



KARISMA®

Rh Collagen

Product Monograph

Bio-restorative filler with a synergistic formula

Collagen R-polypeptide $\alpha 1$ chain

Carboxymethylcellulose

High molecular weight Hyaluronic Acid



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KARISMA FACE Rh Collagen® is a bio-restorative soft filler that thanks to a novel patented natural human collagen precursor (collagen polypeptidic α 1 chain R) promotes the production of type I collagen in dermal human fibroblasts.

These polypeptides, together with high molecular weight hyaluronic acid and CMC (carboxymethylcellulose), act synergistically towards a natural restoration of pre-aging conditions of the skin.

KARISMA FACE Rh Collagen® contains:

1. Collagen Polypeptidic α 1 Chain R

- The polypeptidic α 1 chain R stimulates the synthesis of collagen type I peptide in humane dermal fibroblasts.
- Polypeptidic α 1 chain R stimulates and facilitates the winding of procollagen triple helix chains.

2. High Molecular Weight Hyaluronic Acid

- Hydration and maintenance of an environment favorable to the proliferation and metabolism of fibroblasts.
- Antioxidant action.

3. Carboxymethylcellulose

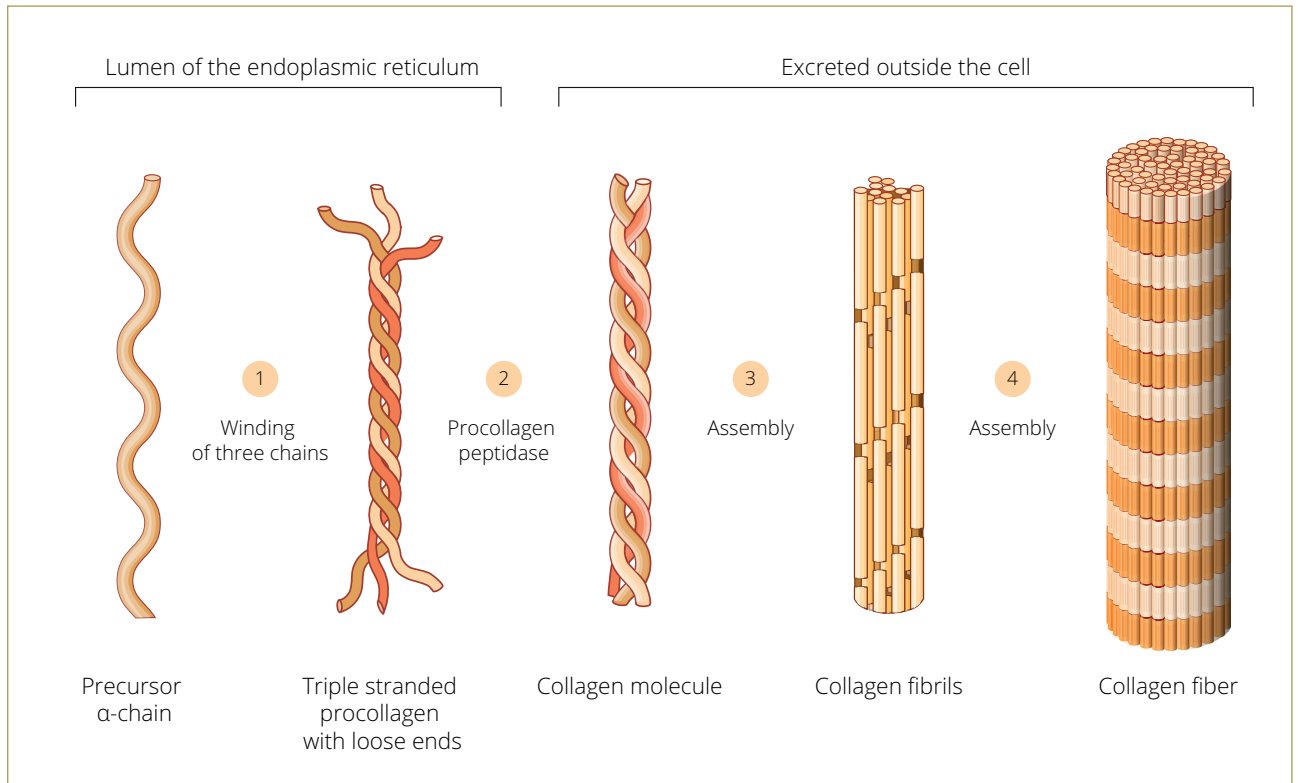
- It inhibits the action of the hyaluronidase enzyme.
- Preserves endogenous hyaluronic acid.
- Slows down the action of MMPs.



Collagen

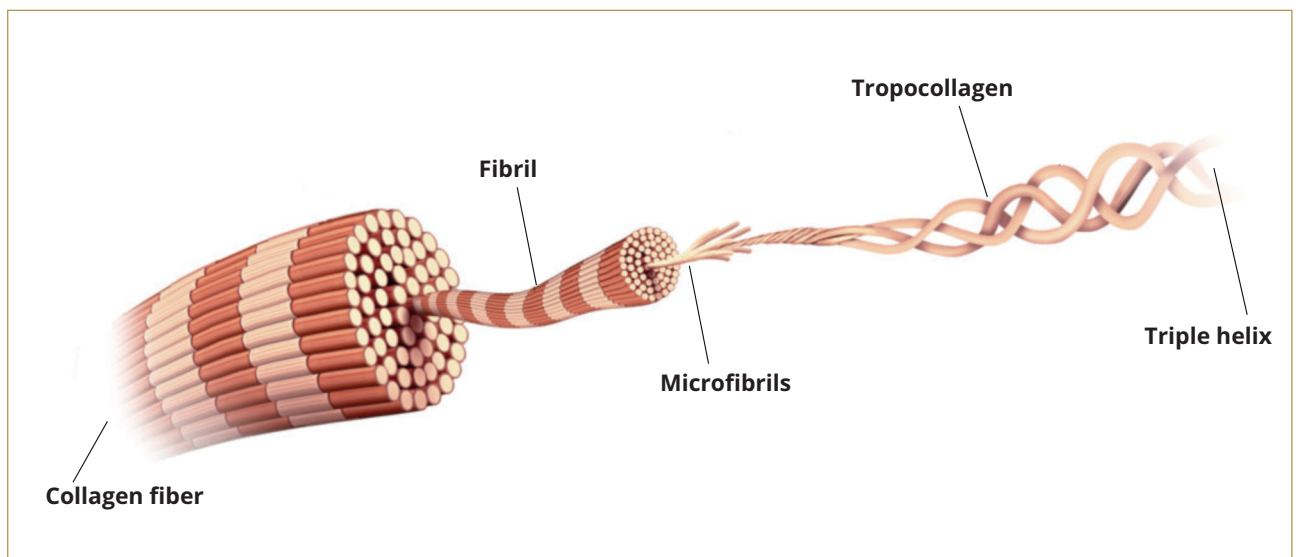
Collagen accounts for 75% of the dry weight of the dermis. Collagen type I is the most abundant form of collagen in human tissues, and is composed by 3 left-handed α chains: **two identical α -1 type I chains and one α -2 type I chain**, organized in a natural occurring triple helical structure.

Biosynthesis of collagen:

