Introduction

The skin is one of the organs that is most affected by the effect of time and a series of physical, chemical and biological insults.

In particular, the epidermis undergoes a progressive reduction in the number, size and turnover of the keratinates, which alone constitute about 80% of its cell population; the result is a progressive reduction of cohesion with the underlying dermis. The latter, made up of 80% collagen - which ensures the firmness of the skin - meets, over time, a progressive wear of its components, with reduction of the quality of hyaluronic acid and degradation of collagen and elastic fibers. Because of these and other mechanisms, primarily dehydration, the skin loses elasticity and firmness and relaxes, favoring the appearance of wrinkles. The quality of hyaluronic acid, the amount of reticular collagen or type 3 produced and the amount of elastorates present can provide valuable information on the extent of damage while functioning as valuable biomarkers for monitoring the effectivenes of any treatments.

In this regard, aesthetic medicine tries to intervene through various techniques, possibly minimally invasive, in order to restore order and balance lost, increasing, each time, the level of oxygenation, promoting cellular metabolism , activating particular signal ways, etc.

In this context, the skin patting is an original and patented technique (Automatic Patting System, APS®) based on the use of a particular multifunctional handpiece equipped with a system of double cones that, rhythmically protruding in a pulsed manner, generate a unique "train" of "pressure" waves, which allow them to penetrate the thickness of the skin in a regular way up to a maximum depth of 0.25 mm, resulting in micro-incision, without apparent loss of substance. The various body areas are covered with two handpieces of different sizes, each "equipped" to perform any ancillary functions (eg iontophoresis, LED, etc.).

The mechanical stimulation induced by the device determines a series of clinically appreciable benefits in the cutaneous areas affected by various pathological conditions, such as relaxation, micro-wrinkles, spots, acne, etc.

These benefits would be due to the combined effect of 3 mechanisms:

A) the increase in blood microcirculation, which facilitates the oxygenation of the treated tissues;

B) stimulation of tissue cellular metabolism which, among other things, favors the transmission of any active ingredients;

C) stimulation of fibroblasts (on both mechanical and respiratory basis), which induces the production of collagen and elastin.

Starting from these assumptions, the present study aims to evaluate the effect of a standard skin patting treatment on the histological skin, in order to contribute to clarify the efficacy, from a clinical point of view, in the different conditions in which the technique finds an indication, alone or in combination with LED therapy.

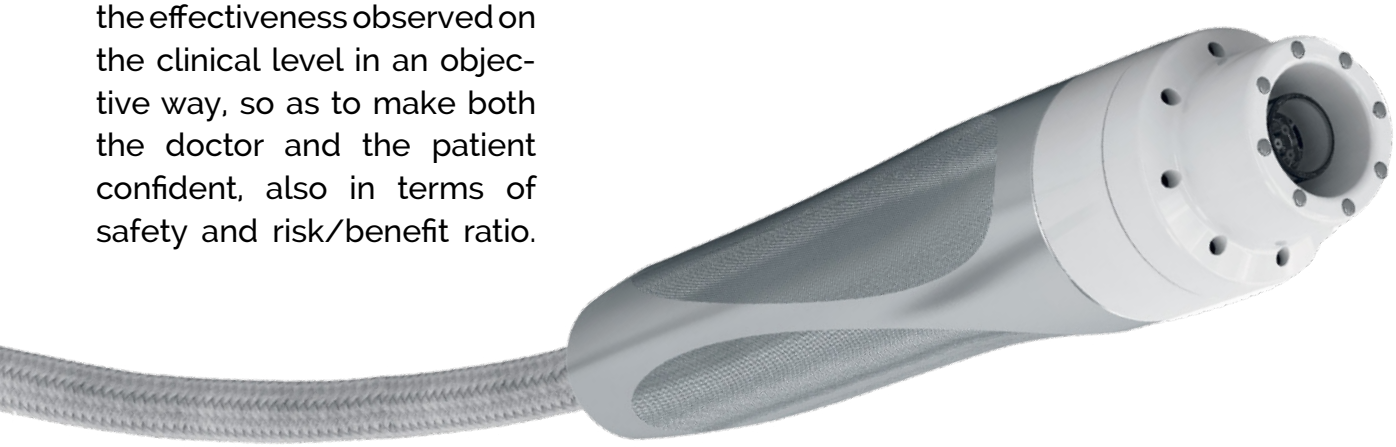
The choice of this experimental approach seems to be the most suitable to document the effectiveness observed on the clinical level in an objective way, so as to make both the doctor and the patient confident, also in terms of safety and risk/benefit ratio.

SCOPE OF WORK

The aim of the study was to evaluate any changes in the cutaneous histological structure - in terms of quality/quantity of the extracellular matrix and of the collagen and elastic fibers - following the APS® versus placebo treatment.

DRAWING, SUBJECTS AND METHODS

The clinical study, developed exclusively and exclusively observational, followed the double blind-randomized-parallel group model. The used device is called APS. It is a multistation medical device produced by APS srl, Via A. Einstein, 8 c/o E. Torricelli Technology Park 48018 Faenza (RA).



THE DEVICE HAS DIFFERENT POSSIBILITIES FOR ACTION THROUGH THE INSTRUMENTS IT IS EQUIPPED WITH:

MICRO-INCISION: a patented method is the innovation of the device and in fact the reason for the clinical study: it works on the principle of Collagen-induction-Therapy; the skin, through tiny devices patented in surgical steel with a double cone shape, is subjected to multiple micro traumas. **ACOUSTIC WAVES:** they are pressure impulses that create a tissue shock; the waves used are generated extracorporeally and applied in a targeted manner; propagation occurs according to the laws of acousto-optics, the discipline that studies the effects and applications of the interaction between a field of efforts produced in a medium by the passage of an elastic wave and electromagnetic radiation; a variation in acoustic properties such as density or sound velocity at the interfaces between different tissues (skin, fat cells or muscles) creates a leap in acoustic impedance with consequent release of energy. **PHOTOBIOMODULATION:** it is a therapy based on communication and interaction between light energy and tissues; the cells are able to absorb the light and the effect of this light communication will be different depending on the physical parameters of light such as (wavelength, frequency of the peaks between the different waves, the intensity of energy) and related factors at the treatment time and the interval between the light pulses; different parameters determine different biological effects: it will be possible to obtain an increase or decrease of the cell activity. **TRANSDERMAL DELIVERY:** allows to convey active ingredients without needles; completely non-invasive and painless, it causes transient permeability and at the same time a tension action of the tissues; after having cleansed the skin, the active ingredients that are to be conveyed are spread directly onto the skin. **IONTOPHORESIS:** one of the different techniques that come within the field of electrotherapies; in this case, a direct current consists of the administration of a drug via a transcutaneous route using a direct current that is produced by a special instrumentation. **VACUUM THERAPY:** it acts through a suction action in the part of the treated fabric. **THE FRACTIONAL BIPOLAR RADIOFREQUENCY:** the controlled and localized heat causes an immediate contracting of the collagen fibers at the level of the deep dermis; the physical law underlying the effects of radiofrequency is given by the modification of the electric field of the treated area with a change in the electric charge and resistance, expressed in ohms, to the movement of ions and molecules, which determines heat, expressed in joules, according to the formula: J = I x R xT, where J = energy, I = current, R = tissue impedance, T = time; the heat produced develops between 3 and 9 mm deep and determines a heating up to 55-65 ° C in a homogeneous way, without thermal diffusion to the surrounding areas; in this way a denaturation of the collagen fibers is obtained (from 5 to 30% of the total fibers) with consequent immediate contraction of the fibers themselves and with a progressive effect in the following 4-6 months; the contraction occurs in a stereoscopic way, at 360°. **PHOTOBIOMODULATION:** it is a therapy based on communication and interaction between light energy and tissues; the cells are able to absorb the light and the effect of this light communication will be different depending on the physical parameters of light such as (wavelength, frequency of the peaks between the different waves, the intensity of energy) and related factors at the treatment time and the interval between the light pulses; different parameters determine different biological effects: it will be possible to obtain an increase or decrease of the cell activity.

