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Mechanisms

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abstract

To explore the field of epigenetics in greater detail, BASF researchers have joined forces and established an interdisciplinary innovation platform. Together with experts from public and private research institutions, they are exploring how modulations to our gene expression, caused by either chronological aging or environmental factors, can be mapped and controlled. BASF's new anti-aging active ingredients harness the platform's findings. Dermagenist®, an extract of *Origanum majorana* leaves, is the first active ingredient that re-activates fibroblasts by protecting them from epigenetic modifications and stimulating their vitality through LOXL1 promoter re-activation. A clinical study demonstrated an 18% improvement in skin density after two months of application, and a 15% improvement in skin firmness after three months. RNAge®, an extract of *Hippophae rhamnoides* seeds, fights chronological microRNA Let-7b dysregulation. BASF has demonstrated that Let-7b is involved in epigenetic regulation and the balancing of proteins in both the extracellular matrix and the proteoglycan network required for a well-balanced dermal structure. RNAge inhibited Let-7b expression by 39% versus the untreated control. The skin rebuilds from deep within, recovering its density (+26%) and firmness (+18.5%) for a healthy and youthful appearance.



Late nights, early starts, and daily stresses – lifestyles can alter our skin cells' functions and accelerate skin aging. The important role that genetics plays in the aging of human skin has been known for some time. Today, we know that epigenetics is just as important: As environmental factors can actually leave their mark on the genome, they can affect skin aging just as much.

DNA methylation is one form of epigenetic modification, in which methyl groups are added to the DNA molecule, changing the DNA segment's activity without changing the sequence. When identified in a gene promoter, DNA methylation typically leads to repression of gene transcription.

Fibroblasts synthesize collagen and other extracellular matrix (ECM) proteins. As they age and undergo epigenetic modifications, such as methylation of their DNA, the cells progressively lose their ability to reorganize the ECM. Their metabolic capacity decreases. The cytoskeleton, which is largely composed of actin fibers, declines, and so does the cells' capacity to contract. To make matters worse, fibroblasts lose their connection to the extracellular matrix. Ultimately, these impairments lead to a lack of collagen and a loss of dermal density.

Reawakening Fibroblasts' Potential

Dermagenist®, a standardized aqueous extract of marjoram, is the first active ingredient that re-activates fibroblasts by protecting them from epigenetic modifications and by stimulating their vitality (INCI: Maltodextrin (and) *Origanum Majorana* Leaf Extract). Its efficacy has been demonstrated both *in vitro* and *in vivo*, with a significant 18% improvement in skin density after two months of application and a 15% improvement in skin firmness after three months of application.

Proof of Concept: *in vitro* Assessments

Effect of Dermagenist on LOXL1 mRNA Expression

Lysyl oxidase-like 1 (LOXL1) is the cross-linking agent responsible for organizing the collagen fibers in the dermis. In aged skin fibroblasts, LOXL1 decreases rapidly. BASF experts demonstrated that a decline in LOXL1 mRNA expression in aged fibroblasts was due to reduced LOXL1 promoter activity. This again resulted from the respective gene's promoter methylation by a specific DNA methyltransferase, DNMT3A. The experts assessed the active's effect on promoter methyla-

tion and found that 0.065 % Dermagenist reduced promoter methylation and boosted LOXL1 mRNA expression by 66 % versus the untreated control (Fig. 1).

Effect of Dermagenist on Pro-collagen I and BMP-1

Additionally, the researchers assessed the effect of Dermagenist on the production of pro-collagen I and its essential partner BMP-1 protein in aged fibroblasts, both of which are crucial for the synthesis of functional collagen fibers. At a concentration of 0.015 %, the active improved pro-collagen I protein synthesis by 36 % and led to a threefold increase in BMP-1 synthesis (Fig. 2). The active's effects on collagen synthesis were also visualized in a reconstructed 3D dermis model (Mimederma®). Dermagenist helped fibroblasts fill extracellular spaces with a fully organized three-dimensional collagen network and stimulated fibrillar

collagen organization (Fig. 3, dotted circle). In the untreated control, collagen was present only around the matrix, and the extracellular spaces were not filled. It can therefore be concluded that the active stimulates the production of deposited collagen I in the matrix and triggers the production of both mature and functional neo-synthesized collagen.

Effect of Dermagenist on Actin Organization

The effects of 0.05 % Dermagenist on the expression of actin and its distribution in fibroblasts were assessed using a micro pattern platform developed by Cytoo (Grenoble, France): Upon adhesion, the cells contract on the substrate, which leads to a reduction in the micropattern area. The active's effects on actin organization were evaluated both at single cell (FibroScreen™ Actin) and multicellular level (FibroScreen™ Actin contractility). At 0.05 %, Dermagenist significantly reinforced actin fibers associated with stretch and adhesion and caused a more concentrated distribution (greater amount of red than in the untreated control, Fig. 4A). Dermagenist also increased the contractility of a group of cells (Fig. 4B).

