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Guidelines for Authors

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All articles in their final version - completed with name, surname, affiliation, address, phone number and e-mail address of the author(s) - must be sent in word format to the Editorial Committee at the following e-mail address: aemj@aestheticmedicinejournal.org. Manuscripts must be written in English, and authors are urged to aim for clarity, brevity, and accuracy of information and language. All manuscripts must include a structured abstract. Authors whose first language is not English should have their manuscripts checked for grammar and stylistic accuracy by a native English speaker.

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- Results
- Discussion and Conclusions
- Acknowledgments
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- Reference list
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Editorial

In modern years, aesthetics has become quite important in every aspect of everyday life: following the hundreds of journals, magazines, blogs and websites pointing their attention towards this interesting and fascinating topic, the request for aesthetic medicine has increased manifolds.

Aesthetic Medicine is a new field of medicine, in which different specialists share the aim of constructing and reconstructing the physical equilibrium of the individual. Treatment of physical aesthetic alterations and unaesthetic sequel of illnesses or injuries, together with the prevention of aging, are perhaps two of the most iconic areas of intervention for Aesthetic Medicine.

However, in order to prevent frailty in the elderly, a program of education is similarly important.

Furthermore, the line between health and beauty is extremely thin: psychosomatic disorders resulting from low self-esteem due to aesthetic reasons are frequent and can not be ignored by a clinician.

It is therefore clear that there is no figure in the field of medicine which is not involved in Aesthetic Medicine: endocrinologists, gynecologists, angiologists, psychologists and psychiatrists, plastic surgeons, dermatologists, dieticians, physiotherapists, orthopedists, physical education instructors, massophysiotherapists, podologists, and rehabilitation therapists are just some of the specialists who are sooner or later going to have to answer their patients' needs for aesthetic interventions.

The involvement of all these specialists fits the description of health as defined by the WHO: "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" for which, undeniably, a team of different physicians is required.

The number of patients requiring medical consultation for esthetic reasons is rapidly increasing: in order to be able to provide adequate feedback, medical and paramedical specialists should be trained and, more importantly, should be taught how to work together. Existing Societies of Aesthetic Medicine from different countries share the aim of creating such teams and provide constant updates to the literature: the creation of an international network of specialists from all around the world under the flag of Aesthetic Medicine represents a challenge, but at the same time it is the proof of the widespread interest in this topic.

The first issue of this Journal represents the results of the efforts of the many national Societies and of the Union Internationale de Medecine Esthetique, now together as one; it is our hope that in years to come this Journal might improve our knowledge in this field, and provide adequate scientific advancement in the field of Aesthetic Medicine.

Francesco Romanelli
MD Editor-in-chief
Associate Professor at "Sapienza" University of Rome
Editors' notes

Aesthetic Medicine, the booming medical activity

Aesthetic Medicine was born in France 40 years ago. The French Society of Aesthetic Medicine was the first of its kind in the world, followed by Italy, Belgium and Spain. Starts were rather difficult as aesthetic procedures in those early years were only surgical. At that time aesthetic doctors and cosmetic dermatologists had very few real medical procedures to offer to their patients for treating aesthetic problems on face and body. At the beginning of the '80s, viable medical procedures started to emerge in Europe for aesthetic and cosmetic purposes. Mostly, at that time, they were imported from the United States: those included collagen injections for wrinkles (Zyderm by Dr. Stegman), and chemical peels (phenol by Dr. Baker, TCA by Dr. Obagi). But, subsequently, European research on Aesthetic Medicine gained momentum. Hyaluronic acid appeared on the market, as it was discovered that it could be used as a dermal filler for wrinkles. During the '90s, the use of lasers offered aesthetic doctors and cosmetic dermatologists new possibilities. The “beam revolution” started with CO2 laser for facial resurfacing. Today, CO2 resurfacing is not used as much anymore, because of the long and difficult postop. CO2 laser was replaced with the gentler Nd-YAG and Erbium lasers and more recently with non invasive photonic devices for facial rejuvenation, including IPL, US and radiofrequency. These new technologies allow today’s aesthetic doctors and cosmetic dermatologists to offer their patients procedures with low risk of post- op complications. Then, Botulinum Toxin has “invaded” both sides of the Atlantic Ocean. Today, Botox injections are the most popular treatment for facial expressive wrinkles. Botox injections are now so common everywhere that many cosmetic surgeons have given up their bistouries for syringes. Last but not least, development in Aesthetic Medicine is shown by mesotherapy and adipolipolysis. About lipolysis, new data and recent publications have explained that radiofrequency, ultrasounds and cryolysé could have positive action to dissolve fat and to improve some unaesthetic disorders like cellulite. These non invasive procedures intend to replace the surgical liposculpture with success. Nowadays, Aesthetic Medicine has the necessary tools to address all major disorders within the aesthetic field. After 40 years, Aesthetic Medicine is now active in 32 countries in the world (France, Italy, Spain, Belgium, Morocco, Poland, Russia, Switzerland, Kazakhstan, Algeria, Brazil, Argentina, Uruguay, Venezuela, Colombia, Chile, Mexico, U.S.A, Canada, South Korea, Ecuador, China, South Africa, Turkey, Ukraine, Georgia and recently Croatia, Portugal, India, Guatemala, Peru and Bolivia). All 32 national Societies are members of the Union Internationale de Médecine Esthétique (U.I.M.E.). Aesthetic Medicine is taught in 7 countries (France, Italy, Spain, Argentina, Mexico, Venezuela, Kazakhstan) in universities that deliver UIME’s diplomas after 3 to 4 years of studies.

What is the future of Aesthetic Medicine?

In the last few decades, patients' desires to look and feel younge, have fueled Aesthetic Medicine and Cosmetic Dermatology: many different procedures have been developed to satisfy the demands. As life-span have increased, patients today are not only asking about aesthetic procedures, they are also asking for a way to stay in good physical conditions in the last decades of their lives. As a direct result, Anti-Aging Medicine, which covers skin aging and general aging, has recently emerged and expanded very quickly. Anti-Aging Medicine can offer senior patients better nutrition, dietary supplementation with vitamins, minerals, antioxidants, and eventually hormone replacement therapy, but only when needed. Today, and in the near future, both Aesthetic Medicine and Anti-Aging Medicine will offer to our patients, who now live longer, better wellness with aesthetic treatments for skin aging and anti-aging treatments for general aging. Aesthetic Medicine is booming, but all medical practitioners should be correctly trained, so its future will be bright.

Jean-Jacques Legrand
Former General Secretary and Honorary President of UIME
Aesthetic Medicine: a bioethic act

When in 1977 the Italian Society of Aesthetic Medicine published the first issue of the magazine “La Medicina Estetica” Carlo Alberto Bartoletti, the Founder, wrote an editorial in which traced the pathway of the discipline and of the Scientific Society, still valid and projected into the future.

Today from that Editorial Board arise an International Journal, which wants to be indexed, in order to give to the doctors practicing Aesthetic Medicine all around the world a solid basis of shared knowledge.

In the late ’60s, what was called in Italy Aesthetic Medicine, moved its first steps thanks to “remise en forme and anti aging projects” imported from the experience the “Institutul de geriatrie Bucuresti”, directed by Dr. Ana Aslan.

For this reason, there is the bioethical imperative that the Discipline should be first prevention, then return to physiology and finally correction.

The worldwide diffusion and the efforts of Industries born on the wave of the phenomenon have often led to choose the fastest route to achieve and maintain the physical aspect in the myth of beauty at all costs, without considering that aesthetic is not synonymous of beauty, but it is a balance between body and mind, and the role of the doctor is to take care of the Person globally and not only focusing on the correction of “a badly accepted blemish”.

Faithful to the teaching of my Master had almost 50 years ago, this new journal will have the task of elevating the human resources, aligning and validating methodologies, but above all affirming the humanitas of the medical art in its purest sense to pursue the good and the graceful for the person who relies on it.

Fulvio Tomaselli, MD
Honorary President of the Italian Society of Aesthetic Medicine

Aesthetic Medicine needs science. All over the world

All Aesthetic Doctors know that science is the basis for safety. Safety is the most important issue in our discipline.

Unfortunately, Aesthetic Medicine is more often surrounded by marketing than by science, despite the hard work done by Scientific Societies all over the World. And, too often doctors working in this field are dealing with sellers that promote products with insufficient scientific studies.

However, they sell it anyway. I think that doctors must learn that the first thing to ask about a medical device is the scientific background regarding that product: patients treated, follow up period, adverse events and, most of all, publications.

With this new International Journal completely dedicated to Aesthetic Medicine, proposed by the Italian Society of Aesthetic Medicine, endorsed by UIME and shared by all the National Societies of Aesthetic Medicine belonging to UIME, World Aesthetic Medicine wants to stimulate scientific production in this discipline to increase safety and quality in aesthetic medical procedures.

Another important goal of the Journal is to catalyze the proposal of new protocols and guidelines in Aesthetic Medicine, with the consensus of the entire Aesthetic Medicine Scientific Community.

What this Journal should achieve in the near future is to improve the number and quality of scientific production in Aesthetic Medicine, in order to allow this discipline to grow in the field of evidence based medicine, not only in the rationale field.

I hope this can be the start of a new era for Aesthetic Medicine, with the commitment of all Scientific Societies all over the world.

Emanuele Bartoletti, MD
Managing Editor
President of the Italian Society of Aesthetic Medicine
General Secretary of UIME
Bio-skin-gineering: a novel method to focus cutaneous aging treatment on each individual layer of the skin specifically and precisely

Riekie Smit

MD, MBChB (UFS), MSc Sports Med (Pret), Adv Dip Aesth Med (FPD) Private Practice, Pretoria, South Africa

Abstract
Facial cutaneous aging changes are clinically visible to both the patient and the physician as progressing wrinkles, cutaneous sagging, textural and tone changes as well as an overall lack of radiance of the skin. Histologically the aging changes are seen within each layer from epidermis, dermo-epidermal junction, papillary dermis, reticular dermis and through to the subdermal layer. In order to effectively treat the entire spectrum of cutaneous aging changes, physicians need to understand that each layer requires therapy and merely single depths of treatment may not recover the pathology in all the layers. The concept of Bio-Skin-Gineering focuses on treating each layer individually with precision of depth and with suitable agents. This article describes the precise injection techniques of reaching each layer of the skin to ensure safe and effective results are obtained when treating cutaneous aging changes.