Patting works on the principle of Collagen-induction-Therapy.
The skin, through tiny devices patented in surgical steel with a double cone shape, and subjected to multiple micro traumas. The method can also be called microncision.

90 patients were selected. Each patient signed an informed consent on the procedure being adopted, but no patient knew which of the three groups he belonged to.
The selected subjects, all female, between the ages of 45 and 65, Body Mass Index (BMI) between 19 and 27, apparently healthy. Subjects normal weight and light overweight not complicated by diagnosis of degenerative diseases and life habits that promote aging, for example: smoking, ethyl abuse, etc.

The input diagnosis was for all cutaneous hypotonia of the face and periocular, frontal and inferior micro-fractures of the face, for which conditions the treatment was performed.
Patients were allocated as follows:

- 30 patients placebo group;
- 30 patients treatment group (skin patting);
- 30 patients treatment group (skin patting + LED).

The action in the placebo group was obtained by applying the non-active device to the affected skin.
Duration of the study 90 days. The protocol included one session per week for 12 consecutive weeks, for a total of 12 treatments. No active ingredient has been added to these to understand the effective therapeutic potential of the device used.

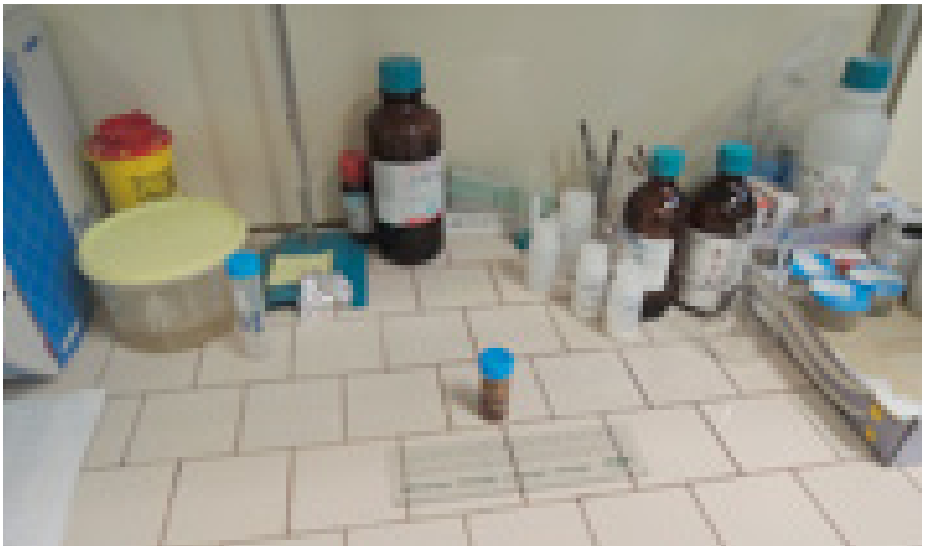
Each patient underwent biopsies at various times: 0 days, 30 days, 60 days and 90 days. The area treated and the location of the biopsy sampling for all patients was the face and the anatomical site of the biopsy, it was the posterior margin of the jaw.
In addition to the histological one, an immunohistochemical evaluation was performed (data available only at 90 days).

Biopsies were performed with a 2 mm diameter circular Japanese punch biopsy. They are devices with very sharp stainless steel blade and plastic handle, on each curette is marked the measure for an easier identification. Individually packed in sterile sachet. The seamless stainless steel blade wire ensures a smooth cutting surface, which allows optimal sample quality. External actionable shutter to facilitate biopsy collection. Products from KAI INDUSTRIES.

HISTOLOGICAL TEST

Were performed and validated at the TEST s.r.l. Modena, via Verdi 36, 41100 Modena From Dr. L. Reggiani Bonetti. And from Dr. A, Martinelli, at different times and without either of them knowing the opinion of the other. Each biopsy sample was fixed using a fixative liquid (4% formaldehyde). At least 10 minutes. Each piece was individually washed to remove the excess fixative by using a phosphate buffered salt solution for a few minutes.

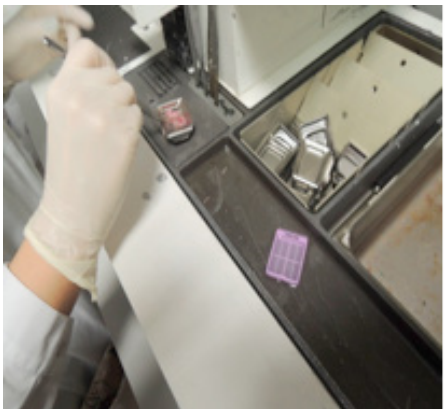
macroscopic evaluation site



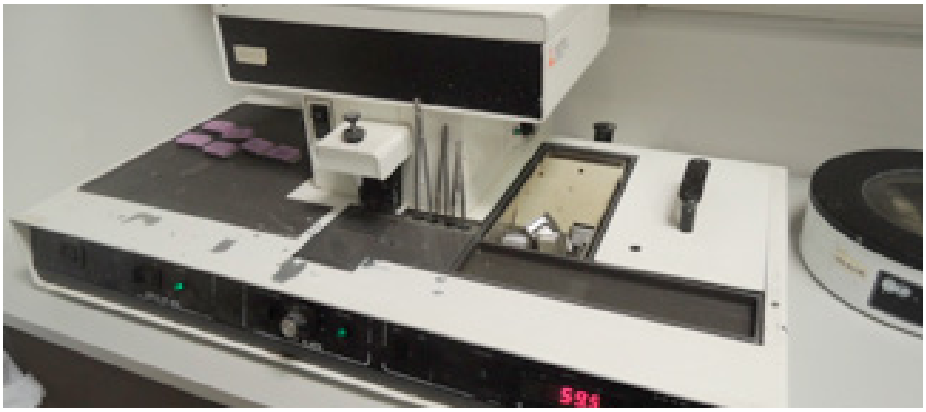
THE PROCESS

The process of dehydration of the piece was carried out using the ascending scale of the alcohols (ethyl alcohol at 70 °, 1 min - 80 °, 1 min - 90 °, 10 min - 100 °, 15 min) to eliminate the aqueous component, 'water does not allow the passage of paraffin in the fabric.

Once this is done the bioptic pieces are diaphanous in paraffin solvents. In fact, the private parts of the water and therefore transparent (diaphanous), are ready to receive the paraffin.

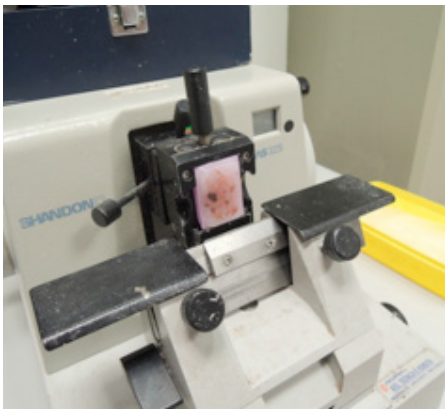


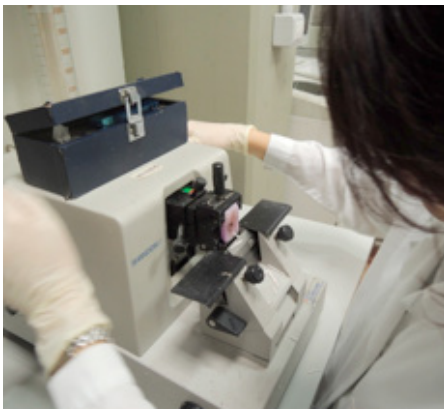
The piece is placed in the paraffin melted at about 60 °, the paraffin is kept constant at the temperature indicated above by means of a stove. For 15 minutes, procedure repeated twice.