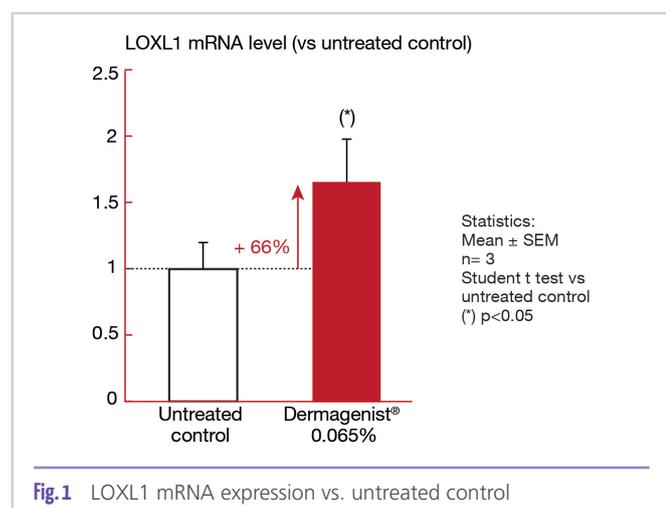


Fig. 1 LOXL1 mRNA expression vs. untreated control

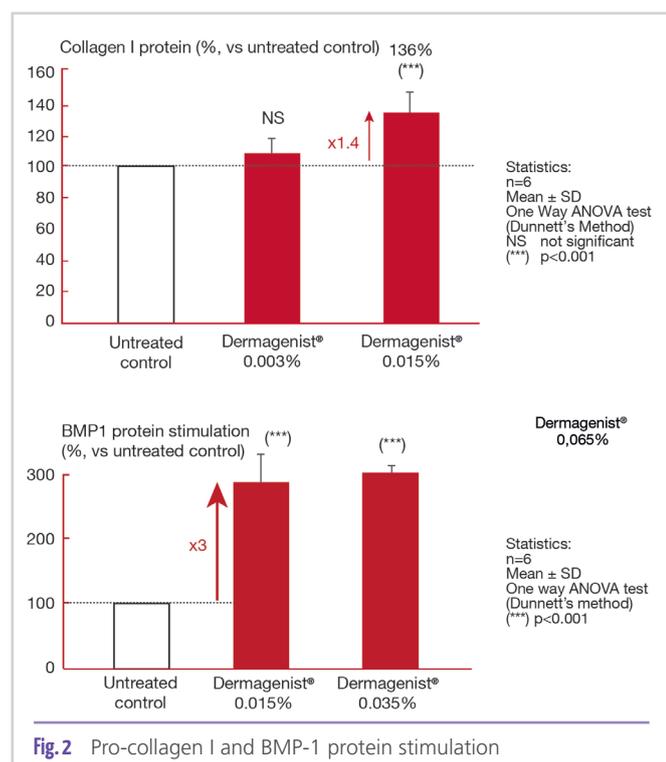


Fig. 2 Pro-collagen I and BMP-1 protein stimulation

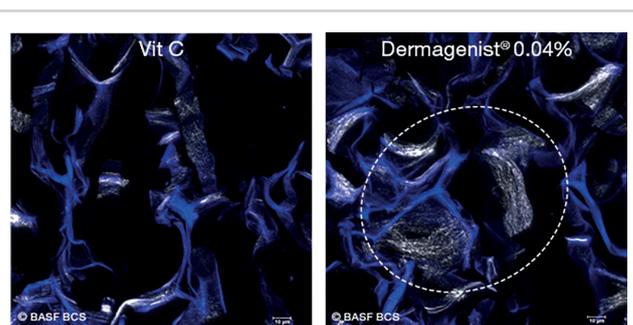


Fig. 3 Two photon excitation fluorescence of scaffold matrix in blue and second harmonic generation of fibrillar neosynthesized collagen in grey. Scale bar = 10µm.

