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Contents

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LARGE INCREASES IN AMERICANS' CONSUMPTION OF sugar-sweetened beverages (SSB) have been a topic of concern. Between 1977 and 2002, the intake of "caloric" beverages doubled in the United States, with most recent data showing that children and adults in the United States consume about 172 and 175 kcal daily, respectively, from SSB,[1] it is estimated that SSB account for about 10% of total energy intake in adults[2,3]. High intake of SSB has....

References Section Example

References

1. Duffey KJ. Popkin BM. Shifts in patterns and consumptions of beverages between 1965 and 2002. *Obesity.* 2007:15(11):2739-2747.
2. Nielsen SJ. Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med.* 2004;27(3):205-210.
3. Drewnowski A. Bellisle F. Liquid calories, sugar, and body weight. *Am J Clin Nutr.* 2007;85(3):651-661.

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Original Article

Efficacy of Dual Low-Level Laser Combined with Bioactive Currents and Focused Functional Skin Massage in Cellulite Treatment

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Abstract

Background: cellulite is a major aesthetic concern for many women, usually affecting the thighs and buttocks. In recent years, several cellulite treatments have been developed, including massage and low-level laser therapy.
Aim: to assess the efficacy of a new body contouring platform combining dual low-level laser and bioactive currents with focused functional skin massage (FFSM) to improve cellulite and evaluate the patients' perception and satisfaction with treatment outcomes.
Methods: case series study of post-pubertal, non-menopausal women with grade 2 or 3 cellulite in the thighs, who underwent ten body contouring sessions with Tanit® device. Before and after treatment, we measured the right leg's fat percentage, thigh circumference and the thickness and stiffness of the subcutaneous panniculus adiposus (SPA). After the last session, patients took a survey that evaluated their perception and satisfaction with the treatment outcomes.
Results: the analysis included 23 women with a mean (SD) age of 39.1 (6.4) years. After ten body contouring sessions, fat content and thigh circumference decreased 1.0% and 2.1 cm, respectively. SPA thickness was reduced around 40%, and, in most patients, the grade of cellulite decreased. Additionally, over 80% of patients perceived an improvement in texture and firmness, and overall satisfaction with the treatment score was high (mean [SD] 9.3 [1.0] out of 10).
Conclusions: in this series of patients, ten body contouring sessions with dual low-level laser, bioactive currents, and FFSM were effective in reducing fat content, thigh circumference, SPA thickness, and cellulite grade, while improving cellulite appearance.

Keywords

Cellulite, body contouring, low-level laser therapy, focused functional skin massage

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Introduction

Cellulite, also known as edematous fibrosclerotic panniculopathy, is a localized metabolic condition of the subcutaneous tissue that alters the skin topography into a surface resembling the one of an orange peel[1]. This disorder usually affects the thighs and buttocks, and sometimes the lower legs and abdomen[2]. Although there is no specific data on its incidence and prevalence, most studies state that cellulite may affect 80%-90% of post-pubertal women[3].

The pathophysiology of cellulite formation is not yet fully understood, but current evidence suggests the involvement of several factors, such as the number and type of fibrous septae, microvascular dysfunction, subcutaneous inflammation and fibrosis, and dermal thickness[2]. In addition, other predisposing factors for cellulite include female gender, age (post-pubertal period), genetic predisposition, caucasian race, increased subcutaneous fat layer, a high-carbohydrate diet, a sedentary lifestyle, and pregnancy[4].

Despite it not being considered a pathological condition, cellulite is a major aesthetic concern for many women[2]. For this reason, in recent years, several techniques and agents have been developed to improve cellulite appearance and skin texture, such as topical and injectable pharmacological agents, as well as mechanical, radiofrequency, laser, or ultrasound therapies[4]. One of the oldest techniques to treat cellulite is mechanical therapy with massages (either manual or device-assisted), which is used to enhance lymphatic drainage and improve subcutaneous tissue microcirculation[5]. Tunay et al. compared the effectiveness of different massage techniques (mechanical massage, manual lymphatic drainage, and connective tissue manipulation) and found that all methods effectively reduced thigh circumference and fat thickness[6]. Low-level laser therapy (LLLT) is another non-invasive technique used in cellulite treatment[7], and a randomized controlled trial showed that it was safe and effective for improving the appearance of the thighs and buttocks[8].

In the last decade, several studies analyzed the efficacy of LLLT combined with massages for the treatment of cellulite and reported good results[9-13]. However, new devices have been developed since their publication. Therefore, the goals of this study were: 1) to assess the effectiveness of a new body contouring platform that combines dual low-level laser and bioactive currents with focused functional skin massage (FFSM) to improve cellulite after ten sessions, and 2) to evaluate the patient's perception and satisfaction with treatment outcomes.

Materials and Methods

Study Design and Patients

This was a case series study of patients who underwent ten sessions of body contouring of the legs with a dual low-level laser combined with bioactive currents and FFSM between January 2021 and February 2021. Non-menopausal women, between 25 and 50 years of age, with grade 2 or 3 cellulite in the thighs, according to the Nürnberger and Müller classification[14], and muscle hypotonia in the inner thighs were included in the study. The exclusion criteria included other cellulite treatments, hormonal disorders, lymphatic or severe vascular diseases, pre-menopause or incipient menopause, and an impaired hormonal profile.

Patients were encouraged to limit or remove their consumption of carbohydrates, fats, and alcohol from 4:00 pm at least one month before the beginning and one month after the end of the study. They were also asked to exercise at low intensity (i.e., fast walking) a minimum of three times a week, on alternate days, during the whole treatment, and two of these exercises had to be performed within the first 24 hours after receiving the treatment.

All patients signed an informed consent form to participate in the study, which followed the ethical standards of the 1964 Helsinki Declaration and its later amendments.

Treatment Device

The treatment device used in this study was a body contouring platform consisting of six pads with a damped, low-frequency current (150-300 Hz) (i.e., bioactive currents) system and 13 laser units (ten with a wavelength of 639 nm and a potency of 25 MW, and three with a wavelength of 830 nm and a potency of 50 MW) each. The device also had a 45-sphere head massage, which allowed a rotational mechanical massage with a maximum speed of 400 rpm.

Procedures

Each session began with 12 minutes of dual low-level laser stimulation combined with bioactive currents in the lateral and posterior thighs (phase 1), followed by a 34-minute massage of both legs with the 45-sphere head at 50-100 rpm (phase 2). The latter consisted of a 22-minute massage with knuckling and rolling maneuvers applied on the back and sides of the legs (10 minutes), the anterior and medial thighs (5 minutes), and the buttocks (7 minutes), and a 12-minute massage with knuckling, rolling, and contouring maneuvers applied on the lumbar area (7 minutes) and the abdomen (5 minutes). The sessions were performed twice a week for five weeks.