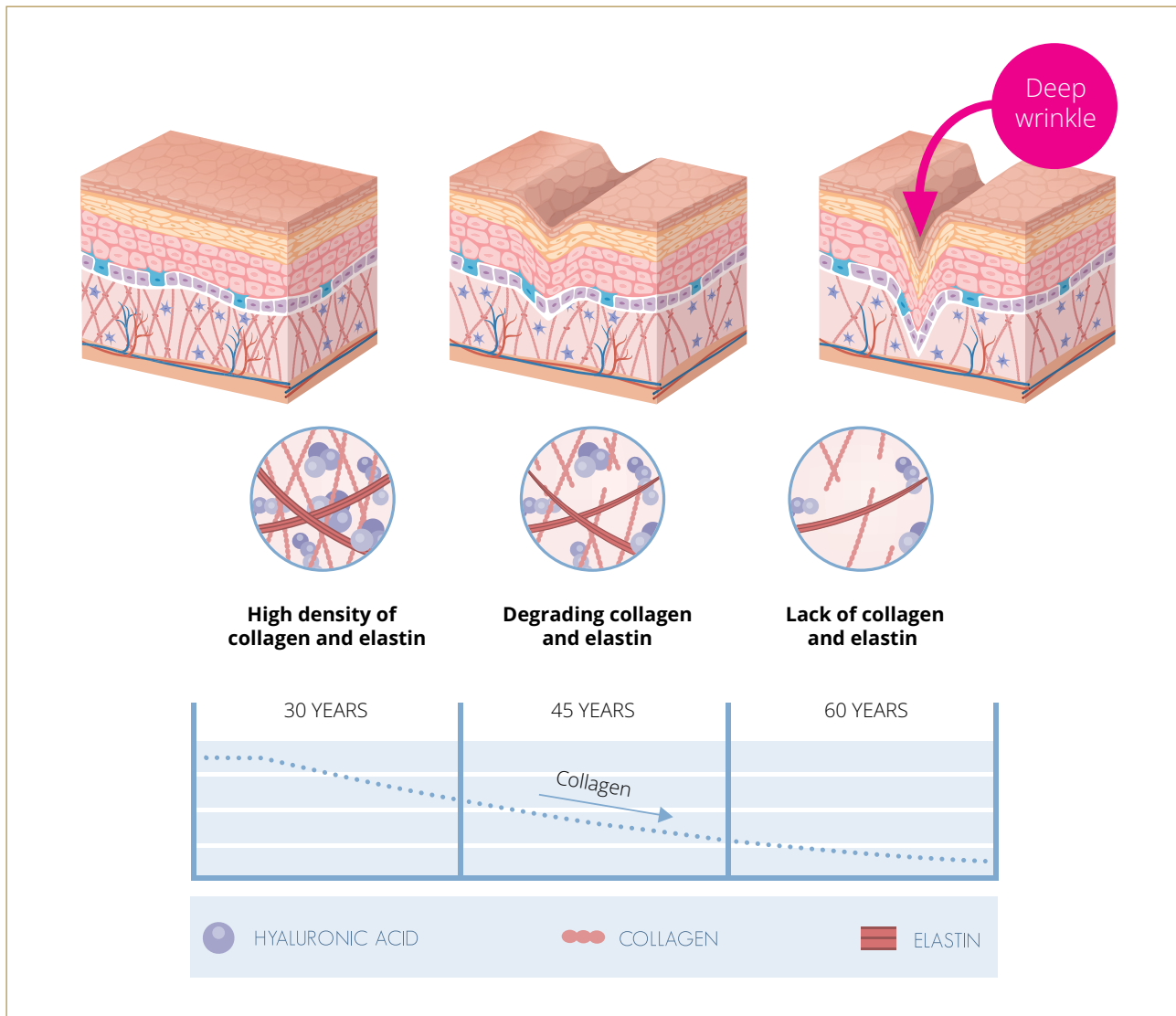


Procollagen is formed by the winding of precursor α -chain in the fibroblast (**step 1**). It is excreted outside the cell where it undergoes further modifications and becomes tropocollagen (**step 2**). Tropocollagen rearranges in parallel rows forming fibrils (**step 3**). Fibrils rearrange in wavy or parallel bundles to form proper collagen fibers (**step 4**).

Structure of collagen:



The mechanical strength of the skin is given by the naturally occurring collagen fibers. Collagen is one of the main components of the dermis: it maintains the skin's structure, tone and elasticity. In the human being, after the age of 40, collagen decreases by 1% every year. The aging process also leads to fragmentation and disorientation of the fibers, which result in qualitative variations in the collagen content, causing an increased stiffness of the skin.



The first type of collagen used for injections, was derived from bovine or human cadavers, which are species-specific and tissue-specific. Certain people could also develop allergic reactions. It may also have hidden risks of animal-derived pathogens, such as the mad cow disease.

For this reason, new clinical and therapeutic approaches, should be developed in order to induce fibroblasts to produce new endogenous type I collagen.



Hyaluronic Acid

Hyaluronic Acid is a naturally occurring molecule that represents from 0,1% to 0,3% of the dry weight of the dermis. **Hyaluronic Acid is an integral component of the extracellular matrix in most mature organisms and is found naturally within most body tissues, including the skin.**

Hyaluronic acid is a natural linear polysaccharide polymer consisting of repeated di-glucan units of N-acetyl-D-glucosaminoglucose and D-glucuronic acid linked by β (1, 4) as well as β (1, 3) glycosidic bonds. It differs from other glycosamino-glycans as it lacks sulfated groups and covalently linked peptides.

Hyaluronic Acid may undergo volume changes due to natural decomposition processes, it has a relatively long shelf-life and it also **attracts moisture into the skin.**

It can also be used to fill scars caused by trauma and surgery, asymmetries resulting from congenital defects, and pits from acne scars.

The therapeutic effect of hyaluronic acid-based preparations directly depends on the molecular weight of this biopolymer.

Hyaluronic acid, produced in high quantities in the extracellular matrix - generating an optimal microenvironment - stimulates the increase, proliferation and migration of fibroblasts, endothelial cells, keratinocytes and promotes neo-angiogenesis, providing a fundamental contribution to the formation of the extracellular matrix, accelerating the regeneration processes.

Hyaluronic Acid:

- Determines tissue hydration.
- Orients the collagen fibers.
- Regulates cell synthesis and differentiation.
- Binds to inflammatory cytokines.

Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a **biosynthetic substance** used as an excipient for many drugs, as a component of adhesion membranes in abdominal surgery and as a carrier in many dermal fillers.

The non-bacterial and non-animal origin of CMC and its anti-inflammatory activity gives this substance unique properties.

CMC has an anti-aging effect in the dermis: it inhibits the action of the hyaluronidase enzyme and preserves the endogenous hyaluronic acid, which, while remaining in the tissues, continues to perform its hydration and tissue repair activity in depth.

CMC also **slows down the action of MMPs** (Matrix Metallo-Proteinases), which are responsible for collagen type I degradation and unwinding of the triple helix chains.



KARISMA FACE Rh Collagen® is a bio-restorative soft filler that induces natural restoration of pre-aging conditions, thanks to its innovative and synergistic formula, by contributing to the production of endogenous type I collagen in human fibroblasts.

KARISMA FACE Rh Collagen® is an injectable MD Class III, that contains:

- R polypeptides $\alpha 1$ chain of type I human collagen (Recombinant Human Collagen)
- High Molecular Weight Hyaluronic Acid
- Carboxymethylcellulose

KARISMA FACE Rh Collagen® is indicated for the medium-term correction of facial skin defects due to skin aging and/or loss of volume and is therefore indicated for those who wish to reduce skin imperfections of the face, lips, redefine their contours, or where there is a particularly unstructured subcutaneous tissue.

KARISMA FACE Rh Collagen® integrates perfectly into the tissues and its corrections respect the naturalness of the face and lip shape.

KARISMA FACE Rh Collagen® formulation, thanks to the presence of the amino acid chain R-polypeptide $\alpha 1$ in synergy with high molecular weight hyaluronic acid and carboxymethylcellulose, fights skin laxity by creating a natural tightening effect.

Cellular stimulation promoted by KARISMA FACE Rh Collagen® restores firmness and structure to the skin, with a gradual, visible and lasting improvement.

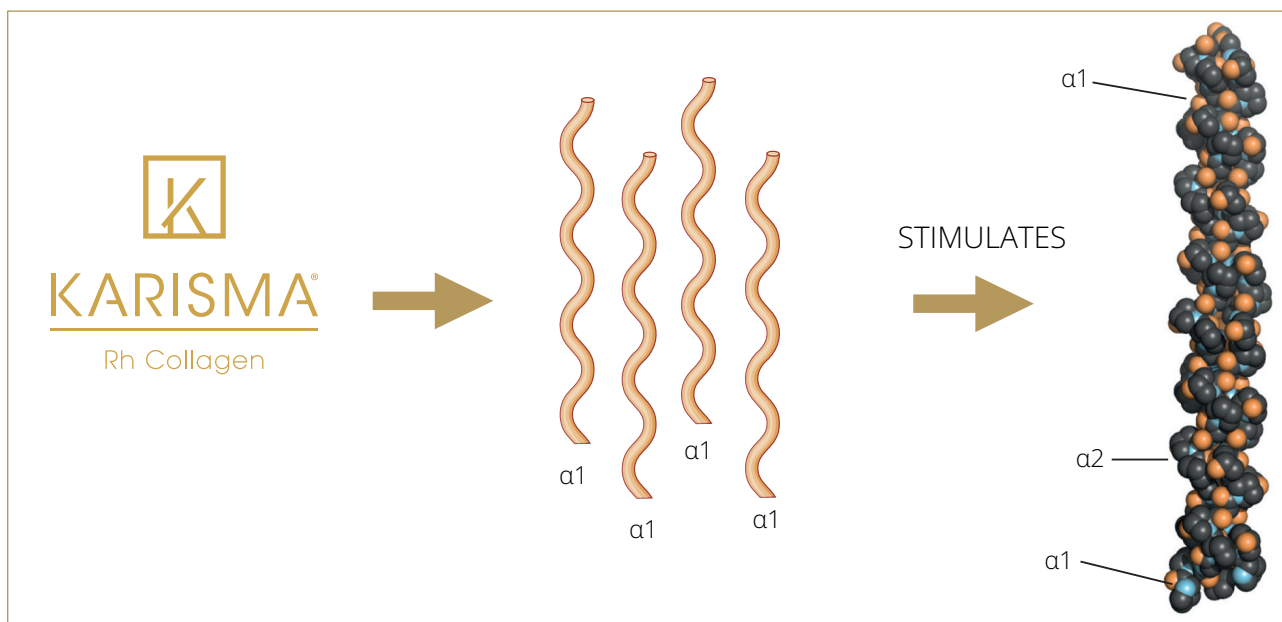
The R polypeptides $\alpha 1$ chain of type I human collagen contained in KARISMA FACE Rh Collagen® (a new patented technology) is synthesized by transgenic silkworm and secreted in their cocoon.

