Biostimulation and Biorestructuration of the Skin

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The process of differentiation of epidemic skin cells is very complexes and regulated by a series of information from the exterior, as well as through complex intercellular enzymatic systems which function as secondary messengers.

Amongst the external informers we must consider the alpha and beta adrenergic mediators, (which work by stimulating activity of the adenylciclasase), and the cholinergic mediators, (which work by stimulating the activity of the guanilciclasic formation of (c-GMP).

Other factors ruling the keratinocitaria differentiation are the Epidermal Growth Factor (EGF) and estrogens. Amongst the intrinsic regulating factors, the calones, substances with simile hormonal activity, have a significant importance. The epidermal calones are produced by keratins in an advanced phase of proliferation and have the function of prohibiting the cellular mitosis of the cells of the basal layer. This regulates skin thickness.

The stimulus of the ECP increases the mitosis of the germinative layer; when the thickness reaches its optimal status, the concentration of the calones produced by the keratinocytes also reaches the necessary level to block the mitosis of the basal layer. When the corneous exfoliation reduces the number of the corneocytes, the concentration of the calones is lowered and the stimulus of the EGF reactivates the mitosis. The correct function of this balance regulates the correct thickness of the skin.

In concluding this report on the dermal functions and bringing it into our aesthetic interventions, we must remember two points: the cholinergic mediators and the calones. This is because treatment with botulinic toxin anticholinergic reduces the effect of this mediator, altering the epidermal function and the peeling treatment exfoliating and reducing the cornecytarius layer determines a reduction of the calones with an increase in the mitotic stimulus and epidermal hypertrophy. The quantity and frequency in the use of botulinic toxin and peeling, requires careful attention.

Below the epidermis there is the derma. The derma is formed primarily by collagen and elastic cells or fibers immersed in a colloidal matrix. The cells are represented essentially by fibroblasts.

The colloidal matrix is formed by the fundamental substance (glycosaminoglycans and proteoglycans) and by fibrous proteins such as collagen and elastin. Collagen, elastin and glycosaminoglycans are produced by fibroblasts and chondrocytes.

The physical status of the dermal matrix is important because, depending on its
consistency, the metabolic exchanges are either facilitated or inhibited.
The state of sol of the colloidal solutions which make up the matrix permits a easier
metabolic exchange, while the more solid state of the gel impedes this. The colloidal
solutions are characterized by solute molecules of considerable dimension, and
thus are unable to disperse in the intermolecular spaces of water, but are
charged with the same electrical current. Due to gravity the first molecules settle on
the bottom but impede others from doing so because the repulsion of the electric charge
of the same sign keep them suspended.
If we saturate the electric charges of the colloidal molecules with charges of opposite
sign, the repulsion force ceases and the various colloidal molecules become compact,
transforming the colloidal solute (sol) into a colloidal gel (gel).
In the derma, the state of sol is maintained by the negative charge present on the
surface of the macromolecules of GAG from which they are made. This negative electric
charge derives from the dissociation of these macromolecules in the slightly alkaline
environment which characterizes the derma (pH:7.4). This pH value is maintained steady
or unchanged by the buffer bicarbonate system.
The normal cellular metabolism produces carbon dioxide. This, in a water solution,
forms carbonic acid that when dissociating frees hydrogen ions which acidify the
solution. The hydrogen ions, positive, neutralize the negative electric charges of the
GAG and determine gasification of the derma with a reduction of the metabolic
exchanges.
Also the inflammatory processes acidify the dermal matrix with consequent biological
damage. Therefore it is important that our aesthetic interventions do not induce
acidification to the derma (inflammation) or a reduction in the buffer bicarbonate
systems.
The fibroblast is the derma’s cell capable of producing all the components of GAG,
collagens and elastin. The productive capacity of the fibroblast differs in
function;
on the age of the cell, on the different stimulated receptors and on the
physicochemical ambiance surrounding it.
In particular, we have also to make a distinction regarding the various types of
collagen being produced. This is because in various aesthetic interventions we speak of
neocollagenogenesis without mentioning the type of collagen being produced and if this
neoproduction corresponds to a real biological rejuvenation of the skin.
We must remember that in young skin the relation collagen type III/type I is much
higher in adults and that this relation tends to diminish with age.
We must also remember that fibroblast produces an immature collagen, tropocollagen,
that is assembled in different ways, utilizing portions of carbossitermal (collagen type I)
or amino terminals (collagen type III). Collagen type III, reticular, is characteristic
of young tissue and maintains the turgidity of the derma. Collagen type I, fibrotic, is
characteristic of older and cicatricial tissue and hardens the derma.
Recent studies indicate the capacity of the fibroblast to be activated towards the
production of one type or the other of collagen and in particular we can distinguish
the fibroblast into two under populations, NF (natural fibroblast) and FF (fibrotic
fibroblast) the latter being characterized by inflamed tissues. The NF mainly produce
reticular collagen, while the FF mainly produce fibrotic collagen.
Considering that the fibrotic collagen is a factor of ageing skin , it is important that
the neocollagenogenesis induced by our aesthetic treatment do not stimulate the
formation, as even if the aesthetic look of the skin could improve, the biological
functions are damaged.
We will therefore utilize neocollagenogenesis treatments of the reticular type to improve
the skin of young patients, while fibrotic
type neocollagenogenesis for older patients, aware that there is aesthetic improvement even at a cost of damage to the physiology of the skin.

