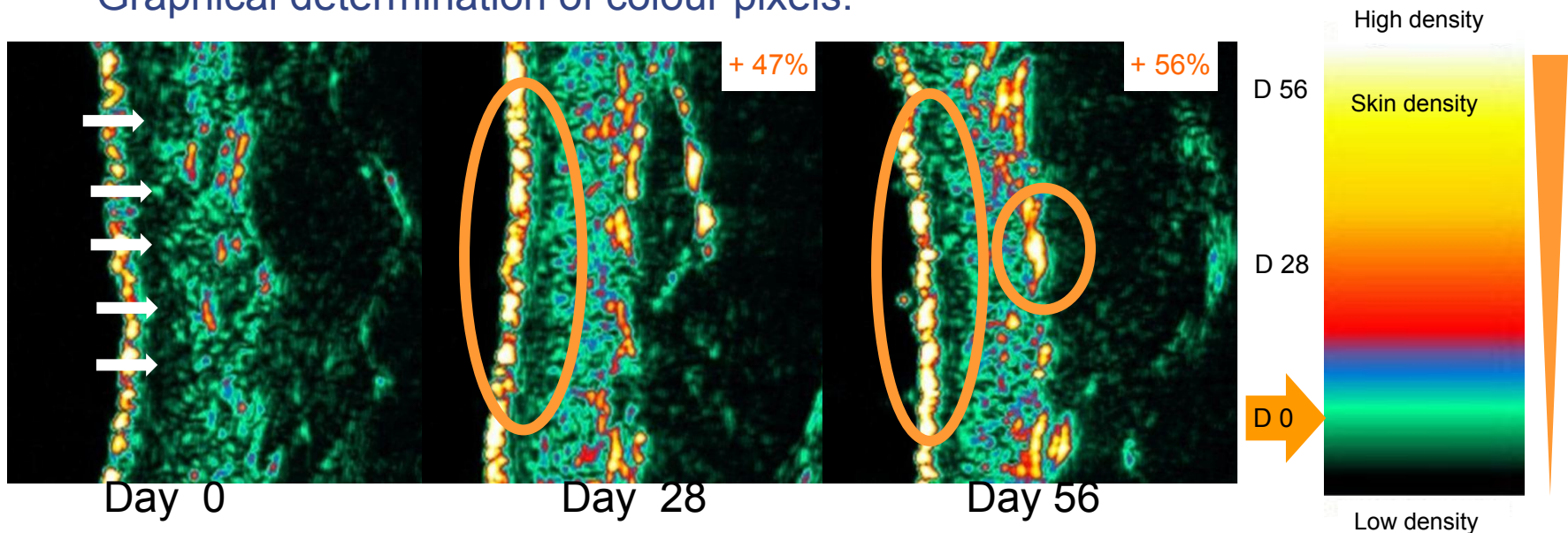
in vivo efficacy – Cream formulation “Young a-Gain” *Eye Contour Cream*

Component	Portion [%]	INCI (CTFA)	Function	Supplier
Water	ad 100.00	Water (Aqua)	Solvent	
Glycerin	3.00	Glycerin	Solvent	
Citric Acid 50%	0.02	Water (Aqua), Citric Acid	pH value regulation	
Tego Care 450	3.00	Polyglyceryl-3 Methylglucose Distearate	O / W emulsifier	Evonik
Tego Alkanol 6855	2.50	Cetearyl alcohol	Consistency	Evonik
Myritol 318	13.00	Caprylic/capric triglycerides	Solvent/Emollient	Evonik
Jojoba Oil cold pressed	6.00	Simmondsia Chinensis (Jojoba) Seed Oil	Emollient	
Colla-Gain™	1.00	Maltodextrin, Alcohol, Punica Granatum Flower Extract, Sodium Ascorbyl Phosphate	Active ingredient	Lipoid-Kosmetik
Preservative	q.s.			