Hydroxylated $\alpha 1$ chain of recombinant type I human collagen acts as a precursor in the synthesis of endogenous collagen. It is assembled from silkworms thanks to the gene sequence of the $\alpha 1$ chain of human collagen and spun into cocoons.



It has a very low risk of allergic reactions (99,9% of similarity), it has 100% solubility with a dimension of 100 kDa and due to its mono-filament structure, it is immediately bioavailable, for collagen production in fibroblasts.

KARISMA FACE Rh Collagen® increases the production of pro-collagen type I C-Peptide in fibroblasts. This type of peptide helps the wrapping of pro-collagen molecules in a triple helix conformation within the endoplasmic reticulum (from procollagen to tropocollagen).

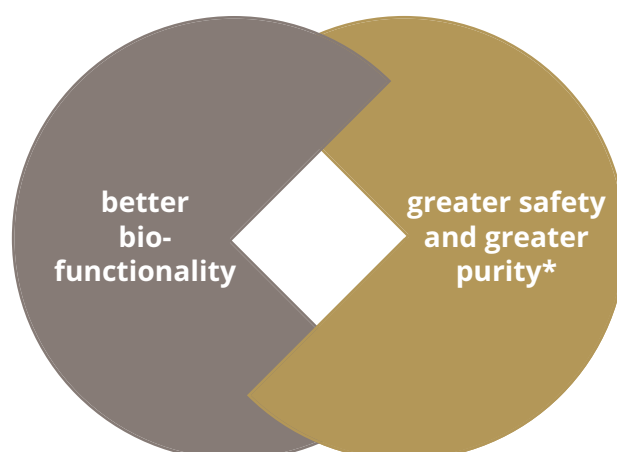


KARISMA FACE Rh Collagen® provides the necessary support for the rapid synthesis of collagen **fibrils**: the amount of free pro-peptides is directly related to the synthesis of the collagen molecule. The R-polypeptide $\alpha 1$ chain contained in KARISMA FACE Rh Collagen® is the ideal building block in regenerative medicine.

KARISMA FACE Rh Collagen® directly stimulates new synthesis of endogenous collagen, simplifying and accelerating the regenerative process.

Unlike other collagens (animal origin) the procollagen (R-polypeptide $\alpha 1$ chain) of KARISMA FACE Rh Collagen® has no potential allergic reactions, has no impurities due to its source, has no need for high purification, and doesn't need to be hydrolyzed in order to lower the molecular weight, because it already has a standardized molecular weight.

KARISMA FACE Rh Collagen® therefore has:



**non-immunogenic,
hypoallergenic, not pathogenic,
no foreign body response.*

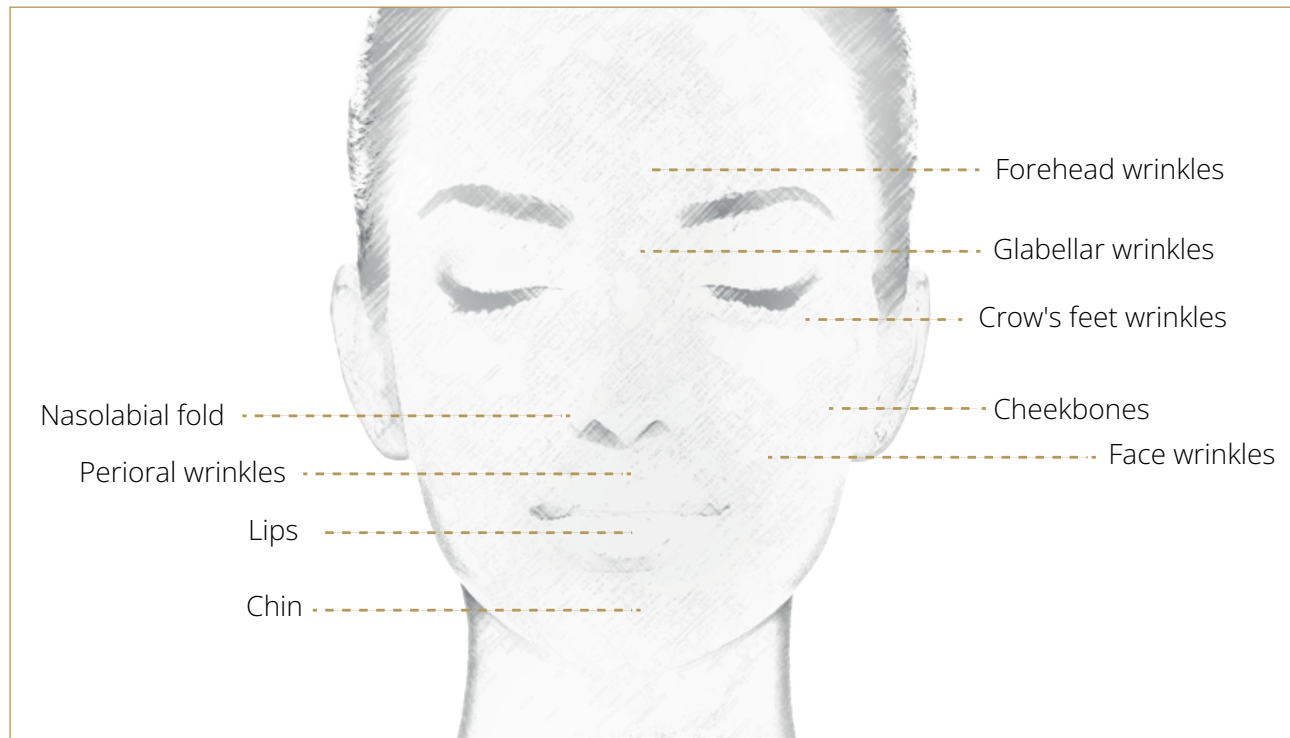
Treatment with KARISMA FACE Rh Collagen® is a restorative and a bio-restructuring process that leads to the production of type I collagen in human dermal fibroblasts, with a consequent lifting effect, greater skin tone and texture, due to a qualitative and quantitative increase in collagen fibers.

Treatment with Rh Collagen (polypeptidic $\alpha 1$ chain R):

- Assembly of collagen fibrils and other multimeric structures.
- Biosynthesis and enzymatic modification of collagen.
- Organization of the extracellular matrix thanks to specific receptors.
- Formation of specific subcellular structures involved in the mediation of extracellular matrix signals.
- Participation of inflammatory response processes.
- Platelet activation.

KARISMA FACE – Rh Collagen® treatment areas

KARISMA FACE Rh Collagen® is a breakthrough bio-restorative injectable filler, that opens a new approach in the treatment of skin aging. KARISMA FACE Rh Collagen® can also be used for the treatment of acne scars and for skin atrophy conditions. KARISMA FACE Rh Collagen® is highly indicated in replenishing skin volume losses.



KARISMA FACE Rh Collagen® restores and directly stimulates α chains of collagen in all those physiological or pathophysiological conditions (like acne scars) thanks to the presence of Rh Collagen and thanks to the help of high molecular weight HA and CMC which stabilize, protect, and hydrate the extracellular matrix, making the environment histologically favorable for the production and maintenance of new endogenous type I collagen.



Treatment protocols

KARISMA FACE Rh Collagen® ensures more effective treatment cycles with a new, clear, and easy-to-follow protocol.

Treatment protocol in pre-aging physiological conditions:

- Point technique
- Linear retrograde technique
- Medium dermis (rarely deep)
- Protocol: T0; T30; Maintenance at 4 months
- 2ml pre-filled syringe

Treatment protocol in anti-aging physiological conditions:

- Linear retrograde technique
- Medium and deep dermis
- T0; T30; Maintenance at 4 months
- 2 ml pre-filled syringe

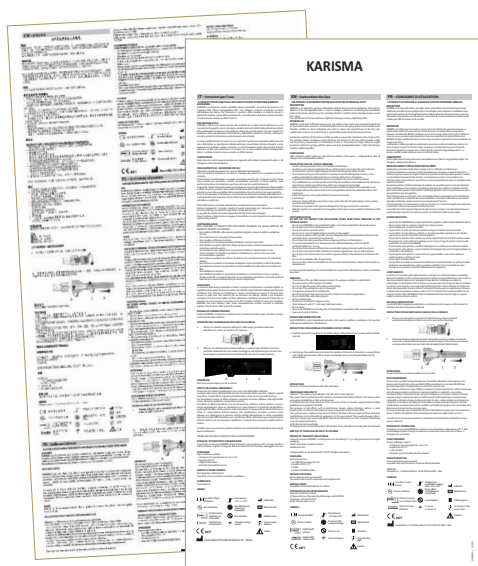
The suggested protocol is 2 sessions, with 30 days interval. Timing of the 2 treatments is very important because **the protocol follows the cellular turnover that usually happens every 28 days.**

It is possible to repeat the treatment or the protocol after 4 months. The treatment lasts approximately 30 mins depending on the area to be treated.