A correct biological state of the derma foresees:
- Maintenance of the colloidal status of the matrix;
- Activation of the metabolism of the fibroblast;
- Stimulation of the neoformation of collagen and elastic fibers.

**Regeneration and reparation**

It is now important to examine closely the concept of neocollagenogenesis, analyzing the process of regeneration and reparation. The regeneration is a physiologic process at the base of a continuous reconstruction of certain tissues, such as those of the skin. In order to maintain functional tissues and apparatus our organism puts into effect a continuous regeneration process based on a dissolution of the preexisting tissue and on its own reconstruction.

In the skin, there are some particular enzymes called metalloproteinase capable of solubilizing through processes of hydrolysis the macromolecules that form the derma. The metalloproteinase distinguish themselves with progressive number indicators of the same molecule on which they effect their action: MMP1 collagenasi, MMP3gelatinasi, etc. The metalloproteinase is present in the derma in an inactive form with its active site blocked by a residual of cysteine: the hydrolysis of this amino acid frees the site containing zinc and permits the action of the enzyme.

As in most parts of the biological systems also the dissolution of the matrix is governed by activators and inhibitors of the MMP. The correct balance between the two apparatus permits the maintenance of a healthy and functional derma matrix. Particular receptors on the cellular wall of the fibroblast are being activated or by the growth factors, or by the lysed components of the derma and bring the sintering of new molecules.

The receptors of the tirosin-kinasi, which are activated by the growth factors (fibroblast growth factor), and the CD 44 (cluster of differentiation), which are being activated by fragments of jaluronic lysed acid, determine the hydrolysis of the poliposphoinositoli of membrane with the liberation of 1-3 of phosphositolo: this reaches the endoplasmatic lysed reticule where, joining up with a specific reticule, induces the entry of calcium ions: the calcium ions activate the proteinkinasi C with the stimulus of early induction the Jun and Fos genes and the subsequent start of the proteic synthesis.

Thus the neoformation of the components of the dermal matrix, and in particular of glycosaminoglycans of reticular collagen (type III) and of elastin.

The reparation is a biological process useful to compensate the loss of part of a tissue as a result of damage. This loss is balanced with the neoformation of a connective tissue called cicatricial tissue. This tissue is richly represented by collagen of type 1.

The cell governing the formation of the cicatricial tissue is always the fibroblast. Obviously in this case we have diverse stimulus to induce the construction of new tissue and not the original tissue.

If previously there were the fragments freed by the hydrolysis of the normal components of the derma to activate the regeneration of the skin, now there are the endocellular components freed by the biological damage and the inflammation mediators, consequent to the biological damage, to induce the activation of the reparation process.

In particular we have the activation of the CD39 on behalf of the fragments of the nucleic acid freed by the nucleus of the damaged cell and the activation of the CD 40 on the part of the mediators of the inflammation (interleukin 4) to stimulate the
formation of the fibrotic tissue rich in collagen of type 1.
From what has been stated above, it is important to point out that: it is sufficient to speak in a general way of fibroblast activation, or we have to be more precise about which receptors we activate?
It is essential to answer the second question because the answer to the stimulation of different receptors could bring a biological improvement or an aesthetic improvement. Biological improvement, useful in all kind of skins; aesthetic improvement useful only in old skins.
Therefore if we speak of fibroblastic biostimulation to be used in a young patient, we have to be sure that the stimulated receptors are only the CD 44. While in fibroblastic stimulation of an older skin, also the stimulus of the CD39 and the CD 40, even if inducing a biological damage, can be accepted because of the aesthetic improvement.
From this, to stimulate the CD 44 we must remember that:
• the proteins derived from the damage of the extracellular matrix, stimulate the synthesis of its components.
• The CD-44, cellular receptors of activation of the synthesis of the Hyaluronic acid shows the biggest activity in the presence of complexes of 20-38 monomers.
While:
- The extracellular nucleotides stimulate the purinergic receptors of type 2
- The adenosine (Purina base) rules the inflammation and the reparation of the tissues
- The adenosine receptors play an active role in the pathogenic of the dermal fibrosis.
- The extracellular nucleotides have been involved as inflammatory mediators in many pathological situations.
- The stimulation of the purinergic receptors 2 of the CD39 is associated with a chronic inflammatory response.
- phlogogen stimulus select the under populations of fibroblasts with an important role in the formation of the fibrosis.
- The interleukin IL-4 is tied up to the CD40 of the fibroblasts with a profibrotic effect and reduction of the antifibrotic effect of the IFN-range.
The stimulus of the CD 44 provides a biological improvement that is seen also with an aesthetic improvement, while the stimulus of the CD 39 and the CD 40 determines only an aesthetic improvement consequent to a fibrosis of the derma and therefore a biological damage.