Efficacy Assessment of Colla-Gain™

Influence of Colla-Gain™ on the density of dermis and epidermis

- Ultrasonographic skin density assessment on the inner forearms
Graphical determination of colour pixels.



Decrease in the number of dark pixels (white arrows) = Increase in skin density

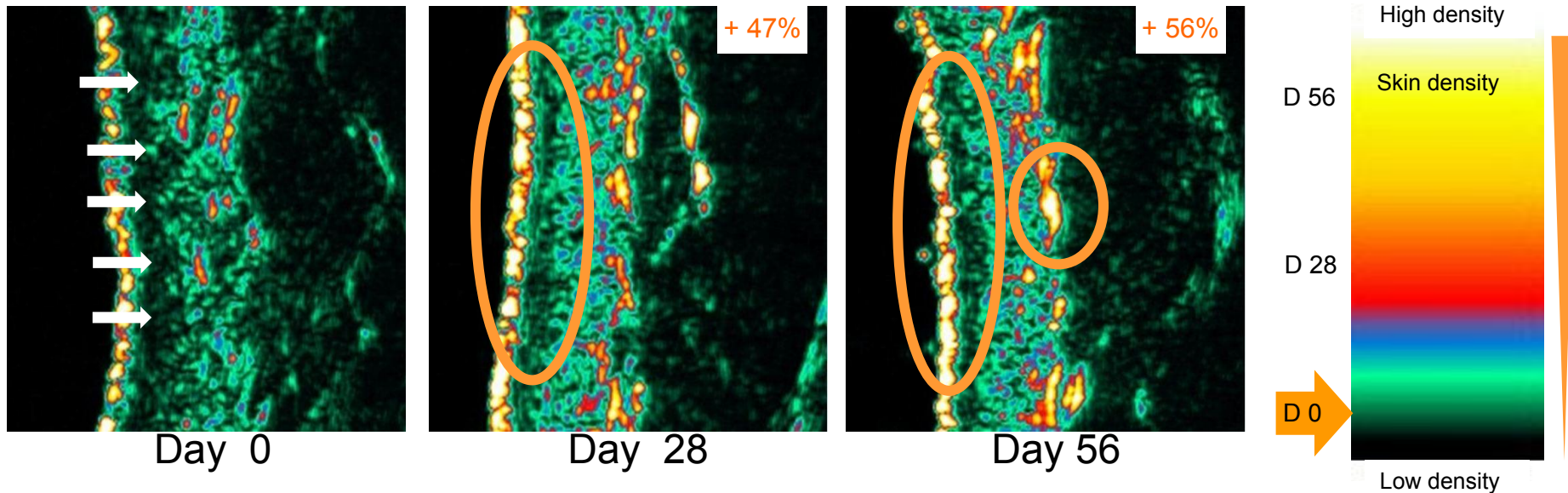
- After 28 days: + 46.8%
- After 56 days: + 56.2% (even the deeper areas of the dermis gained density)

Efficacy Assessment of Colla-Gain™

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Influence of Colla-Gain™ on the density of dermis and epidermis