Packaging and internal leaflet

KARISMA FACE Rh Collagen® packaging contains:

- Bio-restorative Soft Filler (2 ml pre-filled syringe)
- Internal leaflet



To read KARISMA Rh Collagen® internal leaflet, please use this QR Code



Evaluation of the effectiveness of KARISMA Rh Collagen® on Normal or Aged Human Dermal Fibroblasts*

To evaluate the effect of KARISMA Rh Collagen® on aging, viability, growth rate, intracellular collagen levels, extracellular secretion of TGF-β1, wound healing ability, intracellular alpha-SMA levels and fibroblasts contractility, both in normal and aged human dermal fibroblast, *in vitro* studies were performed.

The KARISMA Rh Collagen® *in vitro* studies were performed at: University of L'Aquila (Italy), Department of Life, Health and Environmental Sciences - Plastic and Reconstructive Surgery.

All the results of the *in vitro* studies were presented in two different sessions.

The first part on normal human dermal fibroblasts was presented at **AGORA 2022 - 24th International Congress on Aesthetic Medicine - Milan (Italy) - 6th/8th October 2022.**

The second part of the study on the aged human dermal fibroblasts was presented at: **44th Congress SIME 2023 – Società Italiana di Medicina Estetica** and **18th Congress AIMAA – Accademia Italiana Medicina Anti-Aging – Rome (Italy) – 19th/21st May 2023**

Prof. Maurizio Giuliani, Prof.ssa M.G. Cifone, Prof.ssa B. Cinque, Prof.ssa F. Lombardi, Prof.ssa P. Palumbo, Dr.ssa F.R. Augello, Dr.ssa S. Artone, Tech. Lab. G. De Nuntiis.

*Cell Models

Normal Human Dermal Fibroblasts (NHDFs): Normal human dermal fibroblast (NHDF) cell line was cultured in DMEM supplemented with 10% of fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM glutamine. The cells were maintained in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C. After reaching 80% confluence, the cells were plated and treated as described.

Aged Human Dermal Fibroblasts: To induce the aged phenotype HDFs were treated for 1 h with 25 µM hydrogen peroxide (H₂O₂) solution after which the medium was changed with fresh medium. After 24 h, the cells were treated with KARISMA Rh Collagen® at the concentrations reported below.

• Effect of KARISMA Rh Collagen® on cell viability and growth rate

As evident in Figure 1, in normal HDFs, the treatment with KARISMA Rh Collagen® increased the cell growth in a time- and concentration-dependent manner. In aged HDFs, the proliferation did not increase and remained at constant levels over time.

Of note, when the aged fibroblasts were exposed to KARISMA Rh Collagen®, the cell proliferation enhanced in a time and concentration-dependent manner when compared to relative untreated control, being 5% the concentration most effective in stimulating proliferation of either normal or aged HDFs.

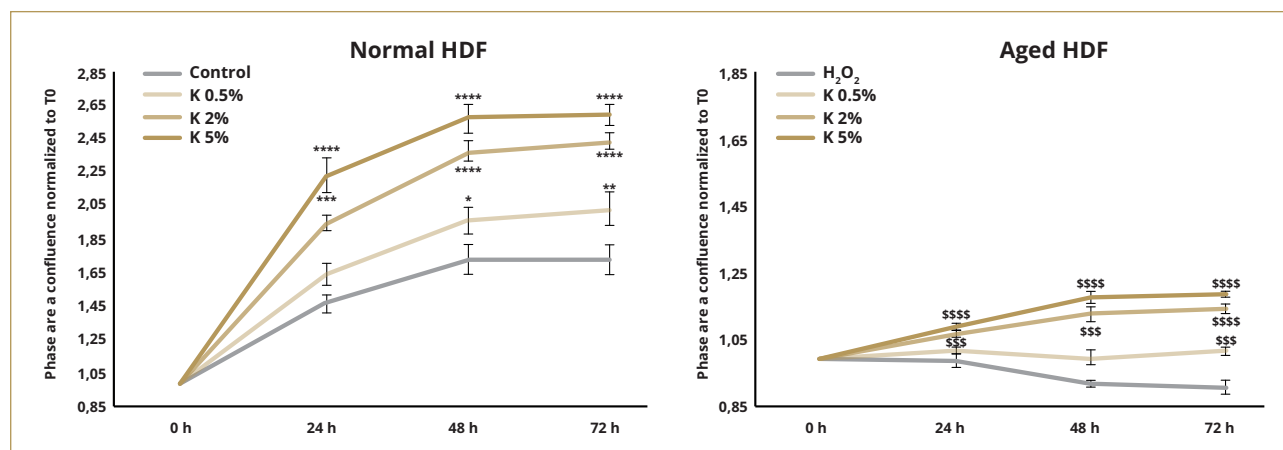


Figure 1. Effect of KARISMA Rh Collagen® (K) on normal or aged HDF growth rate. Growth curves were analyzed as cell confluence through IncuCyte® Live Cell Imager and monitored dynamically from 0 h up to 72 h. Cells were treated with K at indicated concentrations in complete medium. Results relating to one representative out of three independent experiments are expressed as mean ± SEM. For comparative analysis of groups of data, two-way repeated-measures ANOVA followed by Dunnett's post hoc test was used (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs. Control; §§§ $P < 0.001$, §§§§ $P < 0.0001$ vs. aged control).

NHDFs were plated in 24-well plates and incubated with increasing concentrations (0.5, 1, 2, 5, or 10%, final concentration V/V) of KARISMA Rh Collagen® up to 72 h. All tested concentrations of KARISMA did not influence the cell viability assessed by trypan blue dye exclusion test (ever >95-98%). The KARISMA Rh Collagen® concentrations of 0.5% (1 mg/ml high molecular weight HA, 1 µg/ml collagen α1 chain Rh, and 0.2 mg/ml CMC) 2% (4 mg/ml HA, 4 µg/ml collagen α1 chain Rh, and 0.8 mg/ml CMC), and 5% (10 mg/ml HA, 10 µg/ml collagen α1 chain Rh, and 2 mg/ml CMC) were chosen for the subsequent experiments designed to analyze the effects of KARISMA Rh Collagen® on dermal fibroblasts.

The cell growth of HDFs exposed to KARISMA Rh Collagen® was measured using IncuCyte® Live Cell Imager for real time evaluation of cell confluence, dynamically monitored every 6-h over a 72-h time period. Normal and aged HDFs were plated in a 96-multiwell culture plate and then media were replaced with media containing KARISMA Rh Collagen® at different concentrations. At the end of the treatment, the cell confluence (vs. relative T0) analyzed by IncuCyte ZOOM™ software, was used to calculate the proliferation rate.

- **Effect of KARISMA Rh Collagen® on aging of HDF induced by H₂O₂**



To evaluate the effect on the H₂O₂ induced aging process on NHDF, the cells were seeded and senescence was induced via H₂O₂ treatment. Of note, the exposure to KARISMA Rh Collagen® was able to counteract the H₂O₂-induced senescence in a concentration-dependent manner.

In Figure 2 representative images are shown.

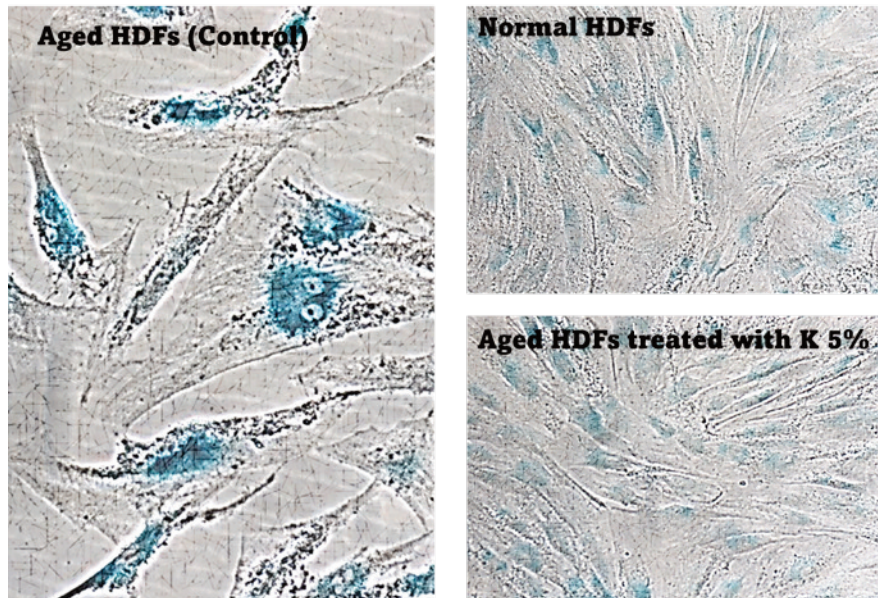


Figure 2. Senescence-associated (SA)-β-galactosidase staining. H₂O₂ promoted an evident SA-β-gal activity, as showed by the blue precipitate. Representative images are shown. The exposure to KARISMA Rh Collagen® at 5% counteracted the H₂O₂-induced senescence.

