GREDECO
Groupe de Recherche et d'Evaluation en Dermatologie et Cosmétique

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ANTI-AGING EFFECT OF TREATMENT BY Perf Action's Airgent IN HUMAN SKIN MAINTAINED IN SURVIVAL

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I. AIM OF THE STUDY

The aim of this ex vivo study was to test the anti-aging effect of Airgent™ on ex-vivo human skin samples harvested from abdominoplasty or lift surgery and maintained in survival conditions. This device has the ability to induce a mechanical stress to the fibroblasts which respond by producing more collagen to repare the dermis. The accelerated dispersion of Hyaluronic Acid increases collagen regeneration.

The **tightening of the skin** was analysed by immunohistochemical analysis of additional of procollagen fibers type III and dosage of collagen synthesis.

The improvement of collagen dermis was analysed by morphometric analysis of collagen bundles.

The improvement of hydratation of the skin was analysed by analysis of biochemical dosage of Glycosaminoglycans.

II. MATERIAL AND METHODS

A) Human skin model maintained in survival

We can evaluate these effects using an ex-vivo human skin model. Human skin samples were obtained from 7 (plastic abdominal surgery or facial lifts). Each skin sample were placed on a porous membrane and positioned in a culture well which will be kept in a sterilizer at 37°C and in atmosphere air/CO2 (95% / 5%). The culture medium, provided by GREDECO research, was placed at the bottom of the well. This medium was renewed 3 times per week.

B) Experimental aging model by UV

To obtain a prematured aging of the skin with alteration of dermis, we used UV A and B radiation, known to induce alterations in middle and profound dermis. The source of ultraviolet radiation is a Vilbert Lourmat stimulator (France) fitted out with a UVA irradiation source (365 nm) composed of tubes T-20.L-365 (no UVB, no UVC emission) mercury vapour tubes, low presure, hot cathodes and then with a UVB irradiation source (312 nm) composed of tubes T-15.M-312 (no UVA, no UVC emission). The radiometer is associated with a microprocessor programmable in energy (J/cm^2), with time basis enabling 6 irradiation measurements per second for controlling the energy received by the skin frament. In this protocol, one session with UVA 12 J/cm^2 and UVB 2 J/cm^2 was made.

A single treatment session with the Airgent™ device was made (High pressure 50% and one shot by cm^2 with 150 μl pf Hyaluronic delivery) . For each donor this treatment was made in duplicate. Then skin fragments were maintained in survival during 21 days.

A comparison was made between :
- aged skin by UV
- aged skin treated by Airgent™

One part was fixed in Formol liquid and embedded in paraffin for histological and immunohistochemical analysis of collagen tissue. One fragment was used for analysis of collagen and Glycosaminoglycans synthesis.

III. ANALYSIS

a) Collagen synthesis

Skin fragments were enzymatically digested in an acetic acid 0.5M solution containing pepsine, overnight at 4°C. The fibroblastic activity for collagen synthesis was evaluated by a spectrocolorimetric method (540 nm) measuring the acido-soluble new collagen synthetized after a specific fixation by sirius red staining (Sircoll Collagen Assay, Interchim). The results were expressed in µg of collagen /mg.

b) Morphometrical analysis of collagen fibers

Serial sections of 4 μm thickness were obtained and specifically stained with a picric acid solution containing 0.1% sirius red Collagen was analyzed by computerized morphometrical analysis . For a quantitative analysis of these macromolecules, a computerized image analysis of each section was made. The stained slides were examined by a microscope (Leitz) (magnification x 160) connected with a camera unit (XC-75 CE type) and with a microprocessor (Q520).

The surface of collagen bundles were measured in μm^2. Then, the relative collagen content of the dermis was expressed as percentage of surface analyzed dermis.

c) Immunohistochemical study of procollagen (type III)

8 μm deep sections were obtained from frozen skin fragments. They were incubated for 45 mins with a monoclonal antibody against Procollagen Type III (SantaCruz) and revealed by using an immunofluorescence staining.