□ Ultrasonography



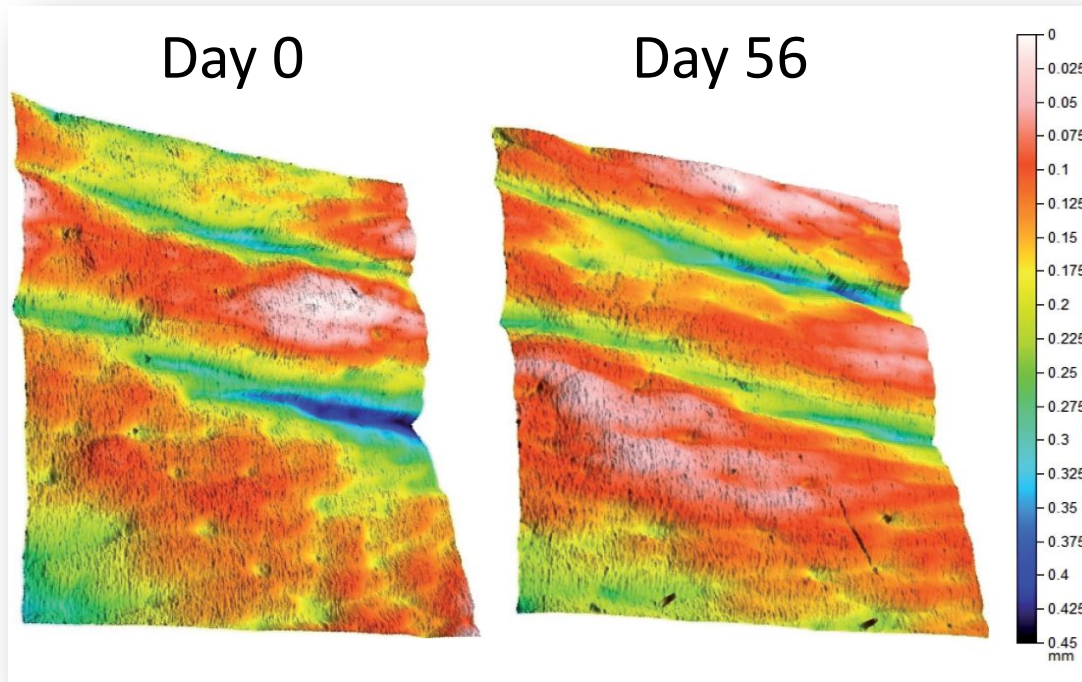
Graphical determination of dark pixels.

Decrease in the number of dark pixels (white arrows) = Increase in skin density

- After 28 days: + 46.8%
- After 56 days: + 56.2%

Efficacy Assessment of Colla-Gain™

Profilometric analysis of silicone imprints of the crow's feet area



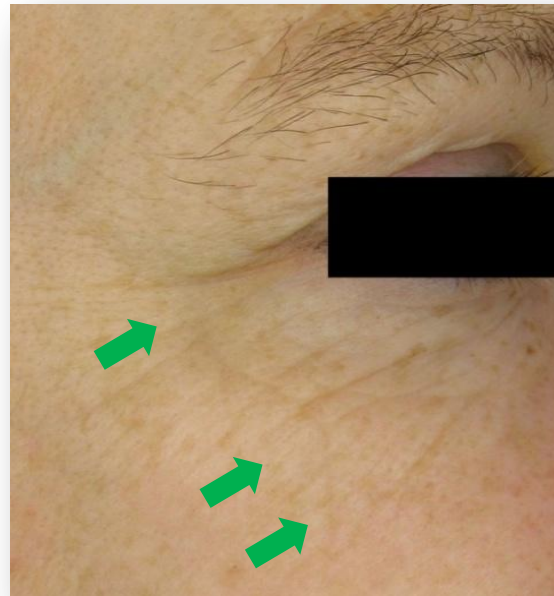
The skin in the crow's feet area around the eye appears plumper and the wrinkles are less deep.

Efficacy Assessment of Colla-Gain™

Photographic documentation of the crow's feet area



Day 0



Day 56

- A clearly visible decrease in the appearance of lines can be observed.