The biostimulation
The proposals, also medical, present in the field of the biostimulation tell us of the use of:
- Vitamins
- Hyaluronic acid
- Fractions of DNA
- Organic silicon
- Radiofrequency
- Laser Energy
It is important before commencing any of these treatments, to consider the real biological effects of each. As doctors freeing ourselves from the simple economic business and choosing science and conscience.
Let’s start to examine closely the concept of biostimulation.
Nowadays this has become one of the most requested treatments of the aesthetic medicine. Many names are utilized in its definition.
- Biostimulation
- Biorestructuration
- Biorigenaration
- Biorivitalisation
We prefer the use of the term biostimulation to indicate an activation of the biological functions of the skin in order to optimize its physiology and achieve aesthetic improvement.
The term biorestructuration is used to indicate an alteration of the normal cutaneous components with damage of the
physiology of the skin even if there is an aesthetic improvement. Therefore, we propose the biostimulation for a young skin to improve its physiology and the aesthetics, while we proposed birestructuration for an older skin to obtain an aesthetic improvement.

This being said, let us see how we can effect a correct biostimulation. The skin functions are activated through a functional improvement of the epidermic and dermal cells which brings a normalization to the condition of the skin. This foresees a regular epidermic renewal and the optimization of the chemical-physical matrix.

The regular epidermic renewal stems from a normalization of the EGF function and of the calones. The chemical-physical optimization of the matrix requires the neoformation of the structural components and the fluidity its colloidal state. The neoformation of structural components of the matrix requires the physiological stimulation of the fibroblast in the regenerative rather than the reparative sense. The dermal regeneration becomes activated through the growth factor or the fragments of the normal components of the matrix. These work on the CD 44 activating the proteinic synthesis in a regenerative sense and improving the neo formation of reticular collagen, Hyaluronic acid and elastin.

The normalization of the colloidal state of the matrix requires the maintenance of the of a physiological ph (7.4). This avoids the transformation of the matricial solution from the state of sol to that of gel to maintain free metabolic exchanges.

The technique of a more physiological biostimulation is today represented by the Treatment with Growth Factors derived from Plasma rich in platelets. This permits the activation of the fibroblast through the use of homologous growth factors and inducing the normal reconstruction of the altered dermal components.

The technique set up in Spain by Prof. Victor Garcia and histological verified by its results, is today particularly widespread in the country of origin and is becoming well known in Europe and South America. Borne from the clinical use of plasma-rich in platelets and of the cellular growth factors connected to them.

A vast bibliography confirms the importance in ophthalmology, dentistry, neurology, orthopedics and in branches of aesthetics. The only problem is the lack of legislation able to regulate this type of transplant. The EU is preparing a law on extracting, conservation and use of any human cell, considering the diffusion of this new type of therapy. In the meantime the use of these off-label activated platelets remains the responsibility of the physician.

Growth factors are small proteinic fragments, belonging to the group of the cytokines, able to join the receptors of membranes to activate or inhibit the cellular functions.

They can be produced by numerous cells and tissues: platelets, fibroblasts, osteoblasts, epidemics cells, liver, kidney, lacrymal glands, etc.

The joining of the tirosin-kinasi receptors to the cellular membrane induces the hydrolysis of poliphosphoinositoli of membrane with the liberation of the 1-3 diphosphoinositol: this reaches the endoplasmatic smooth reticule where, if joined to a specific receptor, induces the entry of calcium jons: the calcium jons activate the proteinkinasi C with the stimulus of the genes at an early induction Jun and Fos and the subsequent start of the proteic synthesis.

Amongst the numerous growth factors freed from our cells, the PDGF was chosen for the ease of its finding, for its specific proliferation activity of the fibroblasts and synthesis of the dermal matrix.

The platelets also free other factors (TGF, EGF, VEGF, IGF) extruded from the big granule alpha after activation. Biologically the activation of the platelets in these cells is by contact with the extravasal connective after a wound (lesion): chemically we obtain this effect with the calcium chloride.
The platelets, furthermore, also transport proteins useful in the reparation and regeneration of the tissues, whether derived from their precursor cell (megakaryocitus) or captured through endocytosis from the plasma.