A semi-quantitative scoring of the intensity of the immunostaining was made on these slides.

d) Analysis of Glycosaminoglycans

Skin fragments were enzymatically digested in a solution containing papaïne, overnight at 60°C. The content of sulfated glycosaminoglycans was evaluated by a spectrocolorimetric method (540 nm) a Blyscan assay kit (Interchim). The results were expressed in ng of glycosaminoglycannes /mg of skin.

e) Statistical analysis

Mean values and standard deviations are calculated for quantitative variables. The statistical significance of changes recorded concerning these parameters is determined with the Student's t-test, meaning that $p < 0.05$ is considered statistically significant.

IV. RESULTS

a) Collagen synthesis

Results are expressed in **Table I.**

After UV aging, we obtained a significant decrease of collagen synthesis (16.3 µg/mg) in comparaison with untreated skin (26.76 µg/mg ; p = 0.03) .

We obtained an increase of collagen synthesis by dermal fibroblasts after Airgent treatment. This increase is statistically significant (24.2 µg/mg) in comparison with UV control skin (that is an increase of 48%; p = 0.01).

b) Histological quantification of dermal collagen by computerized image analysis

Results are expressed in **Table II** and visualized on **figures 1, 2 and 3**.

After UV aging, we obtained a significant decrease of % collagen in superficial and mid dermis : 81.3% in comparison with untreated skin (87.5% ; p = 0.0025).

After undergoing treatment with Airgent™ device, the skin statistically significantly increased its collagen levels at 90.3% in the superficial-mid dermis versus 81.3% for UV control skin (that is an increase of 11%; p=0.006).

c) Immunohistochemical study of procollagen (type III)

After UV aging, we obtained in superficial dermis a decrease of immunostaining of procollagen type III of approximately 70% in comparison with untreated skin . After Airgent™ treatment , a stimulation of procollagen type III synthesis by the fibroblast was obtained with nearly the same level of immunostaining of normal skin (increase of 73% versus UV skin) (**figures 7 and 8).**

d) Sulfated gycosaminoglycans dosage

Results are expressed in **Table III.**

We obtained an increase in sulfated glycosaminoglycans content after Airgent™ treatment. This increase is statistically significant (1658.4 ng/mg) in

comparison with UV control skin (1054.7 ng/mg; p = 0.02), that is an increase of 57%.

A visualistion of total glycosaminogycans made by histological Hale staining confirmed these results, as seen on **figures 4 to 6**.

V. CONCLUSION

By using an ex vivo human skin model, we have observed the Airgent™ technology to be effective for treatment of aged skin. Experimental results showed significant increase in fibroblasts activity (collagen and sulfated glycosaminoglycans synthesis) and collagen regeneration.

In photodamaged skin, the loss of mechanical tension between fibroblasts and collagen bundles appears to be the major factor underlying decreased collagen synthesis by these cells. A part of the mecanism of action of this device can be explained by restoration of connexion between fibroblasts and collagen bundles via hyaluronic acid injection. The second part of action of this device is the strong controlled trauma that induces mechanical stress to the fibroblasts, which respond by producing more collagen and glycosaminoglycans.

Made in Paris , october 5th 2010

Dr Boisnic Sylvie

Tableau I:

Histological and morphometrical analysis of collagen bundles (sirius red staining)

	% in superficial and mid dermis
Untreated Skin	**87,5** ± 5.8
Skin + UV	**81,3** ± 8.2 * p=0,0025
Skin + UV + **Airgent™**	**90.3** ± 3.8 # p=0,006

*: statistical significant difference in comparison with untreated skin (paired Student's T test, $p < 0.05$)

: statistical significant difference in comparison to Skin + UV (paired Student's T test, $p < 0.05$)

Tableau II:

Biochemical dosage of collagen

	μg collagen / mg of skin biopsy.
Untreated Skin	**26,7** ± 11
Skin + UV	**16,3** ± 11.1 * p=0,03
Skin + UV + **Airgent™**	**24.2** ± 10.8 # p=0,01

