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DECORINYL®

CODE: PD090

Date: December 2009 Revision: 11

GENERAL DESCRIPTION

The extracellular matrix (ECM) consists of three major types of macromolecules - fibres, proteoglycans and glycoproteins - each of which is synthesized and maintained by cells specific to the tissue type. The two most important fibrous components of the ECM are collagen and elastin.

A characteristic property of collagens is to form highly organized polymers. In the fibrillar collagens (such as type I collagen), the polypeptide chains, called α-chains, are synthesized in the shape of procollagen, a triple helix with long loose ends. Procollagen is secreted into the extracellular space where most of the nonhelical ends are enzimatically removed. This allows the shortened molecules, now called tropocollagen, to assemble into ordered polymers called collagen fibrils, which are thin structures (<300 nm in diameter), many hundreds of micrometers long in mature tissues, and clearly visible in electron micrographs. The collagen fibrils often aggregate into larger, cable-like bundles, which can be seen in the electron microscope as collagen fibres several micrometers in diameter. This process is called fibrillogenesis.

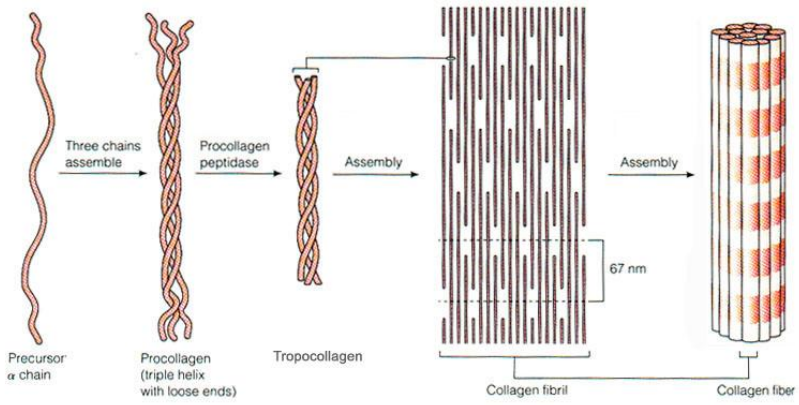


Fig. 1. Formation of Collagen fibres

Fibrillogenesis is an essential process in tissue formation, but must be controlled and regulated in order to avoid excessive bundle-like aggregation of collagen. The molecule responsible for fibrillogenesis control is a proteoglycan called ***decorin***.

The second major components of the ECM are the proteoglycans, a diverse group of soluble macromolecules that have both structural and metabolic roles. Proteoglycans act as tissue organizers, influence cell growth and maturation of specialized tissues, play a role as biological filters and modulate growth-factor activities.

Decorin belongs to a growing family of structurally related proteoglycans, grouped as the small leucine-rich proteoglycans (SLRPs), that are directly involved in the control of matrix organization and cell growth.

Decorin works by inserting itself between two parallel neighbouring collagen molecules in the fibril, helping to stabilise them and orient fibrillogenesis. As decorin binds to the surface of collagen fibrils, it delays the lateral assembly of individual triple helical collagen molecules, and the diameter of the fibrils is decreased. [Schönherr E. J. Biol. Chem. 270:8877-8883, 1995]. This controls fibril dimensions, uniformity of their diameter and their regular spacing, thus helping to establish and maintain tissue shape.

The binding of one collagen triple helix to decorin is proposed to play a major role in the formation of the staggered arrangement of collagen molecule within the microfibrils by preventing lateral fusion of collagen molecules. Decorin governs collagen fibril growth and influences higher order matrix assembly.

Structurally, decorin is similar to a horseshoe where the ß-sheets form the inner concave surface and the α-helices make up the outer convex face. The inner concave surface of decorin is of suitable size to accommodate a single triple helix of collagen.