NHDFs were seeded at 7000 cell/cm² in 6-well plates. Senescence was induced via treatment with H₂O₂ (25 μM) for 1 h in PBS. After a single 1 h treatment, H₂O₂ solution was substituted with fresh culture medium, and the cells were allowed to recover for a period of 24 h. Then, HDFs were treated with Karisma at 0.5-5% for 72 h. After washing twice with PBS, the cells were fixed with 1x fixative solution, and SA-β-gal staining was performed using a senescence-β-galactosidase staining kit (Cell Signaling Technology). Finally, the cells were observed using a light microscope (Nikon).

• Effect of KARISMA Rh Collagen® on intracellular collagen I levels in normal and aged HDFs

Normal and aged HDF cells were incubated for 72 h with increasing concentrations of KARISMA Rh Collagen® (0.5, 2, and 5%), after which the intracellular collagen I levels were analyzed by immunoblotting.

As shown in Figure 3, immunoblotting analysis indicated that intracellular type I collagen expression was significantly up-regulated in NHDFs exposed to KARISMA Rh Collagen® at all tested concentrations.

Of note, also aged HDFs were influenced by treatment with KARISMA Rh Collagen® which, just at 2%, was able to induce an increase of more than 100% in intracellular type I collagen levels.

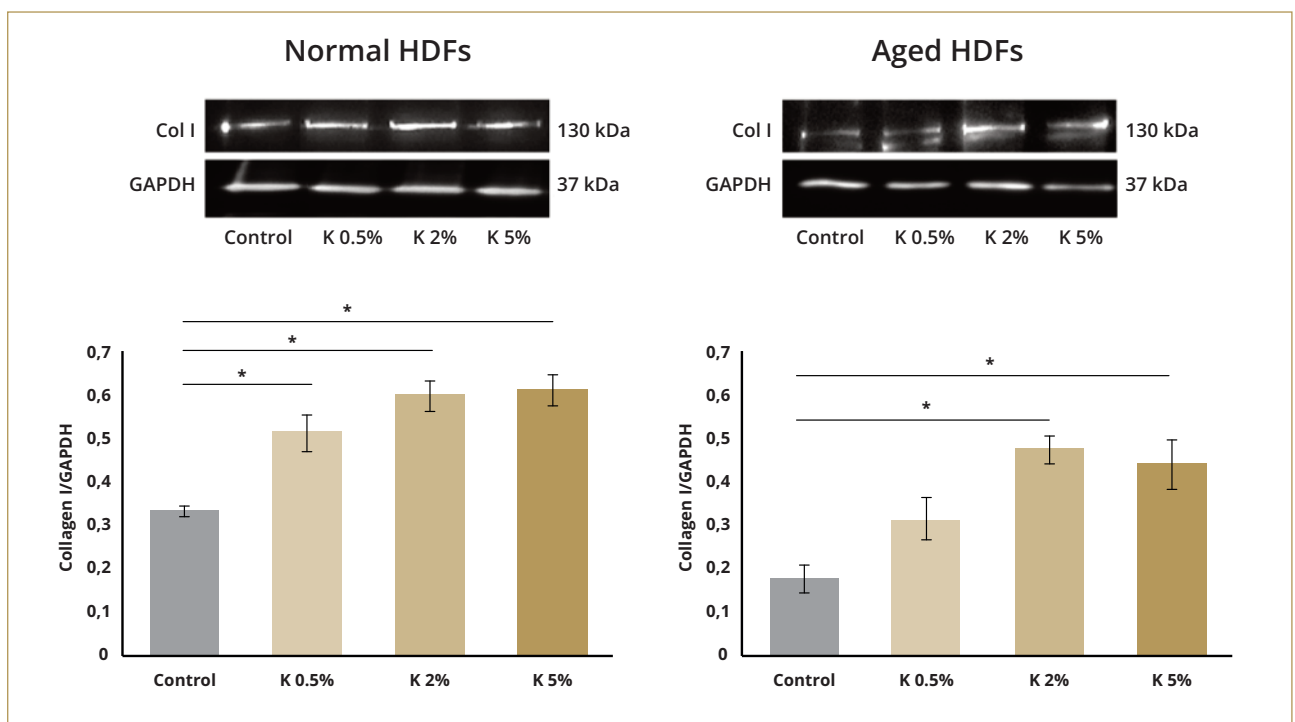


Figure 3. Influence of KARISMA Rh Collagen® treatment on intracellular collagen I levels in normal and aged HDFs. Immunoblotting assay for collagen I was performed on normal and aged HDFs treated with increasing concentrations of KARISMA Rh Collagen® for 72 h. Densitometric analysis is presented normalizing vs. GAPDH protein. The data from three independent experiments are shown as the mean $\pm$ SEM. For comparative analysis of groups of data, one-way ANOVA followed by Dunnett's post hoc test was used (* $P < 0.05$). Immunoblotting images from one representative are shown.

The effects of KARISMA Rh Collagen® on type I collagen production were also evaluated by immunofluorescence in normal or aged HDFs treated with KARISMA Rh Collagen® 5% for 72h.

As shown in the representative images reported in Figure 4, the normal fibroblasts treated with KARISMA Rh Collagen® showed more intense and widespread collagen type I staining compared to untreated cells.

Moreover, the exposure to KARISMA Rh Collagen® led to prominent cytoskeletal rearrangement in treated cells, as shown by phalloidin staining.



The aged cells treated with KARISMA Rh Collagen® showed a more intense collagen type I staining than untreated cells, thus confirming the results obtained by immunoblotting.

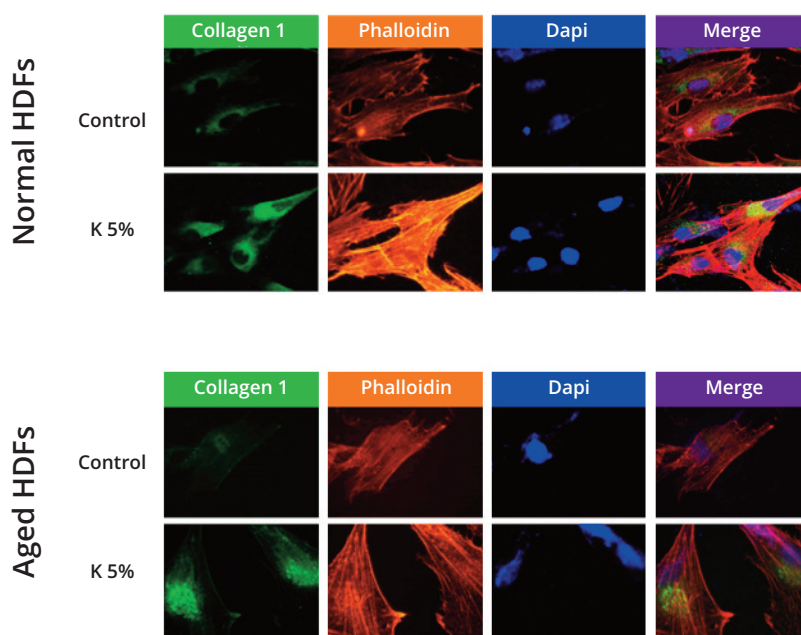


Figure 4. Influence of KARISMA Rh Collagen® (5%) on collagen I expression in normal or aged HDFs. Representative immunofluorescence images of HDFs stained with anti-collagen I antibody (green) and with TRITC-phalloidin (red) to reveal F-actin. Nuclei were counterstained with DAPI (blue) (magnification 100x). The images are representative of three independent experiments in duplicate. All images were acquired at 100x magnification.



- **Effect of KARISMA Rh Collagen® on extracellular secretion of collagen I by normal and aged HDFs.**

A dose-dependent increase in extracellular collagen I was observed in the conditioned medium of normal HDFs treated with KARISMA Rh Collagen® as compared to control cells (Figure 5).

As expected, the aged HDFs secreted lower (~35-40%) basal levels of collagen I than normal control HDFs. When the aged fibroblasts were exposed to KARISMA Rh Collagen®, the extracellular collagen I levels significantly increased in a concentration-dependent manner, assuming values comparable to those of normal HDFs.