Data Collection and Measurements

The patients' age was registered at enrollment. Before the first session and 72 hours after the last session, their body weight, body mass index (BMI), and fat percentage of the right leg were determined through a bioimpedance analysis. Thigh circumference was assessed using a measuring tape, always at the same point for each patient (at length, from the floor to the more prominent spot of the right thigh, measured before starting treatment with the patient barefooted, leaning against the wall with both feet together). Moreover, in the posterior right thigh, the thickness of the subcutaneous panniculus adiposus (SPA) and its two layers (areolar and lamellar) was assessed using a 6-to-18 MHz linear-probe ultrasound, and the stiffness of SPA and its layers was also assessed using the same ultrasound device with a sonoelastography software that allowed to classify SPA into four categories (range: 1 [soft tissue, or absence of cellulite] to 4 [stiff tissue,

or grade 3 cellulite, according to the Nürnberger and Müller classification[14]).
After the last session, patients took a survey that asked whether they had perceived an improvement in terms of texture, fluid drainage, volume, and firmness; the number of sessions before they noticed these results; and overall satisfaction with treatment outcomes (range: 0 [not at all satisfied] to 10 [completely satisfied]). The survey also asked whether they had experienced adverse events, such as bruises, pain, and delayed onset muscle soreness (DOMS) (range: 0 [absent] to 10 [severe]), as well as compliance with dietary and physical activity recommendations (yes/no).

Statistical Analysis

Categorical variables were presented as frequency and percentage of patients in each category, and quantitative variables as the mean and standard deviation (SD). A paired Student's t-test was used to assess any statistically significant differences between pre- and post-treatment values of body weight, BMI, fat percentage, thigh circumference, and SPA thickness. Another paired Student's t-test was also performed in the subpopulations of patients who had followed the exercise and diet recommendations. The threshold for statistical significance was set at a two-sided alpha value of 0.05. All analyses were performed with Excel (Microsoft) or the GraphPad QuickCalcs website (https://www.graphpad.com/quickcalcs/).

Results

Patients' Characteristics

A total of 24 women started the treatment. However, one could not complete all ten sessions and was excluded from the study. Therefore, the analysis included 23 patients with a mean (SD) age of 39.1 (6.4) years, ranging from 26 to 49 years. Mean (SD) of body weight and BMI was 62.6 (8.1) kg and 23.0 (3.5) kg/m2, respectively.

Changes Observed Before and After Treatment

The main changes observed before and after treatment are summarized in *Table 1*.

After all ten body contouring sessions, the amount of body weight was slightly reduced, although the difference did not reach a statistical significance.
The fat content of the right leg and the thigh circumference decreased 1.0% and 2.1 cm, respectively. In addition, the thickness of SPA and its two layers was reduced by approximately 35%-40%.
According to the sonoelastography results, at baseline, most patients had grade 3 cellulite in both the areolar and lamellar layers of the assessed SPA. However, after ten sessions, almost all patients demonstrated grade 1 or 2 cellulite (*Figure 1*).
Regarding patient-perceived improvements after all ten sessions of body contouring, 14 (60.9%) and 12 (52.2%) patients considered that the volume and fluid drainage of their thighs was ameliorated, respectively, whereas most patients noticed an improvement in texture (n=20, 87.0%) and firmness (n=19, 82.6%). In addition, 16 (69.6%) patients observed results within five sessions or less. Overall satisfaction scores were: 10 (n=13, 56.5%), 9 (n=4, 17.4%), 8 (n=5, 21.7%), and 7 (n=1, 4.4%), resulting in a mean (SD) score of 9.3 (1.0) out of 10.
Survey results also showed that 11 (47.8%) and 14 (60.9%) patients had followed the dietary and physical activity recommendations stated at the beginning of the study, respectively. When we analyzed the general and right-leg measurements of the subpopulations of patients who had followed the diet and/or exercise recommendations, we obtained similar results to those found in all the patients (*Tables 2* and *3*; *Figures 2* and *3*).

Adverse Events

Regarding adverse events, 12 (52.2%) patients reported bruises, 16 (69.6%) reported DOMS, and 19 (82.6%) reported pain. In most cases (n=9 [75.0%], n=14 [87.5%], and n=17 [89.5%] for bruises, DOMS, and pain, respectively), these events were mild to moderate (scores ≤5).

	Before treatment	After treatment	*P*
General measurements			
Body weight (kg)	62.6 (8.1)	62.0 (7.7)	0.0804
BMI (kg/m2)	23.0 (3.5)	22.7 (3.5)	0.3363
Right-leg measurements			
Fat content (%)	35.1 (5.0)	34.1 (4.7)	**0.0024**
Thigh circumference (cm)	59.5 (4.9)	57.4 (4.6)	**<0.0001**
Thigh SPA (mm)	15.5 (4.4)	9.2 (2.6)	**<0.0001**
Areolar SPA (mm)	5.7 (2.0)	3.5 (1.5)	**<0.0001**
Lamellar SPA (mm)	9.8 (3.4)	5.6 (1.7)	**<0.0001**

SPA: subcutaneous panniculus adiposus.

Table 1 *- Measurements before and after ten sessions of treatment, mean (SD), n=23.*