Keywords
Skin aging, skin quality, hyaluronic acid fillers, mesotherapy

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Bio-skin-gineering: a novel method to focus cutaneous aging treatment on each individual layer of the skin specifically and precisely

Introduction
Techniques of skin rejuvenation most commonly remain the ablative and exfoliative types of treatments such as peelings, lasers and micro-dermabrasions. In contrast, the revitalizing treatments focus on replenishment and nourishment to reverse the cutaneous aging signs. This concept, unique to the technique of skin rejuvenation mesotherapy or revitalization, focuses on replacement and nourishment of the vital components of the skin with mostly poly-vitamin solutions and especially non-cross-linked hyaluronic acid.

Also, soft tissue filler injections have evolved to unique formulations adapted to various depths of injections by altering the rheology of the hyaluronic acid (HA) fillers with various concentrations, cross-linking degree and chain lengths to adapt product injection to unique depths and indications.

HA fillers are being used for skin rejuvenation purposes by utilizing the low density, low cross-linked HA products suited for dermal injections and not purely sub-dermal as with past and traditional HA fillers used mostly for contouring and volumizing.

Many doctors will perform skin rejuvenation with either fine line filler products (minimal cross-linked HA) placed dermally or just sub-dermal by manual or device injection or some may prefer to treat with poly-vitamin products with mesotherapy techniques of injection. Only recently have we realized the importance of combining both techniques for optimal results.

The technique, which I have named “Bio-skin-gineering”, focuses on treating each individual layer of the skin, with knowledge of exact depths and angle of injection with various products according to the suitability of the product for the specific layer. The rationale behind this concept is that each layer from epidermal, DEJ, dermal to subdermal needs to be treated individually in order to reduce aging changes in the layers individually and to optimize results.

Indications
This protocol is a natural, safe and effective therapeutic intervention to improve the external cutaneous signs of solar elastosis and photo-aging of the face, neck, décolleté region and for the hands.

Patient profile
The protocol is especially suitable for patients with:
- Thin or dehydrated skin
- Fine wrinkling (crepe paper appearance)
- Superficial lines or skin coarseness
- Textural skin aging (due to solar elastosis or poor lifestyle habits)
- Need for preventative intervention

The specific indications that can benefit from this treatment includes:
- Peri-orbital loose skin and wrinkling
- Peri-oral wrinkles
- Cheek wrinkles
- Forehead lines
- Neck and décolleté skin aging
- Skin aging on hands

Treatment rationale
The proposed protocol focuses on replenishment of the decrease in HA (free HA) within the layers; supplementation of the necessary anti-oxidants, vitamins, minerals and agents that will stimulate cell renewal and reduce oxidative aging process; and lastly to replace lost volume within the deep dermal and sub-dermal layers with minimally cross-linked HA to obtain a cushioning effect. The bio-skin-gineering technique opinion aims to emphasize the importance of knowledge of the precise depths of each layer of the skin and how to accurately reach each layer to optimize treatment outcomes.

Methods
Treatment techniques
The technique is performed in 4 steps, all done in the same treatment session.

1. Epidermal rejuvenation (0.05 - 0.1mm thick on face to décolleté)

The epidermis is avascular and not too sensitive. The techniques used can be either with the epidermal needle technique or with epidermal skin needling. The epidermic technique, as described in mesotherapy practice, is performed with a 30G needle of 12mm length at an angle of 10° or less to the skin, with only the bevel entering this superficial layer (Figures 1A and B). The movement is rapid and almost ‘tremor - like’ to ensure that the needle does not enter too deep. Soft pressure on the syringe plunger will ensure droplets of the product are deposited on the surface and reach the epidermis.

Alternatively, the technique of epidermal skin needling with a device containing short 0.5mm - 1.0mm needles and very slight pressure (minimal bleeding) can be used to rejuvenate the epidermis. The product is applied to the skin before and during the rolling technique on the skin (Figures 1C and D). Rejuvenation of the epidermis is performed using a sterile injectable poly-vitamin solution with only free hyaluronic acid (not cross-linked at all) that is suitable for epidermal level of injection or needling. An example of a suitable product for this technique is NCTF135HA, due to its optimal suitability within the epidermis and dermis, and due to the numerous scientific safety and efficacy data.

Role of epidermal technique: enhance cell turnover, improve moisture balance, nourishment of the avascular epidermis and intense cutaneous stimulation. The epidermic technique contributes greatly to the radiance that patients notice from the 1st session.

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Role of epidermal technique: enhance cell turnover, improve moisture balance, nourishment of the avascular epidermis and intense cutaneous stimulation. The epidermic technique contributes greatly to the radiance that patients notice from the 1st session.
2. Dermo-epidermal Junction (DEJ) Rejuvenation (±0.5mm)
This technique has been described in mesotherapy practice as the papule technique\textsuperscript{13}. This layer can only be reached with a manual injection with a 30G - 32G needle of about 4mm length. Separating the epidermis from the dermis, with a papule forming, is the aim of the technique. A typical wheel or papule has to be visible during injection, avoiding too deep injection as this will reach deeper dermis or subdermal level. The technique is focused on problem areas such as inside wrinkles, scars, eyelid skin or areas where maximal lifting is required. The same product as mentioned in epidermal technique and not at all with a cross-linked HA, as the papule will remain. Product choice is essential and should be water soluble, neutral pH and sterile for injection purposes to minimize pain and complications. Role of papule technique in DEJ: optimizes transfer of nutrients, enhanced microcirculation and improvement of the upper part of the papillary dermis (DEJ).

3. Dermal Rejuvenation (±0.5mm - 1mm)
The dermis is most often treated with aesthetic treatments. Low viscosity and minimal cross-linked HA fillers have become popular for dermal injection to enhance collagen quality and quantity\textsuperscript{14}. Non cross-linked HA solutions are often used for intra-dermal injection. The purpose of dermal injections: enhancing collagen content and quality, extracellular matrix enhancement and also improvement of fibroblast and elastin quality\textsuperscript{14-18}.

Dermal rejuvenation with cross-linked HA fillers (low degree cross-linking) are done with a different technique to avoid nodules and other possible complications. Very small amounts of product should be injected per point. The techniques that I have found to cause the least amount of side effects for the patients are not papules, but rather very fine ‘worms’ or lines of product in a mesh/grid pattern. Alternatively a micro-cannula (25 - 27G) can be used intradermal or immediately sub-dermal which is associated with more resistance due to the density of the dermis and more pain compared to sub-dermal. Role of cross-linked HA in dermis: maximizes collagen stimulation, support and enhancement of a thin and dehydrated dermis.
4. Subdermal rejuvenation (1.0 - 1.5mm)

The deepest injection of this protocol could either be performed last or one could also start with this layer, especially when using a product that has lidocaine incorporated within the product. This would minimize the discomfort of the previous techniques in a sensitive patient. The sub-dermal layer, which is the superficial fat pad directly below the dermis, forms a ‘cushioning’ effect to plump a thin and fragile skin. The depth ranges from 1 - 1.5 mm in facial skin and is reached with at least 45° angle of injection.

Use a product containing low degree cross-linked HA and also with free HA that is suitable for superficial injection and should not leave nodule formation or uneven lumps or bumps.

The use of low viscosity hyaluronic acid fillers as a cushioning effect and enhancement of the superficial fat pads under the dermis have increased in popularity over the past few years (Figure 4A).

This technique is ideal to use in patients with loss of sub-dermal fat pads and a skinny or skeletal face. The loss of superficial as well as deep fat pads leads to more excessive wrinkling and dehydration of the skin and dermis.

Subdermal cross-linked HA injections will give an instant plumping of wrinkling, as well as continued stimulation of collagen and elastin production. Though, the revitalizing, hydrating and long-term improvement of the dermis and epidermis is only seen when combined with the prior techniques. The technique used can be either with a micro-cannula (27G or 30G) with a fanned technique (less bruising and reduced risk for vascular placement) or with sub-dermal multipuncture technique with needles (Figures 5A, B and C). The technique choice varies according to the physician’s comfort in skills and according to the product used (Figure 6).

In high-risk regions such as peri-orbital and forehead region, the use of a 25G cannula is vital to reduce the risk of intravascular placement. When using a needle technique, it is recommended to perform aspiration on the syringe before injection of a cross-linked HA filler. Most side effects are transient, mild and technique dependent. These may include mild erythema, bruising and swelling. Using exclusively HA soft tissue fillers for this directly subdermal level will ensure safety, reversibility and reduced lumpiness, especially with a very low viscosity product.

Clinical notes

- Prior treatment with anaesthetic cream improves patient comfort
- In practice, I sometimes start from the deepest technique and end with the most superficial technique. This reduces pain for sensitive patients because the fine line filler contains lidocaine, which then makes the following techniques almost pain free.
- Finish the treatment with an intensive massage with a suitable post procedure product.

The bio-skin-engineering technique can be scheduled in a course of treatments ranging from 2 - 3 treatments depending on the severity of cutaneous aging of the patient. The treatments can be spaced apart from 4 - 6 weeks apart with ideally 3 sessions per year.

**Figures 4A** - Intradermal placement of low viscosity hyaluronic acid filler with a 30G needle.

**Figures 5A, B and C** - Sub-dermal rejuvenation with Art-filler Fine Lines with cannula and needle.

**Figures 6** - Summary of the combined injection techniques in the Bio-skin-engineering protocol.
Bio-skin-gineering: a novel method to focus cutaneous aging treatment on each individual layer of the skin specifically and precisely

Results
Clinical results are visible from the first session, which includes improved hydration, smoothness and plumpness (Figures 7A and B). Continued improvement from 6 weeks to 6 months includes wrinkle reduction and firmness, this can be explained by understanding the wound healing phases of neocollagenesis starting from 4 to 6 weeks only (Figures 8A, B and C).

In a clinical practice it is not possible for physicians to confirm histological improvement on each of our patients following procedures, but we can rely on the studies performed by the manufacturers for efficacy and safety. In general we understand from clinical data that HA soft tissue fillers have a good safety profile, especially when performed with quality products and meticulously safe and sterile techniques\textsuperscript{18}.

Various manufacturers of HA fillers have further shown the improvement of collagen quality and quantity following sub- and intra-dermal placement\textsuperscript{14,18}. Gathering from these studies, we understand that this protocol will therefore improve collagen quantity and quality. Injectable polyrevitalizing solutions used with good mesotherapy techniques have also clinically proven the benefits of treatment with quality products on skin hydration, glow, evenness and further collagen stimulation\textsuperscript{11-13,15-17}.