The use of the PRP (plasma rich in platelets) in clinic has always been directed towards the improvement of the reparation process. The merit of Prof Garcia and of the studies at the Barcelona University is in the verification of the use also in regenerative processes. The histological studies have shown that the introduction of the PRP induces, for a nine month period, the neoformation of reticular collagen (type III) in the derma justifying the affirmation of a biological rejuvenation of the skin of the patient.

The treatment is carried out on the face, neck, décolleté and hands in three sessions (the first, then after three months and after six months). It is essential to note that the biological effect is related to the concentration of the platelets, therefore the plasma, before the somministration, must be enriched. The other certified technique that takes to the use of the PRP is the Biostimulation effected with the SKIN-B product.

This, a medical device of III level certified CE by the Italian Health Superior Institute contains:
- fragments of Hyaluronic acid of 20-38 monomers to activate the Cd44 of the fibroblast;
- Aminoacid precursor of the components of the matrix
- bicarbonate buffer to keep the condition of sol of the matrix

The biostimulation with SKIN-B is effected during the intervals of the treatments with PRP.

These two treatments represent the base for the biological rejuvenation of the patients skin and can be integrated with

The physical biostimulation with LED

Recently light, and in particular photo stimulation, has been approved by the American FDA for the treatment of wrinkles. The principle is based on the fact that the LED releases photons with a low power but able however to give a positive effect on the cells at a morphological and molecular level. The treatment is today placed at an international level amongst the non ablative technologies and in particular as photo rejuvenation with light emitted by diodes, without thermal effect.

The difference between laser light and LED light is:
The non collimation leads to the divergence of rays with a consequent decrease of the intensity at the point of irradiation. In fact while the intensity of the laser is measured in watts, the LED is measured in microwatts.

The absorption of the power of incident light is different according to the wavelength and the material met. The wavelength between 600 and 900 nm is not absorbed by the biological molecules. In this range (600-900) the longer the wavelength the deeper penetration of the skin.

But which is the site for action of the photobiostimulation?

We know that in nature, whether in the vegetable or the animal world, there are molecules considered photosensitive, they change their functions on the basis of the light stimulation. The light activates the photo systems of the vegetable cell by splitting the water and use the hydro genes to activate the synthesis complex ATP and produce energy necessary for the biological synthesis.

Also at an animal level we have some biological structures activated by the light. The most evident example is that given by the rhodopsin contained at a retinal level, the activation of which is at the basis of the mechanism of vision. But also the melanocytes of the skin are cells activated by the light for the production of melanin bodies.

The light develops also an important function in the LIGHT REPAIR of the cellular DNA.
The enzyme photoliasis is a flavoprotein that, activated by the light, repairs the portions of the damaged DNA. But the most interesting point in our discussion is represented by the rings tetrapyrrolics rings present in the mitochondrial cytochromes. At the mitochondrial level, the Chain of Transport of the Electrons consents the formation of the ATP molecules. The enzymes of the chain are represented by the cytochroms. The scheme of the electronic transfer foresees the:
- transfer of the electrons from NADH to the cytochrome;
- transfer of the electrons from the FADH to the cytochrome;
- transfer of the electrons from the cytochrome Q to the cytochrome;
- transfer of the electrons from the cytochrome C to the oxygen as per action of the cytochromoxidasia.

Essential in the chain of transport of the electrons is the protonic flux of the hydrogen ions. This flux consents the formation, by way of the ATP-synthetasis, of the ATP molecules. The ATP-synthetasis mitochondrial has a particular stereochemical structure equipped with a clockwise and anti-clockwise motion on the basis of the protonic flux. The ATP or adenosin-triphosphate is a particular molecule formed by an adenosinic nucleus (adenine plus pentose) with three combined phosphoric radicals. The bond of the last phosphoric group is a one with a high energetic content the breaking of which gives off a high quantity of energy. This said, we can now understand how the Photobiostimulation with LED could be useful in the prevention of the ageing process. Scientific studies confirm that the cytochromoxidasia is the primary accepter of light between the red and the infrared and this light LED improves the electronic movement in the cytochromoxidasia, heart of the formation of the free radicals of oxygen, capable of yielding up to 4 electrons to the molecule of oxygen.

We have to point out the delicacy of this process because the mechanism of oxygen reduction foresees a necessary amount of time for the inversion of the spin of one of the two additional electrons. In fact oxygen at two electrons with spin parallel in the last orbit and the addition of another two antiparallel spin electrons, must be proceeded by an inversion of the spin. If this does not occur at precise times, there can be an escape of the free radicals free from the oxygen; the base of cellular ageing:
- The first target is the mitochondrial DNA where only one deletion results in a loss of the function of the whole filament.
- The damaging of the telomeres in the DNA, results in the non disjunction of the chromosomes during the crossing over, with consequent cellular death.
- The lipoperoxidation of the biological membranes results in a loss of function with cellular death. The loss of double ties of the phospholipids determines a rigidity of the membranes with loss of fluidity and an alteration in the functions of receptorial expressions.
- Finally, the liberation of the free radicals from the oxygen of the cytochromoxidasia, results in the activation of the caspasi with induction of the cellular apoptosis and death. The free radicals of the oxygen liberated by the mitochondria join the APAF 1 (protease activating factor 1) which join the procaspas 9 with successive aggregation and liberation of activated caspasi 9. This activates the cascade of the caspasi with final cellular apoptosis.