Figure 1 A

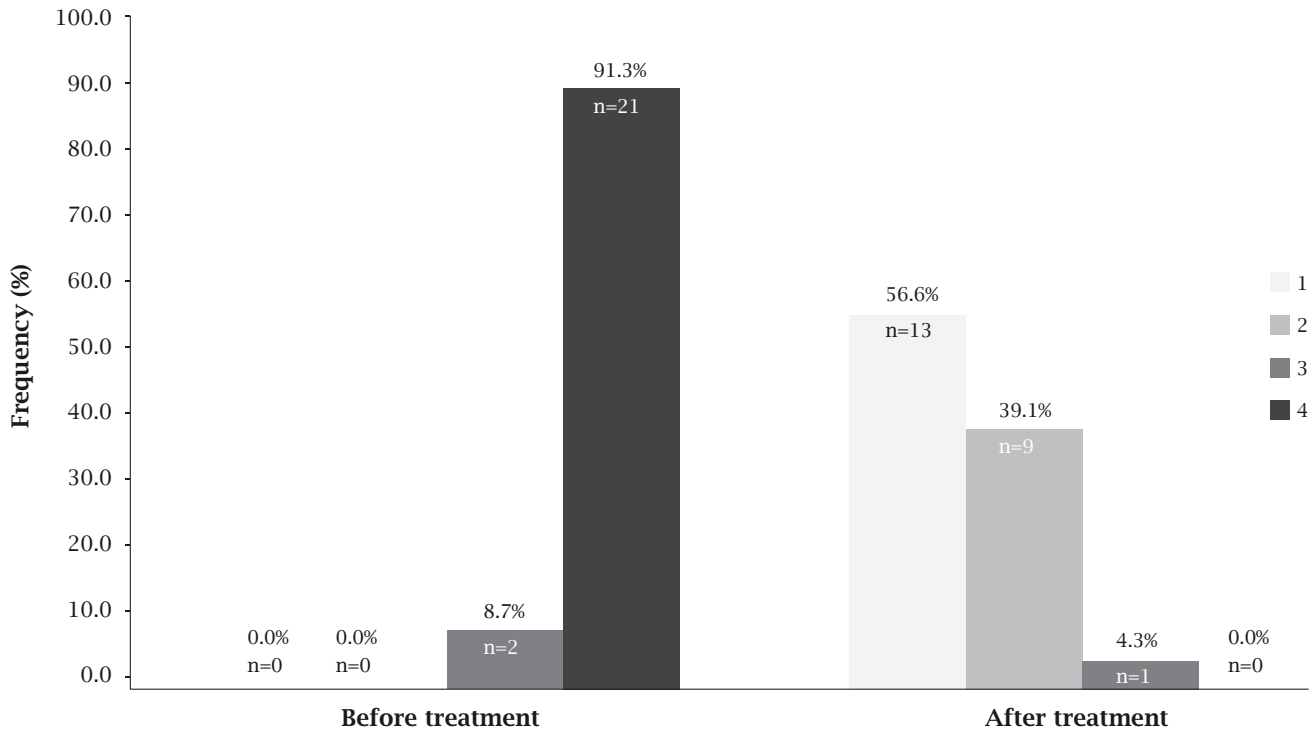


Figure 1 B

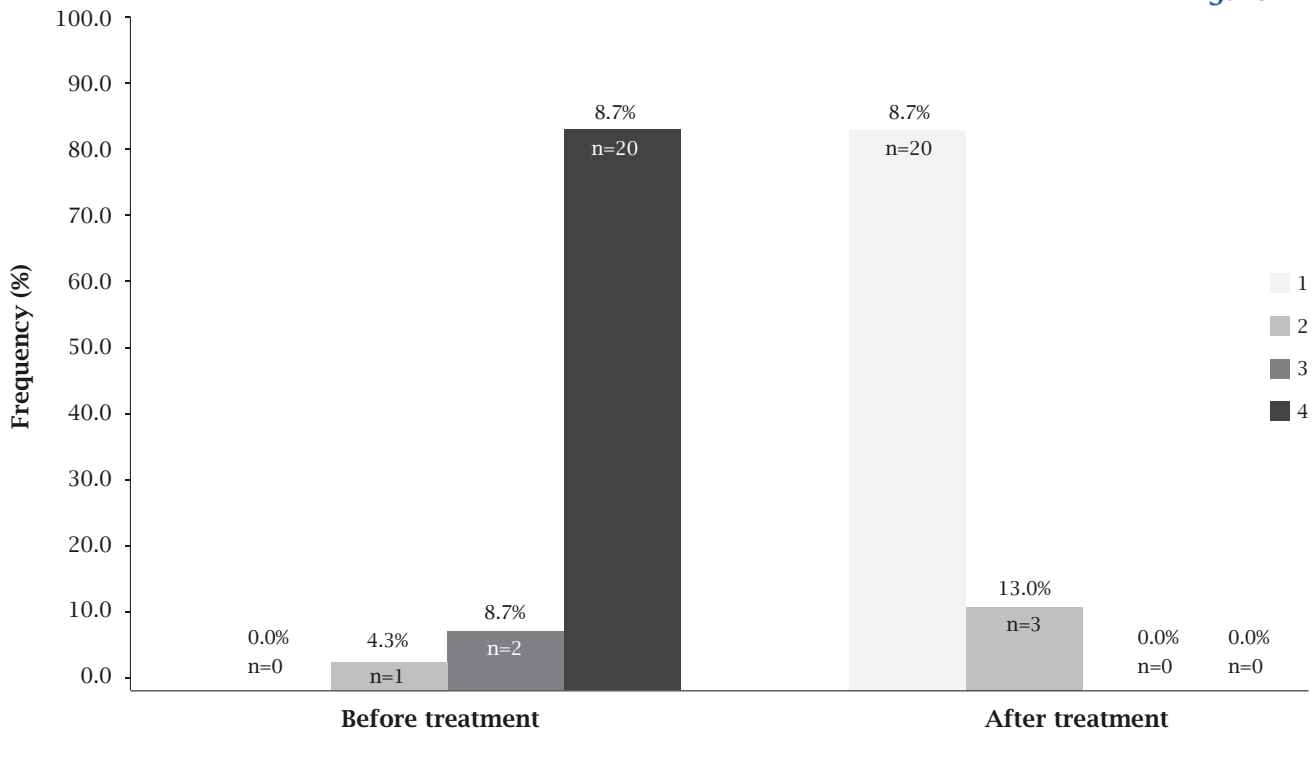


***Figure 1** - Stiffness degree of edematous fibrosclerotic panniculopathy in the alveolar (A) and lamellar (B) layers. 1: Absence of cellulite. 2: Grade 1 cellulite. 3: Grade 2 cellulite. 4: Grade 3 cellulite.*

	Before treatment	After treatment	*P*
General measurements			
Body weight (kg)	64.1 (9.3)	63.4 (8.9)	0.3274
BMI (kg/m2)	24.1 (4.0)	23.8 (3.9)	0.7414
Right-leg measurements			
Fat content (%)	36.3 (5.6)	35.0 (5.3)	**0.0175**
Thigh circumference (cm)	61.0 (5.2)	58.7 (4.9)	**<0.0001**
Thigh SPA (mm)	16.7 (5.1)	9.4 (3.5)	**<0.0001**
Areolar SPA (mm)	5.9 (2.4)	3.9 (1.9)	**0.0013**
Lamellar SPA (mm)	10.7 (3.9)	5.4 (2.2)	**<0.0001**

SPA: subcutaneous panniculus adiposus.

Table 2 *- Measurements before and after ten sessions of treatment in patients who followed diet recommendations, mean (SD), n=11.*

	Before treatment	After treatment	*P*
General measurements			
Body weight (kg)	65.3 (9.1)	64.4 (8.9)	0.1140
BMI (kg/m2)	24.5 (3.7)	24.2 (3.7)	0.5491
Right-leg measurements			
Fat content (%)	36.9 (5.1)	35.9 (4.4)	**0.0286**
Thigh circumference (cm)	61.6 (4.9)	59.4 (4.8)	**<0.0001**
Thigh SPA (mm)	16.2 (4.4)	9.9 (2.7)	**<0.0001**
Areolar SPA (mm)	6.3 (2.1)	4.0 (1.6)	**<0.0001**
Lamellar SPA (mm)	9.9 (3.7)	5.8 (1.8)	**<0.0001**

SPA: subcutaneous panniculus adiposus.

Table 3 *- Measurements before and after ten sessions of treatment in patients who followed exercise recommendations, mean (SD), n=14.*