The piece is waxed with this tool. The piece is made to solidify at room temperature so that the paraffin as well as inside is also present outside and then ready for cutting.

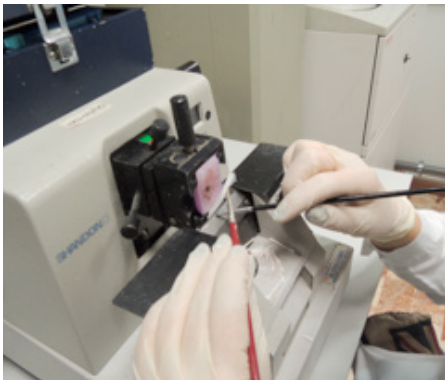
The cut was performed with a rotary microtome, obtaining thin sections of about 4-8 um, then spread in distilled water at a temperature of 40-45 ° and then made to adhere to the slide. Microtome.

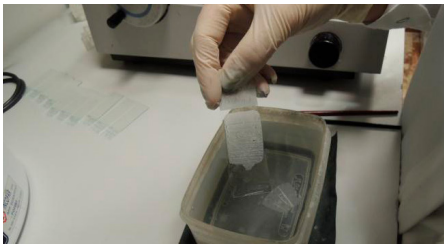




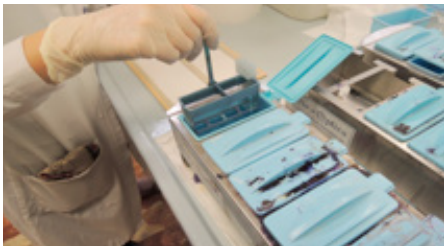
Cells with pieces ready to be cut

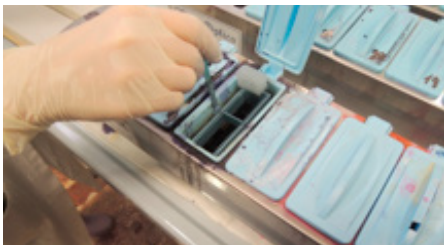
Once the sections were obtained, the paraffin was removed and the rehydration was carried out, followed by steps: Xilol 1, 7 minutes, xilol 2 still 7 min, ethanol 100 ° 1, 5 min, ethanol 100 ° 2, still 5 min, ethanol 90 °, 5 min, ethanol 80 °, 5 min, ethanol 70 °, 5 min, distilled water, washing. Once this was done, the coloring was done.





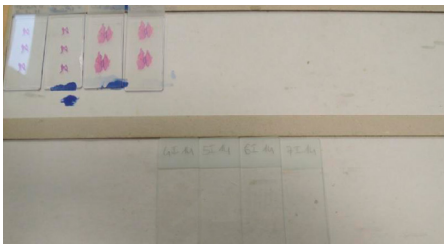
Once the slides were obtained, they passed to their observation, to highlight the aforementioned parameters.





HISTOLOGY

Hematoxylin Eosin - Weigert - Masson's Trichrome - Gomori - PAS - GIEMSA Immunohistochemistry Ki67 - cytokeratins - bcl2 – EPGF.



RESULTS

Subjective evaluation

Questioned on possible changes of the skin of the face, of the 90 patients, 27 patients responded that they had not observed any changes, 48 patients an improvement in facial brightness and skin firmness, 15 patients, a noticeable improvement in brightness, skin firmness and tactile sensation. Of the 27 patients who responded that they did not notice any improvement, they were all part of the placebo group. Of the 48 patients who expressed an improvement in facial brightness and skin firmness, 3 were part of the placebo group, 30 of the skin patting group and 14 of the skin patting + LED group. The 15 patients who expressed significant improvement in brightness, firmness and tactile sensation were all part of the skin patting + LED group.

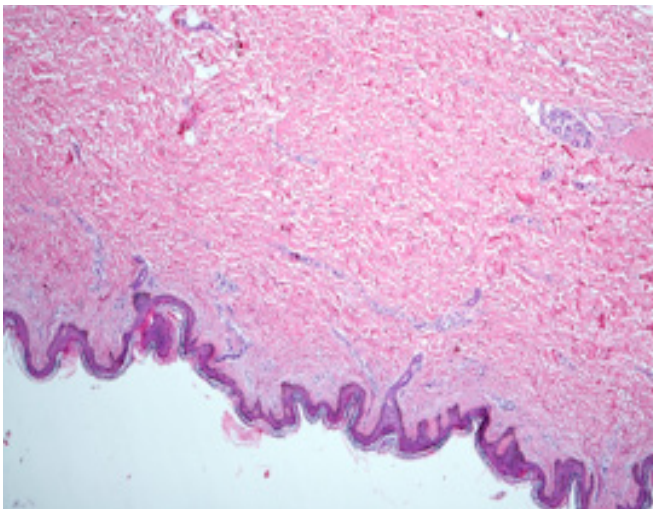
Objective evaluation

PHOTO SELECTION OF PATIENTS TREATED BEFORE DURING AND AFTER.

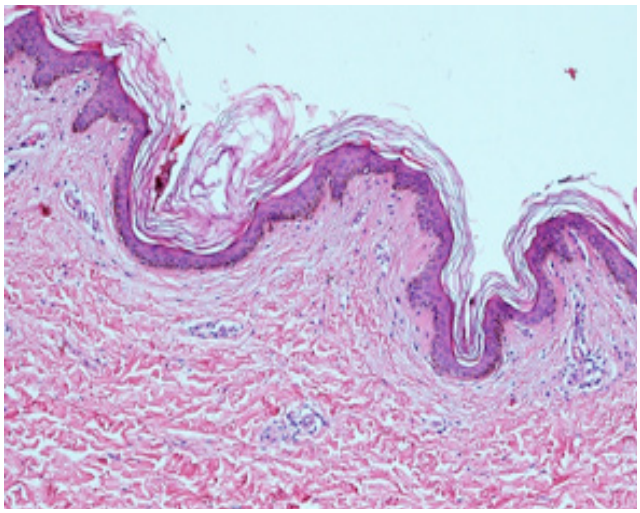
Histological and immuno-histochemical evaluation

After an initial histological evaluation performed with the E.E method, in order to highlight other ultrastructural aspects of the epidermis and the dermis, we proceeded with the preparation of slides obtained using other colors. Given the encouraging results, in terms of stimulus activity both in the epidermis and in the dermis, obtained with the device, we also proceeded to an immuno-histochemical type evaluation, at the end of the treatment (90 days, time 3), always comparing results with conventional histological examination. When evaluating through histochemistry, it must be taken into account that there may be false negatives due to the loss of the antigen due to alterations in the tissue, or to a low concentration of the used antigen, or to the use of inadequate antibodies. or at low concentrations. On the other hand, false positives can also be observed, due to non-specific antibody-tissue bonds, or due to the presence of contaminating antibodies in the solution used, or to a cross-reactivity of the antibody which could react with an antigen different from the one sought. We tried to minimize the effect of these problems by following standardized and controlled procedures.

