Aimtec®
peptides and synthetic molecules

COUNTERACTING THE FORCE OF GRAVITY

uplevity™
peptide

CFDA listed

V. 4

SAGGINESS INDUCERS

Years of exposure to **environmental agents**, personal **habits** and natural **cellular aging** alter the skin, which becomes less smooth and tight.

Gravity plays a special role as a constant force that pulls the skin downwards, forcing it to remain firm and elastic to avoid sagginess.

Collagen and **elastin** fibres are essential components of the Extracellular Matrix (ECM) that together with some **intracellular molecules** maintain the skin flexible but firm.

Enhancing the existing skin elements that provide elasticity and firmness would reduce sagginess, no mater what origin

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WHY DOES SKIN DETERIORATE?

Skin declines as a result of:

- **Intrinsic or biologic aging**: genetically-determined changes occurring from mid-20s onwards, despite their later visible effects.
- **Mechanical aging**: continually repeated muscle movements (smiling, frowning…) that exacerbate expression lines.
- **Extrinsic or environmental aging**: exposure to external sources (UV rays mainly, pollution, gravity...) that limit the ability of functioning properly, leading to premature aging.

Skin gets altered by diverse sources having different effects

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AGING ALTERATIONS ON THE SKIN

General aging changes:

- Slower cellular turnover
- Flattening of the epidermal-dermal interface
- Reduced collagen production
- Elastin fibre disturbances

Additionally, photoaging causes:

- Melanocytes alteration (hyper/hypopigmentation)
- Moisture barrier disruption
- Collagen and elastin loss and fibres breakdown

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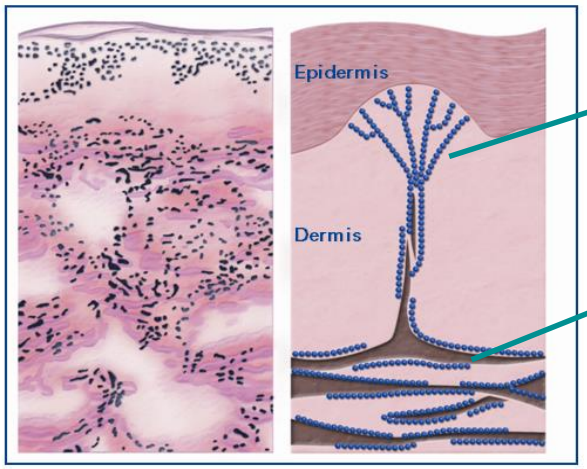
AGING IN ELASTIN FIBRES



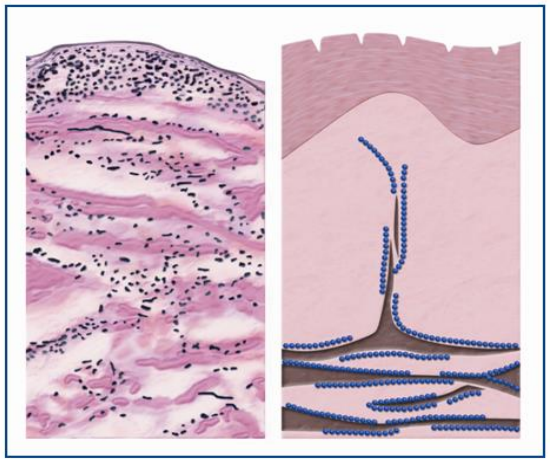
In young skin:

immature fibres course perpendicularly from the epidermal-dermal junction to the top of the reticular dermis.

mature fibres containing deposited elastin run horizontally.

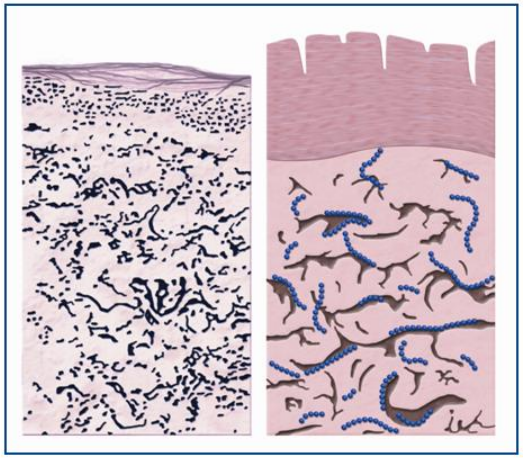
In photoprotected aged skin:

Elastin production naturally decreases: **fewer fibres** and the skin loses resilience.



In photoexposed aged skin:

Accumulation of amorphous elastin protein and breakdown of the typical structure: **thicker and disorganised elastin fibres**.



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KEY ELEMENTS COMBATING SKIN FLACCIDITY

In order to avoid sagginess and flaccidity, several compounds participate in maintaining the skin elements together, providing cohesion and firmness:

- **Elastin fibres**: Tropoelastin (TE)/elastin, Fibulin 5 (**FBLN5**), Lysyl Oxidase-Like 1 (**LOXL1**), microfibrils (fibrillin-1).
- **Collagen fibres**: type I, IV, VI and XIV collagen.
- **Focal Adhesions** (FAs): multiprotein complexes, including **talin** and **zyxin** proteins.
- Integrins: are transmembrane receptors that externally bind to the ECM elements (FBLN5, fibrillin-1, collagen...) and internally to the FAs.

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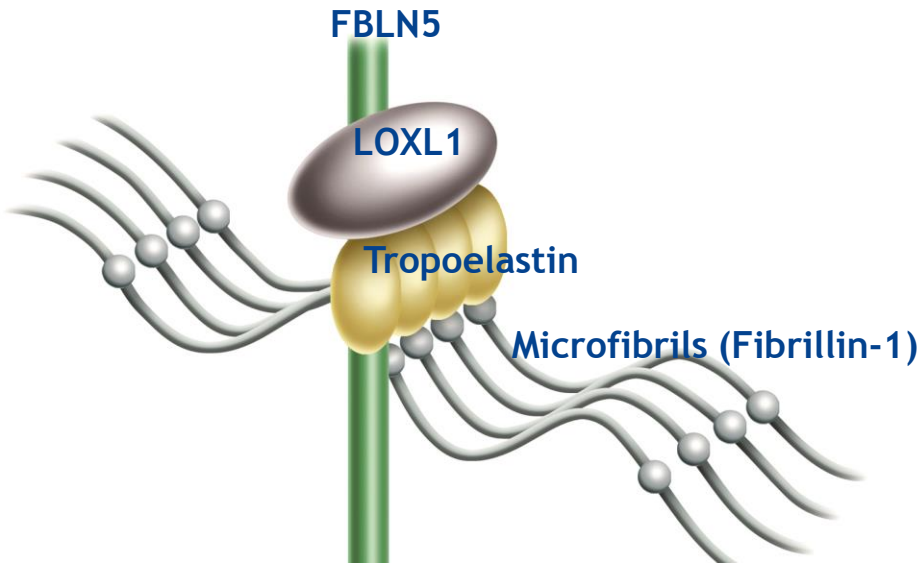
ELASTIN FIBRE FORMATION, FBLN5 AND LOXL1 ROLE

Specific compounds are needed for the last step of this insoluble fibre formation:



- Fibroblasts form TE monomers (elastin precursor), which assemble and aggregate by coacervation: more concentrated and aligned.
- **FBLN5** is an essential glycoprotein for the correct assembly of elastin fibres. It is a bridge molecule that binds to TE, LOXL1, fibrillin-1 and integrins.
- **LOXL1** is an ECM enzyme that catalyses the **union between two adjacent TE** molecules, ensuring spatially defined deposition of elastin and originating the mature **insoluble elastin fibre**.

FBLN5 and LOXL1 become indispensable for an adequate elastin fibre assembly

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COLLAGEN FIBRES FIRMING FUNCTION

Collagen is a well-known key protein for skin cohesion; several types are involved in maintaining skin elements united:

- **Type I**: most abundant protein in the ECM, whose fibres offer a platform for cell attachment and macromolecules anchorage.
- **Type IV:** non-fibrillar protein located in the basement membrane, serving as structural barrier and substrate for cellular interactions. It can bind to type I and VI collagen, forming supramolecular networks that increase cohesion.
- **Type VI**: assembled into microfibrils that function as essential structural elements.
- **Type XIV**: localised near collagen fibrils, regulates fibrillogenesis and it is linked to cellular adhesions and type I collagen.