Figure 2 A

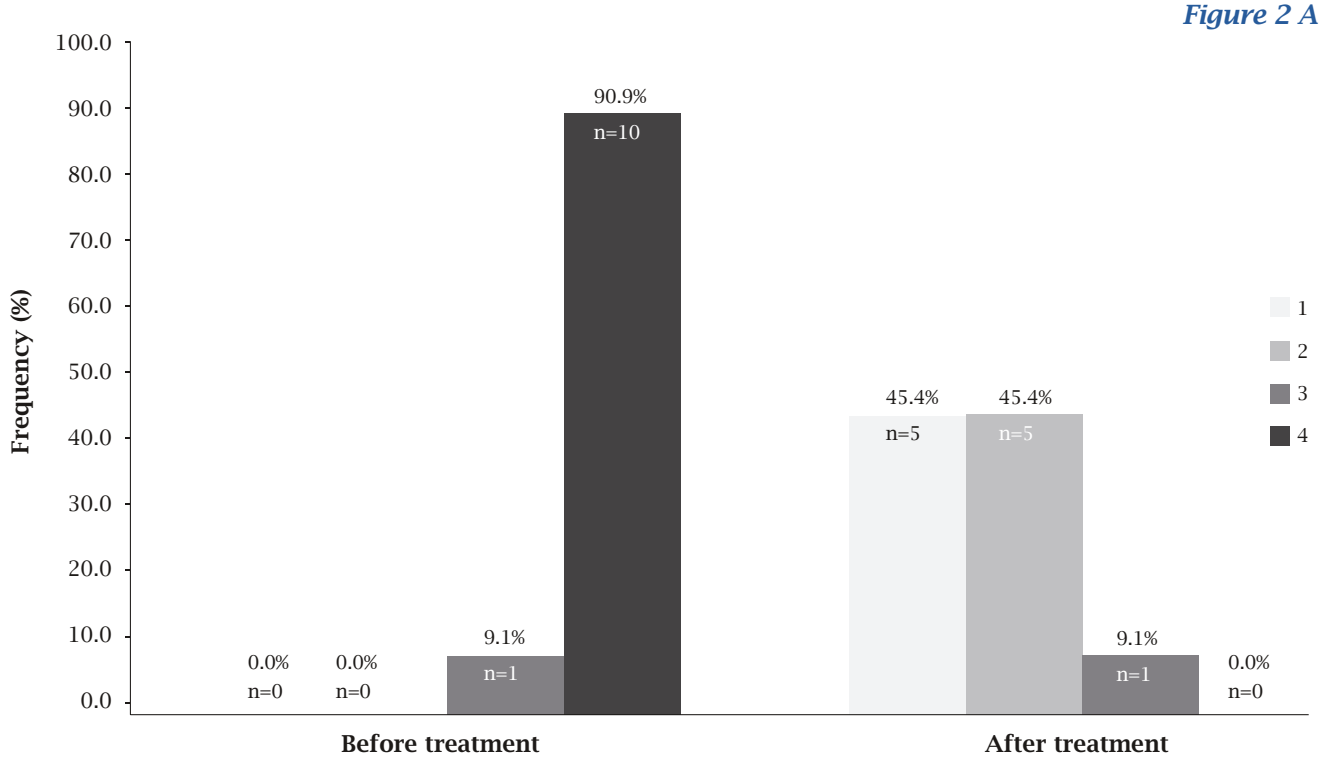


Figure 2 B

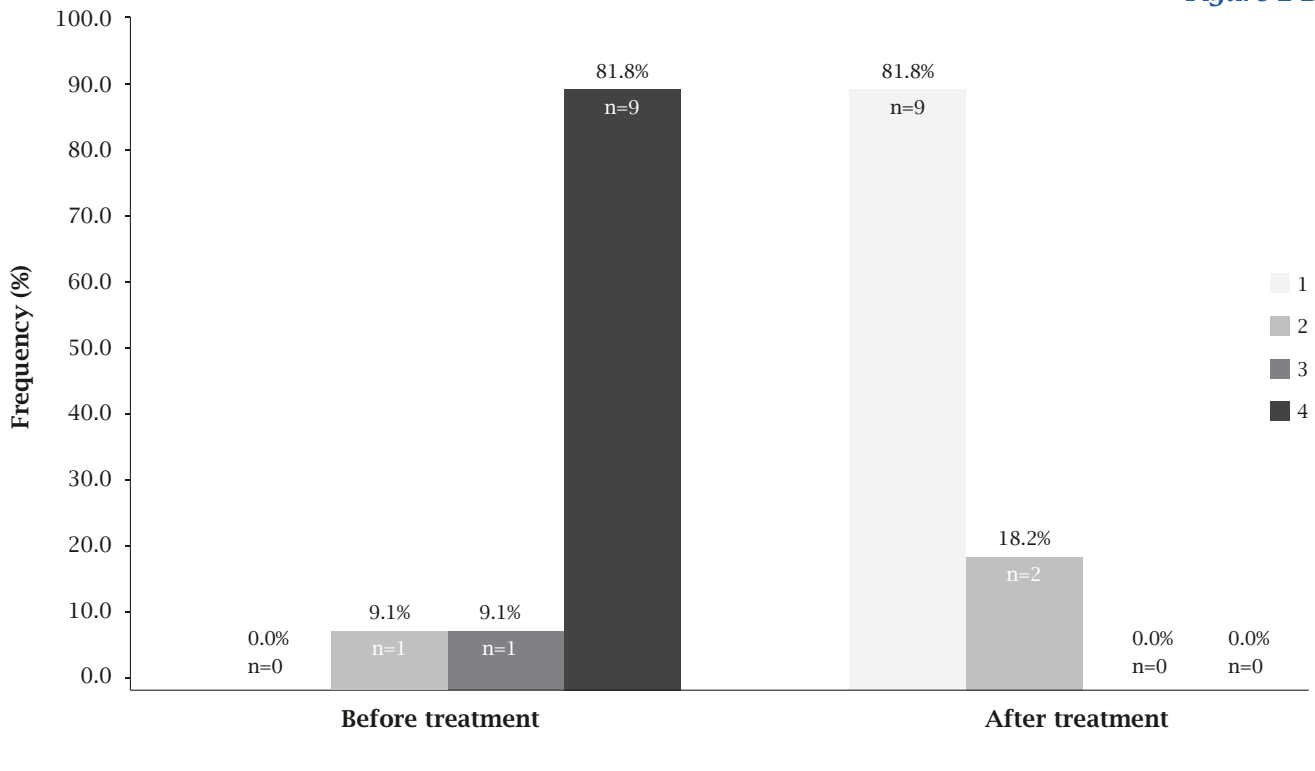
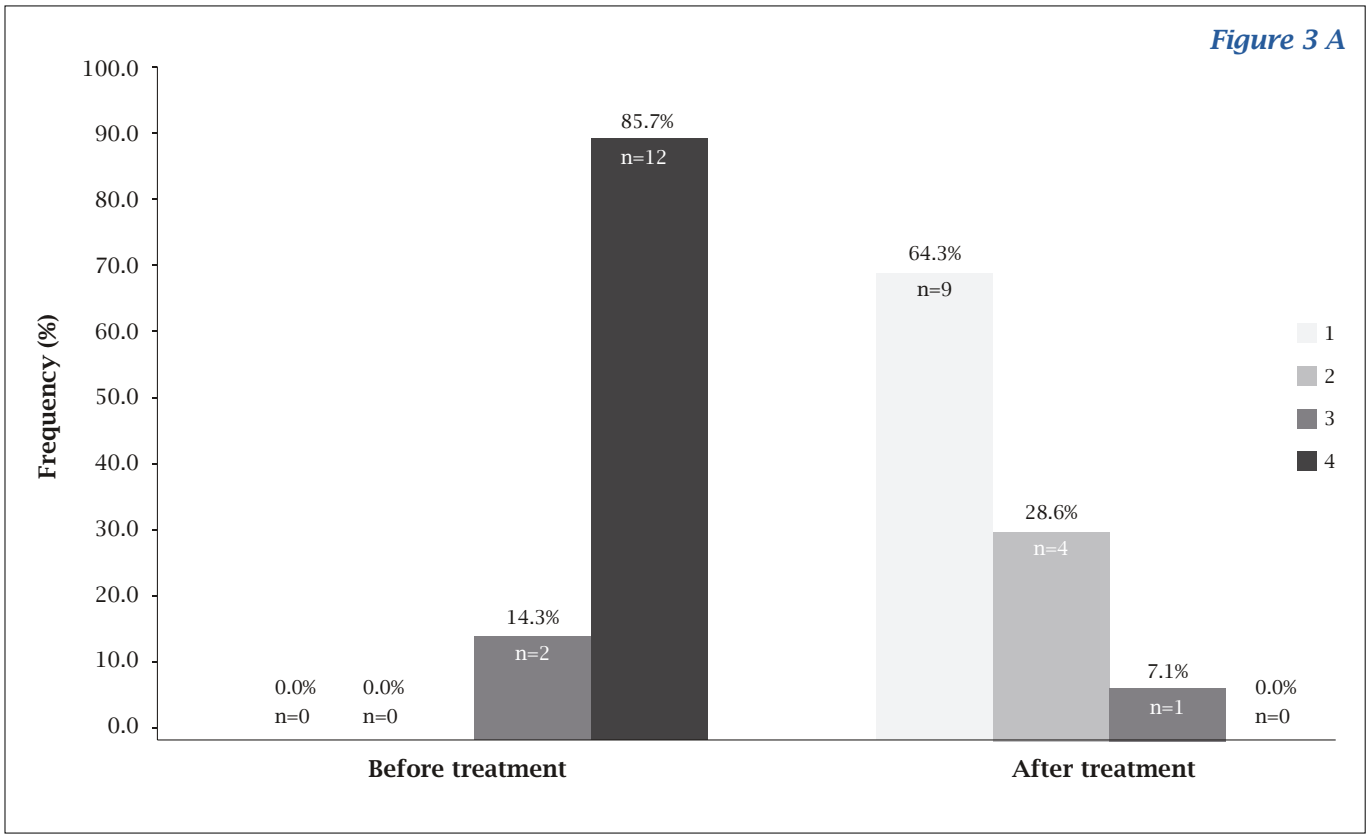