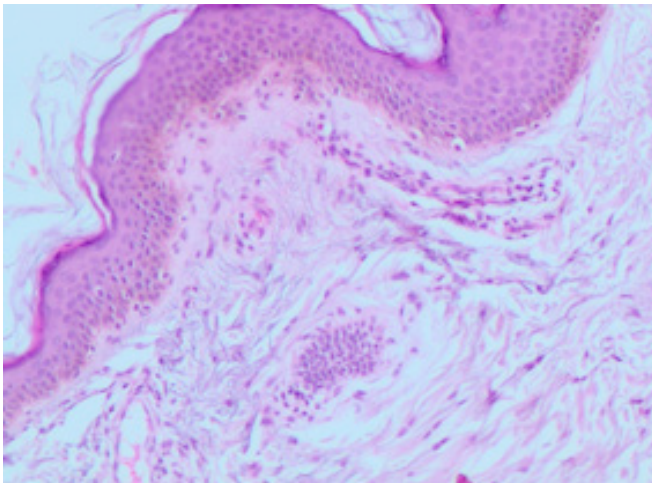
PLACEBO GROUP:



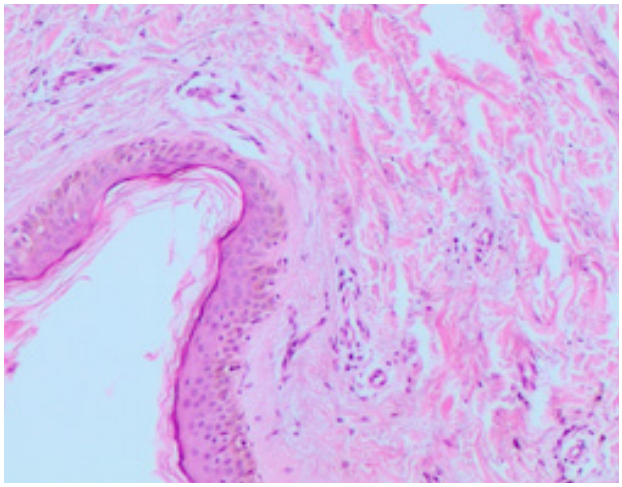
Placebo group. Histological evaluation before the start of treatment (time 0). E. E. Staining 4x. There is a thinning of the epidermis layer and a disorganization of the dermis, with signs of disintegration.



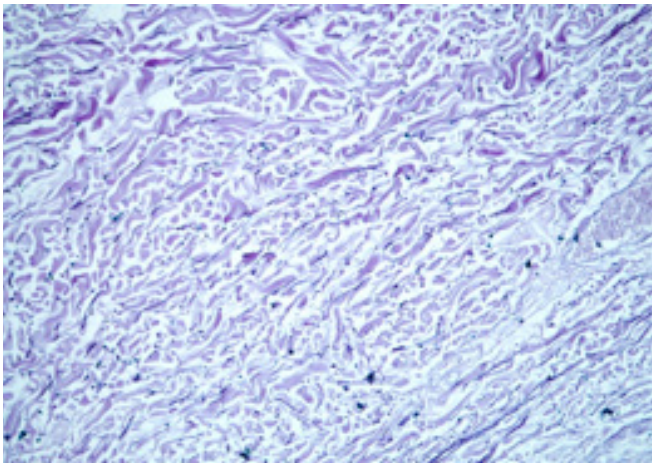
Placebo group. Histological evaluation at 30 days from the start of treatment (time 1). E. E. Staining 4x. The epidermis appears renewed with a good stratum corneum, while the dermis maintains the characteristics of the disorganization and the evident disintegration.



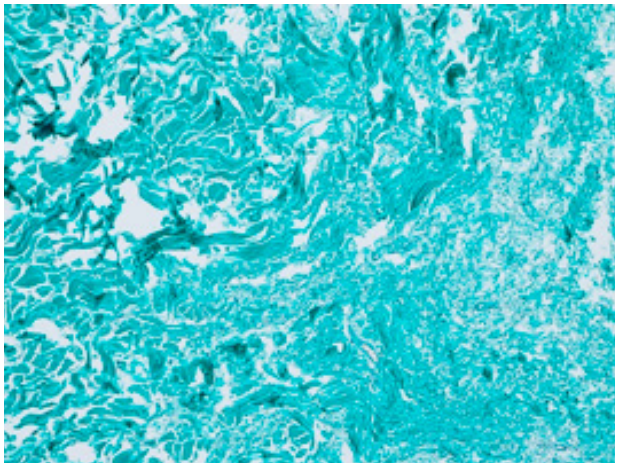
Placebo group. Histological evaluation at 60 days from the start of treatment (time 2). E. E. Staining 10x magnification. The epidermis is thinner. Also with its horny state, the dermis maintains the above characteristics intact.



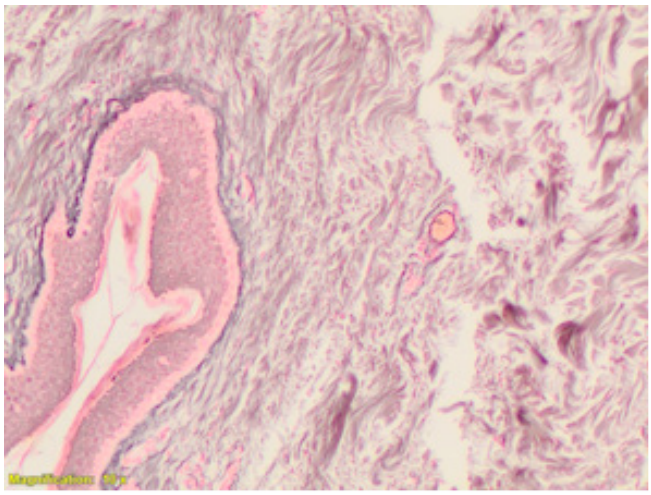
Placebo group. Histological evaluation at 90 days from the start of treatment (time 3). E. E. Staining 10x magnification. The appearance of the dermis and epidermis unchanged in the previous slides is unchanged.



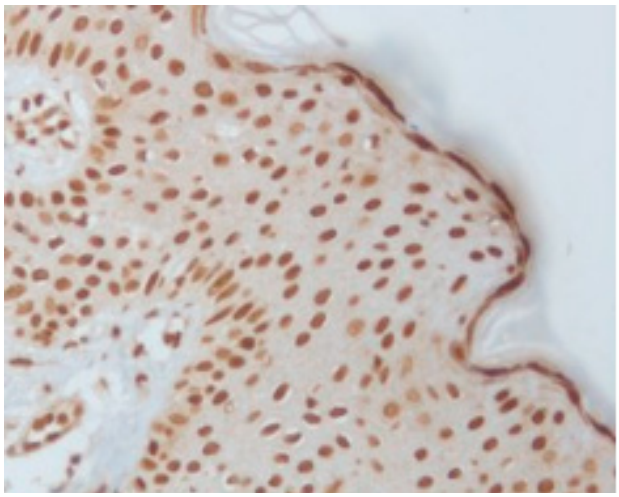
Placebo group. Histological evaluation at 90 days from the start of treatment (time 3). Weigert coloring. 10x magnification. Collagen appears disorganized and disrupted, while the elastic fibers are almost completely absent, except in the lower part of the slide where they appear to be barely mentioned.



Placebo group. Histological evaluation at 90 days from the start of treatment (time 3). Masson's Trichrome Coloring. 10x magnification. Collagen disorganized and disrupted, almost complete absence of elastic fibers; only on the left side of the slide is a hint of active collagen fibers.