Furthermore, the photobiostimulation with LED (red-infrared) results in the activation of the respiratory chain of the mitochondrial with activation of the synthesis of ATP and a functional cellular improvement. The synthesis di ATP is guided by the gradient protonic. In fact, the electronic flux moving along the mitochondrial crests is accompanied by a proteinic flux in the intermembranal space. After the cessation of the electrons to the oxygen the protons pass into the ATP synthetasis, supplying the
force for the formation of the ATP. So we have the passage of a diphosphoric radical in combined to a proton. The radical will join a new proton forming phosphoric acid which terminates its reaction and joins itself to the adenosin-dishosphate, forming ATP. The clockwise rotation consents the synthesis of ATP. The rotation anticlockwise results in the hydrolysis of the ATP. The liberated energy from the ATP is utilized by the cells for the proteic synthesis, for the pumps of sodium and calcium and for the synthesis of DNA and RNA. The application times for the photo modulation, per session, range from 15-20 minutes. The number of the sessions can vary from 1 to 2 for a total of 8-10 treatments.

Biorestructuration
We continue now with the list of other products detailing their mechanisms of action and placing them according to their biological and aesthetic functions.

The macromolecular Hyaluronic acid is a polymer set up by the repetition of monomers formed by the union of Hyaluronic acid with acetyl-glucosamine. This union is permitted by the binding of the Hyaluronic acid and the uridin-triphasphate.

We cannot speak of a biostimulant effect for the macromolecular Hyaluronic acid, because as stated in the scientific literature:

- The presence of Hyaluronic acid does not have effect on the production of endogenous Hyaluronic acid.
- 0,5-1 micromoles of Hyaluronic acid limit induce the reduction of the proteic synthesis.
- High concentration of Hyaluronic acid limit the formation of extracellular matrix.
- 1mg/ml of Hyaluronic acid increases the expression of the metalloproteinase (MMP) and activates those which are latent in the extracellular matrix (MMPs).

We can only speak of an antioxidant and hydrating effect of the macromolecular Hyaluronic acid. In fact literature reports:

- Recent reports described antioxidant properties of glycosaminoglycans (GAGs).
- Since several have shown that Hyaluronic acid (HYA) and chondroitin-4-sulphate (C4S) may act as antioxidant molecules.
- Since Hyaluronic acid and chondroitin-4-sulphte possess antioxidant properties
- Hyaluronan has been assigned various physiological functions in the intercellular matrix, e.g. in water and plasma protein homeostasis.

Therefore, we do not have the stimulation of the fibroblasts and the neocollagenogenesis but only a passive hydration and of antioxidant effect.

The fragments of nucleic acid.
The nucleic acid is an intercellular component contained in the nucleus, but present also in the cytoplasm, in the mitochondrions and in the wrinkled endoplasmic reticule. So the contact of this material with the surface of the fibroblast foresees the cellular rupture due to a biological damage. In the derma the fibroblast receives the information of a biological damage or from the endocellular materials produced by the damage or by the mediators of the inflammation to the damage itself. The joining of fragments of nucleic acid to the CD39, activates the reparative process with formation of a cicatricial tissue. Scientific works with the PDRN tell us of the increase of the fibroblastic activity of the 30% with an increase of collagen, fibronectine and dermal filling. This neocollagenogenesis is relevant to the formation of fibrotic collagen characteristic of a reparative cicatricial tissue.

We furthermore remember the literature which asserts:

- The extracellular nucleotides (PDRN) stimulate the purinergic receptors of type 2.
- The adenosine (Purina basis) regulates the inflammation and the reparation of the tissues.
- The adenosine receptors play an active role in the pathogen of the dermal fibrosis.
The extracellular nucleotides have been involved as inflammatory mediators in many pathological situations.

The stimulation of the purinergic receptors 2 of the CD39 is associated with a chronic inflammatory response.

Phlogogen stimulus select some under populations of fibroblast with an important role in the formation of the fibrosis.

The interleukin IL-4 is tied to the CD40 of the fibroblasts with profibrotic effect and reduction of the antifibrotic effect of the IFN-gamma.

This time we cannot speak of the biological rejuvenation but only of the aesthetic; therefore we can only use this technique in older patients.