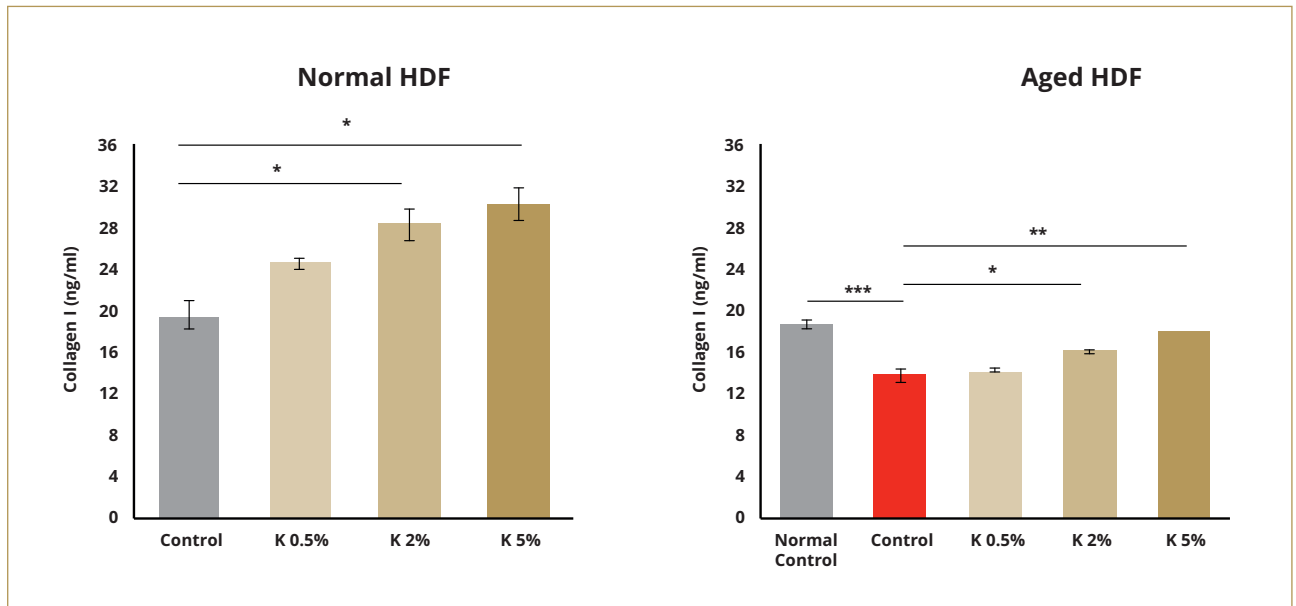


Figure 5. Effect of KARISMA Rh Collagen® on collagen I levels secreted by normal and aged HDFs. Collagen levels in HDFs untreated (Control) or treated for 72 h with KARISMA Rh Collagen® at indicated concentrations were assayed in cell supernatants by collagen I Elisa kit. Results, expressed as ng/ml, are relative to mean values $\pm$ SEM of three experiments performed in duplicate. For comparative analysis of data, the one-way ANOVA followed by Dunnet's post hoc test was performed (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

- **Effect of KARISMA Rh Collagen® on Prolyl-4-hydroxylase A1 (P4HA1), a “rate-limiting enzyme” of collagen production in normal and aged HDFs**

P4HA1 (prolyl-4-hydroxylase A1), the enzyme which catalyzes proline hydroxylation of procollagen, is essential for collagen synthesis and maturation in fibroblasts; it is considered as a “rate-limiting enzyme” in collagen production.

In normal HDFs, the treatment with KARISMA Rh Collagen® led to a significant dose-dependent increase of P4HA1 levels.

In the aged fibroblasts that expressed lower basal levels of P4HA1 with respect to normal cells, the treatment induced an increase in P4HA1 levels at all tested concentrations even where the effect was statistically significant at 2 and 5% (Figure 6).

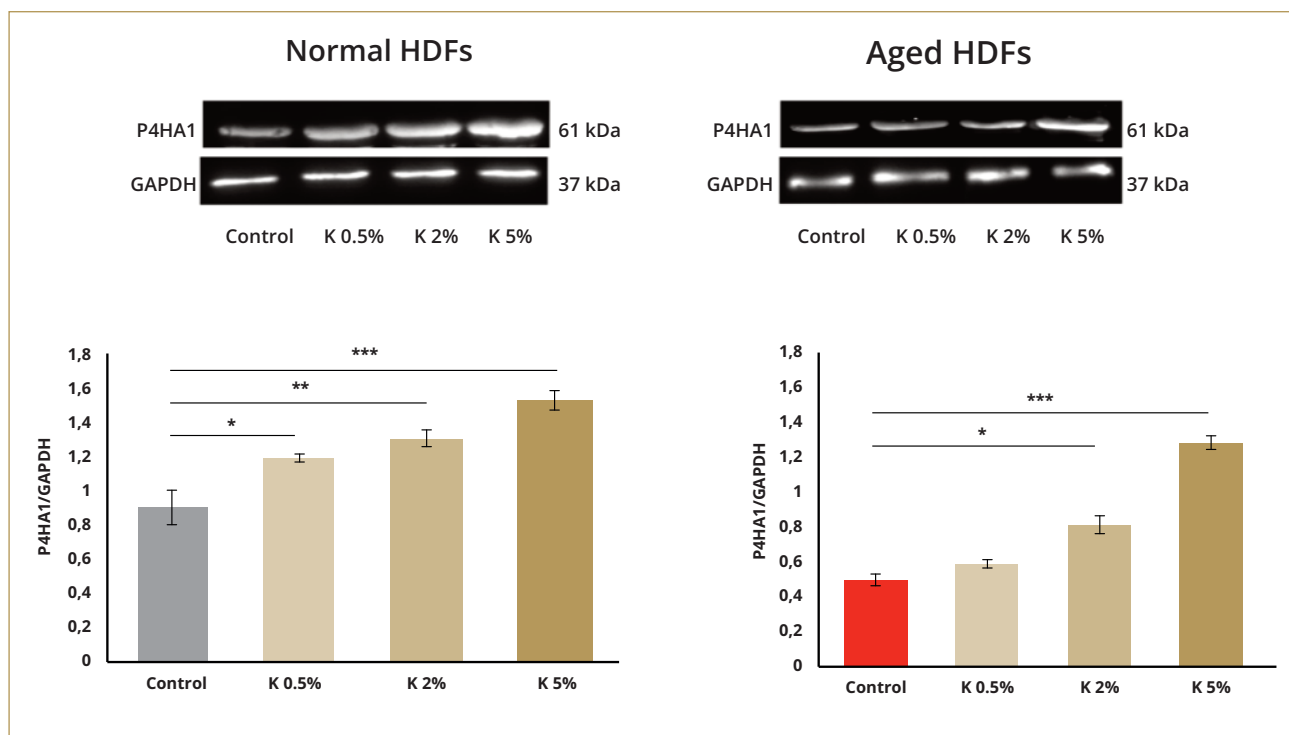


Figure 6. Influence of KARISMA treatment on P4HA1 levels in normal and aged HDFs. Normal and aged HDFs were cultured for 72 h with or without KARISMA Rh Collagen® at 0.5, 2, and 5%, and the P4HA1 protein, key player in collagen synthesis, was evaluated. Immunoblotting assay for prolyl 4-hydroxylase (P4HA1) was performed on normal and aged HDFs treated with increasing concentrations of KARISMA FACE Rh Collagen® for 72 h. Densitometric analysis is presented normalizing vs. GAPDH protein. The data from three independent experiments are shown as the mean $\pm$ SEM. For comparative analysis of groups of data, one-way ANOVA followed by Dunnett's post hoc test was used (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Immunoblotting images from one representative are shown.

• Effect of KARISMA Rh Collagen® on extracellular secretion of TGF- β 1 by normal and aged HDFs

TGF- β 1 represents a key regulator in collagen I synthesis in human skin.

The levels of TGF- β 1 in normal and aged HDFs untreated (control) or treated for 72 h with increasing concentrations of KARISMA Rh Collagen®, were quantified.

TGF- β 1 levels in the extracellular medium of both normal and aged fibroblasts increased significantly after the addition of KARISMA Rh Collagen®, in a concentration-dependent manner (Figure 7).