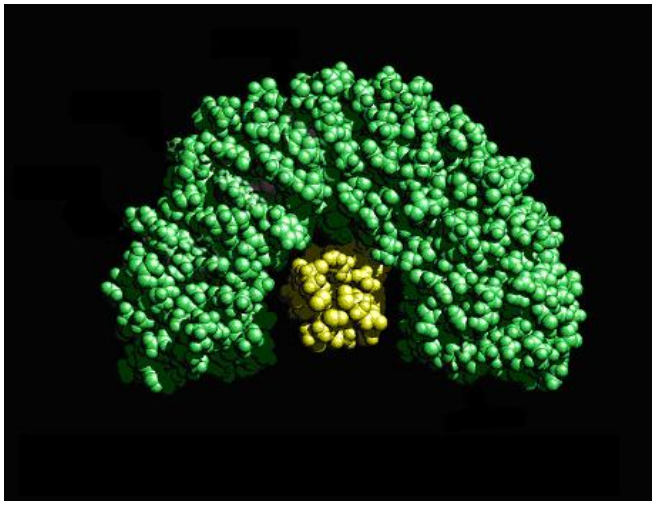


Fig. 2. Model of decorin (green) complexed with a triple helix of collagen (yellow)

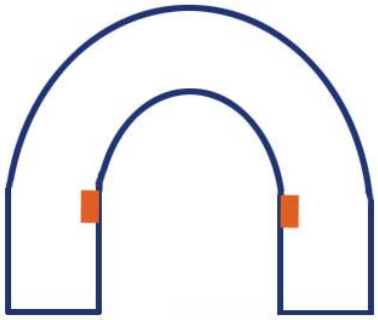
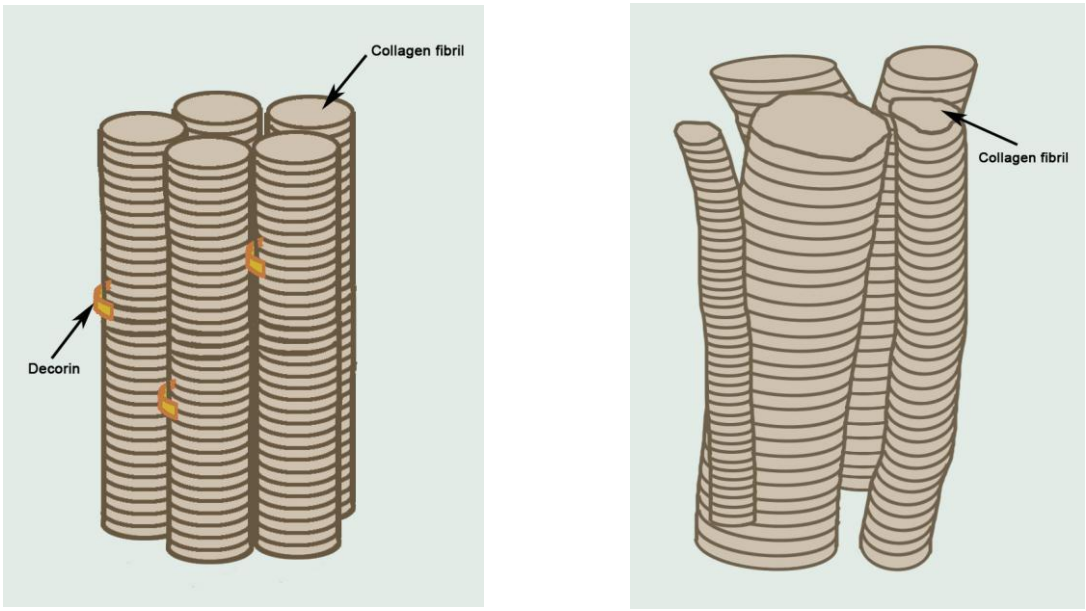


Fig. 3. Collagen binding sites in decorin

Decorin is associated with collagen molecules at specific binding sites in the protein core. There are two collagen binding sites on decorin, one on each arm of the

horseshoe. Two tetrapeptide sequences have been identified in the literature as the specific binding sites of decorin to collagen fibrils [Scott JE. *Biochemistry* 35:8795-99, 1996].



a) Fibrils with uniform diameter (young skin) b) Fibrils with irregular shape (old skin)

Fig. 3. Fibril assembling a) with decorin, and b) without decorin

The importance of decorin modulation in collagen fibrillogenesis has been shown in decorin null mice: homozygous animals are characterised by skin with reduced tensile strength, containing collagen fibrils with irregular profiles due to lateral fusion. [Danielson KG et al. J. Cell Biol. 136:729-743, 1997]. Therefore, decorin is essential for the skin in order to control collagen aggregation and to homogenise fibril diametres and dimensions in order to assure its functional morphology and physical characteristics. Absence of decorin induces fragile skin.