The collagen family is crucial to maintain skin firmness

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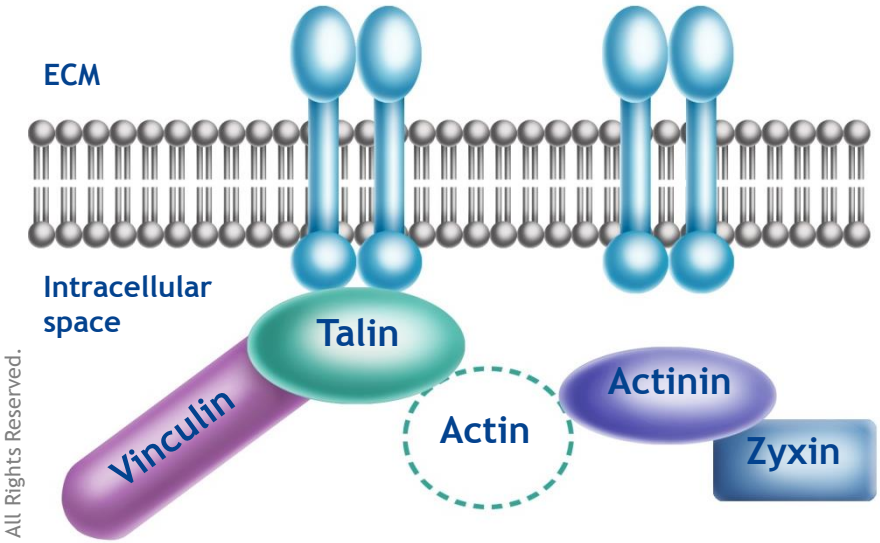
FOCAL ADHESIONS AND SKIN UNION

Inside the cells, integrin receptors are able to attach to FAs that act as interface between the ECM and the cytoskeleton of actin. Talin and zyxin proteins have key roles in this process.



- **Talin**: rapidly accumulates in focal contacts, upon cellular adhesion. It binds to integrins, vinculin and actin.
- **Vinculin**: protein that stabilises cell-cell and cell-matrix junctions.
- **Actinin**: necessary protein for the binding of actin filaments to the membrane.
- **Actin**: essential in cellular movement, contraction and shape maintenance.
- **Zyxin:** facilitates the actin filament assembly, cytoskeleton organisation and gene expression changes induced by adhesions.

Tensile and mechanical forces enhance the recruitment of zyxin, the talin-vinculin union and FA assembly, reinforcing the global binding function of the FAs

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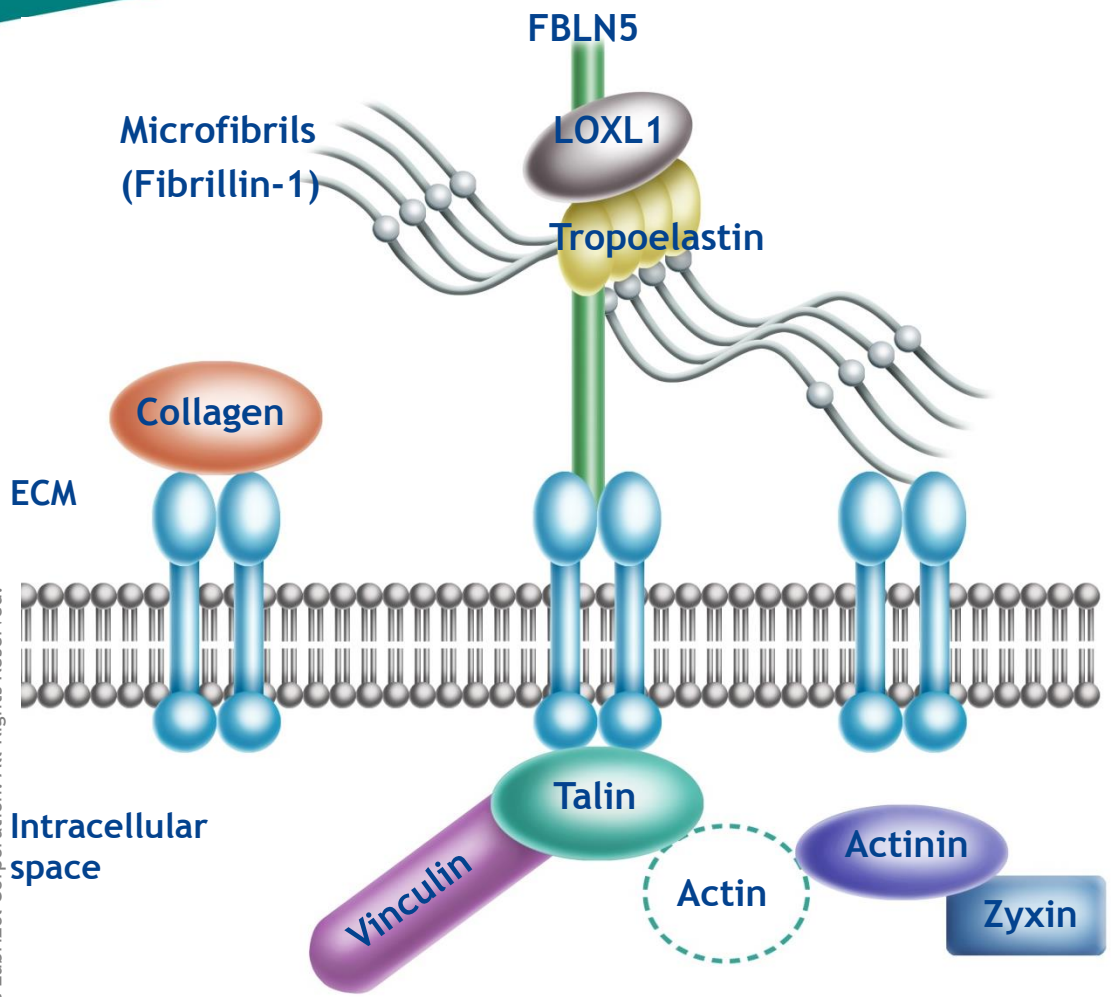
COHESIVE FINAL EFFECT



- Elastin fibres are linked to the cell by FBLN5 and fibrillin-1, which bind to integrins.
- Collagen fibres are associated with integrins and the basement membrane.
- FAs are linked to the ECM elements via integrins as well.

FBLN5, LOXL1, collagen fibres and FAs (talin and zyxin) are key compounds for skin firmness and resistance

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COUNTERACTING THE FORCE OF GRAVITY

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Tetrapeptide designed to fight the undesired effects of a lack of skin firmness and cohesion by enhancing the natural elements that maintain collagen levels and elastin fibres correctly assembled, and facilitating the union between cells and the ECM.

- Activates the FBLN5 and LOXL1 promoters and increases both protein levels *in vitro*: necessary for a correct assembly of elastin fibres.
- Increases elastin synthesis *in vitro*.
- Upregulates genes related to FAs and collagen synthesis, highly inducing type I collagen synthesis too.
- *In vivo*, UPLEVITY™ *peptide:*
  - Reduced specific parameters linked to skin flaccidity and dermal disorganization.
  - Enhanced the general elasticity and firmness of the skin and reshaped the face contour, providing a lifting effect.

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UPLEVITY™ *peptide* EFFICACY

IN VITRO EFFICACY

- Activation of FBLN5 and LOXL1 promoters
- Increase of FBLN5 and LOXL1 protein levels
- Microarray analysis
- Elastin induction
- Type I collagen induction

IN VIVO EFFICACY

- Increase of firmness
- Restructuration of the dermis
- Tensor efficacy

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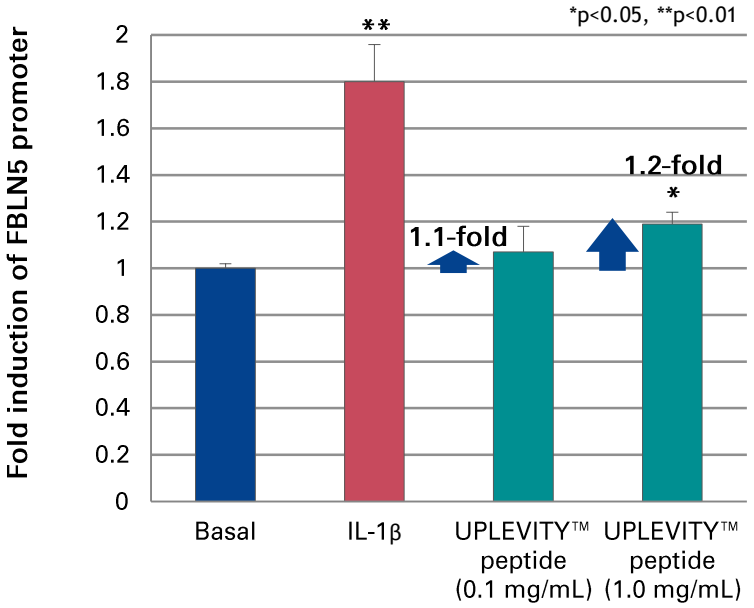
ACTIVATION OF FBLN5 AND LOXL1 PROMOTERS (I)

- Double-transfected human epithelial FBLN5/LOXL1-reporter cells expressing 2 luciferase genes upon activation of FBLN5 and LOXL1 promoters respectively were used to confirm their activation.
- Cells were incubated with medium for 6 h and treated with IL-1β (10 ng/mL, positive control) or UPLEVITY™ *peptide*, incubating them for 16-24 h. Then, luciferase substrates were added to quantify the reactions with their respective luciferases using a multiplate luminometer.

A different set of plates was stained with crystal violet to normalise luciferase units.
Cells only treated with medium were used as a basal negative control.

The tetrapeptide **activated** the **FBLN5 promoter** up to **1.2-fold** compared to cells only cultured with medium.