***Figure 2** - Stiffness degree of edematous fibrosclerotic panniculopathy in the alveolar (A) and lamellar (B) layers in patients who followed diet recommendations. 1: Absence of cellulite. 2: Grade 1 cellulite. 3: Grade 2 cellulite. 4: Grade 3 cellulite.*



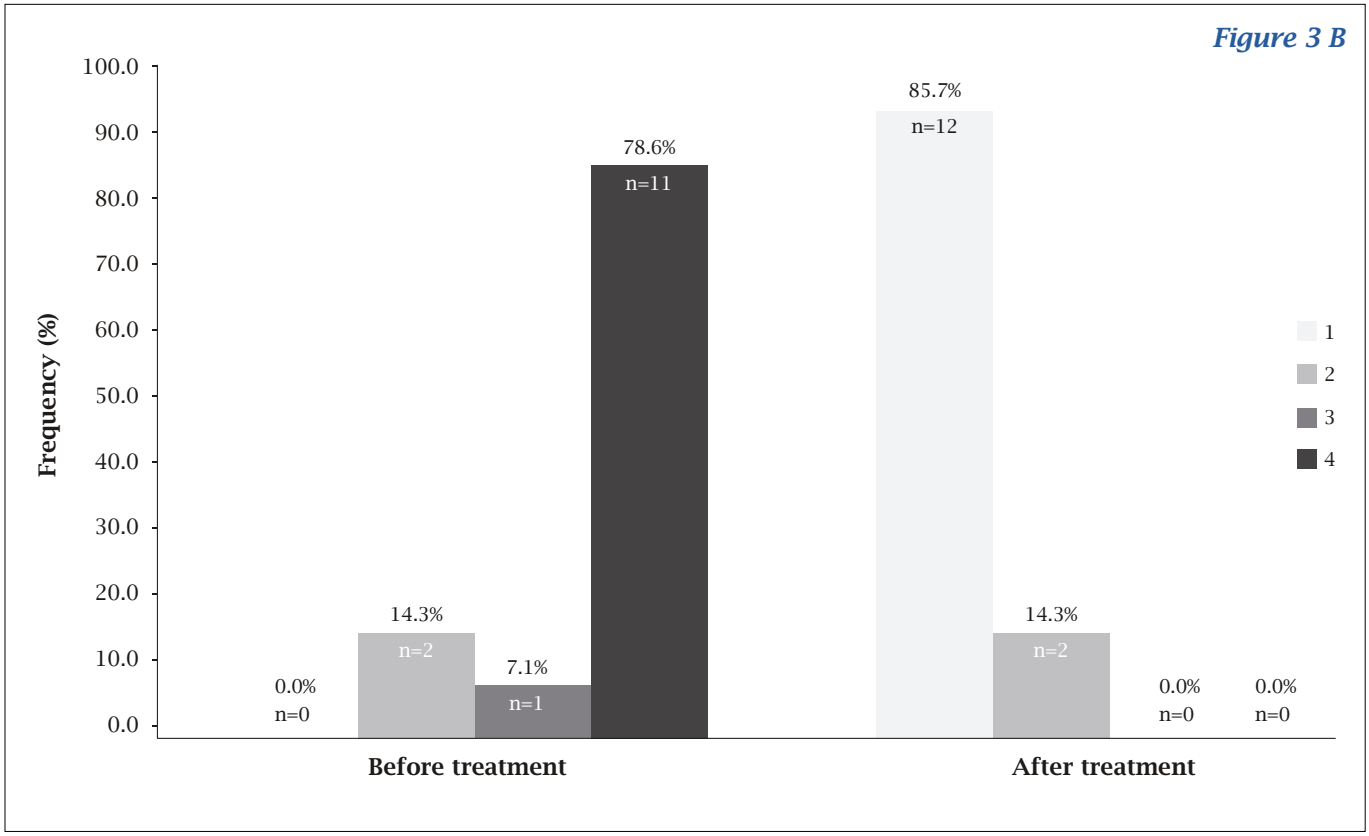


***Figure 3** - Stiffness degree of edematous fibrosclerotic panniculopathy in the alveolar (A) and lamellar (B) layers in patients who followed exercise recommendations. 1: Absence of cellulite. 2: Grade 1 cellulite. 3: Grade 2 cellulite. 4: Grade 3 cellulite.*

Discussion

In this study, we found that after ten body contouring sessions with a dual low-level laser combined with bioactive currents and FFSM, the leg fat content and thigh circumference decreased an average of 1.0% and 2.1 cm, respectively. Moreover, SPA thickness was reduced by 40%, and almost all patients experienced a decrease in SPA stiffness and, consequently, in cellulite grade. Regarding the patients' experience, more than half perceived an improvement in the amount of thigh volume and fluid drainage, and over 80% noticed an improvement in texture and firmness. The patients' overall satisfaction with the treatment outcomes was high, with a mean (SD) score of 9.3 (1.0) out of 10, and all reported adverse events were mild or moderate.

The decrease in thigh circumference observed after treatment is consistent with other studies that analyzed the effect of massages[6], LLLT[8], or both techniques done over time[12,13], although the magnitude of the reduction was lower than that found in our study. Some of these analyses also evaluated the effect on fat layer thickness, with similar results to those we found: Tunay et al. described a reduction in subcutaneous fat thickness after different massage techniques[6], whereas Lach only observed this effect with massages combined with LLLT, but not with massages alone[10]. Furthermore, other authors also described a reduction in cellulite grades, either with LLLT alone[8] or combined with massages[13].

We observed a decrease in the patients' body weight after completing all sessions. Although it did not reach any level of statistical significance, it can be seen as a possible explanation for some of our results. However, all patients experienced a decrease in thigh circumference and SPA thickness, even those who gained weight. This suggests that other factors excluding weight loss are also involved. In this regard, Neira et al. described that LLLT produces temporary microscopic pores in adipocyte membranes, allowing the release of lipids into the interstitial space[15], which was further supported by the findings of Caruso-Davis et al.[16]. Additionally, according to Lach, the massage may help move fat to the lymphatic system[10]. Together, these two mechanisms may explain improvements observed in SPA thickness, thigh circumference, and even fat content found in our study. In this regard, the 40% reduction in SPA thickness of the thigh could be responsible for the small but statistically significant decrease in fat percentage of the whole leg. Moreover, concerning the possible influence of diet and exercise recommendations on our results, we found that those patients who had followed them obtained similar results to the whole study population. This observation suggests that diet and exercise may not be necessary to benefit from the treatment.