However, the aging process induces significant changes in the proteoglycans present in the skin. The most pronounced change in skin proteoglycans is the appearance in mature skin of a catabolic fragment of decorin [Carrino D. *Journal Biological Chemistry* 278:17566-17572, 2003]. Adult human skin contains a truncated form of decorin, which lacks regions of decorin previously shown to be important for interaction with collagen. The appearance of this catabolic product may have significant effect on skin elasticity and morphologic differences between collagen fibres of young and mature skin. So, we need to provide the skin with a substitute for the non functional decorin we encounter as we age.

Lipotec S.A. has synthesised a tetrapeptide, DECORINYL®, which is a mimic of the sequences of decorin that specifically bind to collagen fibrils. [Scott JE. *Biochemistry* 35:8795-99, 1996]. DECORINYL® has proved to regulate fibrillogenesis, control collagen fibril growth and increase skin suppleness, due to a better cohesion of collagen fibres which provides higher resiliency, improving skin appearance.

DECORINYL® has been incorporated into a liposomal system for enhanced penetration and increased efficacy.

PROPERTIES AND APPLICATIONS

- DECORINYL® makes up for the lack of functional decorin as skin ages

- DECORINYL® binds to collagen fibrils and regulates collagen fibrillogenesis, also enhancing collagen fibril stability

- DECORINYL® ensures uniformity of fibril diameter and the regular spacing of collagen fibrils, maintaining tissue shape and giving suppleness to the skin

- The liposomes confer a high and sustained moisturising profile

DECORINYL® can be incorporated in cosmetic formulations such as emulsions, gels, sera, etc., where suppleness and strength of skin is desired.

TECHNICAL INFORMATION

PRODUCT SPECIFICATIONS

Code:	PD090
Appearance:	Suspension
Colour:	Off white to Amber
Active ingredient content:	0.2% Tripeptide-10 Citrulline
Preservative:	0.9 % Phenoxyethanol

PROCESSING AND DOSAGE

DECORINYL® can be incorporated to the formulation in the final stage of the manufacturing process, when the temperature reaches 35 - 40 °C. Care should be taken not to exceed 50 °C.

It is recommended that 5% of DECORINYL® is present in the final formula.

STORAGE AND SHELF LIFE

Keep in a clean, cool and dark place. If product is stored as recommended it will remain stable for 12 months.

SAFETY

The toxicological profile of DECORINYL® for cosmetic purposes was assessed *in vitro* and *in vivo*. A full toxicological report and a summary of all the safety tests performed are available on request.

In vitro tests

Citotoxicity test on 3T3 fibroblasts
The results showed no signs of citotoxicity at the concentrations assayed.

Citotoxicity test on human epidermal keratinocytes
The results showed no signs of citotoxicity at the concentrations assayed.

Mutagenicity test (Ames test)
The results showed no mutagenical activity under the conditions assayed.

Ocular Irritation (HET-CAM test)
The product is potentially not irritating for the eyes.

In vivo tests

Skin sensitisation (Hypoallergenicity)
An HRIPT (Human Repeated Insult Patch Test) was performed on 100 volunteers aged 25 to 59. Decorinyl® did not cause sensitisation in any volunteer so it can be classified as “harmless” as regards of the possibility of skin irritation.

EFFICACY

In vitro

Collagen Fibrillogenesis Assay

Type I is the principal collagen of skin and its molecules assemble into ordered polymers called collagen fibrils, which are thin structures (<300 nm in diameter). Collagen fibrils often aggregate into larger, cable-like bundles, collagen fibres (several micrometers in diameter). This process, called fibrillogenesis**,** results in formation of collagen bundles that are responsible for the strength and resiliency of the skin.

As a function of age, skin undergoes dramatic changes in its mechanical properties, including changes in tissue hydration and resiliency, both related to a decrease in the proportion of proteoglycans, such as decorin, that induce changes in collagen fibrils or fibres.

