THE PAPILLARY DERMIS

L'Oréal Research has substantiated a major breakthrough by developing a new generation of anti-aging active ingredients

The targeted area, which lies just beneath the epidermis, is known as the ***papillary dermis***: the most vulnerable when confronted with the aging process. L'Oreal scientists pooled their expertise to identify and study this shield that retains youthful skin and to discover the means for preserving it.

This wonderful scientific adventure brought together three fields of excellence: enterprising, fundamental research, a unique experimental tool that portrays models of reconstructed skin and a tried and tested know-how for formulating active ingredients.

A STRATEGY FOR LONG-LASTING YOUTHFULNESS

THE PAPILLARY DERMIS

Over 20 years exploring the dermis

As people age, skin becomes finer, drier and loses its elasticity. The aging process induces drastic structural changes in the various compartments of the skin which not only modify its appearance but also its physical and mechanical properties.

Increasingly accurate measurements for the effects of aging

L'Oréal Group researchers are taking advantage of the introduction of ***non-invasive biophysical methods*** and of ***advances in cellular biology*** by studying these changes *in vivo* and *in vitro* on a molecular level. This was carried out not only on the epidermis level but also within dermis structures *(see page 3)*.

Ultrasounds, now used for assessing the skin's echostructure, provide information on skin thickness and dermis consistency. The studies show that ***when people age, skin thickness decreases and the superficial (or papillary) dermis structure loses its density***, i.e. its echogenicity.[1] .

In 1993, a team of L'Oréal researchers, using Magnetic Resonance imaging, illustrated that ***the content of free water in the superficial dermis increases with age***[2]. This increase was linked to the decrease of GAGs content and to a weakening in the dermo-epidermal junction network. As people age, this dermo-epidermal junction de-wrinkles, then flattens out. The dermal papillae disappear, which leads to a reduction in the size of the exchange area between the two compartments. The weakening in the dermo-epidermal junction network contributes probably to a loss in elasticity, which plays a role in wrinkle formation.

These results show that the ***papillary dermis becomes a vulnerable area during the aging process***.

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[1] *De RIGAL J. et al, J Invest Dermatol, 93 : 621-625, 1989*

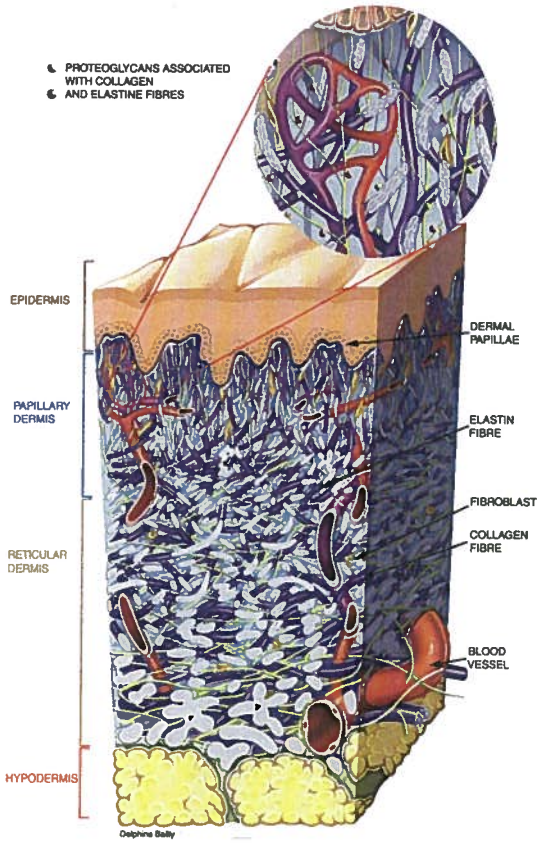
[2] *S. RICHARD, B. QUERLEUX, J. BITTOUN, O. JOLIVET, I. IDY-PERETTI, O. DE LACHARRIERE and JL. LÉVÊQUE.* Characterization of the Skin In Vivo by High Resolution Magnetic Resonance Imaging: Water Behavior and Age-Related Effects. J Invest Dermatol 100: 705-709, 1993

THE DERMIS

The dermis, made up of elastin and collagen fibres which are associated with glycoproteins, is the tissue that supports the skin.