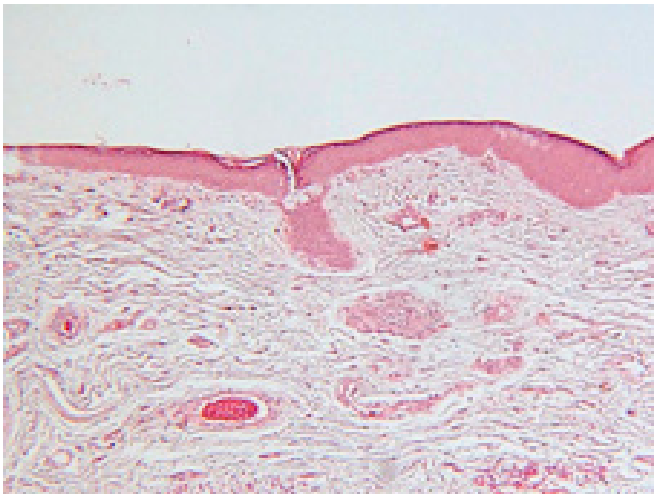


Placebo group. Histological evaluation at 90 days from the start of treatment (time 3). Gomori coloring. 10x magnification. Thin epidermis, disorganized and disaggregated collagen, almost complete absence of elastic fibers, we can also observe the presence of microcirculation vessels almost collapsed.

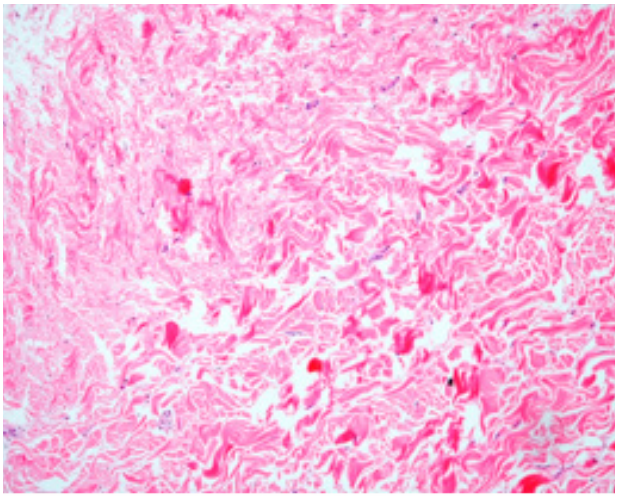


Placebo group. Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). Cellular labeling with anti-Ki-67 antibody. A low cell proliferation index is observed.

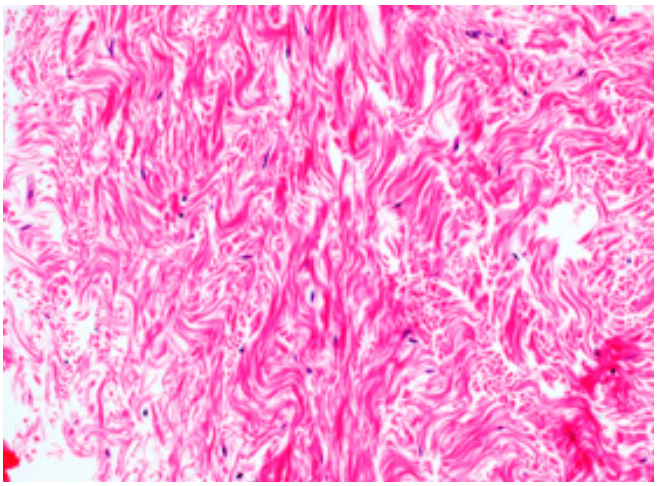
SKIN PATTING GROUP:



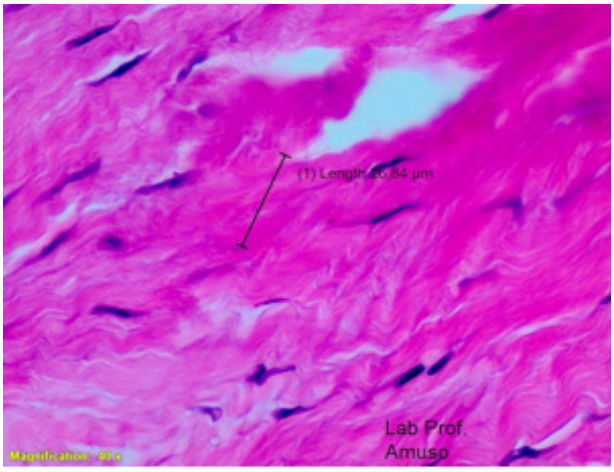
Skin Patting group Histological evaluation before the start of treatment (time 0). E. E. Staining 4x. Thinning of the epidermis layer and disorganization of the dermis, with signs of disintegration.



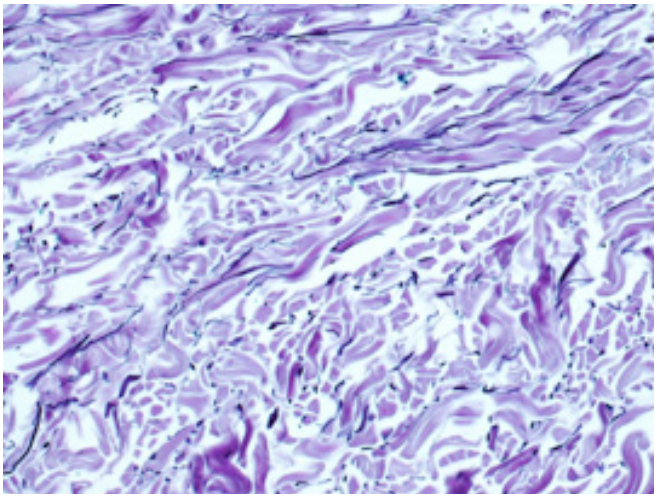
Skin Patting group Histological evaluation at 30 days from the start of treatment (time 1). E. E. Staining 10x magnification. Collagen appears disorganized and disrupted in the upper part of the slide, more dense and organized, with fibers of greater volume, in the lower one.



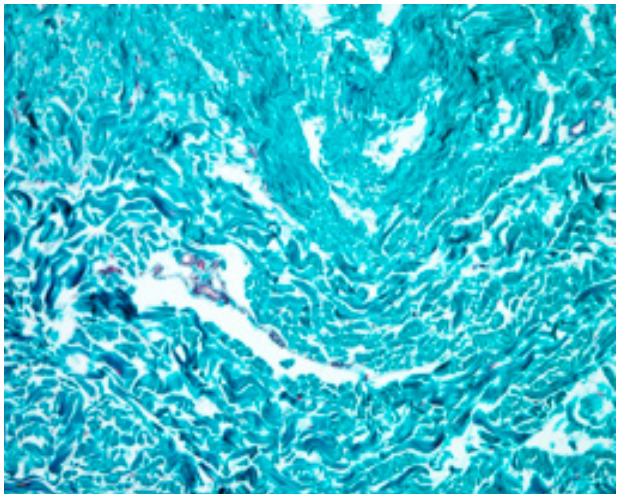
Skin Patting group Histological evaluation at 60 days from the start of treatment (time 2). E. E. Staining 10x magnification. In the derma there is a well-organized collagen, with dense, truncated fibers, arranged perpendicularly in the proximal part of the epidermis, horizontal in the deep one.



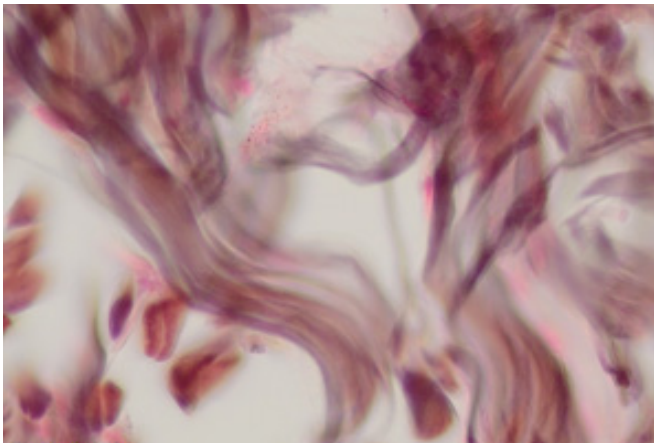
Skin Patting group Histological evaluation at 90 days from the start of treatment (time 3). E. E. staining. 40x magnification. Among the well-known collagen fibers, some of which of type 3, of measurable thickness, whose orientation and structure appear regular, we can distinguish elastic fibers.