Type I Collagen from calf skin (Sigma) samples was treated with DECORINYL® at concentrations of 0.001%, 0.01%, 0.05% and 0.1% (w/v).
The process of fibrillogenesis was measured as turbidity change by monitoring the change in absorbance at 405 nm at 30-min or 1-h intervals.

DECORINYL® presents an inhibitory effect on fibrillogenesis respect to control, as shown in Fig. 4 by lower turbidity readings. Both control and DECORINYL® reach the saturation after around 3 hours.

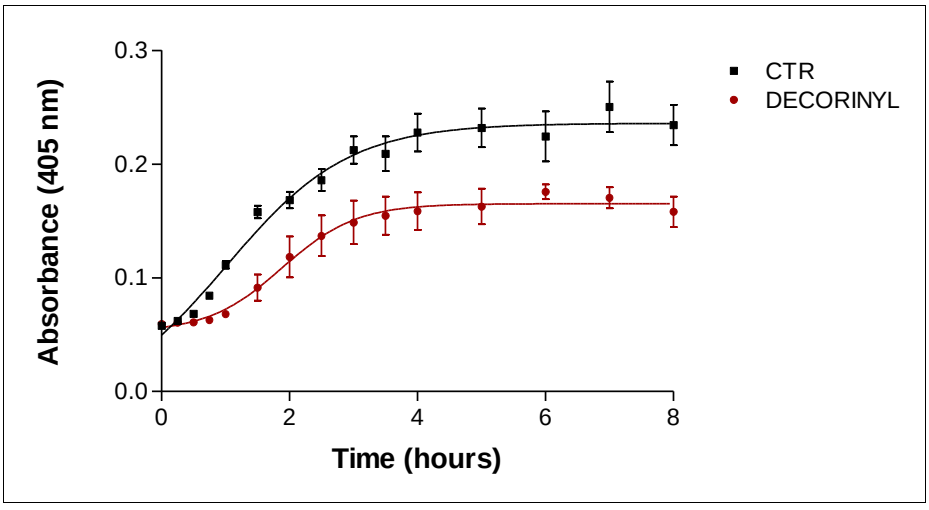


Fig. 4. Effect of DECORINYL® at 0.1% on type I Collagen fibril formation

Fig. 5 represents absorbance readings in percentage respect to control at 2.5 hours (the time point previous to saturation). All tested concentrations of DECORINYL® present significant inhibitory activity on fibrillogenesis respect to control, in a dose-dependent manner.

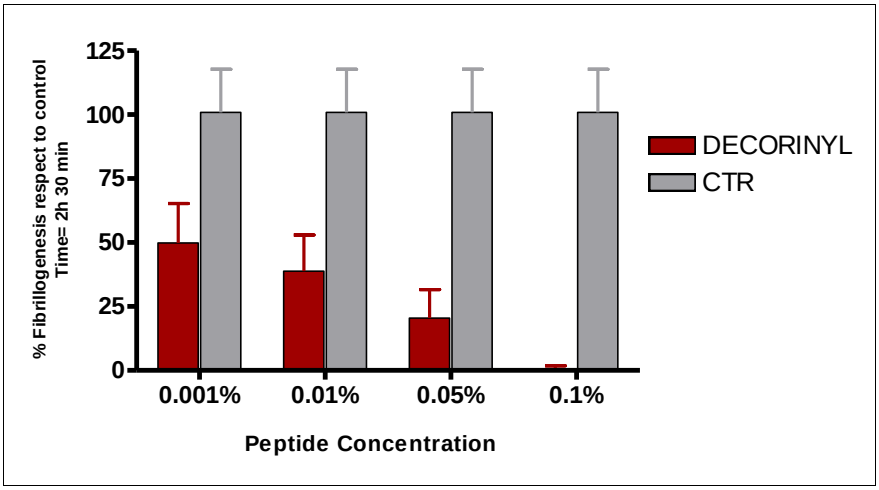
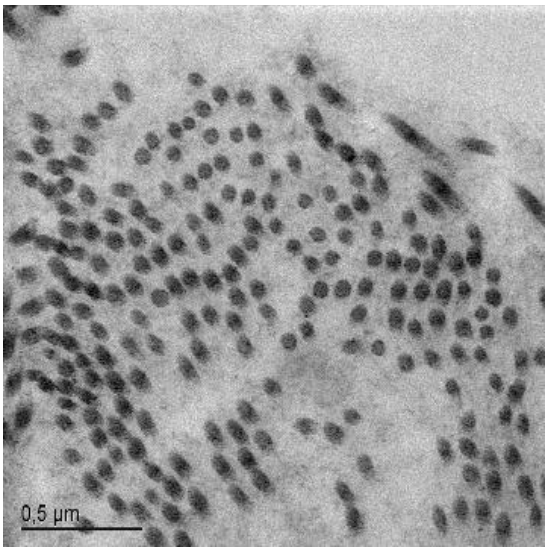