Therefore, we could assume that the combined technique would give us the combined the histological improvement within the skin.

Results are most visible in patients with dehydrated, wrinkled or dull skin requiring an intensive boost. Results are specifically evident on areas with thinner skin such as peri-orbital, neck and décolleté regions.

\textbf{Figures 7A and B} - Before and immediately after the treatment of epidermal technique, papules, multipuncture and subdermal injection. The images show that many patients will require no recovery period.
Bio-skin-gineering: a novel method to focus cutaneous aging treatment on each individual layer of the skin specifically and precisely.

Figures 8A, B and C - Results of Bio-skin-gineering protocol on the forehead of a patient (8A) and the full face (8B and C). The results shown are following 1 treatment session and both images are taken with frontalis muscle contraction.
Bio-skin-gineering: a novel method to focus cutaneous aging treatment on each individual layer of the skin specifically and precisely

Discussion
Revision of aging changes within the individual skin layers
Each layer has specific purposes of functionality and contributing towards the skin’s external appearance (Tables 1 and 2).
Histological Changes seen in Skin Photoaging or solar elastosis.

The importance of Hyaluronic acid (HA)
The use of injectable HA to rejuvenate the skin, rehydrate the dermis and reduce wrinkles are based of the role it plays within the human skin.
Some of the functions of HA within human skin:
- Cushioning and plumping effect (anti-wrinkle)
- Hydrating (retaining water)
- Keeps collagen and elastin moist – youthful skin
- Optimizing wound healing
- Immune function
- UV radiation protection
- Improves collagen synthesis and normal skin function
- Indispensable for the visco-elastic balance of epidermis and dermis
- Role player in keratinization in epidermis

The use of injectable HA to rejuvenate the skin, rehydrate the dermis and reduce wrinkles are based of the role it plays within the human skin.

Table 1 – Summary of the role of each layer in its functionality and aesthetic importance

<table>
<thead>
<tr>
<th>SKIN LAYER</th>
<th>FUNCTION</th>
<th>AESTHETIC IMPORTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>Barrier function (protection from injury, microbial and UV) Water repellant</td>
<td>Keeps skin smooth, soft, supple and prevents water loss.</td>
</tr>
<tr>
<td>Dermo-epidermal junction</td>
<td>Nutrient and moisture transfer from vascular dermis to avascular epidermis</td>
<td>Flattening of DEJ results in skin fragility and dull appearance.</td>
</tr>
<tr>
<td>Dermal</td>
<td>Skin quality and strength: fibroblasts producing extracellular matrix glycoaminoglycans (GAGs), collagen, elastin, hyaluronic acid.</td>
<td>Reduced quality and quantity of GAGs leads to dehydration and wrinkle formation.</td>
</tr>
<tr>
<td>Hypodermis/Subcutis</td>
<td>Adipocytes and collagen fibers for support of dermis and allows dynamic movement.</td>
<td>Gives youthful and plump appearance. Atrophy and gravitational changes contributes to wrinkles and sagging in face.</td>
</tr>
</tbody>
</table>

Table 2 – Summary of the aging changes in the various layers of the skin

<table>
<thead>
<tr>
<th>SKIN LAYER</th>
<th>Aging changes</th>
<th>Symptoms/ Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIDERMIS</td>
<td>Slow cell turnover Atrophy Barrier dysfunction Poor moisture balance Atypical keratinocytes and stratum basale Irregular distribution of melanocytes</td>
<td>Dehydrated surface Rough texture Sensitivity/ Fragility Pigmentary changes</td>
</tr>
<tr>
<td>DEJ</td>
<td>Flattening of DEJ Reduced nutrient transfer form vascular dermis to avascular epidermis</td>
<td>Reduced radiance Fine lines Skin fragility</td>
</tr>
<tr>
<td>DERMIS</td>
<td>ECM health and moisture balance reduces Dermal elastosis Fibroblast dysfunction Reduced collagen and elastin network Disorderly arranged network</td>
<td>Wrinkles Sagging skin Loss of elasticity Crepe-paper appearance</td>
</tr>
<tr>
<td>SUBCUTIS</td>
<td>Atrophy and irregularity of superficial fat pads Reduced support of dermis</td>
<td>Thin, wrinkled skin Loss of volume/ plumpness of skin</td>
</tr>
</tbody>
</table>

Conclusion
The BIO-SKIN-GINEERING protocol is a combination of techniques to ensure rejuvenation from the deepest layer to the most superficial layer to improve the total rejuvenation of the skin and long-term patient satisfaction.
Ideal for wrinkled, dehydrated or dull skin requiring an intensive boost. The protocol is most suitable for peri-orbital, peri-oral, neck and décolleté, or other areas.
Side effects may include some small bruises depending on technique, but other than this no significant risks are involved when using high quality products.
The benefits for the patients include instant radiance and hydration with textural improvement from 4 weeks following the treatment is visible, especially when using high quality products with supporting data. This technique aims to improve the total rejuvenation of the skin and long-term patient satisfaction.
Physicians should be familiar with the techniques to reach each layer separately and have knowledge of the average depth of the layers in facial skin especially. High quality polyrevitalising solution with non-crosslinked hyaluronic acid is used for the epidermal, DEJ and dermal layers. Low degree crosslinking HA fillers are used for the deep dermal injections and sub-dermal placement. Ensure that products used for this technique has sufficient safety and efficacy data. The combined techniques and products ensure combined histological benefits and combined clinically evident benefits for both the patient and the physician.
Although scientific data exists for the use of low viscosity HA fillers and also for the use of certain mesotherapy or revitalizing solutions, this combined technique shows promising results and more research in the combination of these techniques and products would be beneficial.
REFERENCES


Low frequency high intensity ultrasound- and Nd:YAG Laser-assisted liposuction: a comparative study on ex vivo human adipose tissue

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Short Title: Low frequency high intensity ultrasound- vs Nd:YAG Laser-assisted liposuction ex vivo

Abstract
Background: In order to improve the efficacy of liposuction method, several technical updates have been introduced, such as ultrasound (US)- or laser-assisted liposuction. Several papers have reported the effects of these surgical techniques, but no one has given an evidence-based comparison of them.

Aim: The aim was to compare the effects of US and Nd:YAG laser devices on ex vivo adipose tissue.

Methods: Subcutaneous adipose tissue specimen was obtained from one patient (woman, 44ys old, massive weight loss history, requiring a body contouring and abdominoplasty). Tissue sample was divided into 6 portions of the same size and weight. Two portions were used as controls, two treated with the US device (power 100%, ∼19-21 W/cm²; 37.2-42.2 kHz; 10' exposure time) and two with the Nd:YAG laser (10 Watt, 40 Hz, 10’ exposure time). Weight reduction values and lipid profile of fluid portions released from samples after single exposure were registered. Extracted lipid analysis was performed with thin-layer-chromatography. Histological architecture of adipose tissue sections was analyzed by staining and microscopy.

Results: The weight reduction was significant for both US and Nd:YAG laser treatments compared to untreated sample. Of note, the weight reduction was more relevant after US treatment than laser exposure as early as 20’. TLC revealed higher levels of neutral lipids in the fluid portion released by US-treated samples when compared to laser-treated ones. Structural changes were observed after both US and laser exposures.

Conclusions: The results suggest that US appears to be more effective in inducing adipocytolysis as compared to laser.

Keywords
Low frequency high intensity ultrasounds, Nd:YAG laser, adipose tissue, weight reduction, adipocytolysis

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Introduction
In recent years, the increasing demand for minimally aggressive techniques for body contouring, using noninvasive devices have been developed. The improvement of body contouring using non-invasive liposuction approaches in place of surgical liposuction, is associated with safer procedures, quicker recovery, fewer side effects, and less discomfort, representing one of the most stimulating areas of plastic and aesthetic surgery today. In order to improve the efficacy of liposuction method, several technical updates have been introduced, such as ultrasound-assisted or laser-assisted liposuction.

These improvements have increased the selectivity of instruments for adipose tissue, enlarging the total amount of liposaspirate and reducing total blood loss. In the literature, several papers have been reported on these surgical techniques, analyzing, in particular, how they work as well as the aesthetic outcome, but no one has given an evidence-based comparison of them.

It is known that the advantages of laser treatment include a less bleeding, dermal shrinking and an earlier recovery time and, also, that it does not adversely affect the platelet levels but a limitation of these external laser devices is that they cannot use high energy because it causes thermal burns.

The advantages of ultrasound methods are the selective rupture of adipocytes membranes, leaving the blood vessels, connective tissue or nerves undamaged showing no effects on surrounding tissues as damage is restricted to a small point, the tip of the cannula.

As known, the ultrasonic waves can penetrate through tissue and during the way they lose energy for reflecting, scattering, or absorbing by tissues. It is also reported that ultrasound energy produces molecular vibrations causing a temperature increase above 56° C and the subsequent disruption of the cell membrane and collagen denaturation.

Our group has previously investigated the ex vivo effect of both external and surgical ultrasound-irradiation on human adipose tissue specimens by evaluating the weight change and lipid release over time, the histological architecture as well as apidocyteolysis and apoptotic cell death induced by treatment. In particular, after both external and surgical ultrasound treatment, we observed a significant weight loss, a triglycerides and cholesterol release, a clear alteration of adipose tissue architecture, collagen fiber damaged and a down-modulation of procaspase-9 and an increased level of caspase-3.

In the present work, we report the results of an experimental comparative study between two surgical devices, one based on a low frequency high intensity ultrasound supplied with a harmonic cannula and the second equipped with a Nd:YAG laser cannula, performing ex vivo surgical procedures on samples of human adipose tissue. Sample weight variation as well as amounts of oily fractions released after treatment as well as the relative neutral lipid profile were evaluated at different time intervals. The effect of both treatments on the histology of adipose tissue was also analyzed.

Materials and methods
Materials
Neutral lipids: cholesterol (C), oleic acid (OA), 1,3-dipalmitoyl-2-oleoyllycerol (POP), phosphomolybdic acid hydrate (Cat. 221836) and haematoxylin/eosin, solvents for TLC assay (hexane, diethyleter, acetic acid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aluminium silica plates were acquired from Merck, Darmstadt, Germany. Formaldehyde was purchased from J. T. Baker (Phillipsburg NJ, USA). Sterile catheter tip syringes were acquired from BD Plastipak (Franklin Lakes, NJ USA 07417). 10% neutral buffered formalin ready to use, dehyol absolute, dehyole 95, and paraffin were purchased from Bio-Optica (Milan, Italy). Advanced smart processor (AS300), automatic stainer (5010 Autostainer XL), and rotative microtome (RM2135) were purchased from Leica Microsystems (Nussloch, Germany). Direct light microscopy (Eclipse 50i, Nikon Corporation, Japan) and inverted optical microscope (Nikon Eclipse TS100, Nikon Corporation, Japan) were from Zeiss (Jena, Germany).