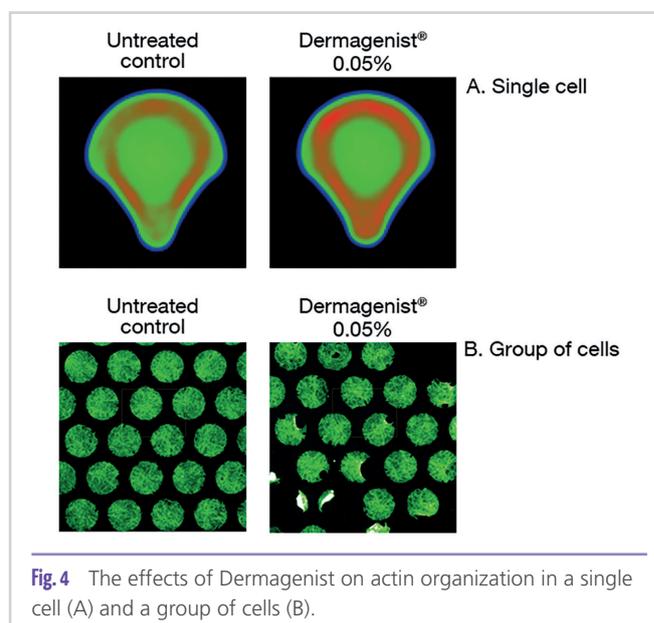


Fig. 4 The effects of Dermagenist on actin organization in a single cell (A) and a group of cells (B).

Protein code	Name	Biological functions
CHSY3	Chondroitin sulfate synthase 3	Sulfation of chondroitin sulfate
XYLT1	Xylosyl transferase-1	GAG chain assembly
TGFBR2	TGFbeta2 receptor	Wound healing and tissue repair
FBN1	Fibrillin1	Skin elasticity
ITGB3	Integrin beta3	Cell shape and ECM deposition

Tab. 1 Five proteins modulated by miRNA Let-7b

Proof of Concept: *in vivo* Assessments

Effect of Dermagenist on Dermal Density and Skin Firmness

Two clinical studies demonstrated the effects of Dermagenist on dermal density and skin firmness.

To assess dermal density, a double-blinded, randomized, split-face study was conducted for a test group consisting of 30 Caucasian females aged 40 to 65 years. For 56 days, the group applied either 0.4% Dermagenist or a placebo formulation on one half of the face, twice daily. The results were evaluated by ultrasound: After 56 days of application, the active significantly increased skin density versus baseline, by 18% (data not shown).

Skin firmness was assessed in another test group, consisting of 30 Caucasian females aged 45 to 60 years. In a double-blinded, randomized and split-face manner, they applied either 0.4% Dermagenist or a placebo formulation on one half of the face, twice daily for 84 days. After 28, 56 and 84 days, an increase in skin firmness of 6.5%, 10.1% and 15.2% respectively was observed, versus baseline. After 84 days of treatment, there was an increase in skin firmness of 9% versus the placebo.

Helping Fibroblasts Renew Vital Proteins

RNAge®, an extract of sea buckthorn seeds (INCI: Maltodextrin (and) Hippophae Rhamnoides Kernel Extract), is another anti-aging ingredient arising from BASF's innovation platform for epigenetics. By downregulating miRNA Let-7b in aged fibroblasts, it helps the skin renew proteins that are vital for maintaining a balanced skin structure. Its effects were observed *in vitro*, while a clinical study illustrated an improvement in half-chin angle as well as a significant increase in dermal density and skin firmness.

Epigenetic Protein Equalizer miRNA Let-7b

With aging, fibroblasts in the dermis increasingly exhibit a state of quiescence, resulting from the repression of gene activity. BASF identified miRNA hsa-Let-7b-5p (Let-7b) as a key miRNA modulating the expression of several proteins involved in renewal of the dermal structure. It can be seen as a specific protein equalizer that is of critical importance for the dermal architecture. In the literature, miRNA Let-7b has been shown to increase with aging and has been associated with fibroblast quiescence. Using the gain and loss-of-function method and Western blot analysis, the experts discovered that miRNA Let-7b epigenetically controls five proteins (Tab. 1), all of which are involved

in collagen and elastic fiber organization and glycosaminoglycans (GAG) synthesis.

By targeting the downregulation of miRNA Let-7b, the scientists aimed to reinforce the biomechanical properties of the dermis to help achieve firmer facial skin.

Proof of Concept: *in vitro* Assessments

Effect of RNAge on miRNA Let-7b Expression

The effect of RNAge on miRNA Let-7b expression in dermal fibroblasts, from donors aged 53 to 68 years, was assessed using real-time quantitative PCR (qRT-PCR). In a dose-dependent manner, the active significantly reduced miRNA Let-7b expression. After 48 hours, at 0.02%, RNAge inhibited miRNA Let-7b expression by 39% versus the untreated control.

Effect of RNAge on Structural Properties of the Dermis

Collagens III and V play a central role in the dermis' collagen network organization. The effect of three days of treatment with RNAge was investigated in an equivalent dermis structure (Mimederma®), which was cultivated for 28 days. After immunostaining (primary antibodies for collagen III and V and secondary antibody: Alexa 488), collagen III and V were observed via confocal microscopy (TCS-SP2, Leica). At 0.02%, the extract clearly improved the organization of fibrillar collagen III and V: After treatment, dark (i.e. empty) areas were filled with collagens (Fig. 5).