Its surface, at the epidermis junction (***dermo-epidermal junction***), bristles with fibrous, vascular and nervous bulges: ***dermal papillae***. These delineate the boundary with the outermost dermal layer: ***the papillary (or superficial) dermis.***

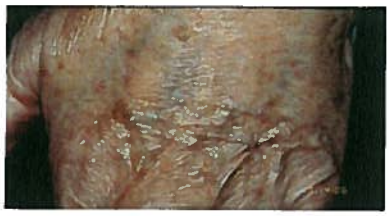
Below this, ***the reticular (or deep) dermis*** constitutes the largest part of the dermis. At this level, the elastin and collagen fibres are dispersed in a multidirectional way whereas in the papillary dermis, the elastin fibres are mainly all positioned perpendicular to the skin's surface.



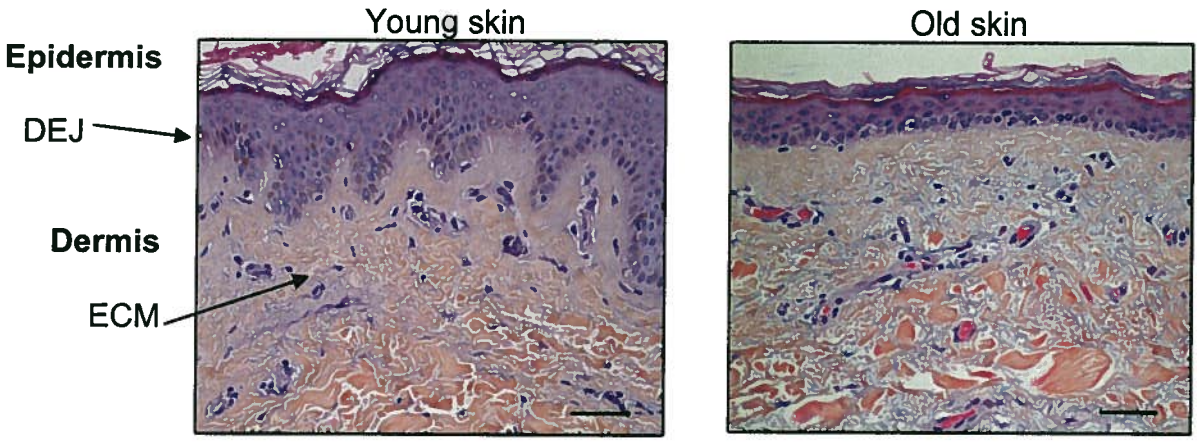
The fibroblasts are the main cells of the dermis. These cells, which are predominantly located in the papillary dermis, and quite rare in the reticular dermis, are specialized in synthesizing the collagen and elastin fibres that constitute ***the extracellular matrix (ECM)***. Collagen fibres provide the ECM with resistance against tension and to traction, whereas elastin fibres provide it with elastic properties.

This fibre network is dispersed within a mixture made up of macromolecules: glycoproteins, **glycosaminoglycans (GAGs)** and proteoglycans (PGs) which capture water and help maintain the cohesion and structure of the dermis. The mecanic properties of the dermis provide the skin with firmness and tone.

Signs of skin aging



The ultimate clinical signs of skin aging are the papyraceous (or cigarette paper) aspect, skin slackening and its loss of consistency: the skin feels like its "creased and crumpled".



On a histological level, skin aging is expressed by a reduction in the thickness of the epidermis, a flattening out of the dermo-epidermal junction (DEJ) and the disappearance of dermal papillae, as well as an atrophy and a disorganization of the extracellular matrix (ECM).

A STRATEGY FOR LONG-LASTING YOUTHFULNESS

Fibroblasts used as models for studying aging

The study of dermis-level aging was centred for a long time on the changes to extracellular matrix macromolecules, such as collagen, but rarely dealt with the constituent cells: ***the fibroblasts***.

In 1979, the American researchers R.A. Harper and G.Grove revealed for the first time the morphological and biological differences between the two populations of dermal fibroblasts[3]: those of the superficial dermis (the papillary fibroblasts) divide more quickly than those of the deep dermis (reticular fibroblasts).