The Radiofrequency in the ageing skin.

The most well known and publicized radiofrequency tool for the ageing of the skin is thus presented: “It is safe, clinically proven way to tighten and contour skin, with improvements in tone contour, and texture occurring naturally through the stimulation of your own collagen”.

Also in this case we speak of neocollagenogenesis without indicating the type of collagen.

Let us investigate the concept of radiofrequency. This permits the transformation of a cold energy at high frequency relevant in heat, with an increase of the internal temperature by way of the Joule effect. All cells of the treated tissue absorb part of this energy, thanks to its grade of resistivity, and is transformed in heat.

It seems important to remember that the law of physics at the base of the effects of radiofrequency, is given by the modification of the electric field of the treated zone with a change of the electrical charge and of the resistance, and to the movement of the jons and molecules which determine heat according to the formula: $J = I \times R \times T$

Where $J$ = Energy, $I$ = Current (voltage) , $R$ = Impedance of the tissue $T$ = time.

Generally the heat produced develops between 3 and 9 mm of depth, according to the tools used, and determines heat up to 55-65 degree centigrade in homogeneous mode, without termic diffusion in surrounding areas.

The biological effect of the heat produced by the radiofrequency is a denaturation of the collagen fibers (from 5 to 30% of total fibers), with a consequent immediate contraction of the fibers themselves, with a progressive effect in the successive 4-6 months.

We must remember that the protein’s structure is characterized in 4 classes: primary, secondary, tertiary and quaternary. The primary formed by a strong covalent bond, units various aminoacids; the others formed by weak bonds permit the tridimensionality of the protein and their functions (structure, enzyme, antibody, etc). The wok bonds break easy with just by an increase of the molecular kinetics energy, (heat). The covalent bond instead requires an enzymatic process of hydrolysis.

This leads us to understand that the increase in heat beyond the physiological value of 37 degrees C, denatures the protein, leading to a loss in biological functions. If the damage continues this results in biological damage and reparative response.

The effects of the RF current are in relation with their frequency and force. Over 1,5 - 2 MHz there is elevated molecular friction which provokes intense heat, enough to induce tissue destruction. Frequencies inferior than 0.3 MHz produce undesirable stimulations in the nervous system.

The official literature confirms:

- Radiofrequency causes movement of charged particles within the tissue, and the resultant molecular motion generates heat. The heat in turn causes collagen shrinkage and new collagen deposition.

- The physical agents (mechanical, thermal, electrical, radiant, etc) determine an inflammatory process of varying degrees, on the biological material, resulting in self damage.
• Phlogogen stimulus select some under populations of fibroblasts with an important role in the formation of the fibrosis.
• The interleukin IL-4 is joined to the CD 40 of fibroblasts with a profibrotic effect and reduction in the antifibrotic effect of the IFN-range.
So, even considering radiofrequency useful for the treatment of the ageing skin, we must use this technique only for older skins because the biological effect is harmful and therefore the results are only aesthetic. An argument similar to that for radiofrequency, is valid for laser treatment for ageing skins.

The laser therapy for ablative cutaneous rejuvenation
Use is made of a controlled vaporization of thin layers of skin. The light emitted by the laser is so intense that in a very short time (90 microseconds) it vaporizes and coagulates a thickness of skin between 40 and 60 micron (the thousandth part of a millimeter).
Resurfacing with laser will produce very good results and the surface of the skin will regenerate, richer in fibrotic collagen and consequently more compact.
A source of energy activates the molecules through a tube containing gas so to determine an atonics excitement followed by a successive release of energy which hits the skin bring about a coagulative or necrotic damage according to the intensity.
The proteic denaturalization or the coagulation is followed by a reparative process that is evidenced by a deposit of cicatrical tissue containing collagen of Type 1. The literature confirms:
• A 1440-nm inducing no ablative neocollagenesis in the remodeling of scars and rhytides. Histological evidence confirms the micro columnar nature of collagen heating using this microarray.
• The physical agents (mechanical, thermal, electrical, radiant, etc) determine on the biological material an inflammatory process of varying degrees, with self damage.

Polylactic acid
Recently this filler has been proposed not only as filler but also as biological stimulus for the rejuvenation of the skin. In fact we read:
The Polylactic acid is different from other fillers. Simply, its action is based not on the filling of the cutaneous defect, but on the increase in derma's volume due to the proliferation of neocollagenesis, induced by the stimulus on the fibroblasts provoked by the Polylactic acid itself.
This is a correct assessment because a permanent filler induces a fibrotic response from a foreign body, and the literature tells:
• Polylactic and microspheres (New-fill) induces a mild inflammatory response. Host defense mechanisms react differently to the various filler materials.
• The chemical agents determine an inflammatory process of varying degrees on the biological material with damage of the same.
• Phlogogen stimulus select some under populations of fibroblasts with an important role in the formation of the fibrosis.
• The interleukin IL-4 ties itself to the CD 40 of the fibroblasts with a profibrotic effect and reduction of the antifibrotic effect of the IFN-gamma.
Therefore the neocollagenogenesis is real but constituted by fibrotic collagen of type I and therefore does not induce a biological rejuvenation but only an aesthetic rejuvenation.
It is also important to remember that the dimensions of the fibrotic capsule which forms around a permanent can be more or less evident. It is important that the use of these products be confined to the deep dermas, avoiding their use on areas of scarce thickness, for example the neck.