Efficacy Assessment of Colla-Gain™

Photographic documentation of the crow's feet area



Day 0



Day 28



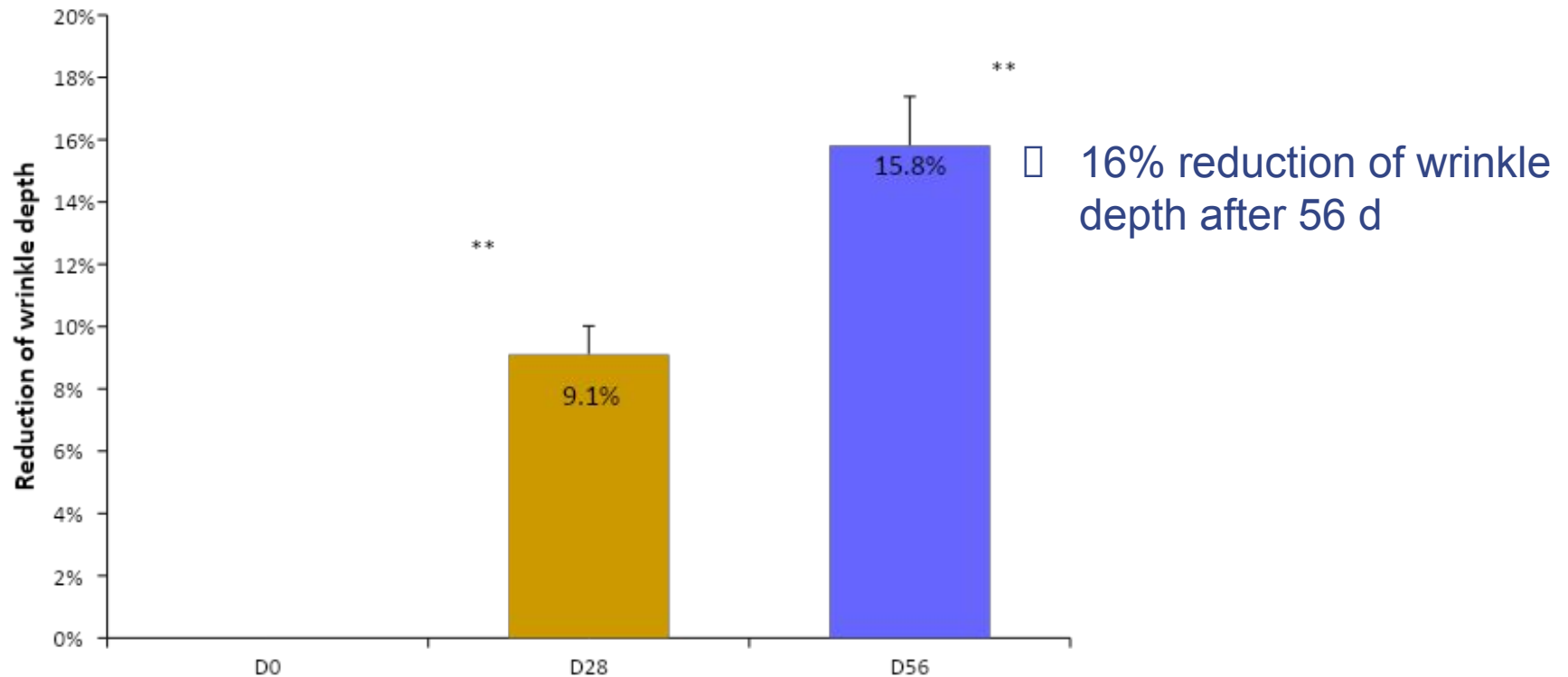
Day 56

- ☐ The frown line / forehead wrinkle is clearly reduced
- ☐ Overall smoothing of the forehead area