L'Oréal Research picked up on this idea and has implemented an incredible tool to pursue dermal exploration: models of ***in vitro skin reconstructed*** from human cell cultures. This experimental method which was initiated over 30 years ago in L'Oréal's laboratories (*see frame*) has been optimized by the team led by Daniel Asselineau, now head of L'Oréal's "Skin Aging" Biological Research group.

In 1992, he developed ***models of reconstructed skin incorporating papillary and reticular fibroblasts***.
From that moment onwards, research increased on these two dermal cell populations as researchers were convinced that they were a fundamental key to the skin aging process.

"For more than 20 years we have been interested in fibroblasts: these cells that are responsible for forming and maintaining dermal structure. We have explored the biological properties and the development with age of these cell populations in the skin, using our reconstructed skin models. Understanding this is the essence for developing new anti-aging strategies". Daniel Asselineau (Ph.D), head of L'Oréal's "Skin Aging" Biological Research group

[3] HARPER R.A., GROVE G., Science, 1979, 204-526 HARPER R.A., GROVE G, J. Cell Sci, 1982, 57, 177-187 Human skin fibroblasts derived from papillary and reticular dermis: difference in growth potential in vitro

A STRATEGY FOR LONG-LASTING YOUTHFULNESS

Studies on reconstructed skin highlight the differences

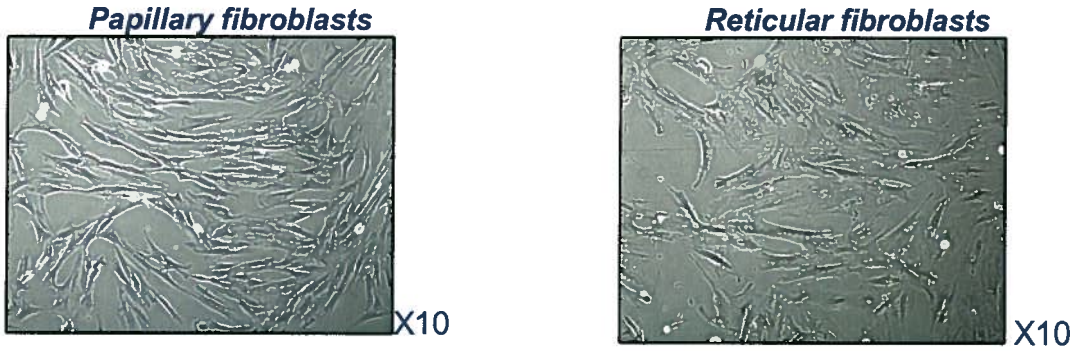
The L'Oréal research teams studied two fibroblast populations through reconstructed skin. The results confirm a certain number of differences on a morphological level relating to the production of growth factors, proteins and other molecules that can be dispersed through the extracellular matrix.

Papillary fibroblasts are characterized by ***a high percentage of small-scale cells*** that are granular and that have ***higher growth potential*** than reticular fibroblasts.

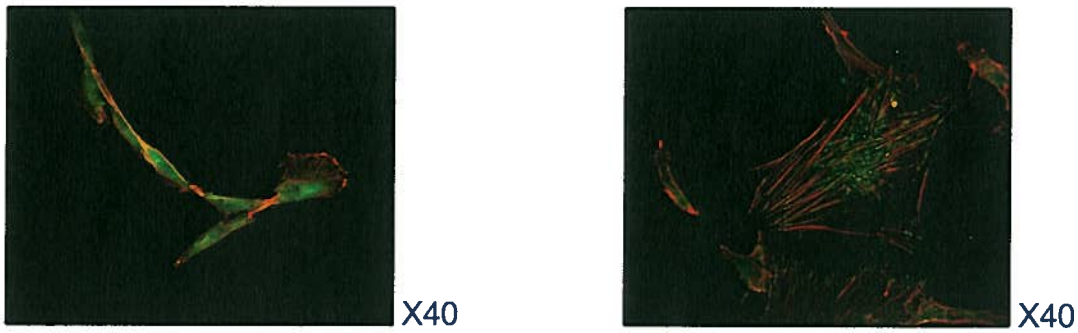
They ***stimulate the epidermal differentiation***: the epidermis of skin reconstructed from papillary fibroblasts is of a higher quality than that reconstructed using reticular fibroblasts.