*: statistical significant difference in comparison with untreated skin (paired Student's T test, $p < 0.05$)

: statistical significant difference in comparison to Skin + UV (paired Student's T test, $p < 0.05$)

Tableau III:

Biochemical dosage of sulfated glycosaminoglycans

	ng / mg of skin biopsy.
Untreated Skin	**1160,6** ± 573,9
Skin + UV	**1054,7** ± 799.5
Skin + UV + **Airgent™**	**1658.4** ± 855.1 # p=0,02

: statistical significant difference in comparison to Skin + UV

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Figure 1:

Collagen in dermis (Sirius red, x 400)

Untreated skin

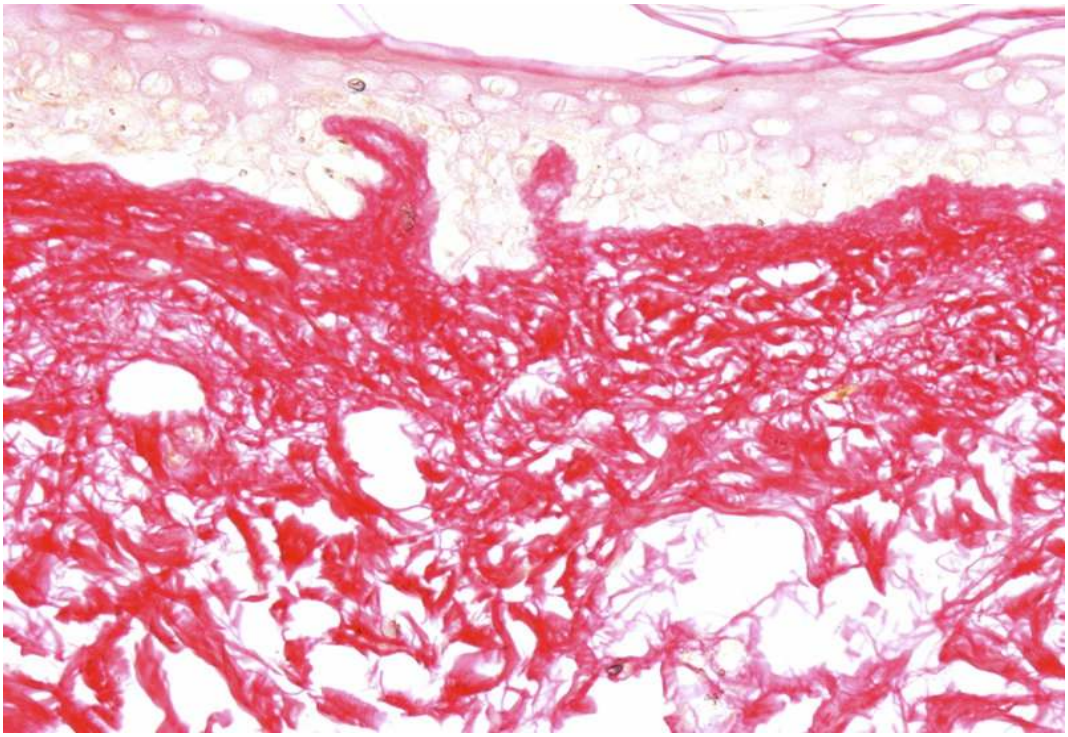

Figure 2:

Collagen in dermis (Sirius red, x 400)