Efficacy Assessment of Colla-Gain™

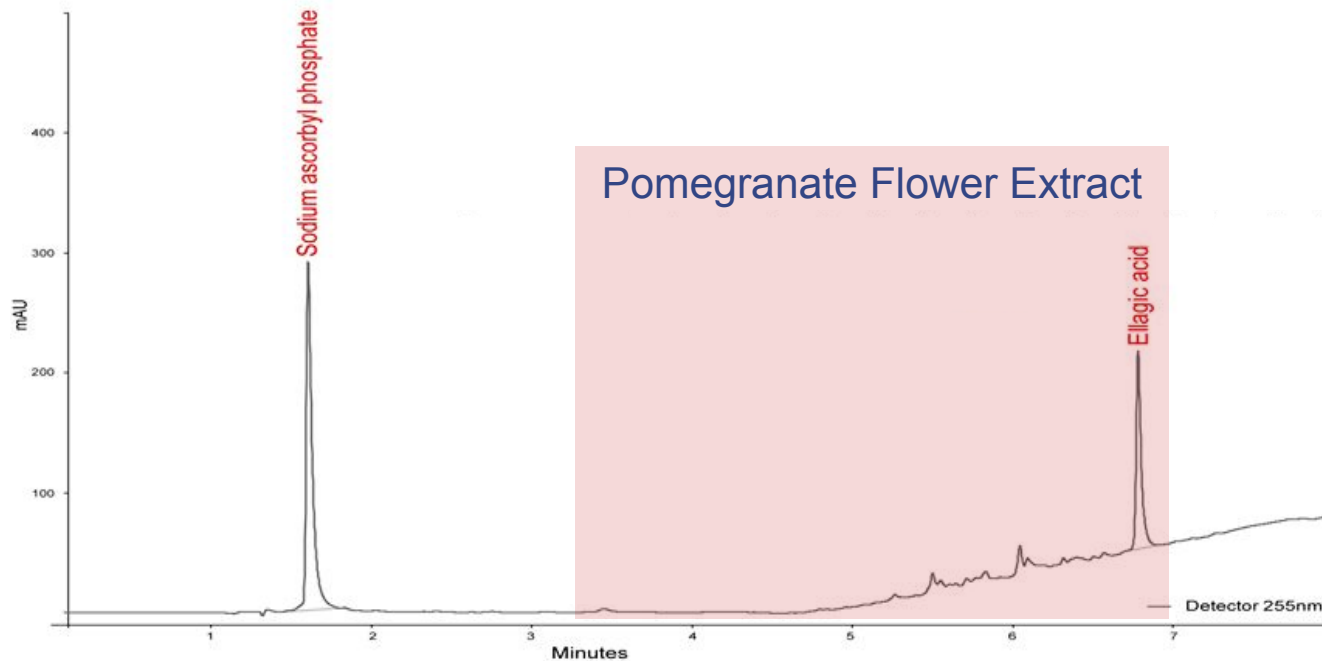
Study 2 Influence of Colla-Gain™ on wrinkle reduction