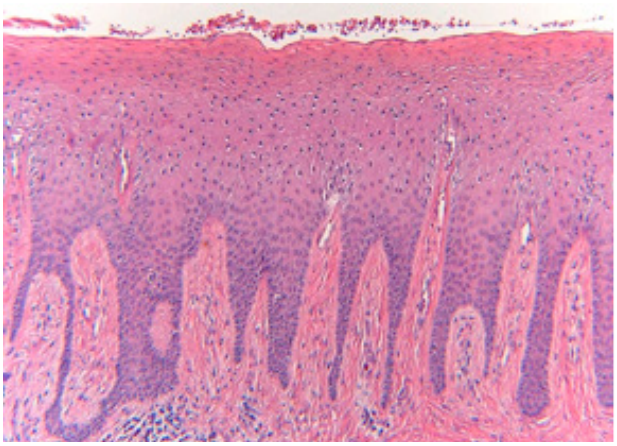
Skin Patting group Histological evaluation at 90 days from the start of treatment (time 3). Weigert coloring. 10x magnification. Well evident collagen fibers and elastic fibers optimally intercalated, well present and long; good angiogenetic activity.



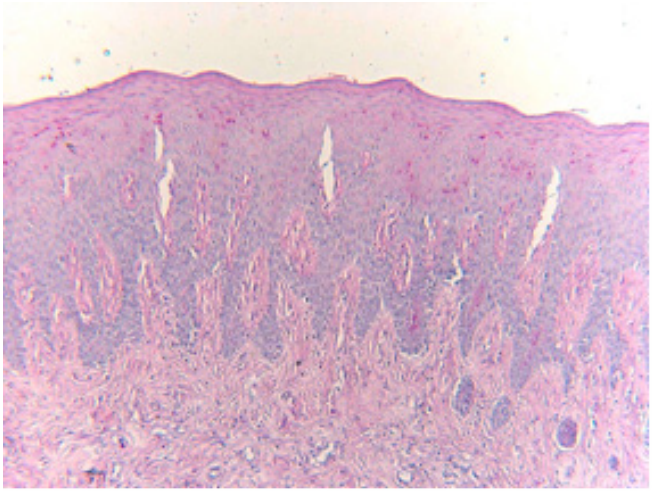
Skin Patting group Histological evaluation at 90 days from the start of treatment (time 3). Masson's Trichrome Coloring. 10x magnification. Collagen fibers well stimulated, well organized and optimally oriented in forming the architecture of the dermis, with the elastic fibers intercalated in the right way, good angiogenic activity.



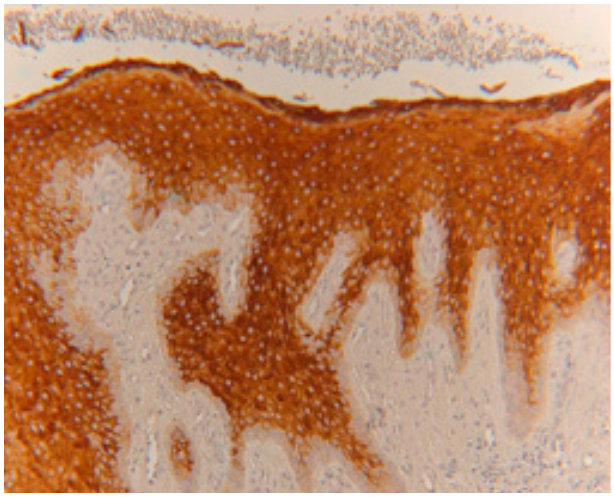
Skin Patting group Histological evaluation at 90 days from the start of treatment (time 3). Gomori coloring. 40x magnification. Collagen fibers in the derma are well represented with the presence of a good angiogenetic activity.



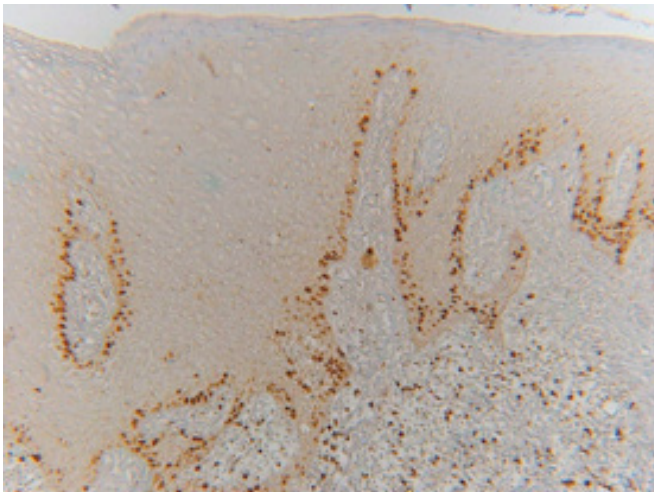
Skin Patting group Histological evaluation at 90 days from the start of treatment (time 3). E. E. staining The basal layer of the epidermis appears very anfrattuoso, with elongated and sometimes intertwined epithelial cords. The superficial layer is partly parakeratosico (contains nuclei). These two phenomena are an expression of vitality of the epidermis, increasing on one side the basal surface for better nutrition and presenting on the other a regeneration with parakeratosis, which will mature with the formation of keratin.



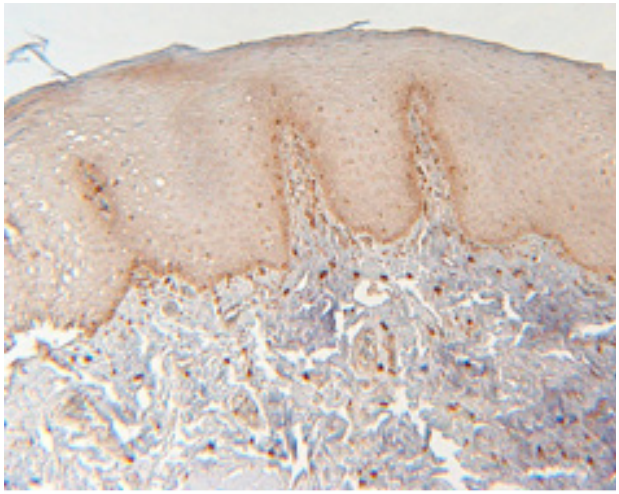
Skin Patting group Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). PAS staining. The slide confirms what we saw in the previous one, but we note the presence of vascular structures in the superficial dermis.



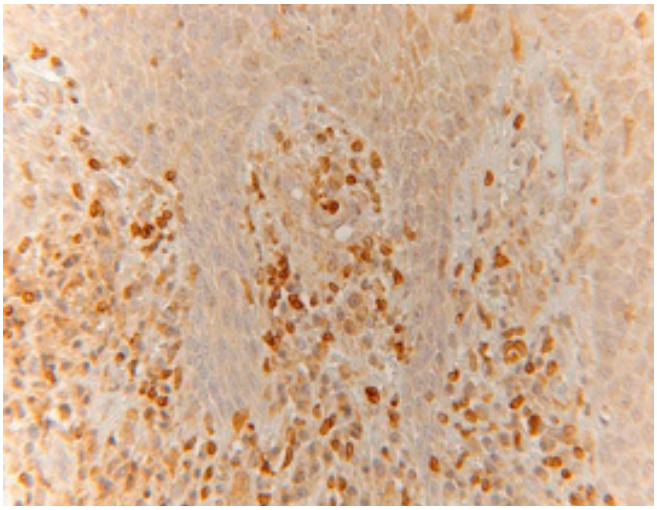
Skin Patting group. Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. The epidermis is marked in brown-brown thanks to the immunohistochemical reaction for high molecular weight cytokeratins (for surface epithelia) and shows a papillary and cordial epithelial hyperplasia of the epidermis basal layers.