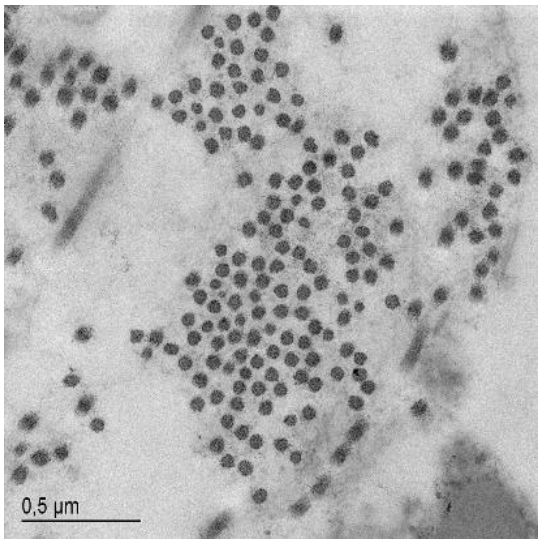
Fig. 5. Dose-response effect of DECORINYL®

Dermal collagen fibrils study in a human skin model

Tissues from a tridimensional human skin model EFT-200 (MatTek Corporation), are treated with DECORINYL® 0.01%. Non treated tissues are used as control. Tissues are sectioned and then observed by Transmission Electron Microscopy (TEM) (Fig. 6.). The diameter of Collagen fibres of two areas randomly chosen from each sample is measured. The data obtained from the measurements is statistically analysed using the *One way ANOVA* analysis (Fig. 7).



Control (untreated)



Treated with DECORINYL®

Fig.6. Images of human skin model sections from which fibres diameters have been measured