UPLEVITY™ *peptide* is able to activate the FBLN5 promoter



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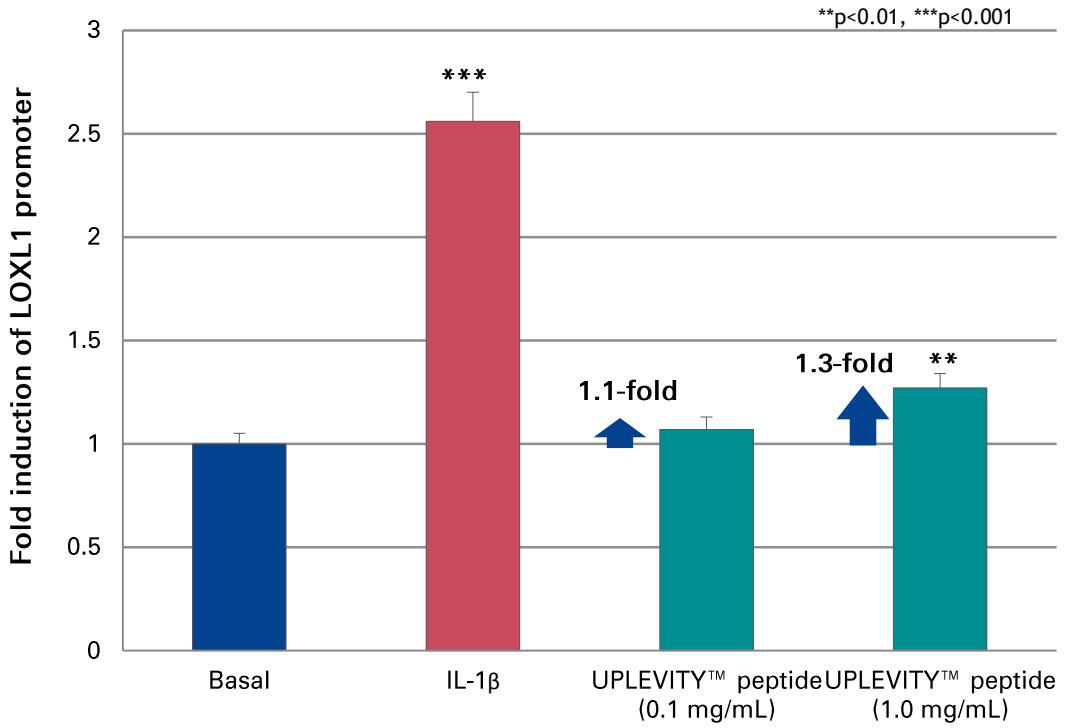
ACTIVATION OF FBLN5 AND LOXL1 PROMOTERS (II)

As the values confirmed, the ingredient **increased** the activity of the **LOXL1 promoter** up to **1.3 times** versus the cells only cultured with medium.

UPLEVITY™ *peptide* activates the LOXL1 promoter



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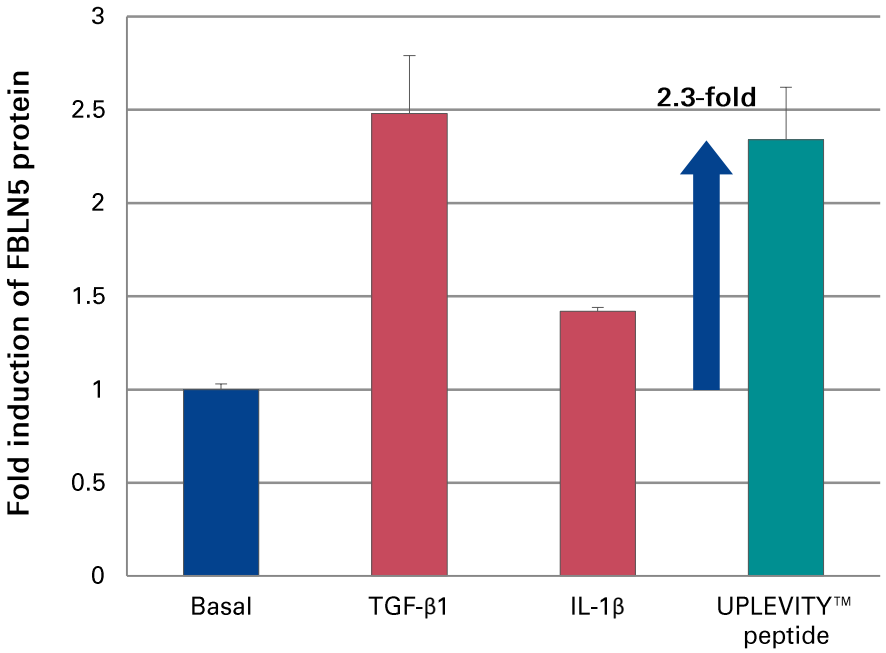
INCREASE OF FBLN5 AND LOXL1 PROTEIN LEVELS (I)

- Human Dermal Fibroblasts (HDFa) were seeded with medium and incubated for 48 h with TGF-β1 (5 ng/mL, positive control), IL-1β (20 ng/mL, positive control) or UPLEVITY™ *peptide* (0.5 mg/mL).
- Then, an immunocytochemistry assay with fluorescent antibodies was performed to detect FBLN5 and LOXL1 proteins. Images were taken and values of Integrated Optical Density were quantified.

Cells incubated only with medium were used as basal negative control.

The active peptide **multiplied FBLN5 protein** levels by **2.3-fold** compared to cells only treated with medium.



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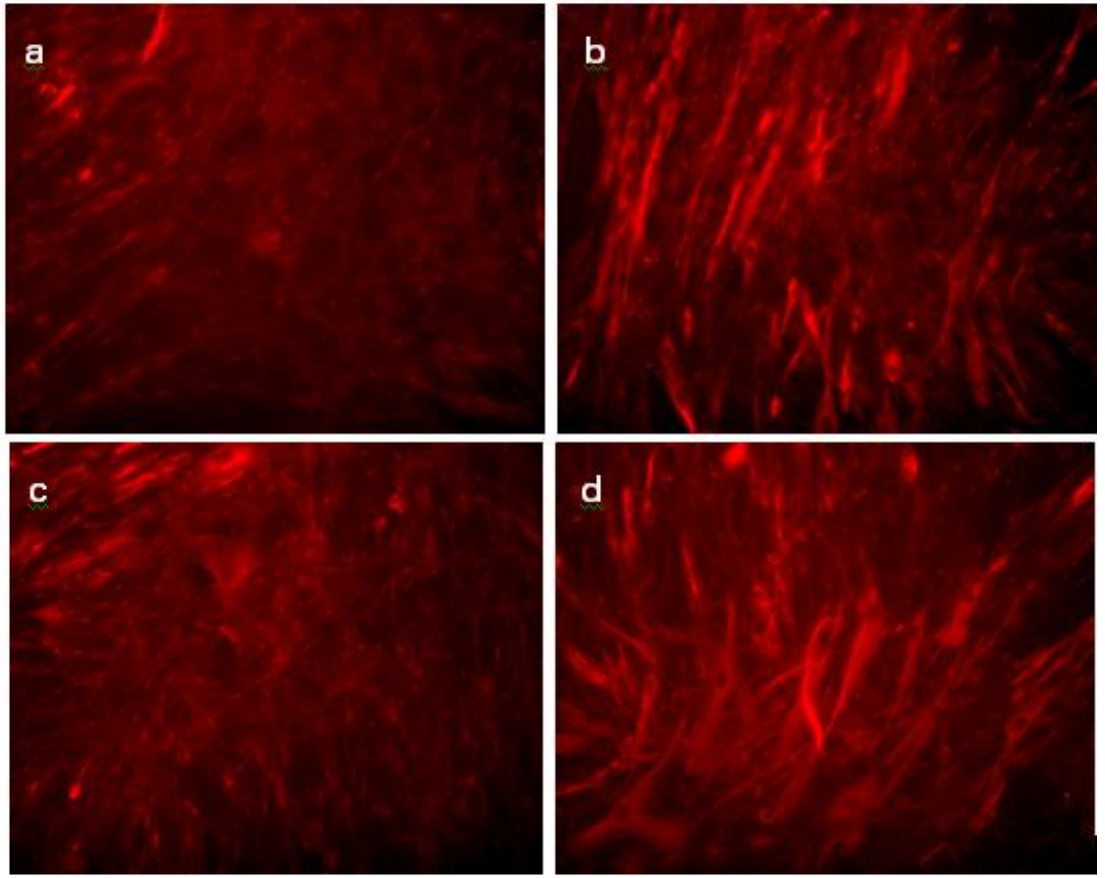
INCREASE OF FBLN5 AND LOXL1 PROTEIN LEVELS (II)

UPLEVITY™ *peptide* is able to raise FBLN5 protein levels



Expression of FBLN5 protein in HDFa: a) Basal conditions, b) TGF-β1, c) IL-1β, d) UPLEVITY™ *peptide*.