Ultrasound and Laser Devices
The ultrasound device “Microlipocavitation” was purchased by Lain Electronic S.r.l., Milan, Italy (Technosinergy Medical Technology, Pomezia - Rome - Italy), composed of a high frequency generator, a radiofrequency transmission cable, and probe with piezoelectric crystal.

The device uses modern microprocessors, able to monitor the session peak cavitation, to manage the enormous amount of energy produced by low frequency ultrasound (US) (37.2-42.2 kHz). The pulsed 1,064 nm Nd:YAG laser “SmartLipo” (DEKA MELA, Calenzano, Florence, Italy), was composed by a laser generator that works in ultra-pulsed mode (pulse time 150 microseconds; frequency 10-40Hz), a transmission cable made in optical fibers (300 micron) and a handpiece equipped with cannula (diameter 1mm) to introduce the optical fiber in tissue thickness.

Adipose tissue source
Subcutaneous tissue specimens of white human fat were obtained from one patient, a 44 year old woman with massive weight loss history (BMI at operation = 29 kg/m2) referred to the Plastic and Reconstructive Surgery Unit (Director: Prof. Maurizio Giuliani), Casa di Cura Di Lorenzo, Avezzano, L'Aquila - Italy, requiring a body contouring and undergoing abdominoplasty. Before treatment, the patient, who gave written informed consent, had an accurate clinical examination to evaluate the general health condition. Patient was normal. In order to obtain a good quality of ex vivo adipose tissue sample, reducing the tissue injury, no adrenaline-based solution was administered before skin incision, and the flap was raised with blade; small vessels were coagulated using a bipolar forceps whereas larger perforators were tied with absorbable braided suture. Immediately after surgery, the sample was kept sterile in saline buffer (NaCl 0.9%) (SB) and sent to the laboratory for ex vivo laser and ultrasound treatment.

Sample preparation
Fresh human adipose tissue explant was divided into 6...
portions of the same size and weight as far as possible (range: 232-235 g) taking care to preserve the integrity of the structural continuity and the junction between the skin layer and subcutaneous fat.

Two tissue portions were used as control (untreated), two were treated with the US device and two were treated with the Nd:YAG laser, as described below. In order to faithfully reproduce the surgical procedure, adipose tissue specimens were infiltrated by syringing with a volume of saline solution equal to sample weight. The amount of SB spontaneously released by the specimens was evaluated. Samples were further weighed at 20 minutes and 2 hours from the end of treatment with US or Nd:YAG laser device for 10 minutes. Control samples (untreated) were also weighed at the same time intervals.

**Protocols for US- and Nd:YAG laser-treatment of adipose tissue samples ex vivo**

As previously described\(^{10}\), US treatment was carried out by a surgeon in our group, with a surgical probe equipped with a harmonic cannula (diameter: 2mm; length: 15cm). The default setting of the ultrasound generator for surgical procedure was chosen to perform the treatment: power 100% (~19-21 W/cm²); frequency 37.2-42.2 kHz; the single exposure time was 10 minutes. To find the optimal resonance frequency, prior to the procedure, the auto-best frequency test was performed according to the manufacturer’s instructions. The treatment was implemented by introducing the cannula directly into fat thickness without skin incision. The single Nd:YAG laser treatment was performed in parallel by a second surgeon of our group on ex vivo adipose tissue samples in the same environmental conditions of US treatment described above. The default setting of the laser generator chosen to execute the treatment was: power 10 Watt, 40 Hz and 10 minutes of exposure time. In order to achieve a uniform energy supply, the optical fiber tip was perpendicularly cut before treatment. The trocar cannula was introduced into fat thickness without skin incision and then the optical fiber was driven 2 mm beyond the trocar cannula tip.

Both procedures were performed within the standards of clinical practice on ex vivo adipose tissue, administering the energy with slow fan-shape movements from the subcutaneous layer moving to the deeper one (Figure 1). In the control group, samples were processed in the same manner without US or Nd:YAG laser irradiation. The experiments were performed at room temperature (~20°C). Each SB-infiltrated sample was weighed before, immediately after exposure (T0) and 20 minutes and 2 hours after US or Nd:YAG laser treatment. The weight reduction percentage was evaluated in each sample with respect to weight registered at T0; fluid portion volumes released from untreated and treated samples were also recorded and collected at 20 minutes and 2 hours. The data obtained from control, US- and laser-treated specimens were compared.

**Thin layer chromatography (TLC)**

Lipids were extracted from the fluid portions released and collected at 2 hours after US and Nd:YAG laser treatments by the sequential addition of 200μl methanol/HCl, 500μl chloroform, and 200μl methanol. Samples were stirred for 2 minutes on a vortex-mixer and centrifuged at 10,000g for 10 minutes. The extraction and centrifugation steps were repeated twice. The organic phases, obtained from different extraction steps, were collected, dried under nitrogen, and then applied to TLC silica gel plates (20x20 cm). Neutral lipids were then resolved using the mixture of hexane/diethyleter/acetic acid (70:25:3 v/v) as solvent. The solvent was allowed to ascend to 1 cm from the top of the plate, and then the plate was removed, air-dried and stained. Cholesterol (C), oleic acid (OA), 1,3-dipalmitoyl-2-oleoyl glycerol (POP), the latter representative of triacylglycerols (TAG), were used as standard lipids. TLC staining was obtained by vaporizing 10% phosphomolybdic acid solution on plates. To perform the plates staining a phosphomolybdic acid solution was prepared by dissolving 10 g in 100 ml ethanol. Dried silica plates were put in the preheated oven for 10 minutes at 80°C and acquired by densitometer (UVtec Limited BTS 20M, Cambridge UK). The densitometric analysis was performed using ImageJ software.

**Histology of ex vivo adipose tissue samples after US and Nd:YAG laser treatment**

Untreated, US- and laser-treated human adipose tissue samples were fixed in 10% neutral buffered formalin at room temperature for 3 days. The samples were washed under running water for 2 hours, and dehydrated in the ethanol ascendant series with an automatic processor (Leica ASP 300) and then manually embedded in paraffin. Tick sections of 4 μm were gained with a rotative microtome and stained with haematoxylin and eosin with an automatic stainer (Leica 5010 Autostainer XL). Glasses were analyzed independently by two pathologists with an Axioskop optical microscope purchased from Zeiss, Germany at 4X magnification.

**Statistical analysis**

Statistical analysis of data was performed using two-way ANOVA followed by Bonferroni post-hoc test (Prism 5.0 GraphPad Software, San Diego, Ca). Data was expressed as mean ± SD. P<0.05 was considered statistically significant.
Results

Weight reduction evaluation of adipose tissue samples treated ex vivo with low frequency high intensity US and Nd:YAG laser devices

The study looked at comparing the ability of low frequency high intensity US and Nd:YAG laser devices to reduce the subcutaneous adipose tissue stores, ex vivo adipose tissue samples, previously infiltrated with SB, and treated for 10 minutes with a single US (Figure 1 upper panel) or laser exposure (Figure 1 lower panel). The sample weight was evaluated immediately after treatment (T0) and at different time points (20 minutes and 2 hours) from the end of treatment and the relative results are shown in Figure 2. The spontaneously released fluid from untreated samples (control) was collected and measured at each time points, generating a relative basic sample weight loss (~3% at T0, ~5% at 20 minutes, ~9% at 2 hours). Both US and laser treatment, either at 20 minutes or 2 hours, led to a significant sample weight loss when compared to untreated samples, with US treatment more relevant than laser exposure in particular at 2 hours. Of note, US treatment caused a sample weight loss significantly greater than the Nd:YAG laser-induced effect already at 20 minutes post-exposure (P<0.001) (Figure 2).

Effect of Nd:YAG laser and US treatment on extracellular lipid

All fluid portions released from untreated, US- or Nd:YAG laser-treated specimens were collected and measured either at 20 minutes or 2 hours from the end of treatment (Table 1). In accordance with the results on weight loss, both US and Nd:YAG laser exposures induced a release of fluid significantly greater than observed in untreated samples either at 20 minutes or 2 hours following treatment (P<0.001). On the other hand, US treatment induced release of a higher fluid volume as compared to laser exposure (P<0.05) (Table 1). Moreover, the fluid released by US-treated samples appeared also visually thicker when compared to those collected from the laser-treated samples. In particular, the amount of the oily fraction in the fluid portions released after US treatment at 2 hours appeared objectively greater in comparison to laser treatment at the same time interval. The representative images of fluid portions collected after 20 minutes or 2 hours from treatments are shown in Figure 3.

To evaluate the effectiveness of low frequency high intensity US treatment and Nd:YAG laser to injure the adipocyte membranes of ex vivo adipose tissue samples, the analysis of neutral lipids’ levels in the fluid portions released from US- or laser-treated or untreated samples at both 20 minutes or 2 hours was performed by TLC. In particular, the levels of cholesterol (CO), oleic acid (OA) and triacylglycerols (TAG) were analyzed after 2 hours from the end of treatment (Figure 4). Both US and Nd:YAG laser treatments were able to cause an increase of lipid released when compared to untreated samples (control) with different levels of statistical significance, and the effect of US irradiation was more relevant. Indeed, the treatment with US caused a release of lipid fraction significantly higher when compared to Nd:YAG.

Table 1 - Total volumes of fluid fractions released from ex vivo infiltrated adipose tissue samples at 20 minutes or 2 hours from the exposure to US or Nd:YAG laser.

*P<0.001 when compared to untreated sample; #P<0.05 when compared to laser-treated samples.
Low frequency high intensity ultrasound- and Nd:YAG Laser-assisted liposuction: a comparative study on ex vivo human adipose tissue

laser exposure (P<0.001 for all analyzed lipids) (Figures 4 A-C). A representative TLC of neutral lipids released in untreated sample (control) and in US- and Nd:YAG laser-treated samples after 20 minutes or 2 hours from exposure is shown in Figures 4 D-E and shows the TLC chromatography of lipid standards: C (Rf=0.38), OA (Rf=0.46), and POP (Rf=0.82).