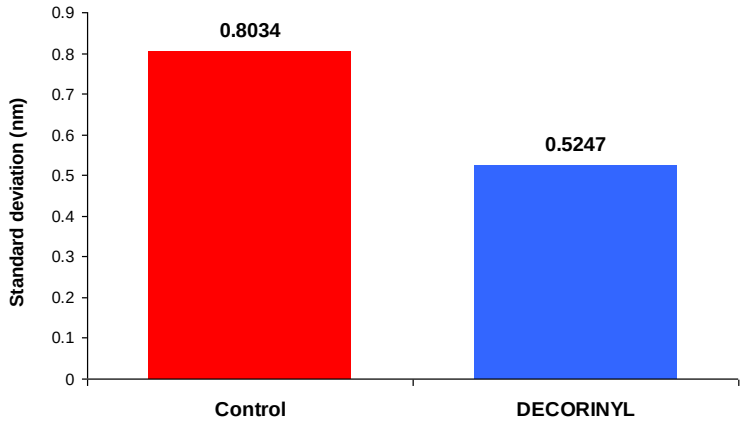


Fig. 7. Standard deviation of collagen fibril diameter measurements

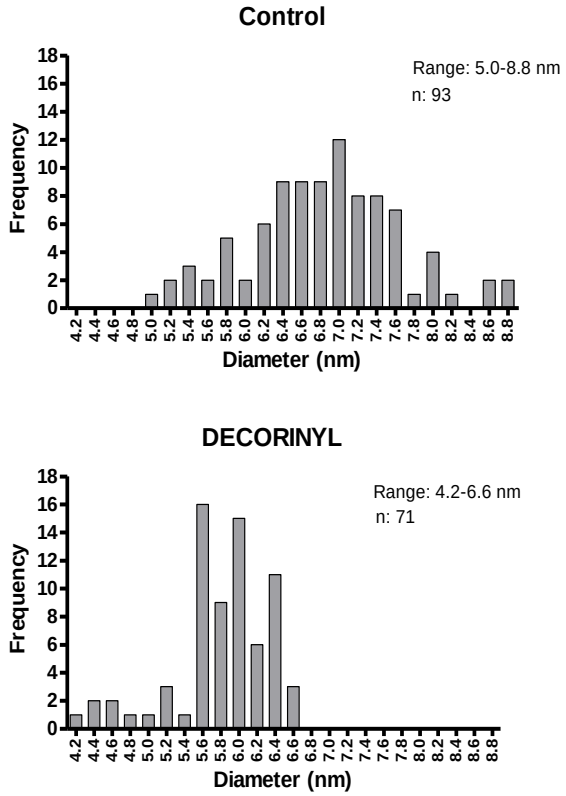


Fig. 8. Distribution of collagen fibril diameter from organotypic human cultures untreated (control) or treated with DECORINYL® 0.01%

The standard deviation is 34.7% lower after the treatment with DECORINYL® 0.01% (Fig. 7). The analysis of variances shows that the fibres treated with DECORINYL® are more uniform, due to its low variability (Fig. 8).

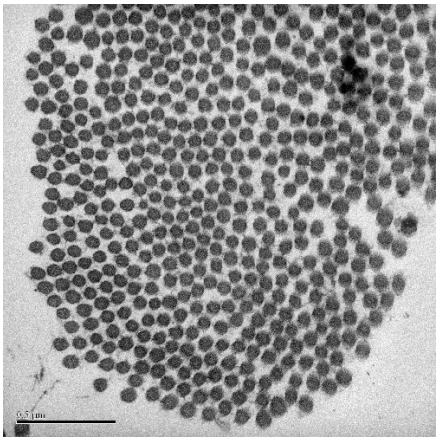
DECORINYL® has proved to influence on the diameter of collagen fibres, making them more uniform.

Ex Vivo

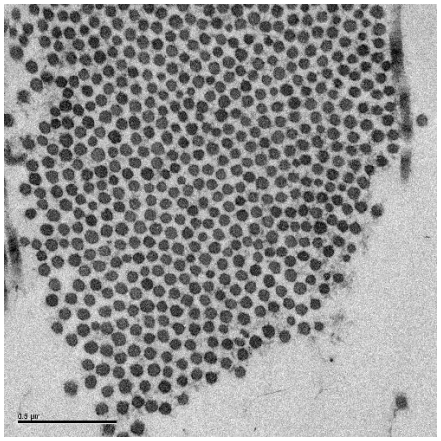
Histochemical study of human skin biopsies

DECORINYL® was tested in order to evaluate its effect in skin collagen fibrils. This test is a replicate of the *in vitro* test, this time using a panel of volunteers.

Skin biopsies of three patients were evaluated before and after a two-month treatment with a cosmetic formulation containing 0.01% DECORINYL®. The collagen fibril diameter was measured from Transmission Electron Micrographs using the AxioVision.AC software (Carl Zeiss) (Fig. 9).



0 months



2 months

Fig.9. Transmission electron micrographs of dermal collagen from skin biopsies of Patient 1

Results demonstrated that the average collagen fibril diameter significantly varies before and after DECORINYL® treatment. After a two-month treatment, collagen fibrils in all patients show a decrease in the standard deviation of the collagen fibril diameter (Fig. 10).

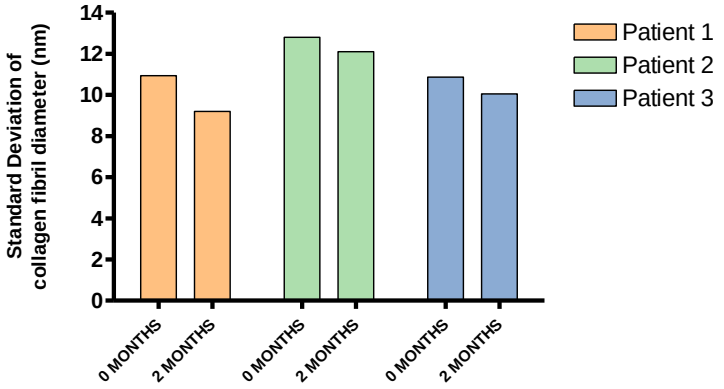


Fig.10. Standard Deviation of collagen fibril diameter in skin biopsies before and after a treatment with DECORINYL®

Analysis of frequency and distribution of collagen fibril diameter reveals that the range and distribution is different before and after treatment with DECORINYL® cosmetic formulation (Fig. 11).