Skin + UV: destruction of collagen fibers in superficial dermis

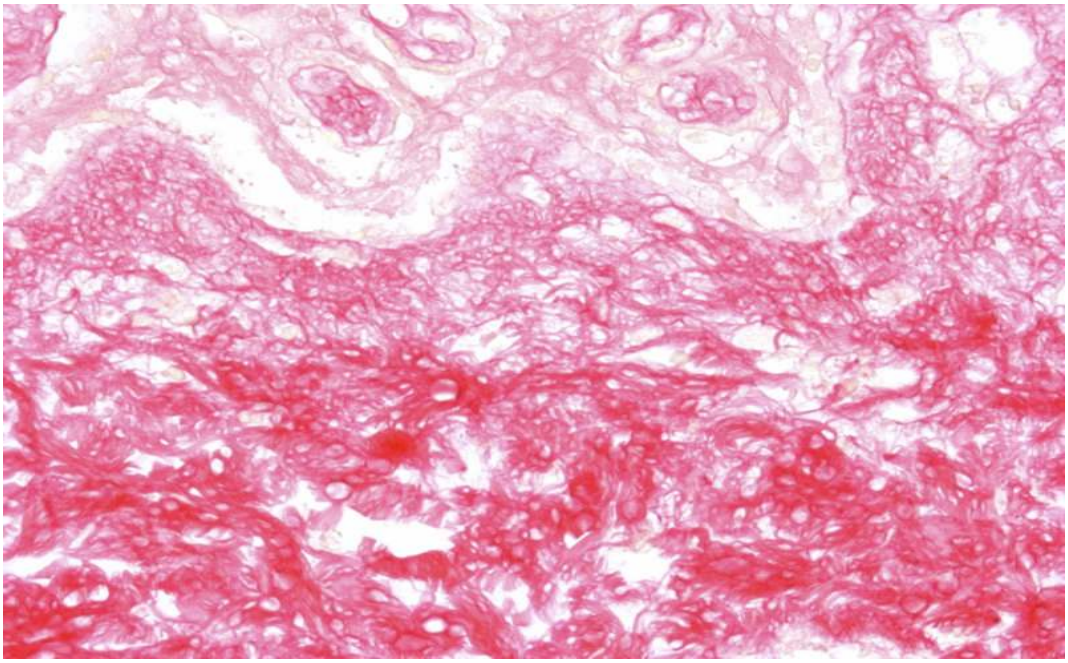

Figure 3:

Collagen in dermis (Sirius red, x 400)

Skin + UV + Airgent™ treatment : repair of collagen fibers in superficial dermis

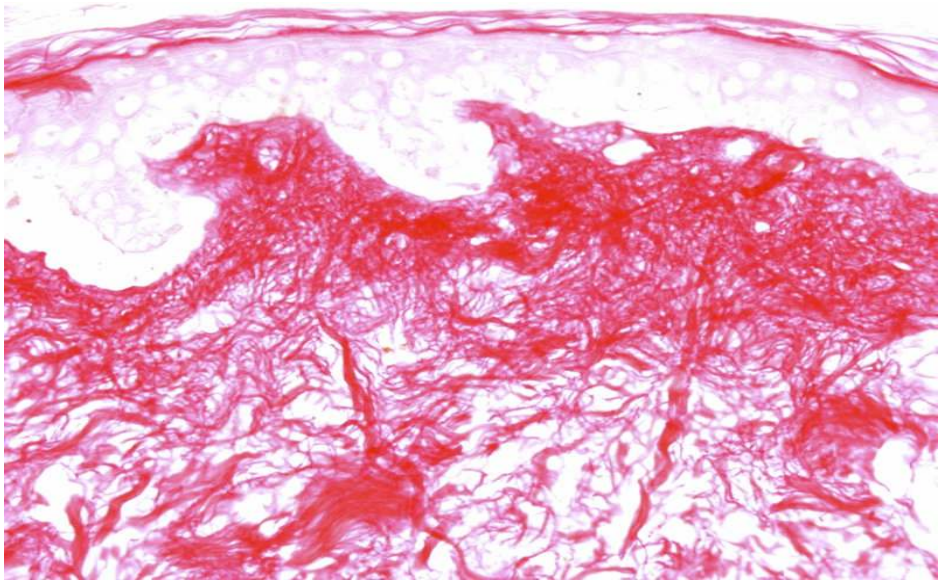

Figure 4:

Histological Hale staining of dermal glycosaminoglycans (x 400)