Effect of US and Nd:YAG laser treatment ex vivo on adipose tissue histology

Histologically, the effect caused by US- and Nd:YAG laser treatments to the adipocyte membranes was also verified. Figure 5 shows representative images of the histology of adipose tissue sections from samples after 2 hours from either US or Nd:YAG laser treatment ex vivo. Sections stained with haematoxylin-eosin displayed a normal lobular architecture with regular and undamaged adipocyte membranes in untreated samples (Figure 5A).

The alterations of adipocyte morphology of US treated samples associated with cell membrane appeared unboundled and broken compared to the histology of Nd:YAG laser-treated sample (Figure 5B and C, respectively).

Figure 3 - Representative images of fluid portions released by the adipose tissue samples and collected after 20 minutes or 2 hours from the Nd:YAG Laser or US treatment are shown.

Figure 4 - Comparative effect of US- and Nd:YAG laser treatment on lipid release. Bar graphs represent percentage increase of cholesterol (C) (A), oleic acid (OA) (B) and triacylglycerols (TAG) (C) in untreated (control), US- and Nd:YAG laser-exposed samples versus the same relative sample before treatment. Shown data were mean values ± SD of two determinations. Representative TLC of neutral lipids released in untreated samples (control), US- and Nd:YAG laser-treated samples before and after 20 min or 2 hours from exposure (D). TLC chromatography of lipid standards: CO (Rf = 0.38), OA (Rf = 0.46), and 1,3-dipalmitolyl, 2-deoylglycerol (POP) (Rf = 0.82) (E).
Low frequency high intensity ultrasound- and Nd:YAG Laser-assisted liposuction: a comparative study on ex vivo human adipose tissue

Discussion and conclusions

Even if the effectiveness of both US-assisted and laser-assisted liposuction has been extensively investigated, comparative studies of these aesthetical surgical procedures have not been reported on and should be required. Indeed, the previous studies have been mainly focused on comparing either US-assisted lipolysis or laser-assisted lipolysis versus conventional liposuction alone\textsuperscript{2,3,11-17}.

To our knowledge, the present study is the first to reproduce in a controlled environment, surgical procedures of clinical practice which demonstrates if both methodologies give overlapping results. In terms of weight reduction and amount of lipid released, US irradiation provided more significant effects within 20 minutes after the exposure. Moreover, the assessment of the released oily fractions following each treatment showed that the US exposure induced a significant increase of neutral lipid levels (C, OA and TAG) when compared to the Nd:YAG laser-treated samples. In addition, US irradiation caused a more effective adipocytolytic effect, which was also supported by microscopic evaluation of adipose tissue sections revealing a high number of injured cell in US treated samples. However, all these results should be confirmed by additional experiments and clinical studies conducted on larger series of patients. Our results highlight that ultrasound-assisted liposuction is more effective to reduce the adipose tissue weight probably due to physical phenomena generated into adipose thickness. As known, an ultrasound device, during lipoaspiration procedure, generates compression and depression cycles of ultrasonic waves at appropriate frequency\textsuperscript{9,18,19}. This action produces a negative pressure in the adipose tissue thickness leading to cavitation that allows a selective tissue lipolysis with adipocyte rupture and triglyceride release from the lipid vacuole to the extracellular space\textsuperscript{10,18}. Of interest, Bani et al demonstrated that, after ultrasound treatment, signs of interstitial inflammation were absent and the cavitation-induced effects seem to be restricted only to adipocytes, without injury to skin, vessels, nerves, or connective tissue\textsuperscript{19}. Moreover, it has been demonstrated that ultrasound-assisted lipoaspiration treatment does not impair the proliferative ability and osteogenic potential of Adipose Stem Cells (ASCs) normally present in adipose tissue\textsuperscript{20}.

On the contrary, a different physical process is generated into adipose tissue using the laser-assisted liposuction. In general, laser device releases a beam that is converted to heat energy through the adipose tissue, connective tissue, and blood vessels. The 1,064...
nm Nd:YAG laser exerts a lipolytic action melting the subcutaneous adipose tissue and, also, the septae that connects the dermal and muscle layers are divided. Anyway, the use of surgical 1,064 nm Nd:YAG laser, even if it is less traumatic than traditional liposuction methods, has the disadvantage to provoke a thermal injury; it is known that thermal energy or heating induces cell morphology and tissue texture alterations when the laser cannula reaches the temperature ranged between 47°C - 52°C. Even if preliminary, all the results from our experimental study could represent a stimulus for appropriate clinical studies designed to comparatively evaluate the efficacy of these two surgical approaches by examining tissue in vivo.

Acknowledgments
The study has been performed in the framework of the "Research Centre for Molecular Diagnostics and Advanced Therapies" supported by the "Abruzzo earthquake relief fund" (Toronto, Ontario). The Authors thank Gasperina De Nuntiis (Department of Life, Health and Environmental Sciences) for technical assistance.

Conflict of Interest statement
The Authors declare that there are no conflicts of interest.

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Low frequency high intensity ultrasound- and Nd:YAG Laser-assisted liposuction: a comparative study on ex vivo human adipose tissue

REFERENCES


Treatment of stretch marks aged more than twenty years with the synergy of electromagnetic field and vacuum. Clinical case studies and subsequent follow-up

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Abstract

Objective: the aim of this study was to verify the efficacy of the synergy between electromagnetic field, electron flow and vacuum realized with Biodermogenesi® method. This synergy was applied on very old (more than twenty years) stretch marks (striae albae - SA) on female patients. The type of stretch marks treated were selected not by location or cause, but by the age of stretch marks. In this case, the study will allow to open a discussion on the possibility to intervene successfully also against the notoriously more difficult types of striae, analyzing the results obtained and the absence of side effects.

Method: 20 women with stretch marks aged more than twenty-years were treated with a treatment program of 9 Biodermogenesi® sessions executed weekly. The treatment was performed with Bi-one® 2.0 MD device, equipped with a generator of electric impulses, electromagnetic fields and vacuum integrated with a bio-feedback system that allows the automatic variation of frequencies and intensities of the electromagnetic field delivered. A subjective evaluation of the outcomes was asked separately to the patients and the researchers; they were asked to evaluate: the filling of the hollow area of the stretch marks, the feeling to the touch of the stretch marks, the discoloration and the eventual tanning of the stretch marks. The evaluation was carried out at T0 (before the sessions - 0%), T1 (after the ninth treatment session) and T2 (after six months from the end of the ninth session cycle). The evaluation scale is as follows:

- Level 0: No improvement (+ 0%)
- Level 1: Poor improvement from +1% to +20%
- Level 2: Minimal improvement from +21% to +40%
- Level 3: Moderate improvement from +41% to +60%
- Level 4: Good improvement from +61% to +80%
- Level 5: Excellent improvement from +81% to +100%

Results: the improvement of the SA has been evidenced with a tangible filling of those same marks, evident both to the touch and to the sight, and with the recovery of its initial color and subsequently uniform with the surrounding skin tissue. In the subsequent or simultaneous exposure to ultraviolet, we appreciated the tanning of the striae with an intensity very similar to the surrounding skin, in total absence of side effects.

There was also a general increase in the elasticity and compactness of the treatment area.

Conclusions: the synergy offered by Biodermogenesi® method has proven to regenerate even very old SA, which are the most difficult ones to treat. These imperfections were significantly reduced, and in some cases were completely eliminated their evidence to touch and sight, thanks to the newfound power to tan the striae with the exposure to ultraviolet rays. The uniformity of outcomes is appreciated, as all patients reacted positively to the therapy in the absence of side effects.

Keywords

Stretch marks, Biodermogenesi, electromagnetic field, vacuum, electroporation, capacitive radiofrequency

Abbreviations

T0 assessment made before the treatment program
T1 assessment made at the end of the treatment program
T2 follow-up made from 6 to 12 months after the end of the treatment program
PIH post-inflammatory hyperpigmentation
SA striae albae
SR striae rubrae
Introduction
Since the second half of the twentieth century, stretch marks have increased exponentially among the female gender and has gradually started to affect young boys as well, becoming probably the most widespread imperfection in the new generations.

Biodermogenesi® has shown remarkable effectiveness in the regeneration of stretch marks and post-surgical scars and burns, favoring the production of collagen and elastic fibers. The aim of this study is to verify the outcomes of the new synergy developed by Biodermogenesi® on a group of female patients, all burdened by very old stretch marks that are white and opaque in color with a deep and rigid structure.

The new synergy, which combines electromagnetic fields with electron flow and vacuum, provides a number of sessions drastically reduced compared to the previous Bi-one® technology, to regenerate a twenty-year stria; before it required at least 20 sessions.

Stretchmarks that are more than twenty years old are more difficult to work on to obtain a significant improvement.

The existing literature has recently focused on the analysis of the results obtained with various types of lasers, facing some consolidated limits: the greater efficacy documented regarding only the red stretchmarks, and therefore newly formed stretch marks. However, we saw that at the end of laser treatment programs the results were varying from moderate to none and were not replicable on all the patients, especially on SA.

Furthermore, the laser procedure caused the constant presence of edema and PIH and a recurrent pain detected by patients.

Briefly analyzing the existing literature in support of laser therapy, we note various evaluations of the results obtained. The results obtained with a non-ablative fractional laser in the experience of Katz et al., limited to only two young patients (12 and 13 years) with stretchmarks dated 3 and 10 months, appear positive.

Other experiences speak instead of appreciable results on a part of the patients treated, compared to others whose outcomes have been minimal or none.

In this sense, we recall the experiences of Bach and his colleagues: "six of the 22 patients (27%) showed good to excellent clinical improvement from baseline, whereas the other 16 (63%) showed various degrees of improvement".

This is confirmed also by Stotland and his colleagues who confirm that: "photographs of 8 randomly selected patients showed an overall improvement of 26% to 50% in 63% (5 of 8 patients)". Even the experience of Tretti Clementoni has obtained greater uniformity of results, he confirms that some patients treated with non-ablative fractional laser do not show significant outcomes: "the volume of SD depressions improved by more than 50% (mean improvement 58%) in the majority of patients (11 of 12 patients) and the color of the lesions improved by more than 50% (mean improvement 54%) in 83.33% of patients (10/12)".

Apart from Katz’s experience, the other researchers do not mention the visual appearance of stretch marks (white or red) and their dating.

The experience of De Angelis et al. is based on 51 patients treated with 1540-nm fractional non ablative Er: Glass laser and the evaluation was done both by researchers and blinded, always with positive outcomes, noting a reduction in striae generally greater than 50%.