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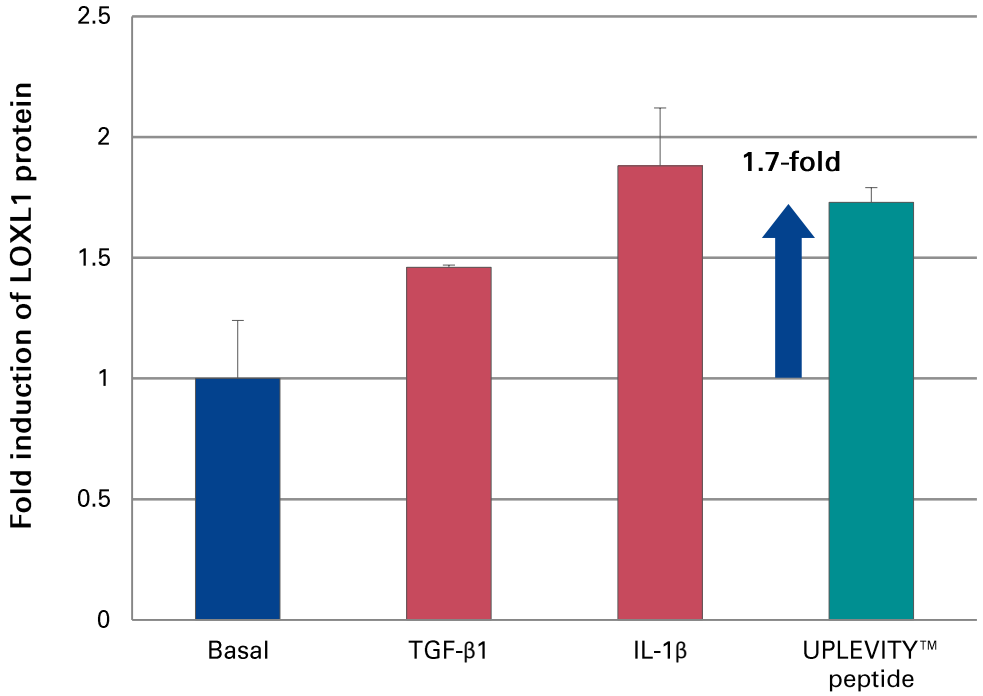
INCREASE OF FBLN5 AND LOXL1 PROTEIN LEVELS (III)

The tetrapeptide **augmented** the expression of **LOXL1 protein** by **1.7-fold** versus the cells only cultured with medium.



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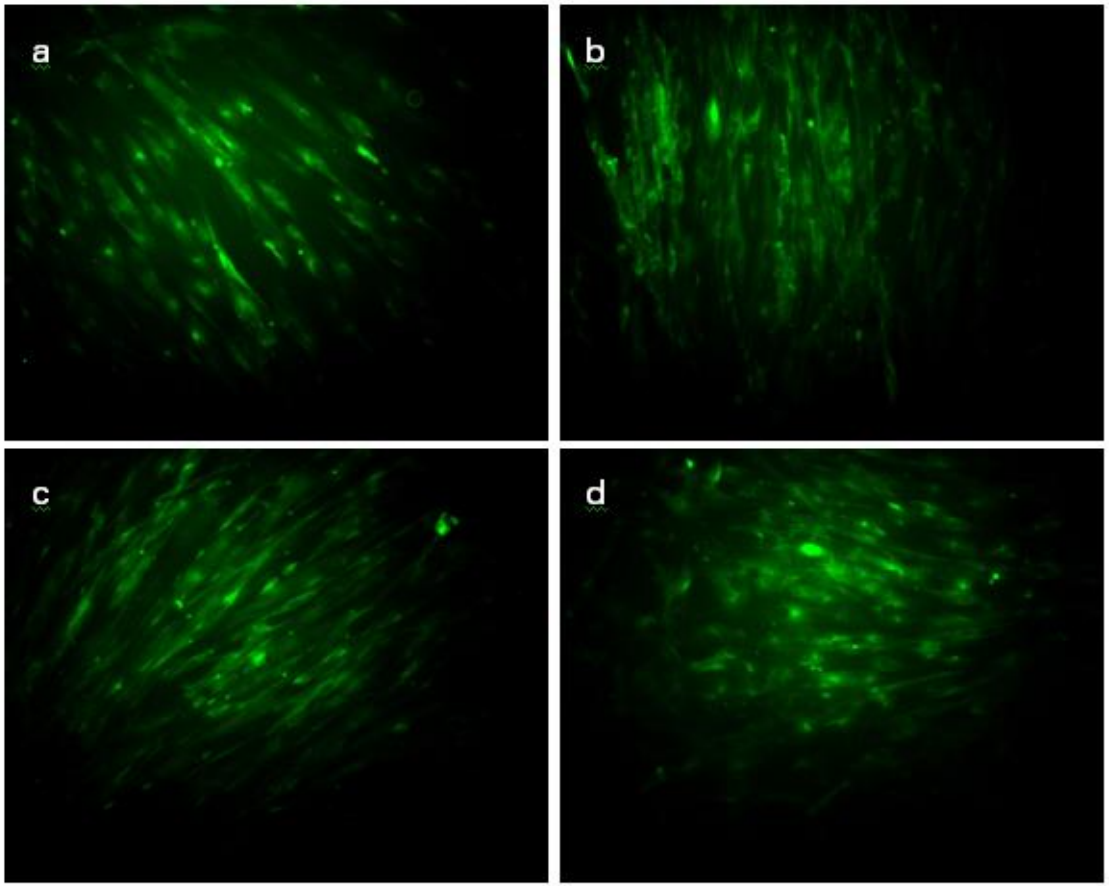
INCREASE OF FBLN5 AND LOXL1 PROTEIN LEVELS (IV)

UPLEVITY™ *peptide* increases LOXL1 protein levels



Expression of LOXL1 protein in HDFa: a) Basal conditions, b) TGF-β1, c) IL-1β, d) UPLEVITY™ *peptide*.

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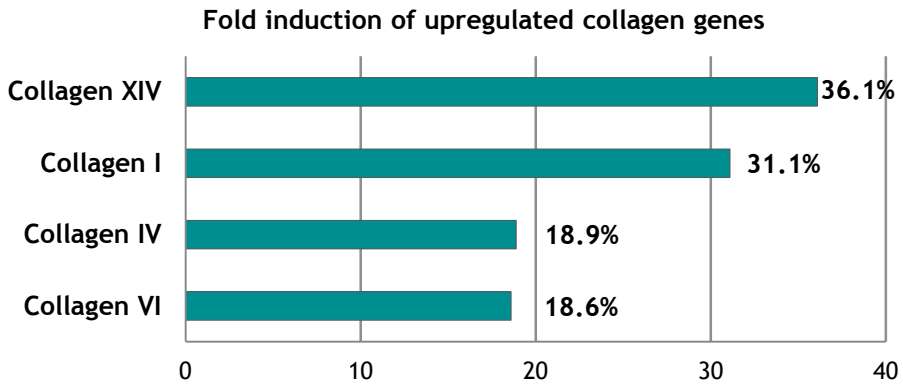
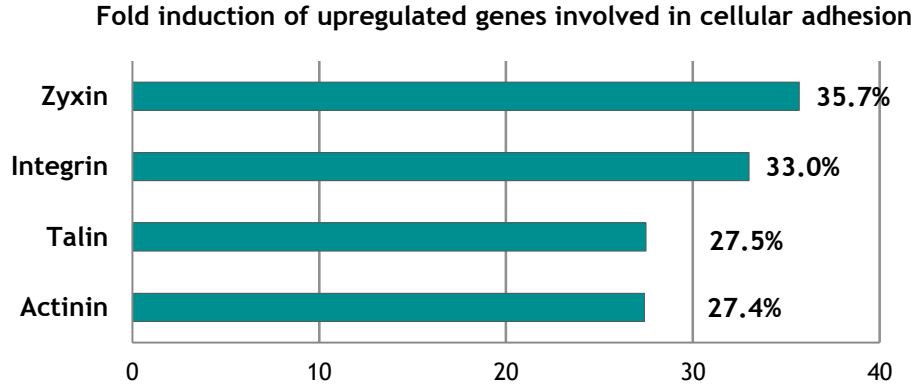
MICROARRAY ANALYSIS

- Microarray analysis (using an ASurePrint G3 Human Gene Expression Microarray v2) was used to detect the genes that were upregulated in HDFa in presence of UPLEVITY™ *peptide*.
- After seeding cells, they were incubated with the peptide (0.05 mg/mL) in supplemented medium for 24 h and lysed to obtain RNA samples. After verifying sample quality and normalising, microarray data determined the genes with differential expression.