The silanolates

A recent proposition from France on an old product made of silanolates (onometiltrisilanolo ortohidroxicibenzato sodium - salicilato of silanolato - pH : 5,7)

In this product the organic silicon is connected to the salicylic acid with hydrogen bonds, permitting the product to remain soluble, thus avoiding the polycondensation of the monometiltrisilanolo. These bonds break once the substance is inserted in the derma. (caution with those patients allergic to the salicylic acid!)

The product is proposed for the treatment of wrinkles, scars, stretch marks and cellulite, thus proposing the biological effect that silicon makes in the skin:

- Bridges of silicon amongst the glycosaminoglycans and the glycoprotein form the skeleton of the intercellular matrix.
- In young people the skin is the tissue that, together with the arteries and the thymus, contains more organic silicon. These values decrease progressively with age.

This affirmation is real but refers to the silicon introduced in the diet, confirmed by official literature:

- Silicon is one of the important oligoelements for the regular metabolism of some of our tissues and in particular for the bony, cartilaginous and connective tissues.
- His principal role is developed in the synthesis of collagen of Type 1 and in the activity of the Proline hydroxilasis.
- Its deficiency is shown with an alteration of the formation of the bony tissue and with a reduced hepatic function of the Ornitina transaminase.
- The exogenous supplementation of silicon in the diet allows, through the normalization of the concentration of orthosilicic acid, to regulate the formation of the extracellular matrix and the calcium metabolism.

But the literature also confirms:

- The hydroxilate silicon or oxydated forms (silanolates) are utilized in the analytic medical technology (Technologies of selective separation) to stop hydrophilic molecules at high molecular weight, such as the Hyaluronic acid, and tying them up, separate them from other components.
- The particles of organic silicon put into the organism induce an inflammatory reaction and a response of fibrotic character. So we cannot credit to an organic silicon put in the derma those actions made by the silicon put in the diet.

From this we can confirm that:

- The silanolates bind themselves to the hydrophilic molecules of the derma.
- The silanolates induce an irritative inflammatory stimulation which stimulates a connective response with a neoformation of collagen of type 1.
- The pH of 5,7 saturates the negative bindings of the colloidal solution of the matrix with the gelation and coagulation of it.
- The salicylic acid regulates the inflammatory process induced by the silanolates thus avoiding an excessive damage.

The use of the silanolates must be permitted only for the aesthetic rejuvenating of old skins.

The therapeutic biostimulation

A bridge of biological stimulation between the physiological stimulation and the aesthetic correction is represented from the so said therapeutic stimulation. This foresees the skin treatment on the basis of the state of the same and has the aim of getting back from an altered state to a normal condition. We have six types of therapeutic biostimulation.
The treatment called Sebum Less is needed to reduce the sebaceous secretion in excess in the seborrhoic skins and uses the botulinic toxin introduced intradermally with small concentrations. We know that the toxin reacts by blocking, in a reversible way, the liberation of acetylcholine at a neuromuscular level and it is useful, for this, in the reduction of the mimic face wrinkles. But the use of this medicine can be enlarged also to other indications.

A first use is that of regulating the epidemic growth stimulus, reducing the effect of the epidermal growth factor. In fact: Acetylcholine (Ach) ...muscarinic receptors activate a metalloproteinase „ which liberates surface-associated heparin-binding epidermal growth factor (HB-EGF) and causes transactivation of epidermal growth factor receptors (EGFRs).

The use of the toxin after the peeling treatment that by espholiating the epidermis move the scale EGF/caloni to a access of EGF, can find an usefulness. We can also attribute to the toxin an antioxidant effect. In fact:

• Acetylcholine (ACh)...opening of mitochondrial (ATP) channels with the generation of reactive oxygen species (ROS). This action can be reduced by blocking the acetylcholine.

We can use the toxine in the seborrhoic skins to reduce the production of sebocyte and to narrow the pores. In fact:

• ....role for Acetylcholine (Ach) in sebum production and as a promoter of sebocyte differentiation.

We can utilize the toxin in the couperosic skins to stimulate the vasoconstriction of the skin. In fact:

• Adrenergic neurons release noradrenalin and ATP to reduce cutaneous blood flow while cholinergic neurons release acetylcholine and a co-transmitter to dilate skin blood vessels.