Untreated skin showing blue staining glycosaminglycans in dermis

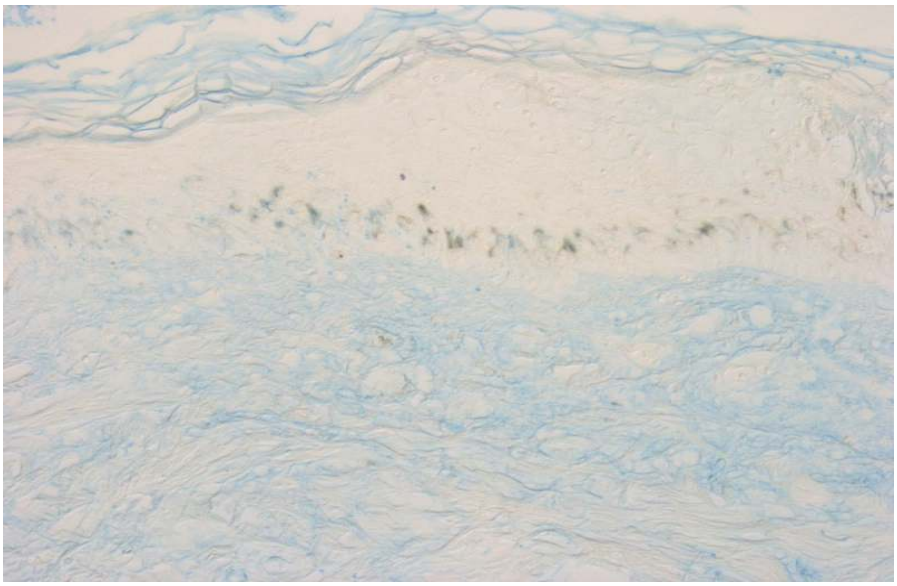

Figure 5:

Histological Hale staining of dermal glycosaminoglycans (x400)

Skin + UV showing decrease of glycosaminglycans in dermis versus untreated skin

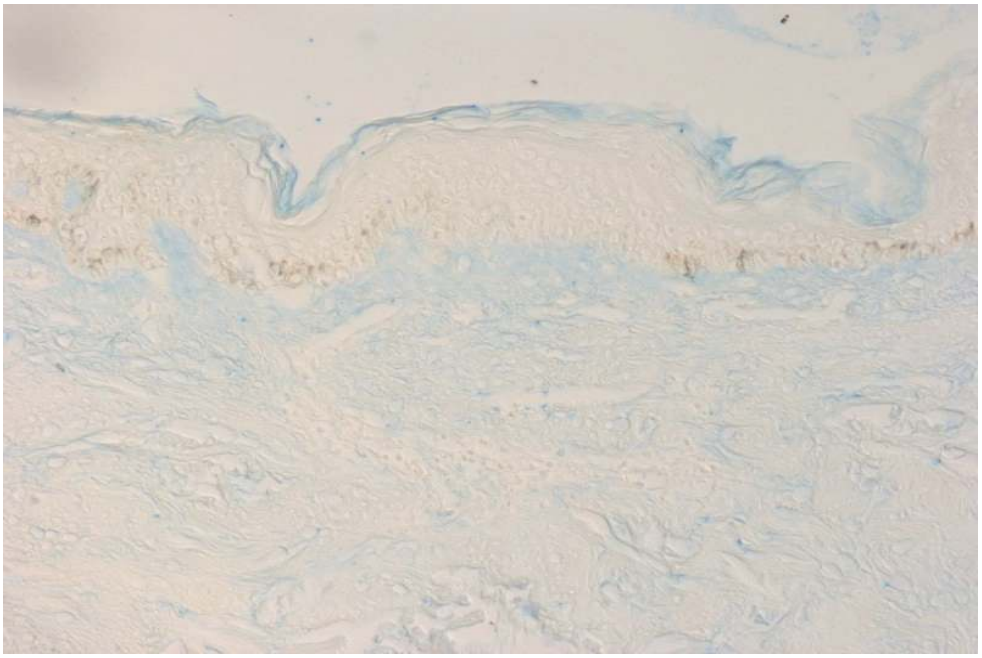

Figure 6:

Histological Hale staining of dermal glycosaminoglycans (x200)