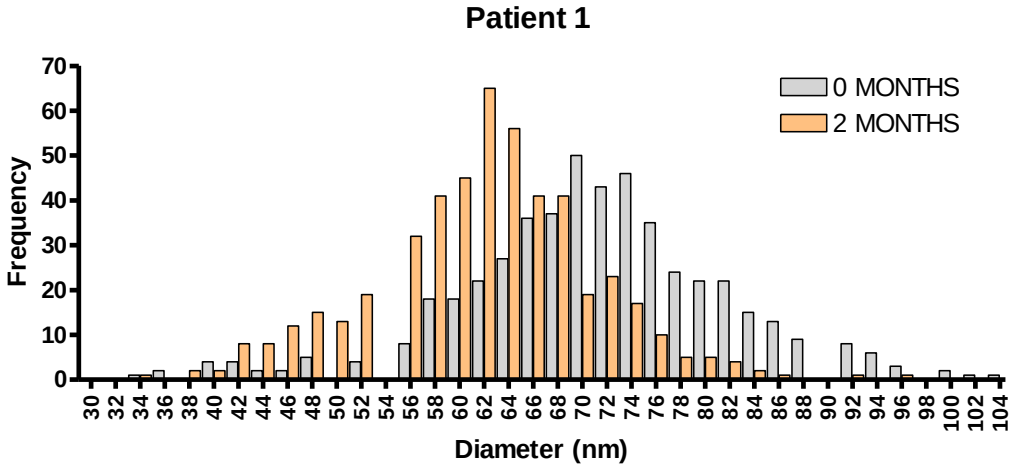


Fig. 11. Distribution of collagen fibril diameter from skin biopsies of Patient 1 before and after a 2-month treatment with DECORINYL®

All three patients show a decrease in the standard deviation of collagen fibril diameter after two-months of treatment with DECORINYL®. This reduction implies a decrease in the variability of the collagen fibril diameters and a higher uniformity of collagen fibrils after the treatment with the peptide. The average decrease of the standard deviation of collagen fibril diameter was 9.63%.

In conclusion, this product is able to mimic the decorin activity and interact with collagen fibrils, regulating the fibrillogenesis process, controlling fibril dimensions and uniformity of its diameter, thus helping to establish and maintain skin mechanical properties and morphology.

In vivo

Measurement of skin biomechanical properties: skin suppleness

DECORINYL® has been tested *in vivo* on a group of 22 female volunteers, aged 40 to 58. A cream containing 5% DECORINYL® was applied daily on the face (temple) during 28 days. Another group of 21 female volunteers was treated with a placebo cream.

Variations on skin suppleness were measured with a MPA 580 Cutometer®. The technique consists of skin aspiration by a measurement probe. The skin is sucked into the orifice of a probe by constant vacuum pressure for a set length of time. The depth to which the skin penetrates into the probe is measured by two optical prisms located at the opening of the probe's orifice to eliminate the effects of friction and mechanical strain. Parameters are obtained after five skin aspirations. This procedure enables the evaluation of variations in the biological extensibility and elasticity of superficial cutaneous layers.