Reticular fibroblasts stimulate matrix synthesis.

In vitro characterization of papillary and reticular fibroblasts



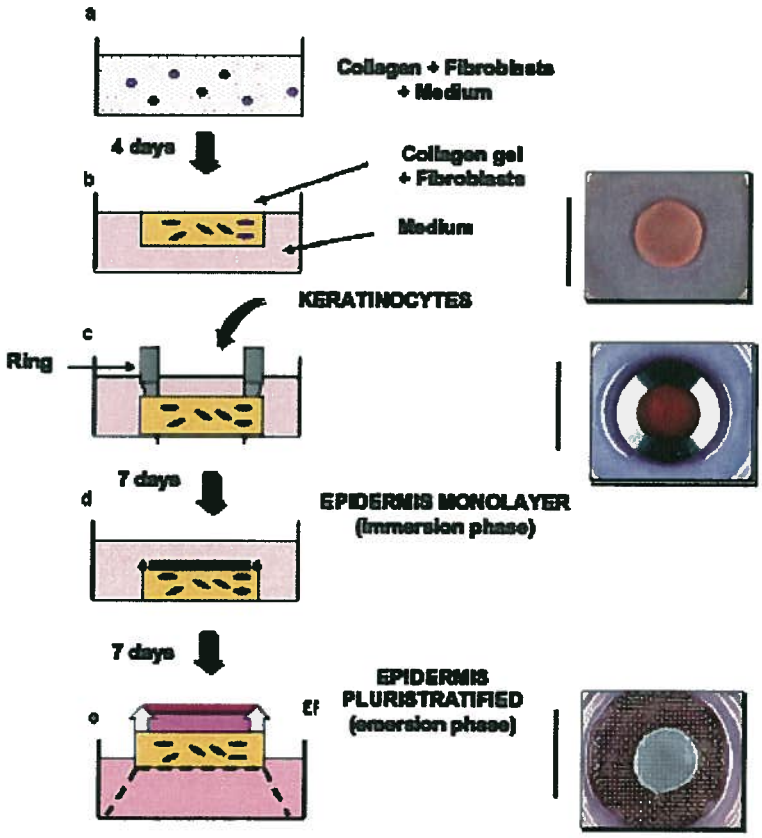
Morphological analysis using phase-contrast microscopy



Actin and Vinculin immunofluorescence indirect marking

RECONSTRUCTED SKIN: A UNIQUE TOOL

Initially developed for grafting severely burned patients, reconstructed skin is also a source of valuable models for testing new dermatological products or cosmetics. Since 1979, the first time epidermis was reconstructed, different types of models made from cultured human skin cells have been developed to reconstruct, in vitro, either the epidermal structure or the dermal structure or the dermo-epidermal structure set.
In 1985, Daniel Asselineau reconstructed a genuine model of "live" human skin. The reconstructed dermis is made up of fibroblasts that are embedded in a collagen I-based matrix. On this dermis, human epidermal cells (keratinocytes) are seeded and cultivated to create corneal layers.



A STRATEGY FOR LONG-LASTING YOUTHFULNESS

The papillary dermis: principal target of aging

Between age and the appearance of aging, the difference is sometimes significant. Aging, caused by the environment and by its main factor, ultraviolet rays, superimposes chronological aging, caused by time and genetic factors. By specifically exploring this "**photoaging**" process, researchers have managed to track the source of skin aging.