UPLEVITY™ *peptide* upregulates the expression of genes involved in collagen synthesis and focal adhesions, all of them implied in skin firmness

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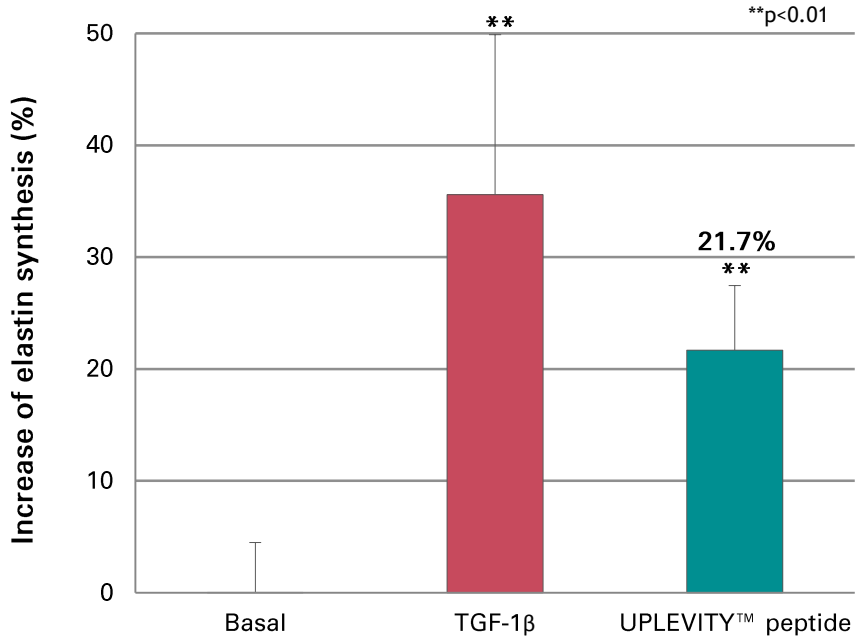
ELASTIN INDUCTION

- HDFa were grown in medium with specific growth factors, seeded and incubated for 72 h. Then, fresh medium containing TGF-1β (10 ng/mL, positive control) or UPLEVITY™ *peptide* (0.1 mg/mL) was added and cells were incubated 48 h more.
- Finally, elastin was extracted and the Fastin Elastin Assay was performed determining its levels using elastin standards previously prepared. Absorbance was read at 540 nm in a microtiter plate reader.

Non-treated cells were used as basal control.



The active ingredient **increased elastin synthesis** by **21.7%** compared to non-treated cells.

UPLEVITY™ *peptide* caused a statistically significant raise of elastin synthesis

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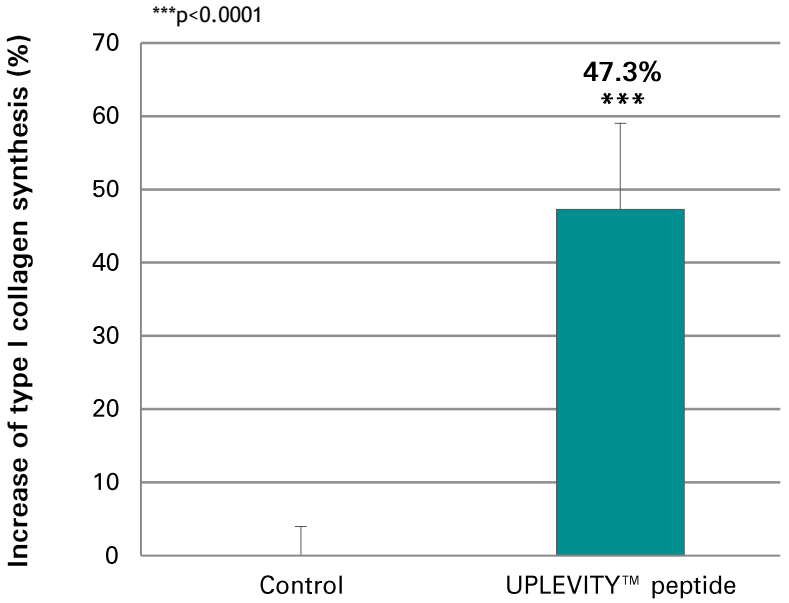
TYPE I COLLAGEN INDUCTION

- HDFa were grown in medium with specific growth factors, seeded and incubated for 24 h. Then, fresh medium containing UPLEVITY™ *peptide* (0.01 µg/mL) was added and cells were incubated 48 h more.
- Finally, well medium was collected and analysed by an ELISA. Absorbance was read at 490 nm in a microtiter plate reader and collagen concentration was determined using a type I collagen standard curve.

Non-treated cells were used as control.

The active ingredient showed to **induce type I collagen synthesis** by **47.3%** versus non-treated cells.

UPLEVITY™ *peptide* provided a clear statistically significant induction of type I collagen synthesis



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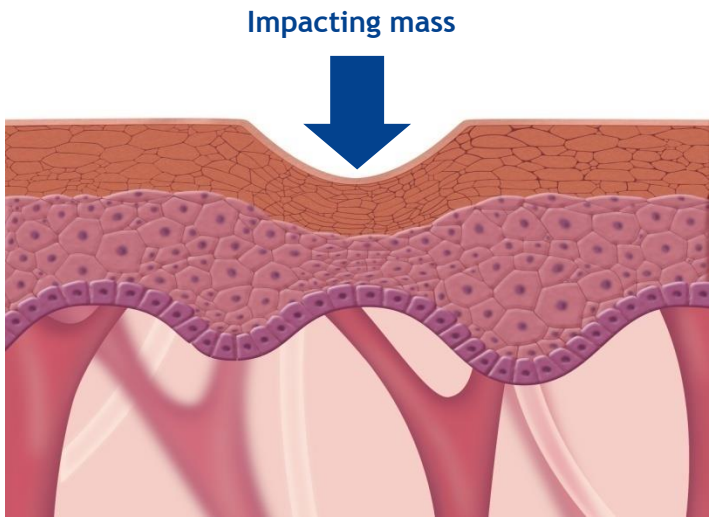
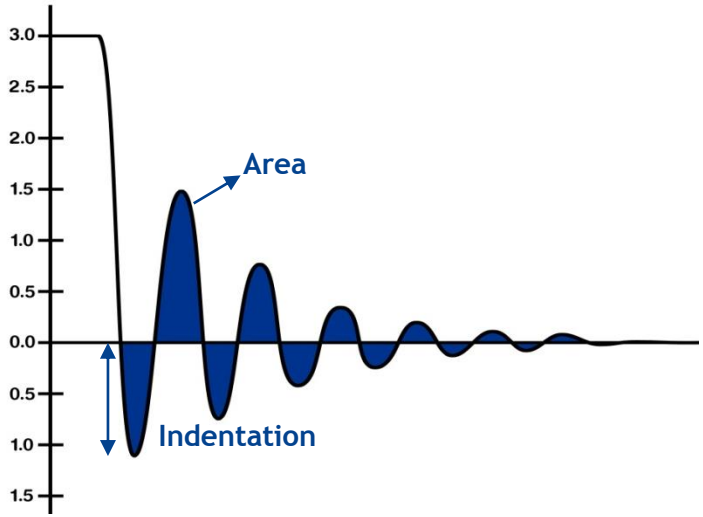
INCREASE OF FIRMNESS (I)

- A panel of 19 female volunteers (50-60 years old) presenting saggy facial skin applied an emulsion containing 2% UPLEVITY™ *peptide solution* on the face twice a day for 55 days.
- Ballistometry was used to measure the mechanical properties of the skin before and after the treatment by analysing the interactions between an impacting mass and the skin surface (cheek), once the induced vibrational movements were converted into electrical signals.
- The indentation (mm) and the area (mm^2) were the studied parameters: the higher the indentation or area values, the higher the skin flaccidity.





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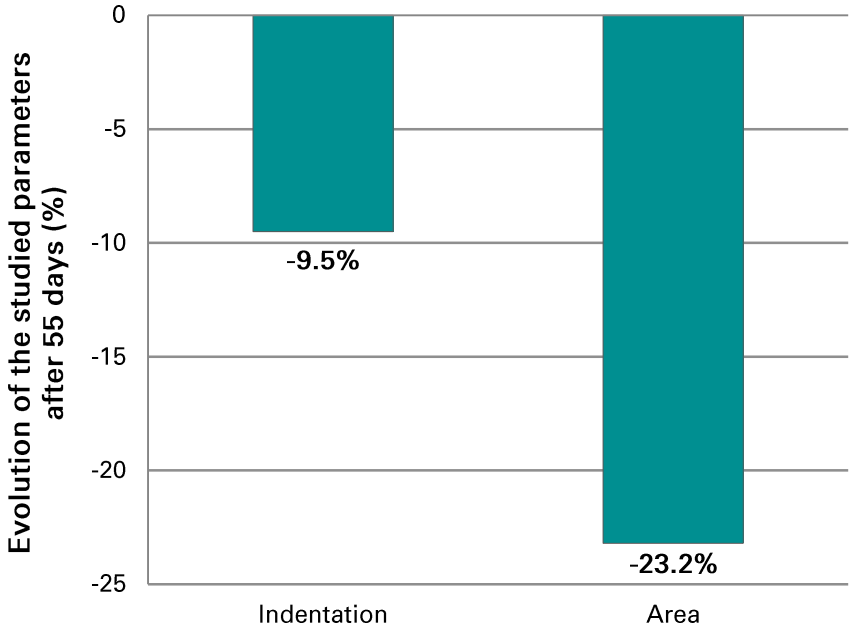
INCREASE OF FIRMNESS (II)

The peptide offered a statistically significant **diminution** ($p \leq 0.05$) of the indentation and area (**-9.5% and -23.2%** respectively), **ameliorating skin cohesion** at the end of the treatment versus the initial time.