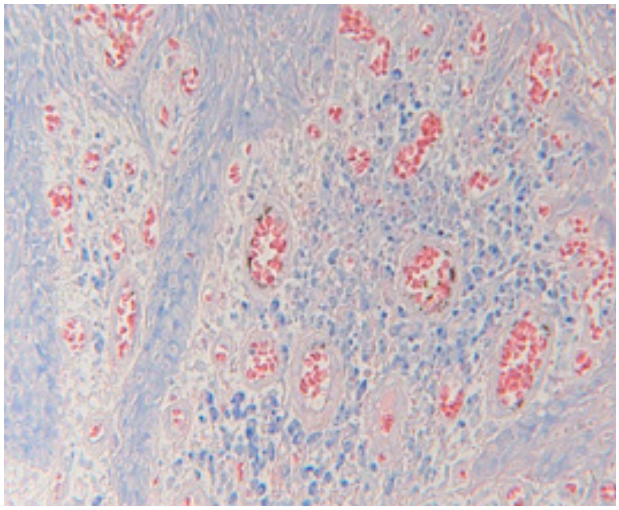
Skin Patting group Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. The cell proliferation index (Ki67) is immunohistochemically positive in the nuclei of the epidermis layer of the epidermis (basal layer) and in the fibroblasts of the dermis.



Skin Patting group. Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. Epithelial-stromal proliferative activity is also evidenced by immunohistochemical labeling of the bcl2 protein.

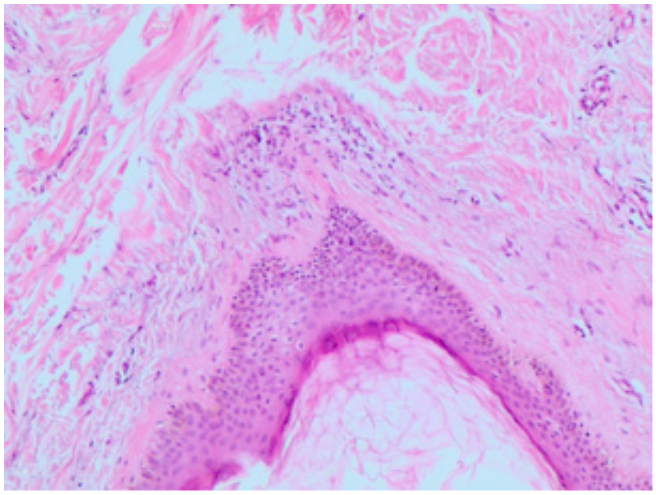


Skin Patting group Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. The immunohistochemical reaction for EPGF (Epidermal Grow Factor) is very evident in stromal cells and some epithelial cells.

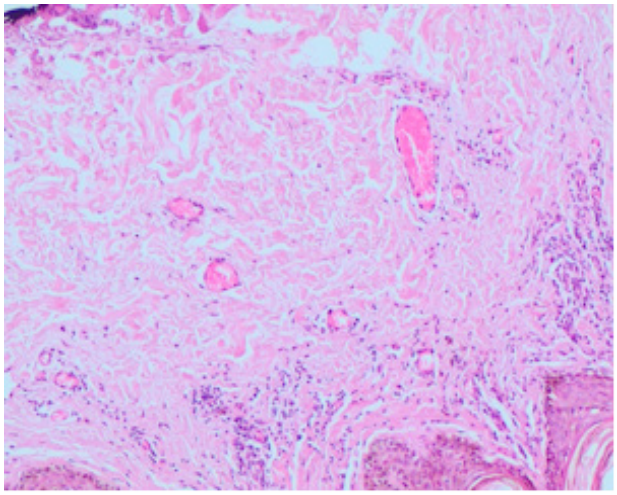


Skin Patting group. Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. Excellent angiogenic activity is evident.

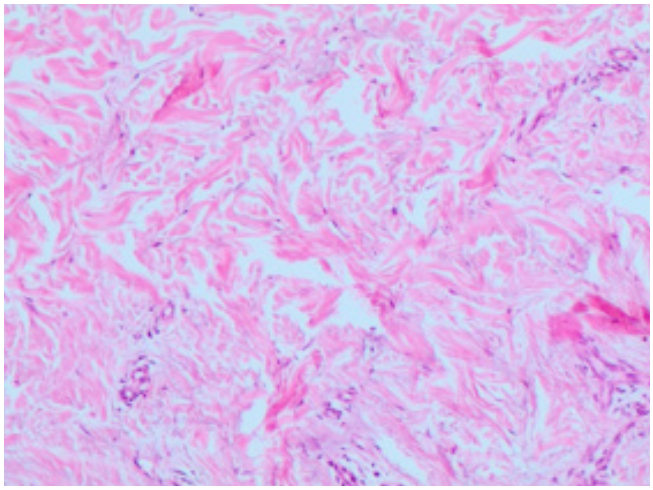
SKIN PATTING GROUP + LED:



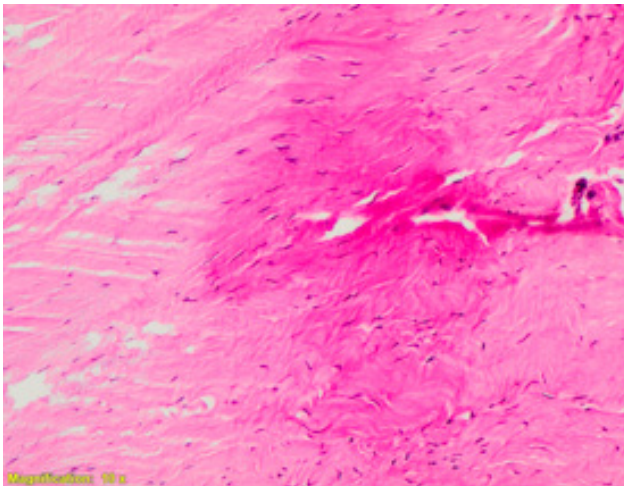
Skin Patting group + LED. Histological evaluation before the start of treatment (time 0). E. E. Staining 10x magnification. There is a thinning of the epidermis layer and a disorganization of the dermis, with signs of disintegration.



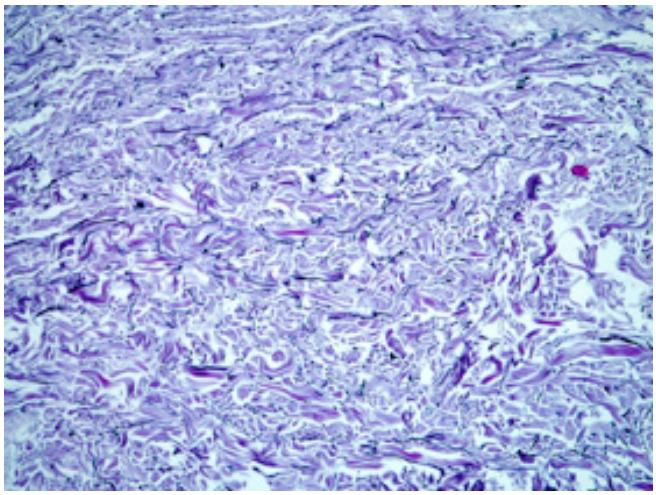
.**Skin Patting group + LED. Histological evaluation at 30 days from the start of treatment (time 1).** E. E. Staining 10x magnification. A good activity at the level of the epidermis can be glimpsed; the dermis appears to be well organized structurally, the collagen fibers appear to be increased in number / thickness (?) and there are evident signs of active angiogenesis, elements that on the whole suggest the effect of stimulation.