Skin + UV + Airgent™ showing increase of glycosaminglycans in dermis versus skin+ **UV**

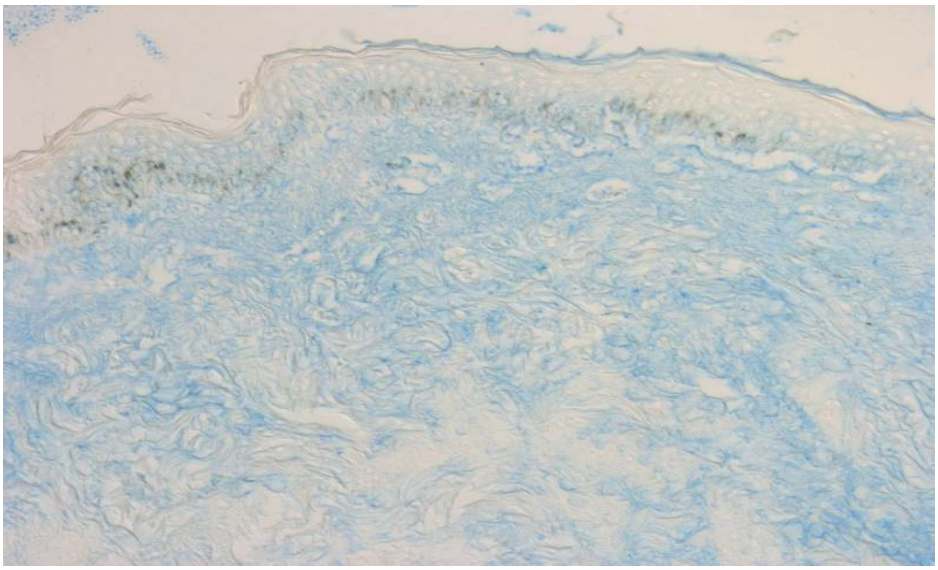

Figure 7:

Immunohistochemical staining of procollagen type III (x400)

Skin + UV showing procollagen type III on fibroblasts (12 points)

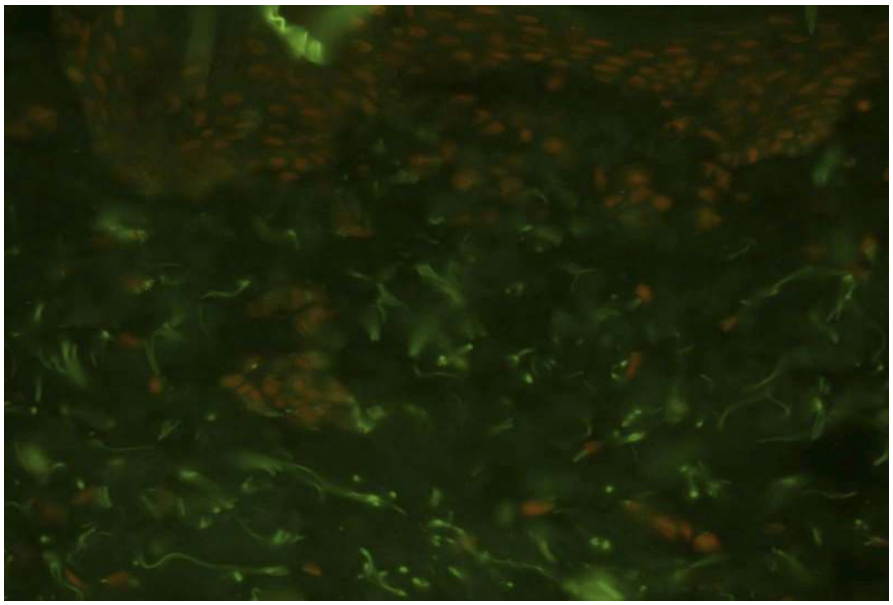

Figure 8:

Immunohistochemical staining of procollagen type III (x400)

Skin + UV + Airgent™ showing increase of procollagen type III on dermal fibroblasts (26 points)