UPLEVITY™ *peptide* improves skin firmness after 55 days



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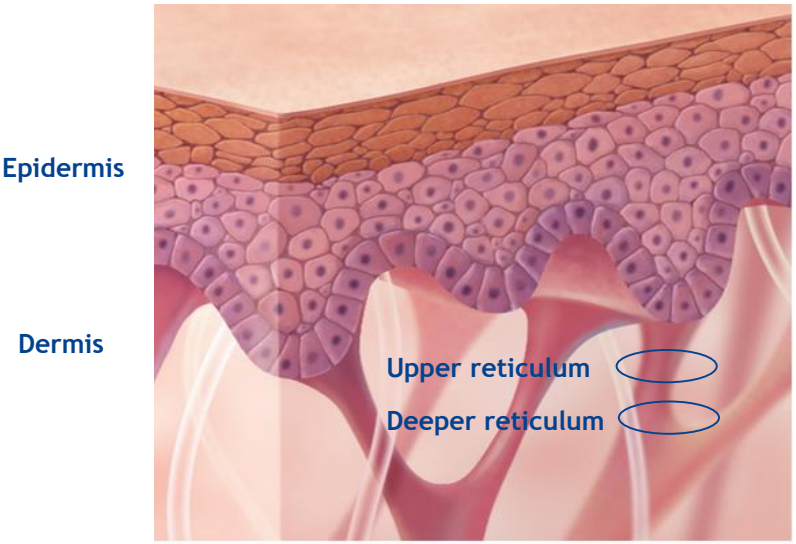
RESTRUCTURATION OF THE DERMIS (I)

- To observe the efficacy in the dermal organisation, 19 volunteers (50-60 years old) presenting saggy facial skin applied a placebo emulsion on half of the face and an emulsion containing 2% UPLEVITY™ *peptide solution* twice a day for 55 days.
- The *in vivo* confocal microscopy was used to quantify the tissular structure of the dermis at two levels: the most superficial part of the reticular dermis (upper reticulum) and 18 µm deeper (deeper reticulum). Two stacks were acquired of each cheek to analyse the fragmentation rate of both reticulums before and after the treatment.
- A VivaScope® was used to carry out the acquisitions and specific software to analyse the digital images, characterising and quantifying the fibres network.

The decrease of both fragmentation rates implies an improvement of the reticular dermis and skin organisation.



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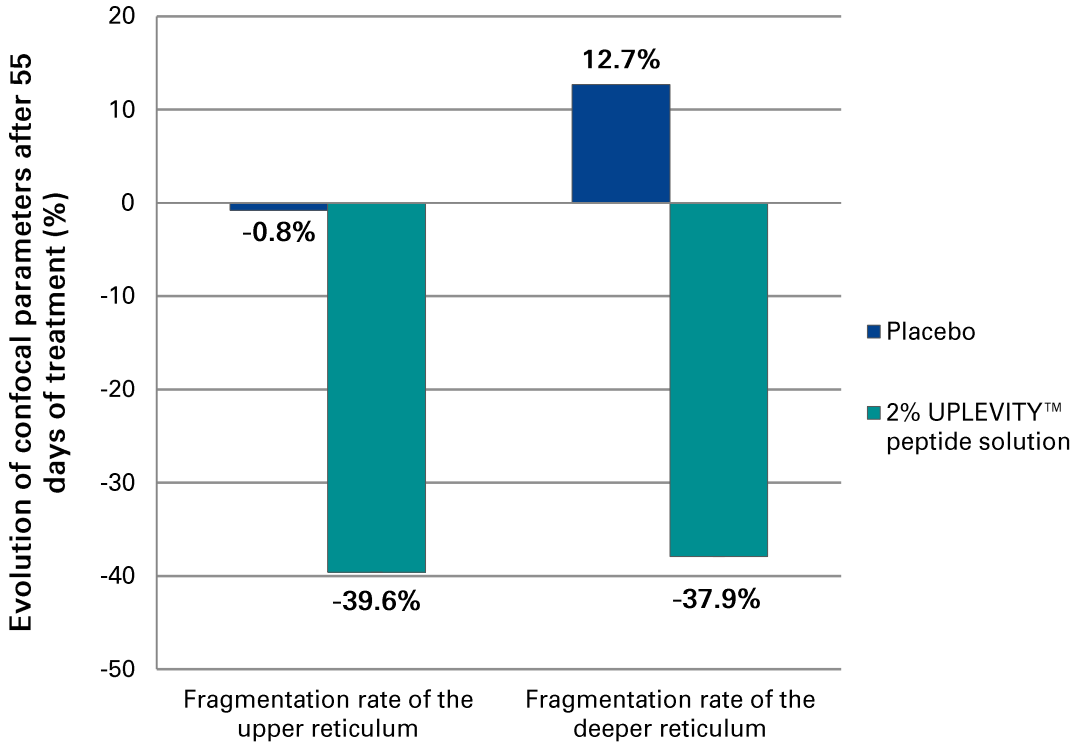
RESTRUCTURATION OF THE DERMIS (II)

The active treatment caused a **statistically significant reduction** of both parameters (**-39.6%** and **-37.9%**), while placebo did not. The difference between the effect of the placebo and active treatment was also statistically significant for both parameters ($p \leq 0.05$).

UPLEVITY™ *peptide* ameliorates skin inner cohesion



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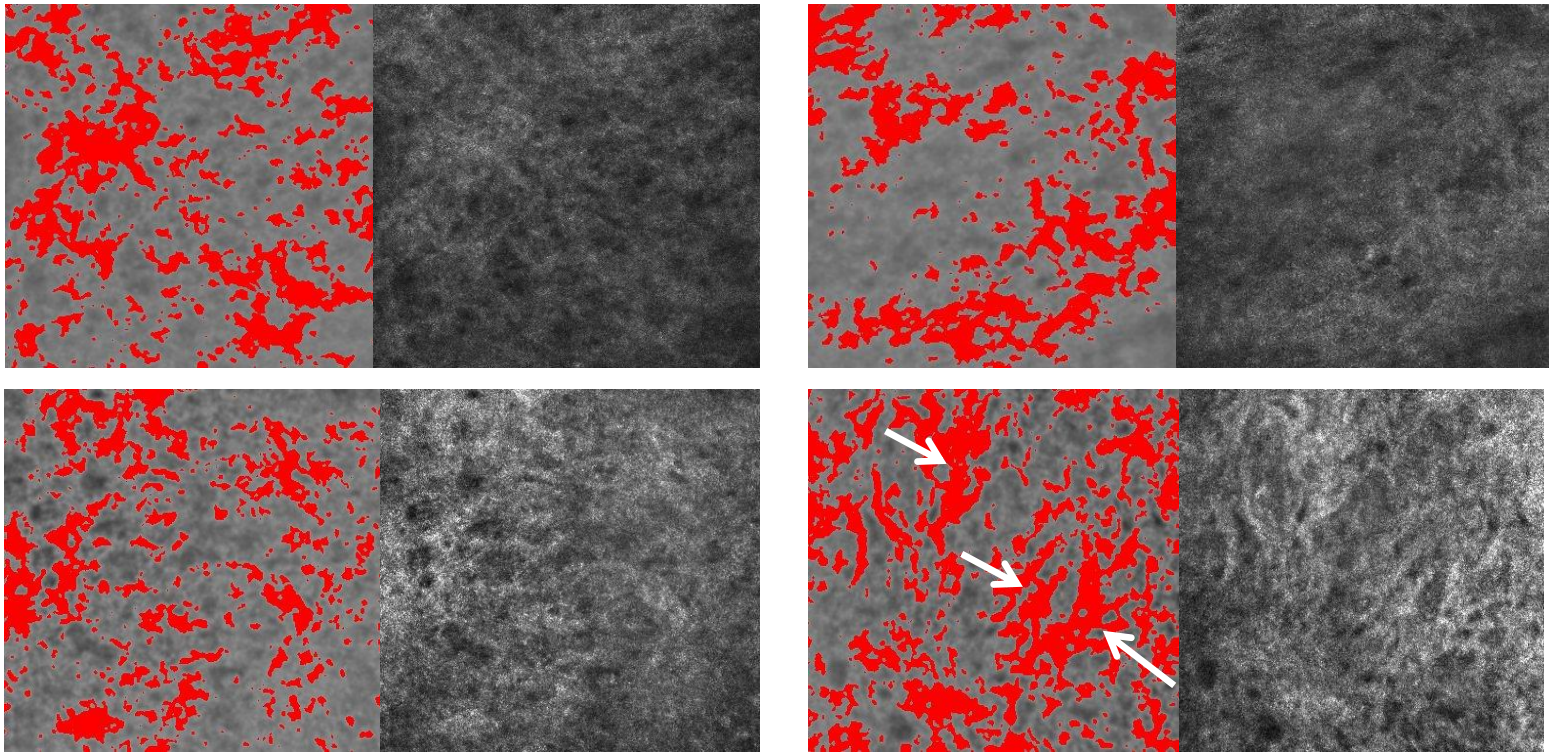
RESTRUCTURATION OF THE DERMIS (III)

Placebo

UPLEVITY™ *peptide solution*



Processed and non-processed images (from the upper reticulum) of a volunteer before (2 left images) and after the treatments (2 right images), highlighting the fibres network.