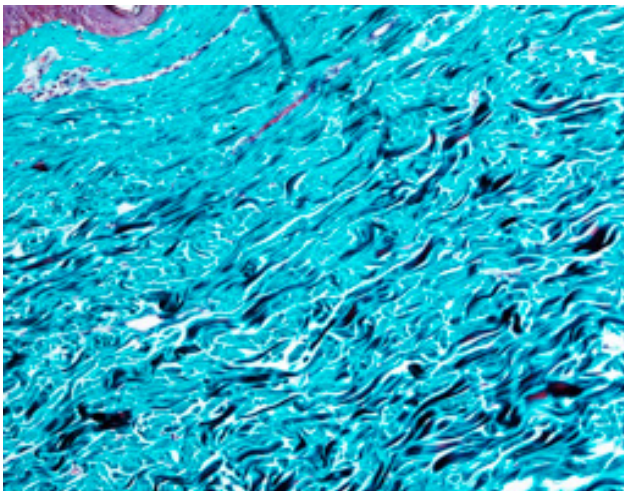
Skin Patting group + LED. Histological evaluation at 60 days from the start of treatment (time 2). E. E. Staining 10x magnification. The dermis appears decidedly stimulated, optimally organized and good angiogenic activity is maintained.



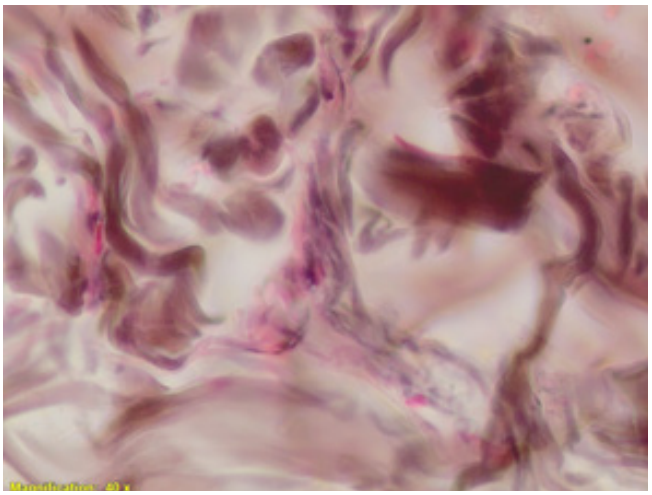
Skin Patting group + LED. Histological evaluation at 90 days from the start of treatment (time 3). E. E. Staining 10x magnification. You notice the perfectly organized and optimally structured dermis with the presence of elastic fibers intercalated between the collagen fibers, as it should be.



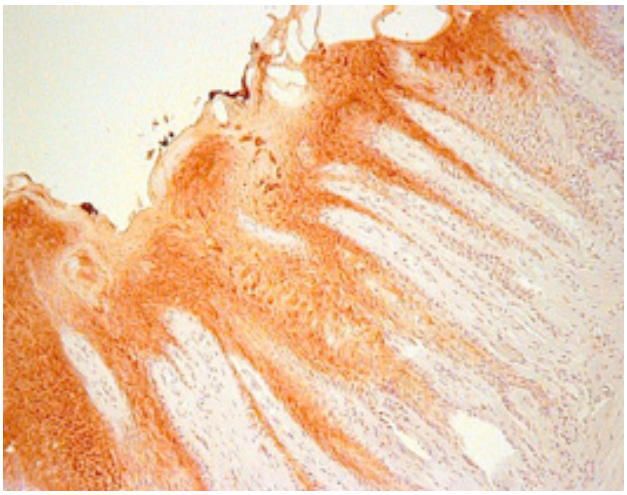
Skin Patting group + LED. Histological evaluation at 90 days from the start of treatment (time 3). Weigert coloring. 10x magnification. Collagen fibers well organized by size and structure and sense of direction; elastic fibers intercalated with the right length are clearly visible.



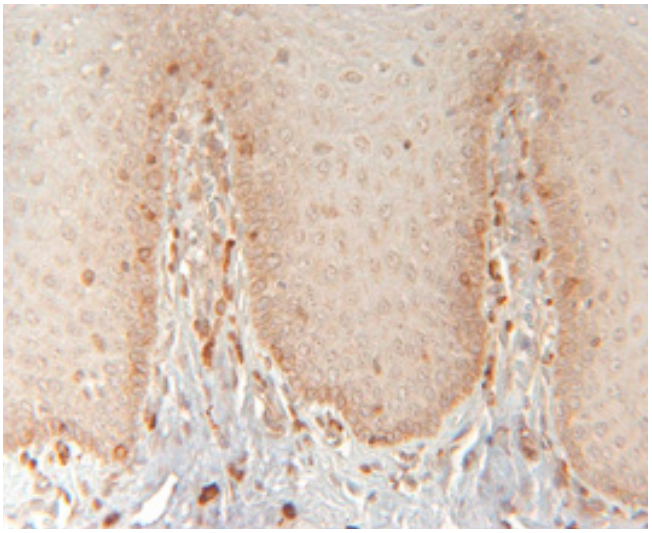
Skin Patting group + LED. Histological evaluation at 90 days from the start of treatment (time 3). Masson's Trichrome Coloring. 10x magnification. At the top we can see the perfectly stimulated epidermis, excellent angiogenetic activity and excellent organization of the dermis with elastic fibers and collagen optimally intercalated.



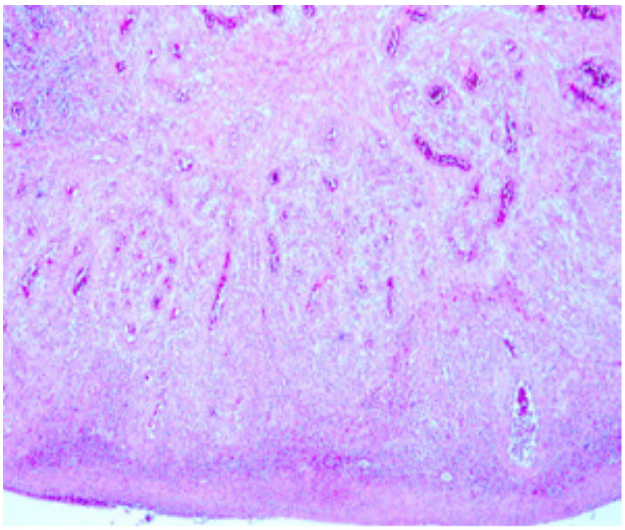
Skin Patting group + LED. Histological evaluation at 90 days from the start of treatment (time 3). Gomori coloring. 40x magnification. Collagen fibers wisely organized and stimulated in the dermis with excellent angiogenic activity.



Skin Patting group + LED. Histological / immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. The epidermis is marked in brown-brown thanks to the immunohistochemical reaction for high molecular weight cytokeratins (for surface epithelia) and highlights a papillary and cordial epithelial hyperplasia of the epidermis basal layers.



Skin Patting group + LED. Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. Epithelial-stromal proliferative activity evidenced by immunohistochemical labeling of the bcl2 protein.



Skin Patting group + LED. Immuno-histochemical evaluation at 90 days from the start of treatment (time 3). METHOD. Blood vessels are well represented in the dermis (PAS, Giemsa).

DISCUSSION AND CONCLUSION:

The evidence presented indicates that the treatment was associated with an increase in collagen in the dermis compared to placebo, with an increase in the quality of hyaluronic acid, to suggest the restoration of cellular metabolism of the epidermis, with cell replication in 28 days. The patients' statements on the improvement of the skin's brightness and its compactness are linked to the above: improvement of the quality of hyaluronic acid, increase in the concentration of collagen type 3 and regularization of the metabolism of the epidermis

BIBLIOGRAPHIC REFERENCES..