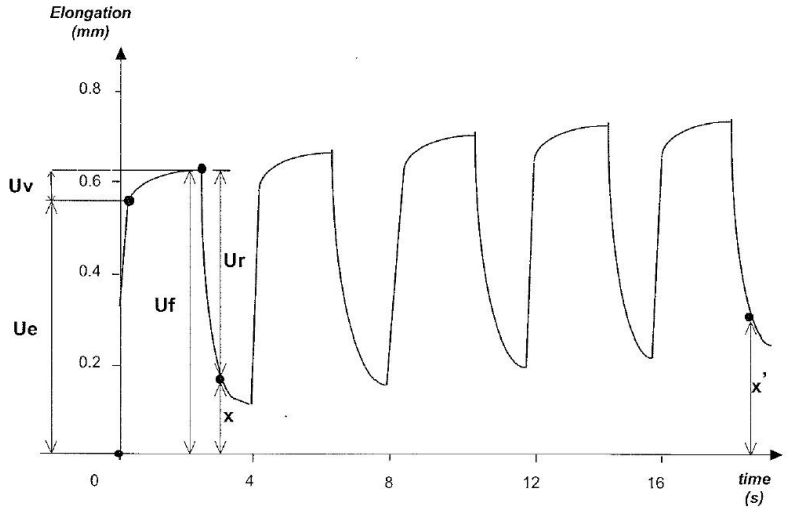
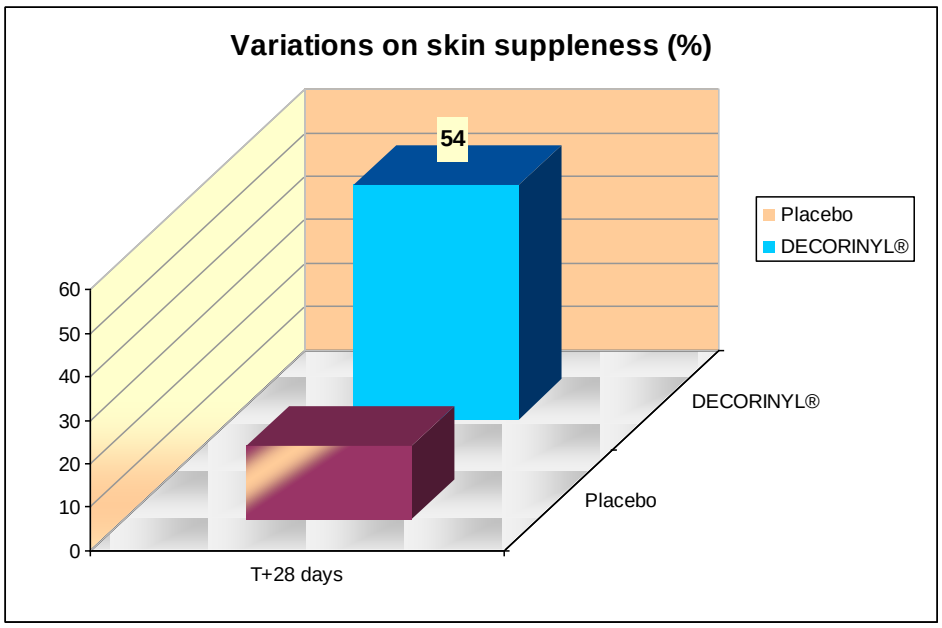


Fig. 12. Cycle of five deformations of the skin measured with the Cutometer®

The rheological parameters obtained after five skin aspirations are:

- immediate distention: Ue (mm)
- delayed distention: Uv (mm)
- total elongation Uf = Ue + Uv (mm)
- immediate retraction Ur (mm)
- residual distention after 1st cycle: X (mm)
- residual distention after 5th cycle: X' (mm)

Skin suppleness is defined as the immediate skin extensibility (Ue): if the immediate extensibility increases, skin is suppler (ΔUe). Variations of skin suppleness were measured at time 0 and after 28 days.



After 28 days, the cream containing DECORINYL® induced a **54% increase** in skin suppleness ($p<0.001$), and this effect was observed in **95% of the volunteers**. No significant increase was observed for the placebo cream.

GENERAL PRODUCT INFORMATION

Trade name	DECORINYL®
Product code	PD090

INGREDIENTS

INCI name	CAS No	EINECS No
WATER (AQUA)	7732-18-5	231-791-2
LECITHIN	8002-43-5	232-307-2
TRIPEPTIDE-10 CITRULLINE	960531-53-7	N.L. [a]
CARBOMER	9003-01-4	N.L. [a]
TRIETHANOLAMINE	102-71-6	203-049-8
PHENOXYETHANOL	122-99-6	204-589-7
CAPRYLYL GLYCOL	1117-86-8	214-254-7

[a] Not Listed

Note: Graphs and photographs 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 Lipotec. Customers are responsible for compliance with local and international advertising regulations.
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