- Profilometric analysis of the crow's feet area with PRIMOS



Looking into Colla-Gain™

HPLC-Plot of Colla-Gain™



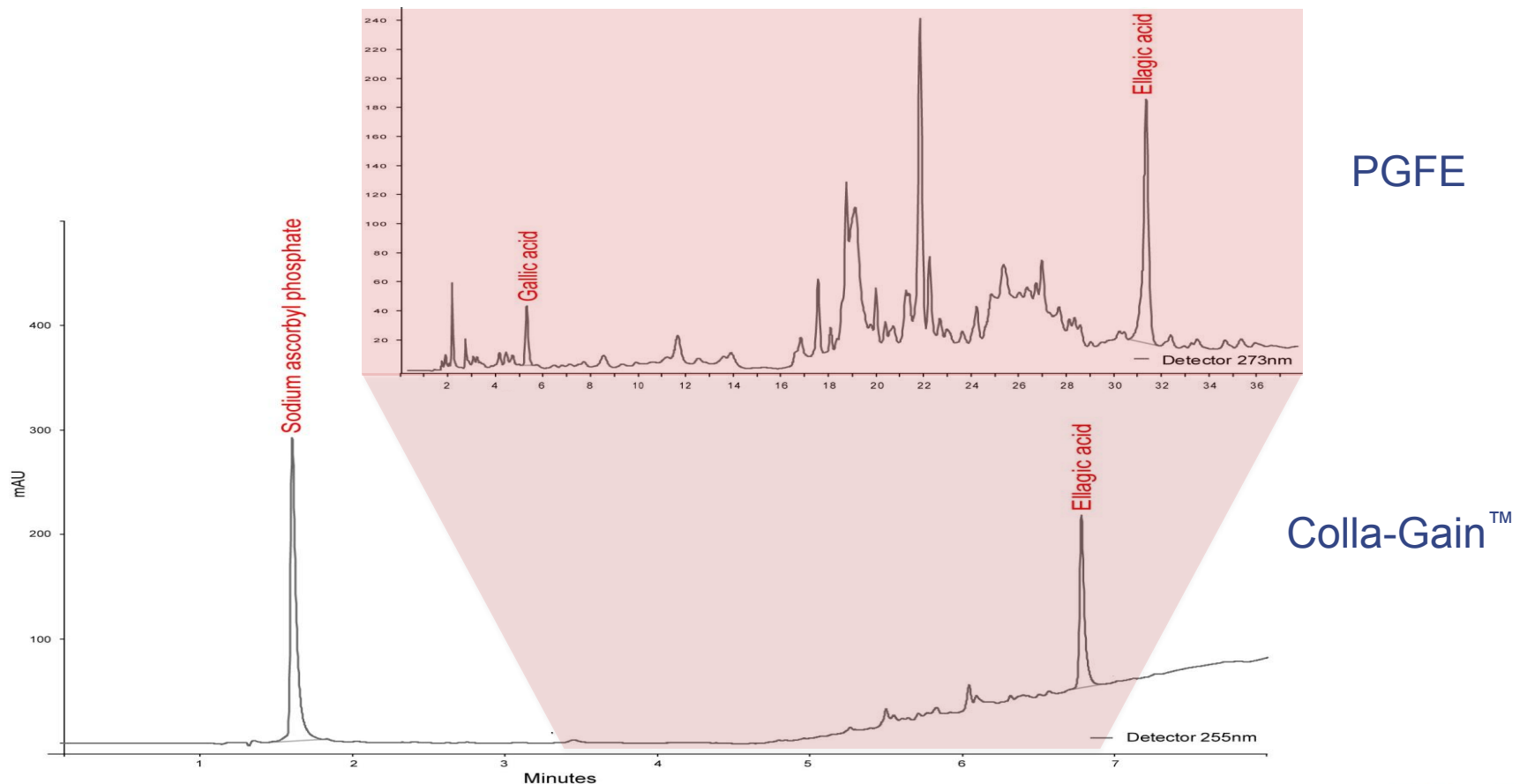
The plot comprises

- Sodium Ascorbyl Phosphate as part of the Colla-Gain™ composition and
- several peaks typical for Pomegranate Flower Extract

Looking into Pomegranate Flower Extract

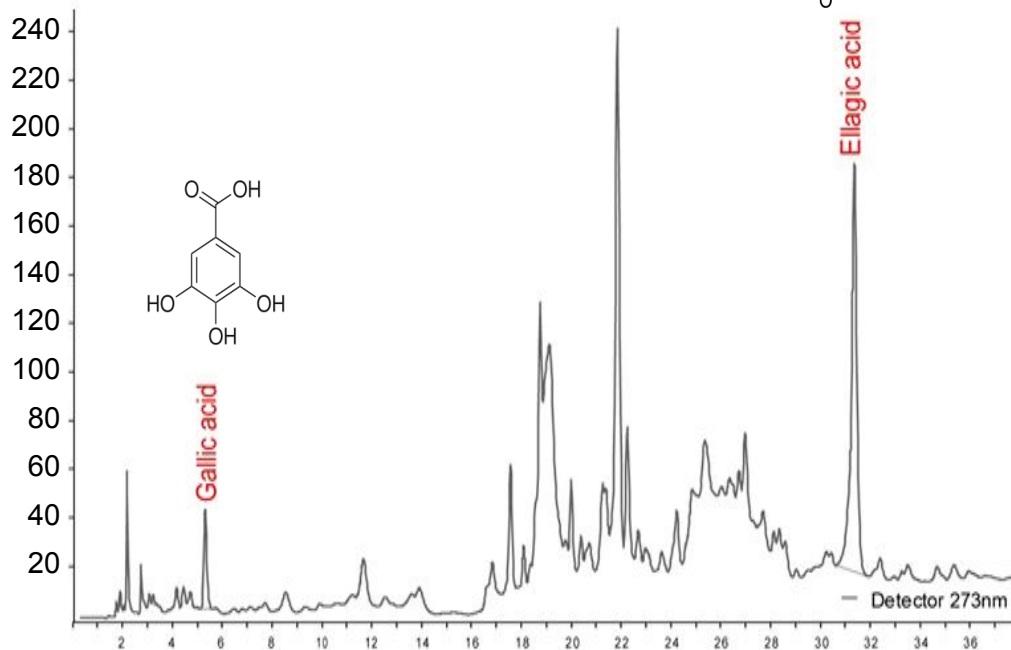
HPLC–Plot of Pomegranate Flower Extract (PGFE)