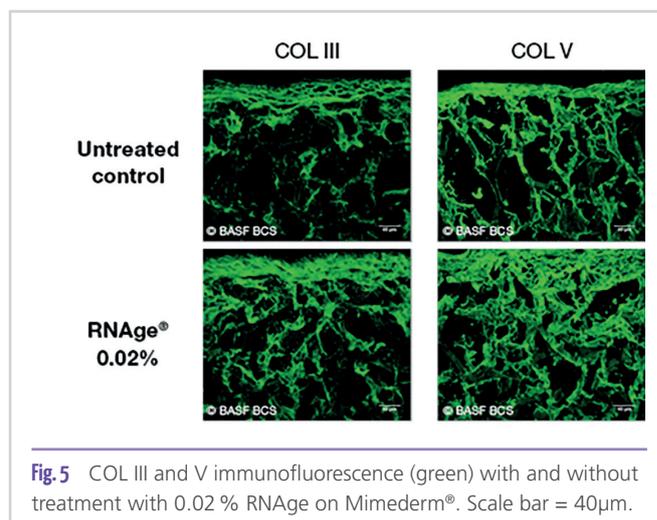


Fig. 5 COL III and V immunofluorescence (green) with and without treatment with 0.02% RNAge on Mimederma®. Scale bar = 40µm.

Proof of Concept: *in vivo* Assessments

Effect of RNAge on Dermal Density, Skin Firmness and Half-chin Angle

A double-blinded, randomized, split-face study confirmed the *in vitro* results. This was conducted on 27 Caucasian females aged 55 to 70 years who presented a loss of firmness and elasticity in facial skin. For a period of 56 days, the group applied either 0.2% RNAge or a placebo formulation twice daily. Subsequently, key signs of a sagging jaw line and chin area were assessed, i.e. dermal density, skin firmness and half-chin angle.

To assess the effect of RNAge on the dermal density of the jaw line, the hyperechogenic density factor was measured by ultrasound (Fig. 6). The echography clearly showed the difference between RNAge (white arrows) and placebo treatment vs D0 (green arrows). Dermal density improved significantly versus placebo and was up to 26% after 56 days of application.

Changes in skin firmness of the jaw line were measured by SkinFibroMeter. After 56 days of treatment, RNAge demonstrated a significant 18.5% increase in skin firmness compared to placebo.

The half-chin angle factor was measured by image analysis on macro-photos. After 56 days, RNAge significantly decreased the half-chin angle versus placebo. On average, the improvement was -2.5%.

Conclusion

By targeting epigenetic mechanisms in fibroblasts, BASF's new anti-aging ingredients positively influence the structural properties of the dermis and offer promising opportunities to counteract the loss of the skin's biomechanical properties observed during aging.

Dermagenist protects cells against LOXL1 promoter DNA methylation, helping them maintain their metabolic functionalities and produce collagens, and retain their contractility and ability to strongly adhere to the extracellular matrix. The active re-induces expression of LOXL1, synthesis of collagen I and BMP1-functionalized collagen protein.

RNAge inhibits the expression of miRNA Let-7b, which is responsible for impairing the synthesis of five dermal proteins involved in both collagen and elastic fiber organization and GAG synthesis.

To conclude, Dermagenist and RNAge help aged skin stay dense and firm, making them suitable for use in a wide range of anti-aging products.

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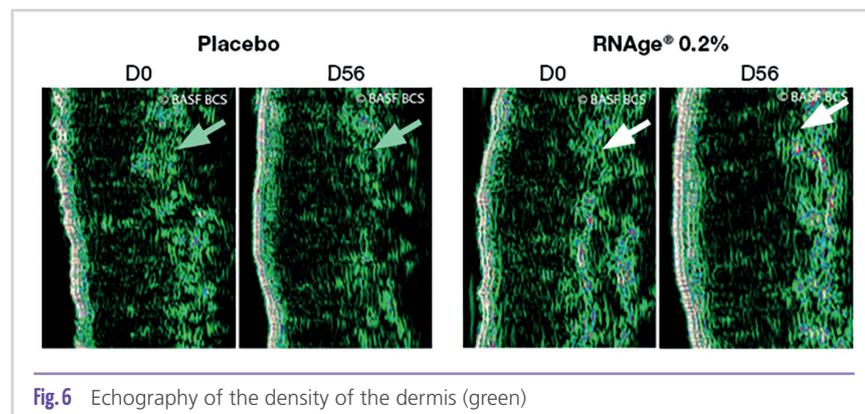


Fig. 6 Echography of the density of the dermis (green)

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