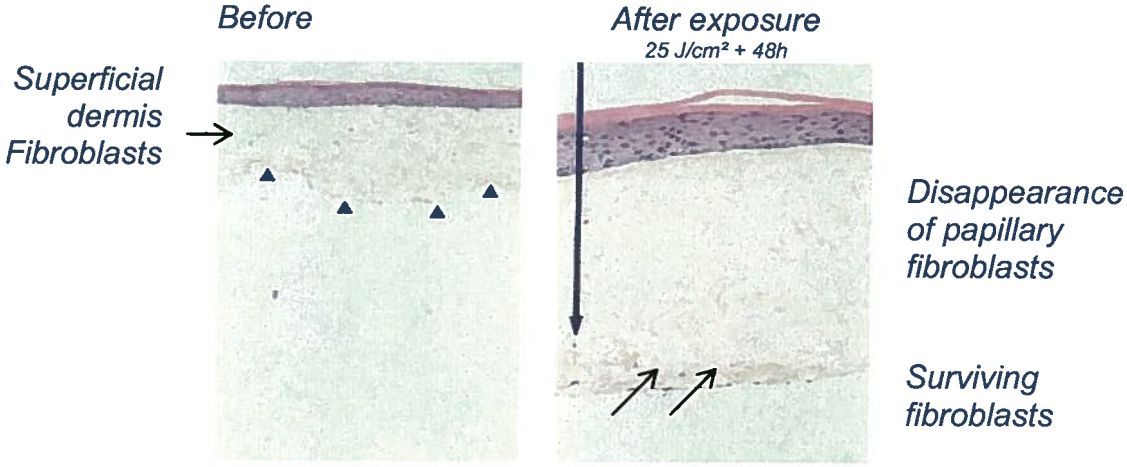
Daniel Asselineau and Françoise Bernerd (Ph.D), head of L'Oréal's "Light and Pigmentation" Biological Research Group, as such, studied the effects of ultraviolet rays on the skin, particularly UVA rays which are the most penetrating. To assess these effects at cellular level, they worked on reconstructed skin subjected to different levels of exposure. In 1998, unprecedented results were published.

The fibroblasts of the superficial dermis disappear following exposure to UVA[4]

A few hours after moderate exposure to UVA (corresponding to 10% of the daily dose absorbed by facial skin in mid April in Paris), ***the fibroblasts of the superficial dermis atrophy (apoptosis phenomenon) and completely disappear within 48 hours***.

Whereas the fibroblasts of the deep dermis remain and recolonize the dermis. This recolonization is associated with ***a reduction in dermal thickness***. It is also characterized by ***a high production of the enzymes which are responsible for damaging collagen*** and of matrix proteins which stick together in clusters adversely affecting elastin fibres. It is for this reason that the skin loses its firmness and elasticity simultaneously.

Effects of UVA on reconstructed skin



[4] BERNERD F., ASSELINEAU D., Cell Death and Diff, 1998, 5, 792-802
UVA exposure of human skin reconstructed in vitro induces apoptosis of dermal fibroblasts.

A STRATEGY FOR LONG-LASTING YOUTHFULNESS

Papillary fibroblasts disappear during aging

In 2008, L'Oréal Research announced ***a major breakthrough***: for the first time ever, it was demonstrated that ***fibroblasts from the papillary dermis and those from the reticular dermis develop differently during the aging process***.

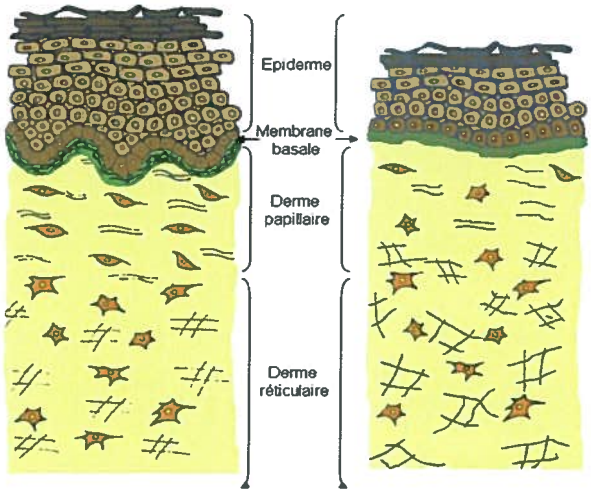
Researchers studied age-related changes on reconstructed skin using specific populations of fibroblasts, isolated on donors of different ages. The papillary and reticular fibroblast pairs from each young and old donor were analyzed and compared.

This work, carried out by L'Oréal illustrated that ***papillary fibroblasts were altered on a functional level and specifically disappeared during the aging process***, whereas reticular fibroblasts were preserved.