The practically zero stabilization of the improvements obtained has limited the spread of this therapy, leaving the problem of hypopigmentation unresolved.

Materials and methods
We analyzed a group of 20 patients aged between 34 and 66 years, all with SA aged between 20 and 35 years, and treated them with a cycle of 9 sessions of Biodermogenesi® on a weekly basis.

The patients were all healthy and did not have any preconditions for being excluded from the trial.

The exclusion criteria are as follows: Pace-Maker users; cancer therapy in progress or during the last 5 years; epilepsy; vascular alterations such as varices, phlebitis and thrombophlebitis; pregnancy or breastfeeding; alterations and hormonal therapies manifested during...
the last 6 months; anti-coagulant therapy; phenomena of anorexia or bulimia during the last 2 years. Biodermogenesi® treatment was performed with an electro-medical device called Bi-one® 2.0 MD, combined with three synergistic active ingredients. Treated stretch marks were present on the breasts, arms, abdomen, hips, buttocks, thighs, calves; for the cases in question we treated a single area on each patient, as required by the official protocols.

Technology
The treatment was performed with a non-invasive electro-medical device “Bi-one® 2.0 MD” and protected by some international patents (Expo Italia S.r.l., via Segantini, 34, Firenze, Italy). The apparatus was equipped with a generator of electromagnetic fields, an electron flow generator, a pair of vacuum pumps and a series of handpieces. The generator of electromagnetic fields emits a capacitive shielded signal at a variable frequency, ranging from 0.5 to 1 MHz ± 10%, and variable intensity up to a maximum value of 6W on a 500 Ohm resistance. The device is equipped with a bio-feedback system that allows to change independently the intensity and frequency of the signal delivered according to the different biological characteristics of each individual patient, increasing the temperature of the treated area between 39° C and 40° C. The electron flow generator emits a 5 Hz square wave signal with a maximum intensity of 0.36 mA on a 500 Ohm load. Generators of the electromagnetic field and of the electron flow are separated mechanically and galvanically from each other and towards the network plant. The brushless vacuum pumps allow delivering negative pressure with absolute precision and stability, with a maximum value of - 0.35 atmospheres.

Procedures
The treatment procedure was divided into two distinct phases, during which several forms of energies are present for different biological actions. The first phase was about a light mechanical peeling. For this phase, we used a single-use abrasive pad placed inside the PEELING handpiece. PEELING handpiece works with a gentle vacuum action, designed to lift the tissue with the stretchmarks, bringing the hollow area of the imperfection outwards. The complete deconstruction of the elastic fibers that characterizes very old striae11,12 allows the striae to extend outwards, bringing the dense and compact corneous layer in relief, favoring a selective reduction. The second phase, called ACTIVE PLUS, provides the synergistic action of vacuum and biocompatible electromagnetic field generated thanks to a capacitive radiofrequency with variable frequency and intensity. The combination of these forms of energy activates a greater action by the arterial capillaries, it increases the caliber and brings to the matrix oxygen and nutritional elements, stimulates the lymphatic microcirculation, and helps to drain part of the toxins present11. The simultaneous flow of electrons, object of the new technology, allows a reduction to the electrical resistance of the skin tissue, effectively amplifying the yield of the electromagnetic field.

As the effectiveness and the useful dose of the applied electromagnetic field is inversely proportional to the electrical resistance; the flow of electrons reduces this value and consequently increases the regenerative efficacy of the electromagnetic field. At the same time we observe a strong pumping of the Na + / K +, able to increase the fibroblast activity, leading to the synthesis of collagen and elastic fibers11,12 and favoring a tissue repair11,12,13.

The technology adopted for the present study provides a platform developed by Philips, able to compare the acceleration obtained with sodium and potassium through the cellular barriers:

Previous Bi-one® technology from 300 to 450 mV
New Bi-one® 2.0 MD technology from 750 to 850 mV

Documenting an effectiveness on average by double with respect to the previous technology. The full treatment session takes about 25 minutes in total. The protocol provides a preliminary evaluation system of stretch marks that determines the level of difficulty and therefore anticipates the patient what the result will be, how many sessions will be needed to obtain this result and how long the treatment cycle will take to be completed. All the patients examined, respected the indications provided by the protocol.

Evaluation
The evaluation of the results of the treatment of stretch marks was carried out by using the VAS (Visual Analogue Scale) scales of the patient and the doctor. Assessments were made before the start of treatment, during the preliminary visit (T0), after the last treatment (T1) and after a period between 6 and 12 months from the end of the sessions (T2). The VAS scale asks the patient to make the most of the following parameters:

- Improvement of the stretch marks to the touch (depth and fibrosis)
- Improvement of the visual stretch marks (color and opacity)
- Increase in the faculty of the stretch mark to get a tan

The values are expressed from 0 = 0% (no improvement), from 1 = 1% to 20% (poor improvement), from 2 = 21% to 40% (minimal improvement), from 3 = 41% to 60% (moderate improvement), from 4 = 61% to 80% (good improvement), from 5 = 81% to 100% (excellent improvement).

Results
The results obtained are summarized in the following Tables 1, 2 (patient’s VAS scale) and Table 3 (doctor’s VAS scale). The scales measure the perceived improvement on the treated stretch marks.

VAS Scale - Patient
At the end of the treatment program (T1), 11 patients (55%) found an improvement between 41% and 60%, while 9 patients (45%) found an improvement between 61% and 80%. The perception of the improvement obtained was increased when the follow-up (T2) was performed.
Treatment of stretch marks aged more than twenty years with the synergy of electromagnetic field and vacuum. Clinical case studies and subsequent follow-up

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient’s Age</th>
<th>The presence of stretch marks in years</th>
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<th>Patient T2</th>
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Table 1 - VAS scale of the doctor and the patient.

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<tr>
<td>HR</td>
<td>5</td>
</tr>
<tr>
<td>PMG</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2 - Patient's VAS scale.

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Table 3 - VAS scale of the doctor.

<table>
<thead>
<tr>
<th></th>
<th>1/20%</th>
<th>21/40%</th>
<th>41/60%</th>
<th>61/80%</th>
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<td>T1 - After therapy</td>
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<td>T2 - After 6 months</td>
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</table>

after more than 6 months from the end of the treatment program: 1 patient (5%) found an improvement between 41% and 60%, 6 patients (30%) found an improvement between 61% and 80%, while 13 patients (65%) found an improvement between 81% and 100%.

VAS scale - Doctors

At the end of the treatment program (T1) the doctors in one case (5%) evaluated an improvement between 21% and 40%, on another patient (5%), between 41% and 60%, on 15 patients (75%), between 61% and 80% and on 3 patients, between 81% and 100%. Also the evaluation of the improvements obtained was increased by the doctors during the follow-up (T2): in one patient (5%) they evaluated an improvement between 41% and 60%, on 8 patients (40%) between 61% and 80% and on 11 patients (55%) between 81% and 100%.

Analyzing the documented results, a noticeable overall improvement of the treated stretch marks is evident, both in the evaluation of the patients and the doctor. In the T1 test, we notice an overall improvement of the SA, which tends to increase in the months after the treatment. The progression of the improvement is due to the activation of virtuous reactions, which we have also found with the previous version of the technology. The reactivation of the sodium and potassium pump allows restoring a better activity of the fibroblast, which physiologically manifests itself in the course of a few weeks after the treatments, during when the maximum regenerative response is obtained by the treated tissue. Another aspect that the patients have greatly appreciated is given by the newfound ability of striae to tan with the sun exposure another aspect that we had found with the previous technology. Early experiences, first of Dr. Artigiani and coll. had documented a recovery of the ability to tan by the stretch marks treated with Biodermogenesi®. This aspect has allowed patients to expose themselves to ultraviolet also during the treatments, highlighting a progressive tanning of the striae. Of course, for the patients living in Sanremo and Palermo who were part of this study, it was much easier to obtain the tanning of the stria as both of the cities are known for their beach attractions.

The stabilization and progression of the outcomes, as shown by the conclusions made in T2, where the appreciation of patients and doctors is consolidated, is confirmed by Bacci, who performed a follow-up after more than 5 years from the end treatment with Biodermogenesi®. In his study, Bacci highlighted a general improvement of the results previously achieved, without any regression of the outcomes obtained on the patients.

Unlike what was found with other technologies, the treatment of striae with Biodermogenesi® did not cause pain, discomfort, or any side effects, even minimal, at the end of each treatment session. Patients were able to regain their lifestyle immediately without any limitation.

The choice of evaluating the results obtained with the present therapy by means of the VAS scale exposes to the risk of subjectivity that would not occur with instrumental or bioptic tests. However, we believe that the type of result obtained, which is the filling of the striae, even if they are present for more than twenty years, and their subsequent tanning makes this assessment acceptable. In fact, the filling and the rediscovered capability of tanning of the stretched skin derives exclusively from a reorganization of the epidermis.
and the dermis, a restored basal membrane, a correct positioning of the melanocytes and from an adequate dermal vascularization. Basically, to completely tan the striae, it is essential to fully regenerate the skin tissue. In our opinion, the results obtained on all patients adopting the VAS scale is certainly subjective, but in the specific case it is not questionable.

Discussion
Biodermogenesi® opens up a new perspective in the treatment of SA by applying for the first time a non-invasive method that is not based on damage and subsequent repair. We know that collagen fibers change between 52° and 55°C and contract at 65°C and come to denature between 60° and 70°C.

The thermal effect induced by Biodermogenesi® stabilizes the dermis temperature between 39° and 40°C and therefore the variation of collagen and elastic fibers documented bioptically by Bacci and by Artigiani et al. gives us a curiosity about the induced regenerative mechanism, presumably related to the Van’t Hoff law.

In the case of Biodermogenesi®, it is believed that the thermal effect is the consequence of the increased activity of Na⁺ / K⁺ across the membranes, favored by the applied electromagnetic field, it determines this reaction for mere friction.

The regenerative faculty of the tissues subject to greater activity by these carriers is amply demonstrated by the literature in sports medicine, in the field of recovery of muscle injuries. However, the evident improvement of the treated striae is obtained in total absence of side effects.

Conclusions
Biodermogenesi® can be used successfully in the treatment of SA, even if they are very old (more than twenty years), favoring both an aesthetic result and an effective regeneration of dermis and epidermis, as evidently demonstrated by the renewed ability to tan by the striae as a result of correct skin reorganization. All patients treated in accordance with the protocols have noticed a significant improvement in the imperfection, also confirmed by the doctors, with no side effects and without limitations to a normal lifestyle.