The patients' survey showed that they were very satisfied with treatment outcomes—especially with skin texture and firmness improvement—while only experiencing some mild or moderate adverse events. Safety results are consistent with those reported in other studies using LLLT and/or massages[6,8,10,12,13].

A possible limitation of our study is the absence of data on a more extensive follow-up period, which could be helpful to assess the duration of outcomes over time. Also, the number of patients was not very large, but is sufficient according to Seidenari et al.[17]; moreover, it allowed us to find statistical differences between before and after ten body contouring sessions. The strength of our study is that improvements in cellulite were not only assessed using visual- and tactile-based classifications, which are subjective and poorly reproducible, but they were also objectively measured through thigh circumference and SPA thickness and stiffness, which are considered suitable methods to evaluate the efficacy of cellulite treatments[17].

In summary, the results of our study indicate that ten body contouring sessions with a dual low-level laser combined with bioactive currents and FFSM are effective and safe in reducing fat content, thigh circumference, SPA thickness, and cellulite grade, while improving cellulite appearance. Further studies are required to see the persistence of these effects over time.

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Conflicts of Interest and Source of Funding

The authors declare that they have no conflict of interest. Novasonix provided financial support to perform specific measurements on patients.

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aesthetic medicine

Original Article

The role of regenerative medicine in androgenetic alopecia treatment: an uncontrolled clinical trial

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Running title: Regenerative medicine in alopecia

Abstract

Background: androgenetic alopecia, a disease that affects 70% of men and 40% of women, is characterized by the gradual miniaturization of the hair follicle, resulting from the alteration of the dynamics of the hair cycle.
Aim: one of the most recent and promising advanced medical therapies is regenerative medicine. The important action of Adipose Derived Stem Cells (ADSCs) on the hair follicle is that of inducing anagen by stimulating the stem cells of the hair follicle. The Fibroblastic growth factor expressed by ADSCs also plays an important role in regulating the bulge and the basal layer of the epidermis, inducing follicular neogenesis.
Methods: this study is aimed at evaluating the effectiveness of the technique used for the treatment of androgenetic alopecia. The protocol involved the association of carboxytherapy and regenerative therapy through the grafting of a vascular stromal fraction and mesenchymal stem cells derived from the adipose tissue.
Results: 15 patients were enrolled (9 men and 6 women). From the scores relating to the satisfaction and satisfaction questionnaire completed by the patients, it is also clear how this type of therapeutic protocol is practically painless, has significantly improved the patient's self-confidence and relationship with others, hair density and how much it has reduced hair loss.
Conclusions: the analysis of the results of the study shows how this therapeutic protocol combining regenerative therapy and carboxytherapy is a valid, promising technique and is part of a multidisciplinary approach more suited to the therapy of a pathology such as androgenetic alopecia.

Keywords

Androgenetic alopecia, regenerative medicine, hair loss, growth factors, SEFFIHair

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Introduction

Androgenetic Alopecia (AGA) is characterized by the gradual miniaturization of the hair follicle, resulting from the alteration of the dynamics of the hair cycle, which leads to the transformation of the terminal hair follicle into fleece[1]. Understanding hair follicle biology over the past 20 years has established the pivotal role of mesenchyme-derived dermal papillae in maintaining hair growth, with multipotent epithelial stem cells in the bulge area resulting in proliferation and differentiation. Other autocrine and paracrine factors and signaling pathways are involved in this cross-talk between the dermal papillae and hair follicle stem cells[2-4]. Many of the studies have accepted the role of androgens and the interaction between the dermal papillae and the hair follicle as critical processes involved in the miniaturization of hair follicles. The higher the concentration of androgens and androgen receptors, the greater the effect on the expression of genes that control the follicular cycle. The transmission of the signal between the dermal papillae and the hair follicle in the bald person causes the premature cessation of the anagen (hair growth) associated with the premature entry into the catagen (follicular regression phase). Catagen occurs as a consequence of the reduced expression of anagen maintenance factors (such as growth factors IGF-1, bFGF and VEGF). Furthermore, an increased expression of cytokines (TGF-beta1, IL-1alpha and TNF alpha) promotes apoptosis[5]. Recently it has been discovered that DKK-1 is a gene upregulated by DHT with the consequent inhibition of the cells of the outer root sheath and triggering apoptosis[6]. The complex of causes that therefore make Androgenetic Alopecia a multifactorial pathology include: genetic predisposition, food related problems, endocrinopathies, use of certain drugs, psychological causes, inflammation of the hair follicles, environmental factors and some microbial toxins[7-8].

Therapy

Over the years, various therapeutic strategies have been developed for androgenetic alopecia; from pharmacological treatments (Minoxidil, 5 alpha reductase inhibitors, hormone therapy), to surgery, botulinum toxin, and carboxytherapy. In recent years it has become necessary to resort to experimentation with some alternative therapeutic strategies.

The use of genes, cells and tissues as new therapeutic resources take part in characterizing contemporary medicine. Advances in regenerative medicine have increased interest in the application of stem cells for the reconstruction of damaged tissues and to develop regenerative therapies for the skin[9,10]. Therapies based on human mesenchymal stem cells (HMSC) have been used in regenerative medicine in various medical areas such as orthopedics, neurology, cardiology and dermatology. HMSCs come from the mesoderm and share their origins with the skin. When these cells are implanted in a damaged tissue, they are able to repair and regenerate the anomalies[11-13]. These cells showed important immunomodulatory activity and are capable of secreting various cytokines and growth factors such as IL-6, IL.8, Vascular Endothelial Growth Factor (VEGF), Basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF) which promotes tissue regeneration[14-17]. Although the first tissue used to obtain HMSC was the bone marrow, studies today have shown that the subcutaneous adipose tissue is an excellent source of HMSC both from a qualitative and quantitative point of view[18-23]. Some preclinical studies have shown promising results using HMSCs in the treatment of AGA. Byun et al. He developed a pilot study to demonstrate the immunomodulatory effect of mesenchymal stem cells in androgenetic alopecia.

In recent years, attention has been paid to the importance of the dermal macro-environment surrounding the hair follicle. Some studies show how follicles function within a large exchange network and not in a closed system; there are many cellular signals of follicle-follicle and macroenvironment-follicle exchange.

The macroenvironment includes cells such as dermal fibroblasts, skin adipocytes, preadipocytes and other extracellular components such as blood vessels and intradermal nerve plexus and immune cells. Therefore, in the light of the previous considerations relating to the pathophysiology of AGA, a therapy with ADSCs is also capable of inducing a transformation of the dermal macroenvironment by amplifying those communication signals between the hair follicle and cells of the macro-environment. When we take adipose tissue, in addition to adipocytes we take Stromal Vascular Fraction (SVF), that part rich in stem cells (ADSCs) which includes fibroblasts, pericytes, pre-adipocytes, hematopoietic cells, immunity cells (granulocytes, endothelial cells, monocytes, lymphocytes) and growth factors. As with all therapies it is very important to know the dose that we administer to patients, so it is important to know that in the 1ml of adipose tissue we take we have, on average, from 100,000 to 1,000,000 mononuclear cells of the SVF and of these, from 1, 10% are real stem cells. However, for the purpose of our therapy, not only the action of stem cells is important but also that of all the cells of the SVF and the growth factors present.