After the active treatment, larger red "objects" were noticeable, due to a minor fragmentation of the fibres network.

UPLEVITY™ *peptide* restructures the dermis, improving the fibres network

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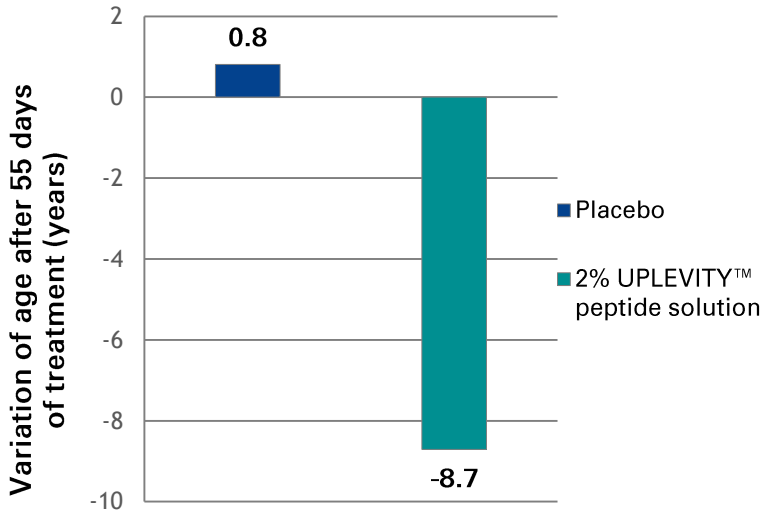
RESTRUCTURATION OF THE DERMIS (IV)

- **AGE CORRELATION**
  - The results of fragmentation rate were used to calculate the equivalent age of the volunteers' skin.
  - This calculation was based on a linear statistical correlation between the real age of the subject and the level of their fragmentation rate.



UPLEVITY™ *peptide* rejuvenated the estimated age of the skin by almost 9 years

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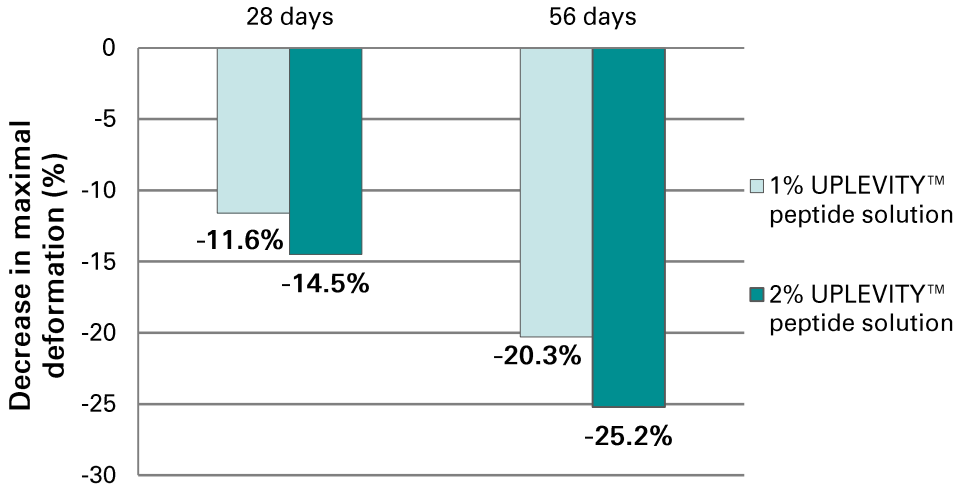
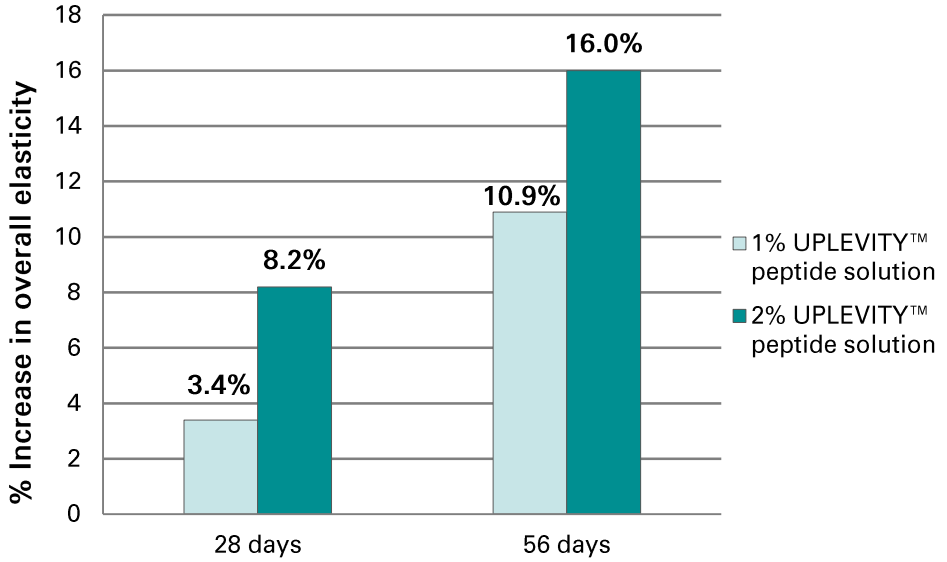
TENSOR EFFECT (I)

- The elasticity of the skin was measured with a cutometer through the parameters maximal deformation (RO) and overall elasticity of the skin (R2).
- A panel of 40 female volunteers (40-60 years old) with signs of sagging skin and loss of elasticity on the face was divided into two different groups, the first one applied a cream with 1% UPLEVITY™ *peptide solution*, while the second, used a cream with 2% UPLEVITY™ *peptide solution*, twice a day for 56 days.





UPLEVITY™ *peptide* improves skin firmness and enhances the general elasticity of the skin

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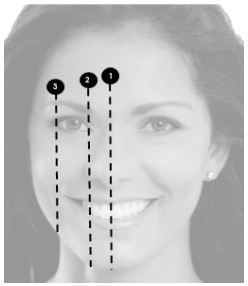
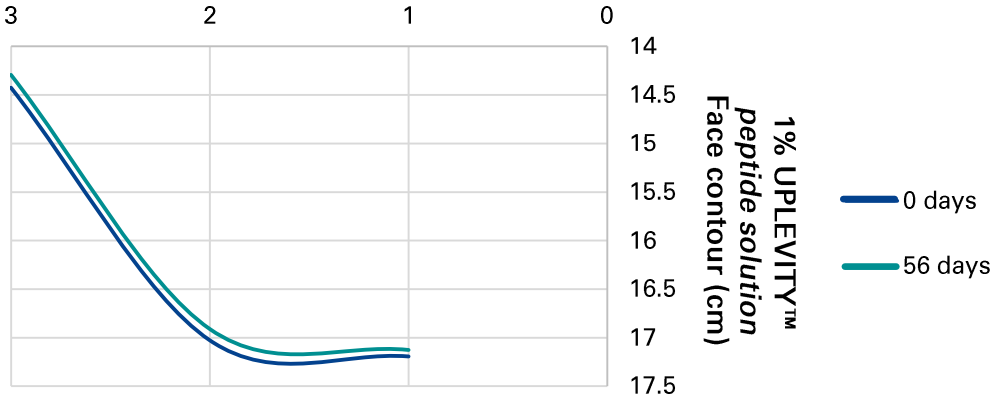
TENSOR EFFECT (II)

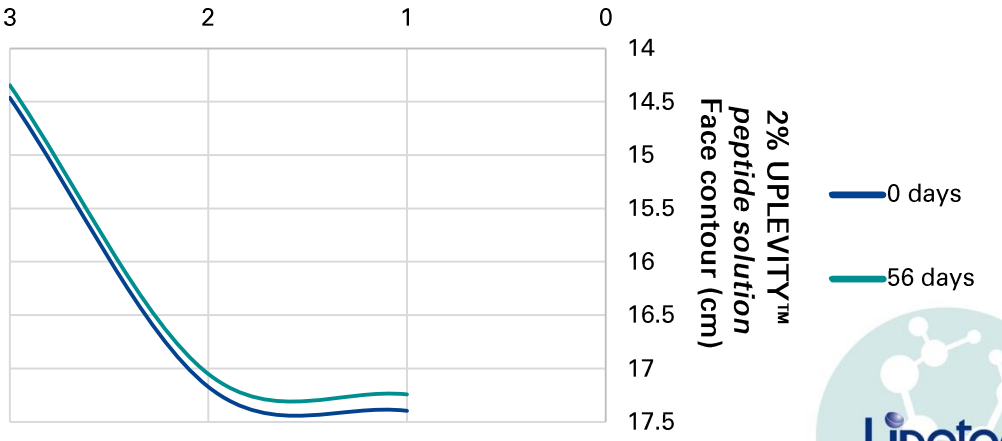
- The tensor and reshaping effect was evaluated on the face by a morphometric image analysis. This effect was measured by taking three different positions of the face into account.