Conflict of interest
The authors declare that they have no conflict of interest.
Treatment of stretch marks aged more than twenty years with the synergy of electromagnetic field and vacuum. Clinical case studies and subsequent follow-up

Case 2

Figure 2 - S.L., a 43-year-old patient with stretch marks present for 20 years. Pictures taken at T0 and T1 + 6 months. Stretch marks are filled and tanned and at the same time, skin tone has improved. The abdomen is more compact and firm and the navel has reopened.

Case 3 dx

Figures 3 (A-B) - P.G.M., a 34-year-old patient with stretch marks present for 21 years. Pictures taken at T0, T1 and T1 + 6 months. In the patient in question we appreciate the filling of stretch marks since T1, where they started to pigment due to limited sun exposure. The photo taken at T1 + 6 months highlights an excellent tanning of stretch marks thanks to subsequent sun exposure and the achievement of substantial uniformity with the surrounding tissue.

Case 3 sx
Treatment of stretch marks aged more than twenty years with the synergy of electromagnetic field and vacuum. Clinical case studies and subsequent follow-up

REFERENCES


Lip augmentation with a new generation of liquid implant: Agarose-gel

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¹MD, Private Practice for Dermatology and Aesthetic Medicine, Frankfurt/Main, Germany

Abstract
Dermal filler that is used in the lip area must be locally stable and have a low particularly potential for side effects. In addition to the previously established substances, stands a new alternative to choose from agarose gel, especially for their biocompatibility scores.

In Aesthetic Medicine, in addition to the body's own substances, such as autologous fat augmentation, are found a variety of dermal fillers for this use. The requirements for such fillers in general extend to the effectiveness, volume, elasticity and the safety profile of the fillers or liquid implants, such as the local resistance and a low side effect profile. Specially interesting are non-permanent fillers, that are stimulating by the fibroblast, that act for a volume-replacement. For the best results, are important, besides anatomical knowledge of the face, the physiology process of aging, and the suitable filler for the intervention. In tissue augmentation lies a central aspect in the three dimensionality of the face, thereby it is an advantage over the often two-dimensional surgical treatment. As we age, our skin loses the ability to preserve moisture, resulting in the visible loss of firmness, pliability, and plumpness. Augmentation means a restoration of the lost volume (age related misplaced skin) in sense of a three-dimensional tissue repositioning.

Keywords
Agarose gel, dermal filler, lip augmentation, lip restoration

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Introduction
Lips are one of the most injected areas on the human body. Hyaluronic acid (HA) is a golden standard mostly used for this procedure. The main aspects in the treatment of lips are comfort (for both the practitioner and for the patient) and a long-lasting effect. In addition, it is necessary to use safe substances with regard to their compatibility and local resistance.

Based on the proven safety of Agarose gel it has been a factor in its increased use. Only rarely are complications reported. Agarose is in principle not a completely new material in medicine. It has been used in the dental field for more than a decade. The substance class is a neutral polysaccharide, it is completely biocompatible and thus degradable.

The aim of this article is to show agarose gel as at least an equivalent to those for treatment of the lips. The following overview describes the possibilities of this treatment method.

Lip augmentation
In the lower part of the face are the lips as a focal point, especially in the foreground of the interest. Lip augmentation is a cosmetic procedure that can give you fuller, plumper lips. These days, an injectable dermal filler is the most commonly used method of lip augmentation. There are many types of dermal filler that can be injected into your lips and around the mouth. But the most common fillers today are products that contain hyaluronic acid. The goal of an ideal injection is to make, improve the appearance of lips by adding shape, structure, volume, but also a natural softness too, without causing a change in movement or in facial expression. The best suited filler for the lip area is on one hand locally resistant, that means the injected substance remains in the addressed region and does not migrate in around tissue. Other unwanted effects are also possible, such as pain, swelling and bruising, lip asymmetry, allergic reaction causing redness or itching around the lips. For this reason, the knowledge of the diversity of different materials and techniques in aesthetic medicine in cases of lip augmentation, is essential to increases the quality of treatment and for the patient satisfaction.

A prerequisite for this process is a good cooperation between patient and doctor, and which techniques or products are best to be used. As a new alternative to the previous substances a new filler is now available: Agarose-gel.

One hundred percent natural
The agarose gel is available on the market fulfilling the expectations and requirements of the most demanding practitioners. Agarose is a polysaccharide from D-galactose and 3,6-anhydro-L- galactose, which are glycosidically linked. It puts the main component on the agarose. As it is made up of 100% natural polysaccharides, it is completely biocompatible and also degradable. It contains for example no cross-linked synthetic chemicals BDDE (1,4-butanediol diglyceridyl ether). The gel is sterile, very viscous and elastic, and clear and transparent. Due to the isotonic property of the gel, this filler is almost painless when injected. It is locally stable and it has very few side effects. There is also an immediate result achievable without expected downtime. The increase in volume is directly visible because no hydrophilic volume process has to be waited for. The human organism has no specific enzyme, to break down agarose. Compared to hyaluronic acid, which is degraded by hyaluronidase, agarose degradation is made slowly by macrophages, before it finally takes place in the pentose cycle, and it is eliminated in the endoplasmic reticulum. Thereby agarose is a long lasting aesthetic effect expected to last.

Proven and compatible
Agarose has been the substance of choice in various studies regarding its biocompatibility and no cytotoxic and genotoxic properties. Due to its biocompatible character agarose gel has been around for over 10 years already in frequent use in the field of dental medicine and in oral surgery. It is described to be a soft tissue augmentation for the perioral region. The occurrence of complications is extremely rare. Another benefit of this new filler is the replacement of lost subcutaneous fat and remodeling of the upper and lower lip contours. Subcutaneous filler is able to achieve a youthful and natural look by filling-out lines around the mouth, the nasolabial fold as well as chin augmentation (Figure 1). Patients are trending to natural and biocompatible dermal fillers for a safe and effective solution to anti-aging.
It is recommended to use before treatment a local disinfection and also to apply anesthetic cream to the injection site to numb the area. In some cases, there is also the need to consider a local injection with anesthetic (lip block). The treatment should be as painless as possible for the clients. For this, besides a topical anesthesia, also the application of a very thin cannula is recommended. Agarose itself is an almost painless injectable because of its isotonic properties, as mentioned above. Only in the expansion in the tissue does a burning symptom come. Therefore, the agarose gel can be mixed with a local anesthetic, such as Lidocaine. Due to the viscosity of the material generally it is possible to use the 30 gauge cannula for the lips. The reduction of hematomas and swelling is avoided largely by the direct compression and cooling, for example with cool packs, for a few minutes after the injection. This minimizes and closes the bleeding. Patients should avoid for about half an hour hot drinks (Coffee and Tea) due to lip anesthesia. A direct reintegration into social life is easily possible due to the fast convalescence. For example, the treatment can also take place during lunch breaks or before important events (Weddings), and patients can return to work or participate in events after the treatment on the same day. According to the principle "What you see is what you get" the result is visible immediately after the injection as well the final result (Figure 2).

### Application

Patients with acute or chronic skin pathologies or direct involvement in or around the lips to be treated were excluded. Pregnancy, lactation and hyaluronic acid treatment less than 3 months earlier were also excluded criteria. In total 11 patients were treated. The patients were between 19 and 38 years old. All patients were female. Nobody had had a treatment with permanent fillers before. Five patients had previously had an injection with hyaluronic acid in the lips (Tables 1 and 2).

<table>
<thead>
<tr>
<th>Treatment area</th>
<th>Initial treatment</th>
<th>Touch-up</th>
<th>Initial and Touch-up in total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean (mL)</td>
<td>N</td>
</tr>
<tr>
<td>Overall</td>
<td>11</td>
<td>0.66</td>
<td>6</td>
</tr>
<tr>
<td>Upper lip</td>
<td>9</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>Lower lip</td>
<td>5</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>Oral commissure</td>
<td>3</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>Perioral line</td>
<td>3</td>
<td>0.1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1 - Injection Volume by treatment area.

<table>
<thead>
<tr>
<th>Treatment areas</th>
<th>Initial Treatment (N=11) % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper lips</td>
<td></td>
</tr>
<tr>
<td>- Vermillion border</td>
<td>81,8% (9/11)</td>
</tr>
<tr>
<td>- Body of the lip</td>
<td>63,6% (7/11)</td>
</tr>
<tr>
<td>Lower lips</td>
<td></td>
</tr>
<tr>
<td>- Vermillion border</td>
<td>45,5% (5/11)</td>
</tr>
<tr>
<td>- Body of the lip</td>
<td>36,4% (4/11)</td>
</tr>
<tr>
<td>Oral commissures</td>
<td>27,3% (3/11)</td>
</tr>
<tr>
<td>Perioral lines</td>
<td>27,3% (3/11)</td>
</tr>
</tbody>
</table>

Table 2 - Treatment areas and techniques.
Lip augmentation with a new generation of liquid implant: Agarose-gel

An additional benefit is the use for patients who have been previously demonstrated intolerance, incompatibility to hyaluronic acid or other ingredients. The only adverse events described were hematoma, redness, bruising and swelling. All adverse events lasted for a maximum of 7 days. The sense of satisfaction by the patients were evaluated with the use of a subjective analog scale from 1 to 10. The mean score of satisfaction of cosmetic result was 7-10 immediately after treatment, and the score decreased after some months (Table 3). The results lasted 5 months with a gradual decline to baseline. The injected Agarose gel was very well tolerated with only a few mild adverse reactions which resolved spontaneously after a few days only. No major complications (e.g. infectious processes, palpable implants, nodularity, overcorrection, allergies) were observed.

<table>
<thead>
<tr>
<th>Immediately after treatment</th>
<th>7-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>7-9</td>
</tr>
<tr>
<td>2 months</td>
<td>6-8</td>
</tr>
<tr>
<td>4 months</td>
<td>3-5</td>
</tr>
<tr>
<td>6 months</td>
<td>1-2</td>
</tr>
</tbody>
</table>

Table 3 - Score of satisfaction of patients.