The rationale used in the development of the SEFFI system (Superficial Enhanced Fluid Fat Injection)[24,25], used to conduct this study, was to take the cellular micro clusters already of the appropriate size, through the use of a specific cannula, to have a fluid tissue ready to be injected with minimal manipulation that does not interfere with cell viability. The fluidity of the tissue is essential to allow grafting into the superficial dermal and subdermal layers, to improve cell engraftment and expand the possibility of using the method.

As for carboxytherapy, it induces a state of relative hypercapnia and decreases the local pH, which causes a strong vasodilator response, ultimately increasing blood flow to the injection site. Since the pathogenesis of hair loss in Androgenetic Alopecia is caused by a combination of factors including a reduced vascularity of the scalp, it seemed interesting to evaluate its efficacy and safety in the treatment of this pathology combined with regenerative medicine[26].

Materials and methods

This observational study aimed at evaluating the efficacy of the technique used for the treatment of androgenetic alopecia.
Before the treatment, a trichoscopy was obtained using the Dino-Lite Polarizer trichoscope. The parameters that were analyzed at the trichoscopy were mainly two: the perifollicular pigmentation and the presence of a yellow shift as these are two typical characteristics of androgenetic alopecia indicative of a lack of adequate nutritional and oxygen supply and consequent follicular asphyxia. In order to standardize the image acquisition technique and to make the evaluations reproducible in the different phases of the study, the "Configuration 10-20" protocol established by the International Federation in Electroencephalography and Clinical Neurophysiology, normally used in the positioning of the electrodes to complete an EEG (*Figure 1*).

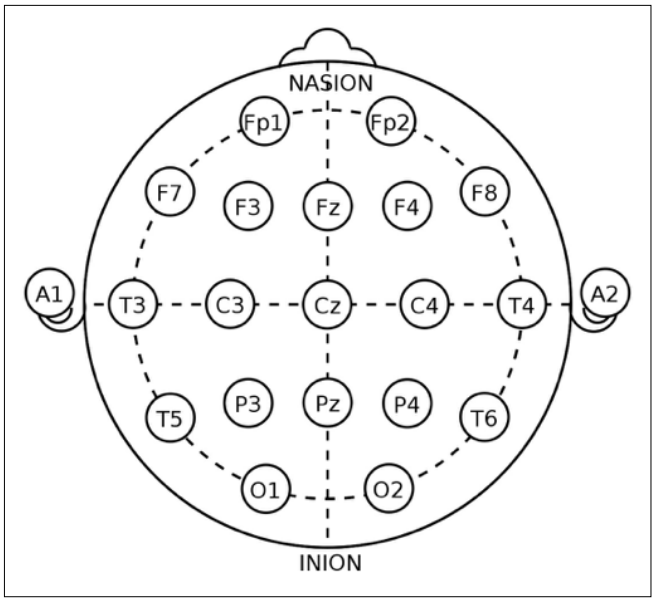


***Figure 1** - Protocol "Configuration 10-20".*

The choice of the image acquisition site was customized according to the distribution of the patient's alopecic areas. Patients enrolled in the study were classified clinically, to assess the degree of alopecia, according to the Hamilton-Northwood scale and the Ludwig scale, respectively for men and for women[27]. Photos were also taken of the overall view of the scalp before carrying out the therapy and 3-4 months after the treatment. The protocol used in this study involved the association of Carboxytherapy and Regenerative Therapy through the grafting of a Vascular Stromal Fraction (SVF) and Adipose derived Mesenchymal Stem Cells (ADSCs). For the regenerative therapy a guided medical device was used in order to standardize the level of tissue sampling, preparation and grafting of the tissue (SEFFIHAIR produced by SEFFILINE srl Bologna Italy). The device has the CE and ISO 13485 mark and is undergoing FDA authorization. In order to prepare the scalp for the grafting of SVF and ADSCs, a treatment with carboxytherapy was carried out using the Carbo2 HP DTA device using the following protocol: Needle 30G / 4mm, Flow 15-20 cc / 15sec, Temperature 40°, total volume 10 -20 cc for single area circumferentially to the skull and inside with treatment in the occipital area of the origin of the vascular axes[26]. After the carboxytherapy treatment, the removal of adipose tissue was performed. Numerous studies have shown that the subcutaneous adipose tissue is composed of adipose cells and the stromal tissue (SVF) containing mesenchymal cells and in particular stem cells deriving from the adipose tissue (ADSCs). This procedure involves grafting the tissue taken and prepared with SEFFIHAIRR in the same patient (autologous graft) in order to obtain the stimulation for hair growth, an improvement in microcirculation (angiogenic action) and an anti-inflammatory and antifibrotic action. The system can be used in different anatomical areas in the same patient and in the same procedure. We considered absolute contraindications to this treatment: Infections in progress in the area of sampling or grafting of the tissue; Presence of malignant neoplasms in the area of sampling and grafting of the tissue; Pregnancy or breastfeeding; Anticoagulant therapy or severe bleeding disorder; Allergy to local anesthetic; Dysmorphophobia; Immunosuppressive therapies in place; Debilitated subjects. No side effects or complications were noted during the study. We believe that the results that can be obtained with autologous regenerative therapy may vary depending on the patient's age, state of health, lifestyle, surgical site and experience of the doctor who performs it. The amount of tissue removed must be limited to what is actually needed. The operating field must be totally sterile. The device is disposable and sterile, not resterilizable. The choice of the tissue sampling area must be made on the basis of the following criteria: An Adequate presence of subcutaneous adipose tissues (pinch test equal to or greater than 4 cm); The Size of the area which must have a minimum diameter of 20cm; The most common sampling areas are abdomen, flank, and the trochanteric region.

Regerative therapy

The adipose tissue harvesting procedure is performed under local anesthesia. The adipose tissue was harvested with a 2 mm diameter microperforated cannula with 1 mm side port holes, mounted inside the special patented guide. Both the cannula and guide are included in the SEFFIHAIRTM medical device (produced by SEFFILINE S.r.l. Bologna-Italy) (*Figure 2*)[28-32].

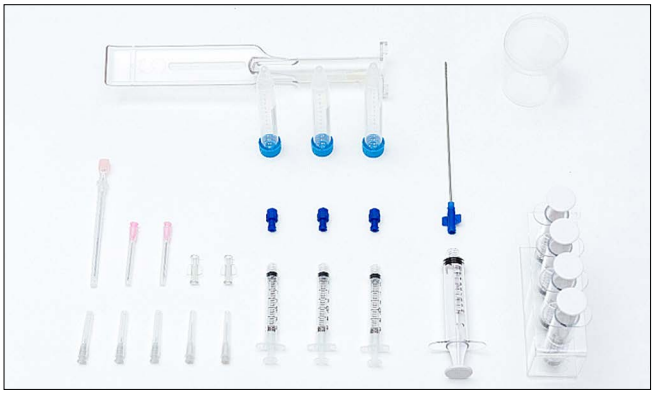


***Figure 2** - Seffihair™ medical device produced by SEFFILINE S.r.l. Bologna-Italy.*

The guide is addressed to standardize the procedure, to guarantee that tunneling is performed in the subcutaneous tissue adjacent to the dermis; this layer has been proven to hold more mesenchymal and vascular stem cells[33,34]. Once the adipose tissue was harvested, it was gently washed. The harvested and washed tissue was emulsified with 20 passages from one syringe to another. Subsequently the tissue was subjected to centrifugation (3500 rpm for 10 minutes) in order to separate the liquid (infranatant) tissue from the adipose stromal part. Once the separation had taken place, the infratant (portion containing the cells of the stromal vascular fraction and adipose derived stem cells) was aspirated and injected with a mesotherapy technique into the scalp (*Figure 3* and *4*).