In fact, while aging the papillary fibroblasts are being transformed. They atrophy and they lose their growth potential, as well as their ability to stimulate the epidermal differentiation. Those fibroblasts enable the synthesis of extracellular matrix proteins.

This breakthrough, acknowledged by the scientific community, was published in PLoS ONE[5]. It provides a new perspective to skin morphogenesis and aging. ***The papillary dermis*** appears as ***the main shield for retaining youthful skin***. And, as such, the target for a new generation of anti-aging active ingredients.

Young skin Old skin



During the skin aging process, the papillary fibroblasts lose their particularities and tend to become similar to reticular fibroblasts on morphological , biochemical and functional levels.

[5] MINE S., FORTUNEL N.O., PAGEON H., ASSELINEAU D., PLoS ONE, 2008, 3 (12) : e 4066 Aging Alters Functionality Human Dermal Papillary Fibroblasts but Not Reticular Fibroblasts: A new View of Skin Morphogenesis and Aging

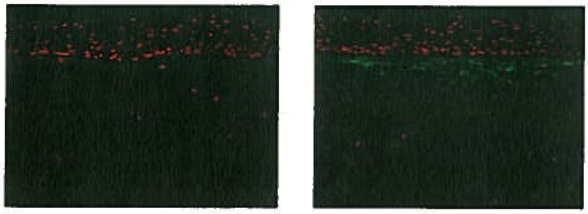
A STRATEGY FOR LONG-LASTING YOUTHFULNESS

Rhamnose, a new active ingredient for molecular purposes

The challenge is to select the most effective molecules to stimulate or to restore papillary fibroblast activity. Apart from elastin and collagen, the papillary dermis is rich in molecules that are related to sugars and more specifically to monosaccharide. Rhamnose was, as such, specifically selected by L'Oréal's biological evaluation unit as an appropriate anti-aging prospect.

The effectiveness of Rhamnose is illustrated in vitro on reconstructed skin
When Rhamnose is added to papillary fibroblast cultures of young or old skin, it proves its effectiveness.

Rhamnose stimulates collagen I production
It favours the production of pro-collagen I, precursor of collagen I, a protein that is fundamental in maintaining skin structure and whose production tends to decrease with age and with photoaging effects.

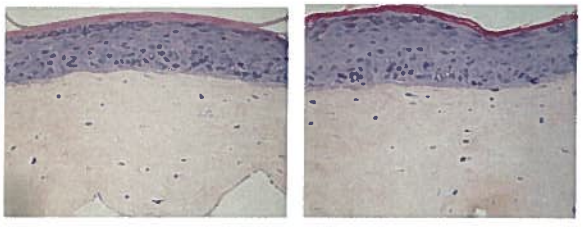


Rh- Rh+

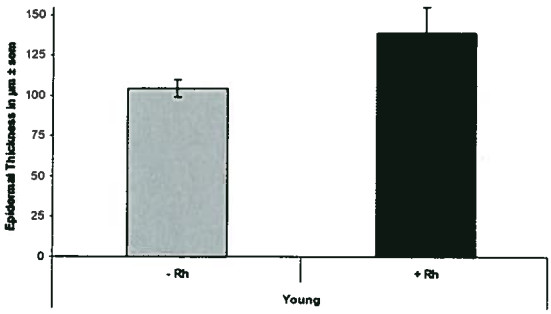
Immunomarking of collagen synthesized by fibroblasts in reconstructed skin. An increase in marking can be perceived (in dark green), i.e. a collagen increase on the skin treated with Rhamnose (Rh+).

Rhamnose reinforces the dermo-epidermal junction
By stimulating the synthesis of certain proteins involved in dermo-epidermal cohesion, particularly collagen IV and collagen VII.

Rhamnose induces an increase in epidermal thickness
This superficial layer of the skin becomes finer over time. Papillary fibroblasts reinforce their action on the differentiation and proliferation of epidermal cells (keratinocytes) under the effect of Rhamnose.



Rh- *Rh+*



Histology after the standard HES (Hemalun Eosine Saffran) staining of reconstructed skin obtained using (Rh+) or without using Rhamnose treatment (Rh-). The epidermis becomes thicker under the effect of Rhamnose.