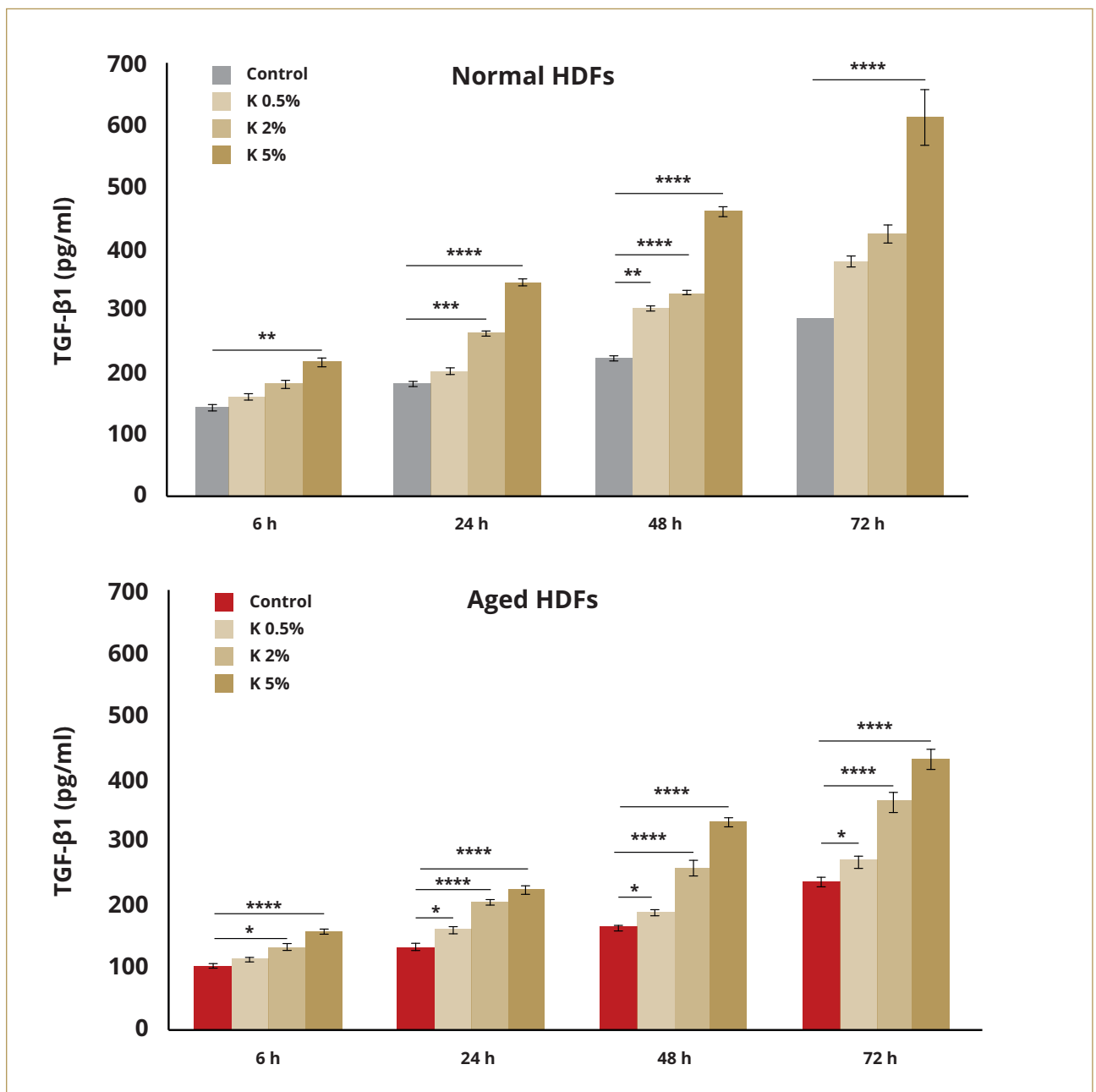


Figure 7. Effect of KARISMA Rh Collagen® on TGF- β 1 levels secreted by normal and aged HDFs. TGF- β 1 levels in fibroblasts untreated (Control) or treated for 72 h with KARISMA Rh Collagen® at indicated percentages were assayed in cell supernatants by TGF- β 1 Elisa kit. Results, expressed as pg/ml, are relative to mean values $\pm$ SEM of three experiments performed in duplicate. For comparative analysis of data, the one-way ANOVA followed by Dunnet's post hoc test was performed (* $P < 0.05$ ** $P < 0.01$, **** $P < 0.0001$).

• Effect of KARISMA Rh Collagen® on wound healing assay



The cell migration is defined as the time taken to close an open wound after a linear “scratch” or wound which is artificially created across a monolayer of cultured cells.

The quantitative in vitro analysis of wound-closure rate showed that the closure percentage at 6, 12, and 24 h increased in normal HDFs treated with KARISMA Rh Collagen® respect to control in a concentration-dependent way.

These results showed that KARISMA Rh Collagen® was effective in stimulating both migration and proliferation rate of normal HDFs (Figure 8).

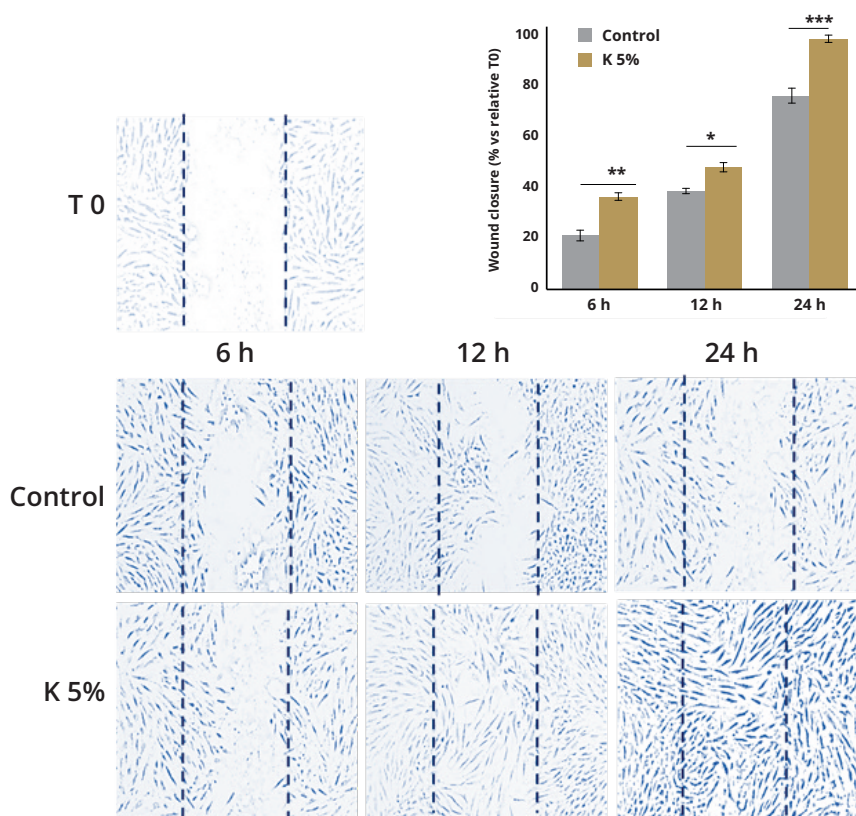


Figure 8. Effect of 5% KARISMA Rh Collagen® on proliferation and migration of NHDFs at different time points. The quantitative results in the histogram are expressed as % of wound closure vs relative T0 (mean ± SEM) and are from three independent experiments in duplicate. For comparative analysis of groups of data, repeated measures two-way ANOVA followed by Dunnet post hoc test was used (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control). Representative microscopy images captured at 0, 6, and 24 h are also shown (10× magnification).

Cell monolayers in 12-well plates were scratched using a 200 µL pipet tip to create a uniform cell-free wound area with reproducible width of wounding. Cell cultures were then incubated in the absence (control) or presence of KARISMA Rh Collagen® at 5%.

Cell migration was monitored by microscopy using an inverted light microscope and images were captured (10× magnification) at different time points after the injury. To calculating the % relative closure, the images acquired were analyzed quantitatively using the TScratch software. Quantification of relative wound closure was performed according to the equation, where Tn is a specific time point (h) after the scratching:

$$\% \text{ Relative Closure} = \frac{(\% \text{ scratched area at T0} - \% \text{ scratched area at Tn})}{(\% \text{ scratched area at T0})} \times 100$$

• Effect of KARISMA Rh Collagen® on intracellular alpha-SMA levels in normal and aged HDFs

Alpha-smooth muscle actin (alpha-SMA) is an actin isoform that contributes to cell-generated mechanical tension and is increased in activated fibroblasts.

Immunoblotting analysis indicated that intracellular alpha-SMA expression in normal HDF cells increased in a KARISMA Rh Collagen® concentration-dependent manner even if the up-regulation was statistically significant at 5%.

In aged fibroblasts the alpha-SMA levels were also increased after treatment with KARISMA Rh Collagen® and resulted significantly higher at 2 and 5% when compared to the relative control (Figure 9).