The mechanism of formation of the sebum foresees the stimulation of the sebaceous cells on behalf od the diidrotestosterone, coming from the reduction of the circulating androgens on behalf of the 5-alpha reductase. The diidrotestosterone reacts at DNA cellular level by stimulating the codification of the RNA necessary to the formation of the sebum. The botulinic toxin, by blocking the secretion of the acetylcholine, reduces the effect of this on the differentiation of the sebocyte and on the liberation of the sebum. It reduces, furthermore the increase of the ematic flux, always induced by the acetylcholine, and consequently to the flux of androgens.

For the treatment there is need of 10 units of toxins diluted in 3 ml of physiologic solution. Some intradermally shots, in those areas interested by the sebaceousipersecretion. The Hydra Plus treatment utilizes some no cross-linked Hyaluronic acid in order to increase the fixation of water at a dermal level.

The Hyaluronic acid contained in the core of the proteoglycans has the capacity of fixing a high number of molecules of water in the matrix. Also in the literature we find this important function in the keeping of the homeostasis of intradermic water.

The intradermic introduction of non cross-linked Hyaluronic acid agrees to increase the fixation of the water molecules, thus reducing the loss for transpiration and improving the hydration and the turgid of the derma.

The treatment is repeated one or more times during the month, introducing the product in the dehydrated areas.

The treatment called Aging Therapy foresees the introduction of antioxidants to block the damage from the free radicals of the oxygen.

The free radicals of the oxygen are normally produced, in the interior of the mitochondrias, through an enzymatic chain called chain of transportation of the electrons, as products in-between of formation of water molecules. The hearth of this mechanism is the cytochromes oxidase, an enzyme capable of tie together electrons to the oxygen molecule and put it together...
later to the atoms of hydrogen in order to form the water molecules.
The mechanism of reduction of the oxygen foresees a necessary time to the inversion of the spin of one of the two to be added electrons.
This can take to the escape of the radical oxygen (with only one electron) before the completion of the orbit. If the escape process exceeds a certain quantity (antioxidant concentration) the free radical of the oxygen can react with many biological structures damaging them.
This damage is compensated, up to a certain level, by the antioxidants' action. First amongst all, the vitamin E and the vitamin C. Also the enzymatic antioxidants (catalase and glutathione peroxidase) develop the blockage function of the lipoperoxidative damage.
On these bases it is useful to give intradermically, some Vitamin C and glutathione with the aim of optimizing the skin defenses to the oxidative damage.
The treatment is repeated once or more times during a month with intradermal shots.
The treatment called Photo aging Therapy prevents the alterations of the matrix related to the inflammatory process produced by the ultraviolet rays with a special buffer solution.
The UV rays hit the skin determining the activation of the cellular phospholipase. This frees from the arachidonic acid membranes reactivating the fall of the ecosanoids with acidification of the dermal matrix.
The status of sol of the colloidal solution of the matrix is permitted by the dissociation of the proteic macromolecules of which they are made. To the physiological pH of 7.4 the radical acid is dissociated, thus determining a negative charge of the macromolecule. The common negativity of the macromolecules bring to the repulsion of the same by the creation of a colloidal solution to the status of sol, permitting the free metabolic exchange. The inflammatory response induced by the ultraviolet rays brings to an acidification of the matrix with liberation of hydrogen protons. The protons, positives, gets together with the carboxylic radical, negative, giving balance to the free charges and transforming the colloidal solution of the matrix from the state of sol to that of gel. The macromolecules of the matrix get compacted due to the loss of the electric repulsion with gelation of the colloidal solution which makes the matrix. The matrix solidifies passing from a status of sol to that of gel and therefore losing its function of metabolic exchange.
The treatment foresees one session or more during the month, with introduction of a bicarbonate buffer with intradermic shots.
The treatment of called Flabby Less consists of the introduction of a medical device for the restructuring of the derma to reduce tissue looseness.
The aim is that of increasing the concentration of the fibrotic collagen and of stretching the hypotonic tissue increasing its rigid component. The scheme is one of activating an inflammatory process and the formation of fibrotic collagen.
The Medical device is constituted by a solution of amino acids, acid and hypertonic. The chemical damage induces a reparative response with the fibrosis of the derma.
The treatment is carried out with intradermic shots, one or more times during the month, in the hypotonic areas.
The treatment called Choline Therapy foresees the introduction of glycerate choline as activator of the cholinergic dermo-epidermic system.
We must remember that with the same motivations we subminister DMAE. This is the precursor of the choline and the direction somministration will anticipate the metabolic passage in the optimization of the concentration of acetylcholine.
The treatment is performed with intradermic shots, once or more times a month, in areas where a metabolic improvement is required.
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