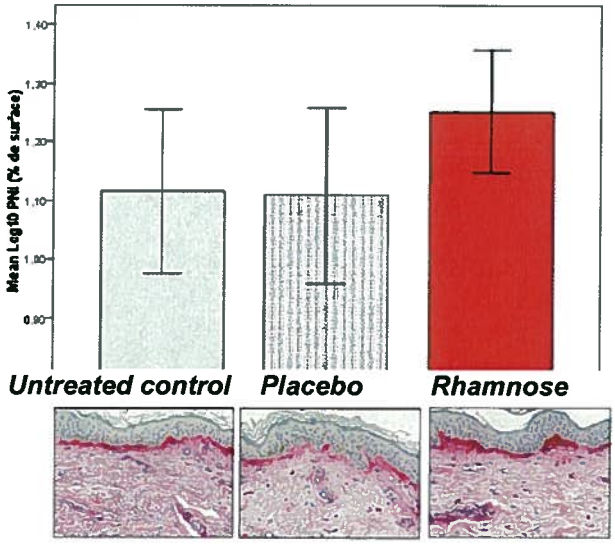
A STRATEGY FOR LONG-LASTING YOUTHFULNESS

The effectiveness of Rhamnose is confirmed *in vivo*

The 5% clinical proof-of-concept studies show that papillary fibroblasts are effective in producing "youthful" molecules.

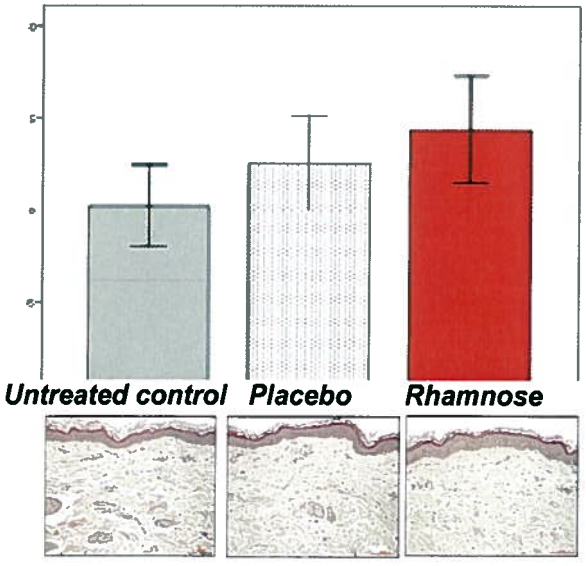
The effectiveness of Rhamnose on skin aging was ***assessed after 8 weeks of a bi-daily application of the treatment*** on the internal side of the forearm of female volunteers aged between 50 and 70 years old. The clinical and histological parameters were measured before and after treatment with Rhamnose or with the placebo.

The results obtained confirm the effects of Rhamnose obtained *in vitro*: after topically applying 5% Rhamnose for 2 months, ***a significant increase of he pro-collagen I expression at papillary dermis level and considerable epidermis thickening*** were measured.



Pro-collagen I expression

The increase is significant on skin treated with Rhamnose (p=0.04 with placebo, p=0.05 with untreated control).



Epidermal thickness

The difference in thickness is significant between the skin treated with Rhamnose and the untreated control (p<0.001).

A STRATEGY FOR LONG-LASTING YOUTHFULNESS

The first of a new generation of anti-aging active ingredients

The increase of the synthesis in the area treated is proof of the effectiveness of Rhamnose. This active ingredient has the ability to protect the skin and to repare its aging damages by increasing the synthesis *in vivo* of macromolecules whose role is fundamental in skin architecture.

The proofs of effectiveness illustrated *in vitro* and *in vivo* make Rhamnose the first of a new generation of active ingredients capable of preserving the cells which are targeted by aging.