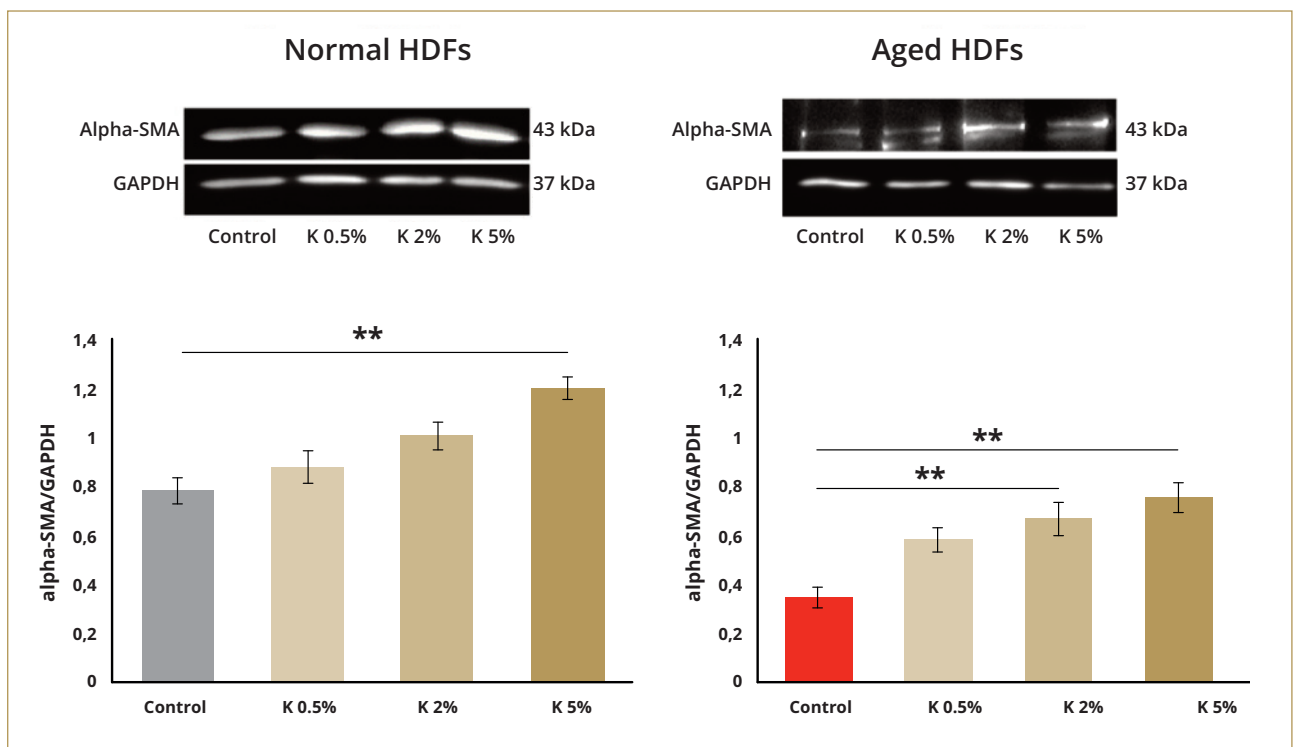


Figure 9. Influence of KARISMA treatment on alpha-SMA levels in normal and aged HDFs. Normal and aged HDFs were incubated for 72 h with increasing concentrations of KARISMA Rh Collagen® (0.5, 2, and 5%), after which the intracellular alpha-SMA levels were analyzed by immunoblotting. Immunoblotting assay for alpha-SMA was performed on normal and aged HDFs treated with increasing concentrations of KARISMA for 72 h. Densitometric analysis is presented normalizing vs. GAPDH protein. The data from three independent experiments are shown as the mean ± SEM. For comparative analysis of groups of data, one-way ANOVA followed by Dunnett's post hoc test was used (** $P < 0.01$). Immunoblotting images from one representative are shown.

• Effect of KARISMA Rh Collagen® on NHDF cells contractility.



To evaluate the efficacy of KARISMA Rh Collagen® on normal and aged dermal fibroblasts contractility, KARISMA Rh Collagen® was added.

The results showed that KARISMA Rh Collagen® treatment for 24 h led to a marked and concentration-dependent decrease in the size of the collagen lattices compared with the control group.

The results indicate that KARISMA Rh Collagen® was able to induce the increased contractility of fibroblasts embedded in collagen lattices.

The aged HDFs showed reduced contractility, as demonstrated by the larger disc size compared to the normal control. Interestingly, this effect was counteracted by the addition of KARISMA Rh Collagen® which was able to induce a relevant concentration-dependent increase of contractility of aged HDFs.

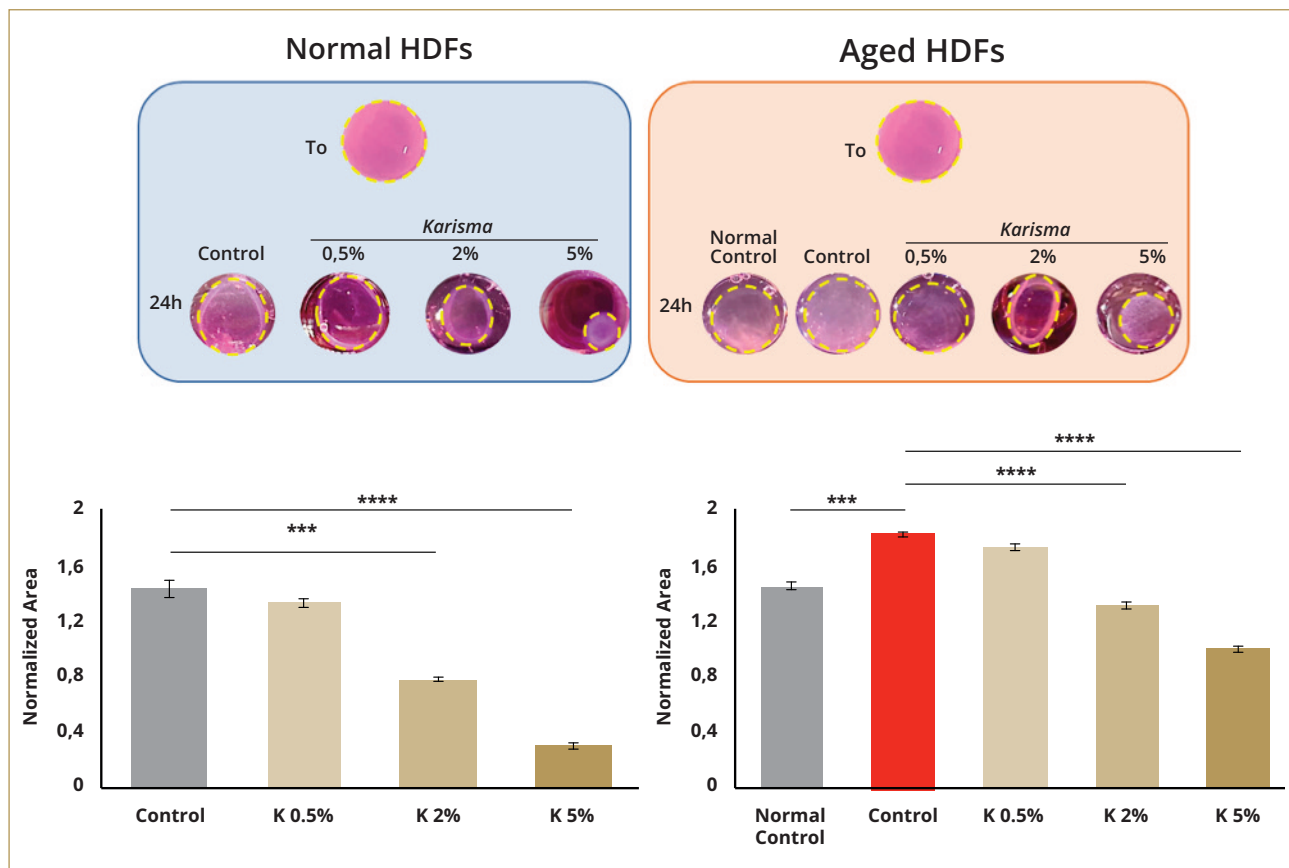


Figure10. Collagen gel retraction assay of normal and aged HDF-populated collagen lattices. To assess the contraction capacity of normal and aged HDFs, the cells were seeded into three-dimensional collagen latex. After latex polymerization, the complete media containing increasing concentrations of KARISMA Rh Collagen® were added. The images of the wells were acquired at T0 and 24 h. The gel area was measured with ImageJ and normalized to pre-release area (T0). Normal and aged HDFs were mixed to collagen solution and seeded in 24-well culture plate. After lattices polymerization, the complete media containing 0.5-5% Karisma was added. The images of the wells were acquired at T0 and 24 h and gel area was measured with ImageJ and normalized to pre-release area (T0). Values of normalized area are expressed as mean $\pm$ SEM of three independent experiments in duplicate. The comparative analysis of data has been carried out by using one-way analysis of variance (ANOVA) followed by post hoc Dunnet's test. (***) $P < 0.001$, (****) $P < 0.0001$.



Conclusion

In vitro studies have shown that KARISMA is able to induce on normal and aged HDFs a significant and dose-dependent effect on parameters crucial for skin bio-revitalization including:

- Cell proliferation and migration.
- Counteraction of the dermal fibroblast-induced senescence.
- Overexpression of intracellular levels of type I collagen and increased secretion of extracellular collagen type I.
- Upregulation of P4HA1, a rate-limiting enzyme in collagen synthesis and maturation.
- Increase of TGF- β 1, a key stimulator factor of collagen I synthesis in human skin.
- Stimulation of migration rate and repair of HDF scratched monolayer.
- Upregulation of alpha-SMA levels and increased dermal fibroblast contractility.

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