Conclusion

Agarose gel is a safe, low-risk, easily applicable therapy option for practitioner and provides a particularly good alternative method for augmentation area of the lips. The application of agarose shows through clinical studies and analysis a high safety. This innovative filler is characterized by local stability and good compatibility. Because of its biocompatible character, it does not matter what material was used previously in the injection area. As this remedy is 100% natural and very viscous substance, patients will achieve a high degree of satisfaction, as their facial expressions remain very natural and harmonious even when they move. Thus, a realistic satisfaction of the patient expectation can be achieved, with an excellent cosmetic effect. In addition, agarose has a very fast convalescent period and subsequent changes in shape do not come after the injection. In summary, treatment with agarose has many benefits. Thanks to its properties, this filler represents an important and successful option in the modern aesthetic medicine. Agarose Gel is available in four different strengths for very soft, moderate, mild and fairly deep wrinkles.
REFERENCE


Ultra-sound Evaluation of Dermis

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Abstract
The ultra-sound evaluation of dermis, along with other instrumental examinations, medical history and traditional clinical examinations aimed at identifying the patient's request, is part of the aesthetic medical checkup. The ultra-sound measurement of dermis is performed by means of a high-frequency probe (20-100 MHz) and helps evaluate age-related dermal change. The ultra-sound cutaneous examination is intended to provide a qualitative and quantitative evaluation of skin layers and surrounding structures. The available data suggest that ultra-sound evaluation is a non-invasive diagnostic technique that supports anti-aging treatment monitoring and can be considered as a valid option for the future evaluation of the efficacy of tailored anti-aging injections and topical therapies. This data seems to be confirmed by a clinical experience conducted with a medical device (Class III) to verify the effects on facial skin aging by means of ultra-sound evaluation. In this article, we discuss and analyze the benefits of the introduction of the qualitative and quantitative diagnostic evaluation of hypodermal ultra-sound imaging.

Keywords
Photoaging, cronoaging, SLEB, Ultra-sound evaluation, dermis

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Ultra-sound evaluation with high-frequency probe

The ultra-sound evaluation of dermis, along with other instrumental examinations, medical history and traditional clinical examinations aimed at identifying the patient's request, is included in the aesthetic and medical checkup.

This non-invasive diagnostic technique is considered as a powerful tool in the diagnosis and management of dermatological conditions in the clinical practice, since it provides clinical data that would not be available at naked eye examination\(^1\,2\).

With regard to this, it is also important to highlight that ultra-sound evaluation with high-frequency probe (20-100 MHz) can play a crucial role in the evaluation of age-related dermal change in the clinical practice. Moreover, the presence and degree of a typical Subepidermal Low-Echogenic Band (SLEB) is strictly related to photoaging: the lower is SLEB echogenicity, the greater is photoaging\(^3\).

The high-frequency probe helps evaluate the structural characteristics and dermal thickness. The aim of the cutaneous ultra-sound examination is to provide a qualitative and quantitative evaluation of skin layers and surrounding structures, being an additional and reliable instrument in the diagnostic phase as well as in the evaluation of the activity and severity of cutaneous diseases\(^4\).

Photoaging initially produces an increase in elastic fibers with consequent dermal hyperechogenicity, which then results in the loss of collagen and elastic fibers and a decrease in dermal thickness and echogenicity\(^5\). Several studies support the relationship between cutaneous thickness and skin aging. A study by Gniadecka M et al. 1998\(^6\) showed the thickness and echogenicity of dermal layers by means of ultra-sound technique. The author showed that changes in dermal layers differ according to which body region is evaluated. In fact, in photo-exposed body regions, the most superficial dermal layers are characterized by a progressive reduction in echogenicity, while in the body regions that are not exposed to sun rays the author observed an increase in echogenicity\(^7\).

The deepest layers of dermis have an increased echogenicity in all body regions. The author concluded that although photoaging and chrono-aging increase and/or reduce skin thickness depending on the body region studied, no strict correlation was generally observed between skin thickness and age.

In a later study, Gniadecka M 2001 analyzed skin aging-related dermal change observed by means of ultra-sound evaluation with high-frequency probe. The author showed that the presence of SLEB in photo-aging is strictly related firstly to the degeneration of elastic fibers in papillary dermis, secondly to the basophil degradation of collagen and thirdly to the accumulation of glycosaminoglycans (GAGs) and water in papillary dermis\(^8\).

Based on these considerations, SLEB could be an ultra-sound manifestation of elastosis and edema in papillary dermis. The correlation between age, SLEB thickness and echogenicity makes it possible to use these parameters to evaluate the level of cutaneous photoaging\(^9\,10\). Based on a number of available scientific data, ultra-sound evaluation is a non-invasive diagnostic technique that supports anti-aging treatment monitoring and can be considered as a valid option for the future evaluation of the efficacy of tailored anti-aging injections and topical therapies\(^11\,12\).

All the above is supported by a clinical study conducted in 28 patients eligible for biostimulation treatment with a medical device (Sunekos 200) in injectable intradermal sterile solution, containing a functional complex of 6 amino acids (glycine, L-proline, L-lysine, L-leucine, L-valine, L-alanine) in association with high-purity hyaluronic acid, of non-animal origin, at a concentration of 10 mg/ml.

It has been documented that the morphological structure of elastin is characterized by a prevalence of L-alanine and L-valine\(^13\,14\) that make this product particularly active on the turnover of the proteins of the ExtraCellular Matrix in case of facial skin laxity\(^15\).

According to the recommendations of the Italian Society of Mesotherapy (Società Italiana di Mesoterapia, SIM), the investigators administered one session for 1 month (4 infiltrations); one session every 15 days for 2 months (4 infiltrations) and one monthly maintenance session. Monitoring by ultra-sound technique was performed just before the first administration, before the fifth administration and one month after the last maintenance administration, in the zygomatic and mandibular region on the right and left hemilates (Figure 1).

In the study group (Figure 1) an improvement was observed in the structure, as well as an increase in the dermal thickness, a hypoechogenicity of dermis and an improvement in SLEB with matrix reorganization, and these factors were maintained until the end of treatment. The most important factor is matrix reorganization.

**Figure 1** - Ultra sound results of the right and left zygomatic regions.

In conclusion, all subjects showed a reduction in the echogenicity of the dermis, associated with an increase in the thickness after only 4 weekly sessions; one month after the last treatment, an increase in the thickness was observed, as well as a normalization of echogenicity related to patient's age, matrix reorganization and SLEB.
Conclusions
In summary, the ultra-sound evaluation of dermis, along with other instrumental examinations, medical history and traditional clinical examinations aimed at identifying the patient's request, is part of the aesthetic medical checkup.

This non-invasive diagnostic technique supports anti-aging treatment monitoring and can be considered as a valid option for the future evaluation of the efficacy of tailored anti-aging injections and topical therapies. It is also an additional reliable instrument in the diagnosis and management of dermatological conditions in daily clinical practice. Based on these considerations, in a recent clinical experience 28 patients were treated with a product containing hyaluronic acid and AA and showed a qualitative and quantitative improvement in most patients (Figure 1). After 4 weekly administrations, the ultra-sound evaluation showed a reduction in echogenicity that could be related to the deposit of hyaluronic acid and an increase in edema in the acute phase. At the end of treatment, the increase in thickness was maintained and echogenicity restored. The evaluation performed in this undoubtedly positive experience should be further studied in a systematic clinical study, since the results could be extremely relevant.

Figure 2 – Ultra-sound evaluation of the right and left zygomatic regions; T1=first administration, T2=after 4 weekly administrations, T3=one month after the last administration.
REFERENCES


Courses and Congresses

2019

21-23 February - Malaga (Spain)
34th National Congress SEME
Spanish Society of Aesthetic Medicine
Palacio de Ferias y Congresos
President: P. Vega
Email: seme2019@pacifico-meetings.com
Web: www.seme2019.org

26 - 27 April - Brussels (Belgium)
Congress SBME - BVEG 2019
Belgian Society of Aesthetic Medicine
Radisson Blu Royal Hotel
President: J. Hebrant
Web: www.radissonblu.com

17 - 19 May - Rome (Italy)
40th SIME Congress
Italian Society of Aesthetic Medicine
Rome Cavalieri Congress Center
President: E. Bartoletti
E-mail: congresso@lamedicinaestetica.it
Web: www.lamedicinaestetica.it

14 - 15 June - Basel (Switzerland)
16th Congress of the Swiss Society of Aesthetic Medicine
7th Congress of the Swiss Society of Aesthetic Surgery
Safran Zunft, Basel
President: S. Le Huu
Email: info@ssme.ch
Web: www.ssme.ch

15 - 16 June - Opatija (Croatia)
2nd Congress of the Croatian Society of Aesthetic Medicine (HUEM)
Hotel Milenij Opatija
President: E. Bunar
Web: huem.eu/congress 2019

13 - 14 September - Paris (France)
40th National Congress SFME
French Society of Aesthetic Medicine
Palais des Congres de Paris
President: J.J. Legrand
Email: congress@sfme.info
Web: www.sfme.info

26 - 29 September - Warsaw (Poland)
22nd World Congress of Aesthetic Medicine
Organized by: Polish Society of Aesthetic and Anti-Aging Medicine
Hilton Warsaw Hotel and Convention Center
President: A. Ignaciuk
Web: www.icaam.pl

2020

6 - 8 November - La Paz (Bolivia)
2nd Bolivian Congress of Aesthetic Medicine
Bolivian Association of Aesthetic Medicine (ASOBOME)
Hotel Atix La Paz
President: D. Hurtado Terrazas
Facebook page

8 - 10 November - Long Beach California (USA)
16th AAAM Congress
American Academy of Aesthetic Medicine - AAAM
President: M. Delune
Email: delegate@aaamed.org
Web: www.aamceed.org

24 November - Montevideo (Uruguay)
XVIII Congress of the Aesthetic Medicine Society of Uruguay
President: A. Elbaum
Email: medicinaesteticacongreso@gmail.com
Web: www.sume.com.uy

28 November - 1 December - Belek (Turkey)
3rd National Medical Aesthetic Congress
Turkish Association of Medical Aesthetic Medicine
Kaya Palazzo Golf Resort Hotel, Belek - Antalya
President: H. Subasi
Email: mesterder@opteamist.com
Web: mesterder2019.org

2 - 3 March - New Delhi (India)
International Congress of Indian Society of Aesthetic Medicine
President: A. Rana

15 - 17 October - Quito (Ecuador)
XIII Pan American Congress of Aesthetic Medicine - UIME
Organised by: Ecuatorian Society of Aesthetic Medicine
President: V. Tinoco Kirby
Email: medesteticaapanam2020@gmail.com
Web: www.seem.com.ec