Rendez-vous at the 2011 WORLD CONGRESS OF DERMATOLOGY in SEOUL
May 24/29, 2011

Communications :

- Isolation and description of two fibroblast subpopulations in the dermis of human adult skin.
  Asselineau D. , Pageon H.
- Different roles potentially played by papillary and reticular fibroblasts in skin investigated through the reconstruction of skin in vitro.
  Asselineau D. , Pageon H.
- Specific alteration of the papillary fibroblast population during aging of human skin.
  Asselineau D. , Mine S. , Fortunel N.,
- Beneficial effect of Rhamnose on reconstructed skin made with a dermal compartment containing papillary fibroblasts from young and old donors.
  Asselineau D. , Zucchi H. , Pageon H.
- Topical L-Rhamnose especifically induces pro-collagen-I in vivo in human aged skin
  Tran C. , Azouaoui A. , Bredoux C. , De Lacharrière O.

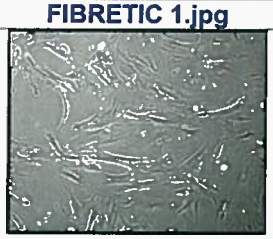
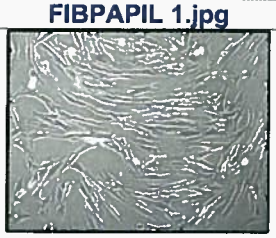
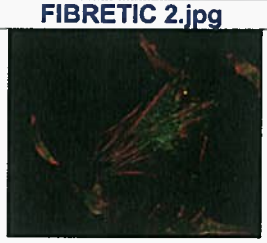
Symposium : *A new view on skin aging*

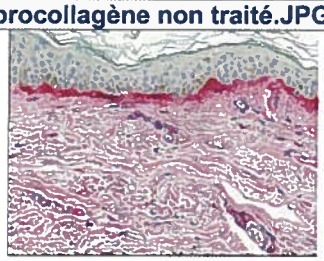
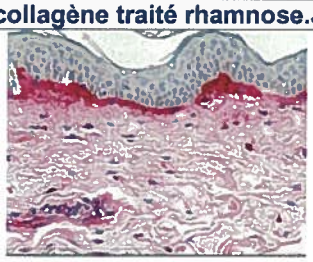
- Stimulating effects of Rhamnose on papillary fibroblast define a new skin anti-aging molecule
  Asselineau D.

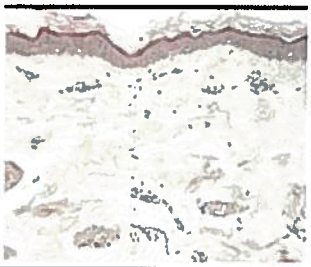
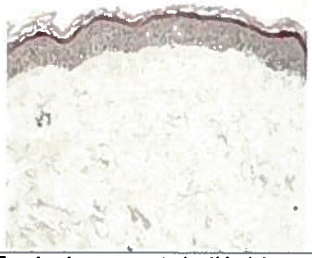
- The effects of Rhamnose on Asian reconstructed skin
  Cai A.

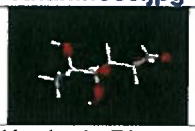
DERME PAPILLAIRE & RHAMNOSE
PAPILLARY DERMIS & RHAMNOSE

IMAGES, & LEGENDES IMAGES, & CAPTIONS

FIBRETIC 1.jpg	FIBPAPIL 1.jpg	FIBRETIC 2.jpg	FIBPAPIL 2.jpg
 X10	 X10	 X40	 X40
Fibroblastes réticulaires *Reticular fibroblasts*	*Fibroblastes papillaires* *Papillary fibroblasts*	*Fibroblastes réticulaires* *Reticular fibroblasts*	*Fibroblastes papillaires* *Papillary fibroblasts*

procollagène non traité.JPG	procollagène traité rhamnose.JPG
	
Expression du pro collagène I *Echantillon non traité* *Procollagen 1 expression* *Untraited control skin sample*	*Expression du pro collagène I* *Echantillon traité au Rhamnose* *Procollagen 1 expression* *Skin sample traited with Rhamnose*

Epaississement non traité.jpg	Epaississement traité rhamnose.jpg
	
Epaississement de l'épiderme *Echantillon non traité* *Epidermal thickness* *Untraited control skin sample*	*Epaississement de l'épiderme* *Echantillon traité au Rhamnose* *Epidermal thickness* *Skin sample traited with Rhamnose*

Rhamnose.jpg

Molécule de Rhamnose *Rhamnose molecula*