UPLEVITY™ *peptide* provides a lifting effect

A **decrease** of **0.10 cm** and **0.13 cm** was observed after 56 days for the 1% and 2% UPLEVITY™ *peptide solution* respectively.



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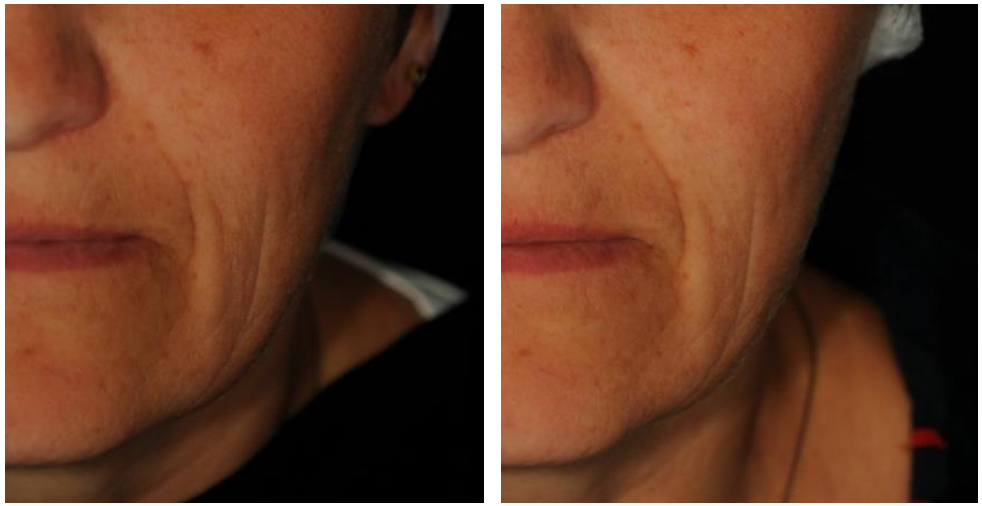
TENSOR EFFECT (III)

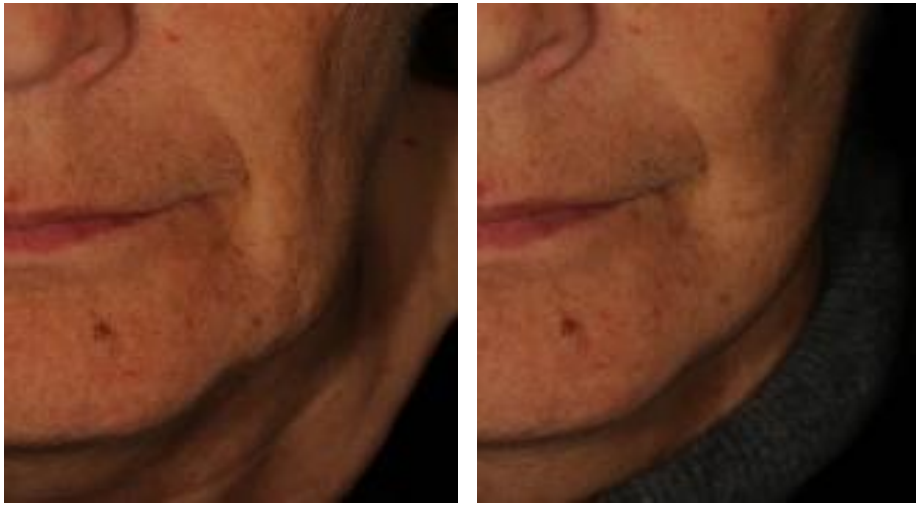
- Digital pictures of two different volunteers.

0 days | 56 days



1% UPLEVITY™
peptide solution

0 days | 56 days



2% UPLEVITY™
peptide solution

UPLEVITY™ *peptide solution* visibly reshapes the contour of the face

uplevity™ peptide

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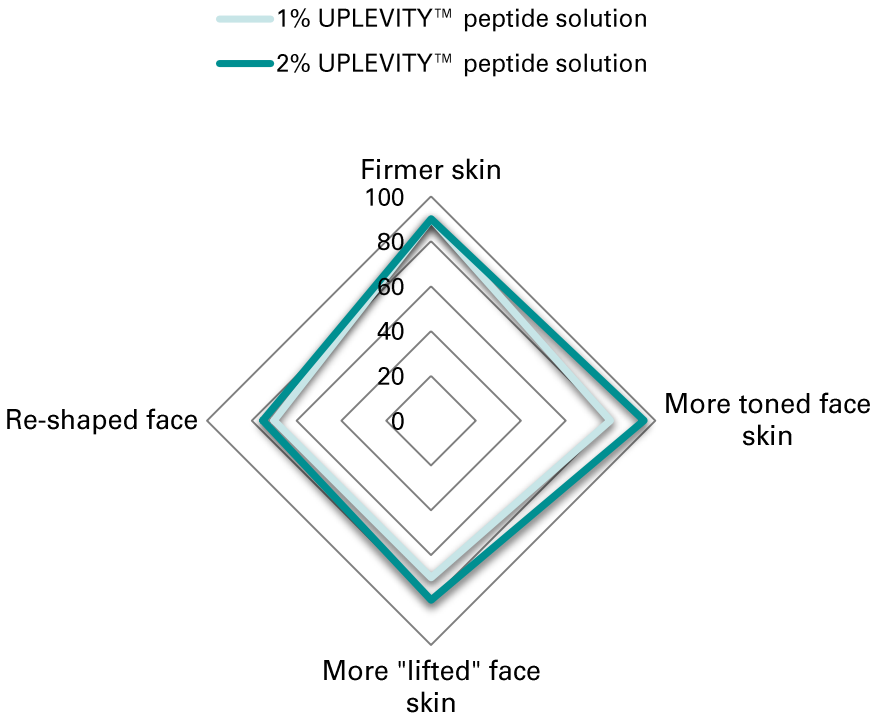
SELF ASSESSMENT

- A self-assessment regarding the efficacy of the product was filled out by the volunteers and an analysis of their opinion was made.



✓ **95%** of volunteers noticed a **more toned skin** after using the cream with 2% UPLEVITY™ *peptide solution*.

✓ **90%** believed that the 2% treatment offered a **firming effect**.

uplevity™ peptide

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CONCLUSIONS

uplevity™ peptide

CFDA listed

- **augments FBLN5 and LOXL1 protein** levels, which help elastin fibres to be assembled accurately.
- **upregulates** the **expression of genes involved** in collagen synthesis and FAs, all of them beneficial elements that improve **skin cohesion**.
- provided a statistically significant **induction** of **elastin synthesis** (**21.7%**), protein directly linked to skin elasticity and recoil.
- **increased type I collagen synthesis** by **47.3%** (statistically significant value), helping to improve skin firmness.
- **improved skin cohesion** and **firmness**, as *in vivo* it reduced the indentation and area parameters and it decreased the fragmentation of the fibres network. Calculations extrapolate a **decrease** in the **apparent age** of the volunteers of **almost 9 years**.
- **enhanced** the **general elasticity** of the skin and **improved** skin **firmness**, as at 2% reduced maximal deformation (-25.2%) and increased overall elasticity (16%), after 56 days.
- assisted in visibly **reshaping** the **face contour**, providing a **lifting** effect.

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TECHNICAL INFORMATION

DESCRIPTION

Tetrapeptide that fights sagginess by enhancing fundamental elements like collagen and elastin, and key proteins such as fibulin 5 (FBLN5) and lysyl oxydase-like 1 (LOXL1) for the assembly of functional elastin. UPLEVITY™ *peptide* further contributes to skin firmness by overexpressing genes involved in cellular cohesion due to focal adhesions (FAs), that are the interface between the actin cytoskeleton and extracellular matrix (ECM).

APPEARANCE

Translucent solution containing 0.05% Acetyl Tetrapeptide-2.

INCI

Water (Aqua), Acetyl Tetrapeptide-2, Caprylyl Glycol.
Preservative free.

PROPERTIES

UPLEVITY™ *peptide* counteracts the effects of the force of gravity by increasing collagen and functional elastin synthesis, and cellular support. It provides a better structure to the dermis improving the quality of mature skin.

APPLICATIONS

UPLEVITY™ *peptide* can be incorporated into cosmetic formulations and any treatment to fight sagginess and loss of firmness, especially in mature skin.

DOSAGE 1-2%

pH Recommended pH range between 5.0 and 8.0.

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COUNTERACTING THE FORCE OF GRAVITY

Note: Graphs and photographs of efficacy tests are available for customer use provided that the final product contains the same concentration of active as the formulations in our tests. Customers must request written permission for use of the graphic material and/or ingredient tradenames to Lubrizol. Customers are responsible for compliance with local and international advertising regulations.

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