□ Besides Ellagic acid, Gallic acid is another typical marker substance



Ellagic Acid & Gallic Acid – not only markers

HPLC–Plot of PGFE



Ellagic acid and gallic acid
are both phenolic compounds

- Ellagic acid is a natural phenol, i.e. an antioxidant. It is found in numerous fruits and vegetables.
- Gallic acid is a phenolic acid found in e.g. gallnuts or witch hazel. As an antioxidant it helps to protect human cells against oxidative damage.

□ The presence of anti-oxidative components in PGFE is certainly supportive for the anti-ageing capabilities of Colla-Gain™.

Consumer Test with Colla-Gain™



Checking a cream formulation with 1% Colla-Gain™ over a period of 7 weeks (starting panel: 63 consumers)

After 7 weeks the remaining 56 consumers noticed:

- The eye contour skin seems smoothed out (88% of 56 consumers)
- The crow's feet wrinkles look reduced (82%)
- The fine lines and dry lines look less visible (89%)
- The eye contour skin feels smoother (89%) and firmer (86%)
- The eye contour skin looks plumped up (75%), refreshed (89%)
more radiant/glowy (86%), more tightened, as lifted (86%)
- The eye contour looks younger (80%)
- The eye seems rejuvenated (79%)

Cosmetics with Colla-Gain™

Colla-Gain™ can be used in any kind of anti-ageing and anti-wrinkle applications, e.g.



- Anti-wrinkle products for improved skin density
- Face creams with smoothing capabilities
- Eye area creams with collagen stimulation properties
- BB Creams with rejuvenating activity
- Day & night creams to support healthy and fresh looking skin
- After sun creams with skin regeneration properties
- After beauty salon treatment / after botox supplement
- Neck and décolleté creams with the power of pomegranate flower
- Hand creams with collagen stimulation abilities
- Lightening / brightening products

Easy Formulating with Colla-Gain™

Colla-Gain™ is compatible with cosmetic ingredients

- preparation of an aqueous solution and addition to the water phase at $< 40^{\circ}\text{C}$
- optimum pH range 5 – 8

Recommended use concentration: 0.3 – 2%

for example

0.3 – 0.5 % for prevention purposes in age group 20 – 35 years

1 – 1.5 % for skin forming and toning of age group 30 – 49 years

up to 2 % for rejuvenation of 50+ consumers

Summary

Colla-Gain™

an anti-ageing solution inspired by nature

- Reduces wrinkles
- Regenerates the skin
- Improves the skin's density and smoothness
- Protects against the impact of UV-A exposure
- Increases cell viability
- Generates skin lightening



Literature



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2. Quan, T. et al. (2009) Matrix-degrading metalloproteinases in photoaging J. Investig. Dermatol. Symp. Proc. 2009 14 81): 20-4.
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Documentation on Colla-Gain™

Article No. 400941.00.2

Publication in Personal Care 3-2012 available on request

Frame formulations available on request

Consumer study available on request

Standard documentation (MSDS, composition, specification, etc.)

MMP-1 = Matrix Metalloproteinase 1, an enzyme responsible for collagen breakdown
(e.g. induced by environmental factors like UV irradiation)

PRIMOS = Phaseshift Rapid in vivo Measurement of Skin,
a non-invasive 3D profilometry

For further information please consult our webpage
www.lipoid-kosmetik.com

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