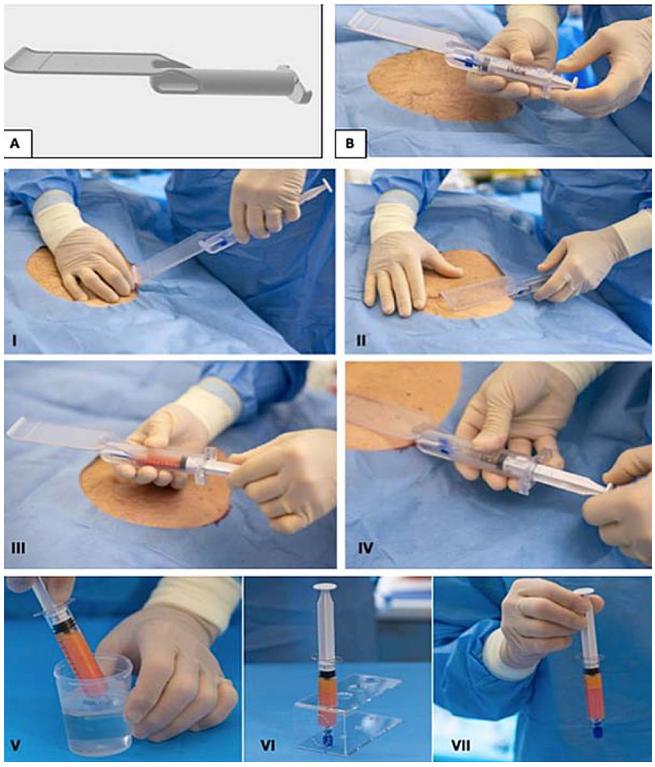


Figure 3 *- SVF and ADSCs pickup procedure.*

Figure 4 *- Extraction and grafting of stromal vascular fraction cells.*

Results

15 patients were enrolled, 9 men and 6 women who have undergone regenerative therapy with the use of mesenchymal stem cells derived from the adipose tissue and carboxytherapy for the treatment of androgenetic alopecia. The following pre and post treatment trichoscopy-related characteristics were analyzed (*Table 1a* and *1b*): Perifollicular pigmentation, yellow spots and hair density.

MALE PATIENTS	PERIFOLLICULAR PIGMENTATION REDUCTION	YELLOW SPOTS REDUCTION	DENSITY INCREASING
1	YES	YES	YES
2	YES	NO	YES
3	YES	YES	YES
4	YES	YES	YES
5	NO	YES	YES
6	YES	YES	YES
7	NO	YES	YES
8	YES	YES	NO
9	NO	NO	YES

Table 1a: *Trichoscopic modification pre and post therapy in male patients.*

FEMALE PATIENTS	PERIFOLLICULAR PIGMENTATION REDUCTION	YELLOW SPOTS REDUCTION	DENSITY INCREASING
1	YES	YES	NO
2	NO	NO	YES
3	YES	YES	NO
4	YES	YES	YES
5	NO	YES	NO
6	YES	NO	YES

Table 1b: *Trichoscopic modification pre and post therapy in female patients.*

In 6 out of 9 men and 4 out of 6 women there was a reduction in perifollicular pigmentation after treatment; In 7 out of 9 men and 4 out of 6 women there was a reduction in the presence of yellow spots; In 8 out of 9 men and 3 out of 6 women there was an increase in hair density. Clinical improvement was also analyzed taking into consideration the starting degree of the scales relating to the classification of androgenetic alopecia (Norwood in men and Ludwig in women) (*Figure 5* and *6*, *Table 2a* and *2b*, *Graph 1a* and *1b*) and it was seen how in 6 out of 9 men and in 3 out of 6 women there was an improvement of one degree in the respective scales. As a percentage, then (*Table 3*) we see how, in all the patients treated, considering these characteristics, there was a reduction in perifollicular pigmentation in 67% of cases; a reduction in the presence of yellow spots in 73% of cases and an increase in hair density in 73% of cases. On the other hand, considering the clinical characteristics, on all patients treated there was an improvement in 60% of patients (*Graph 2*).

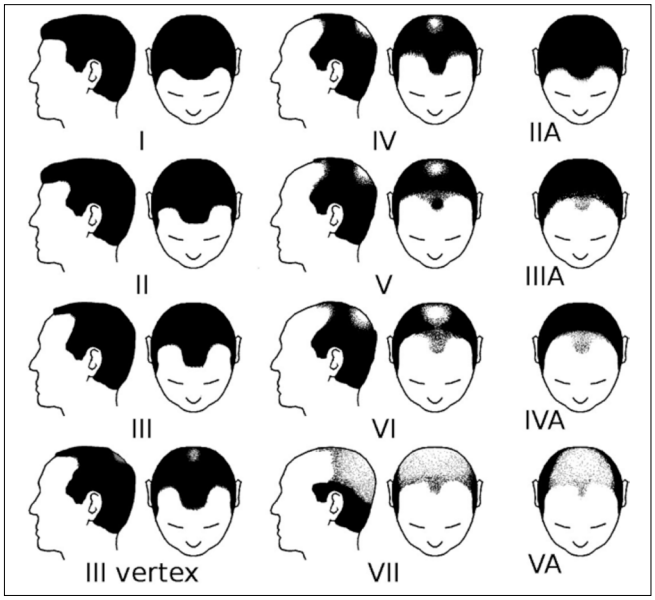


Figure 5: Male androgenetic alopecia classification table.

MALE PATIENTS	PRE THERAPY	POST THERAPY
1	6	5A
2	3 VERTEX	3
3	4	3 VERTEX
4	4A	4A
5	4	4
6	4	3 VERTEX
7	4	3 VERTEX
8	4	3A
9	3 VERTEX	3 VERTEX

Table 2a: Clinical modification (Norwood scale) pre and post therapy in male patients.

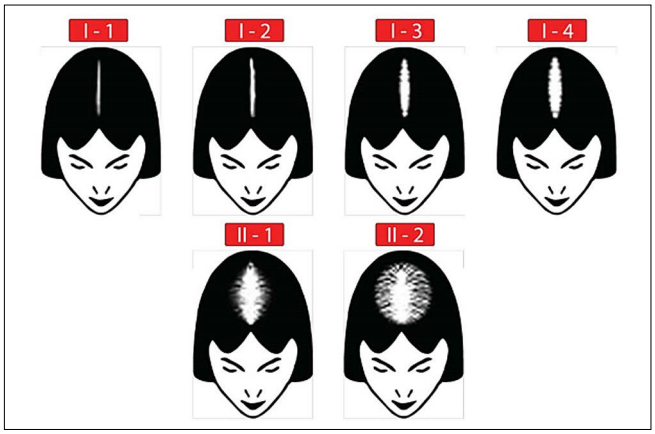
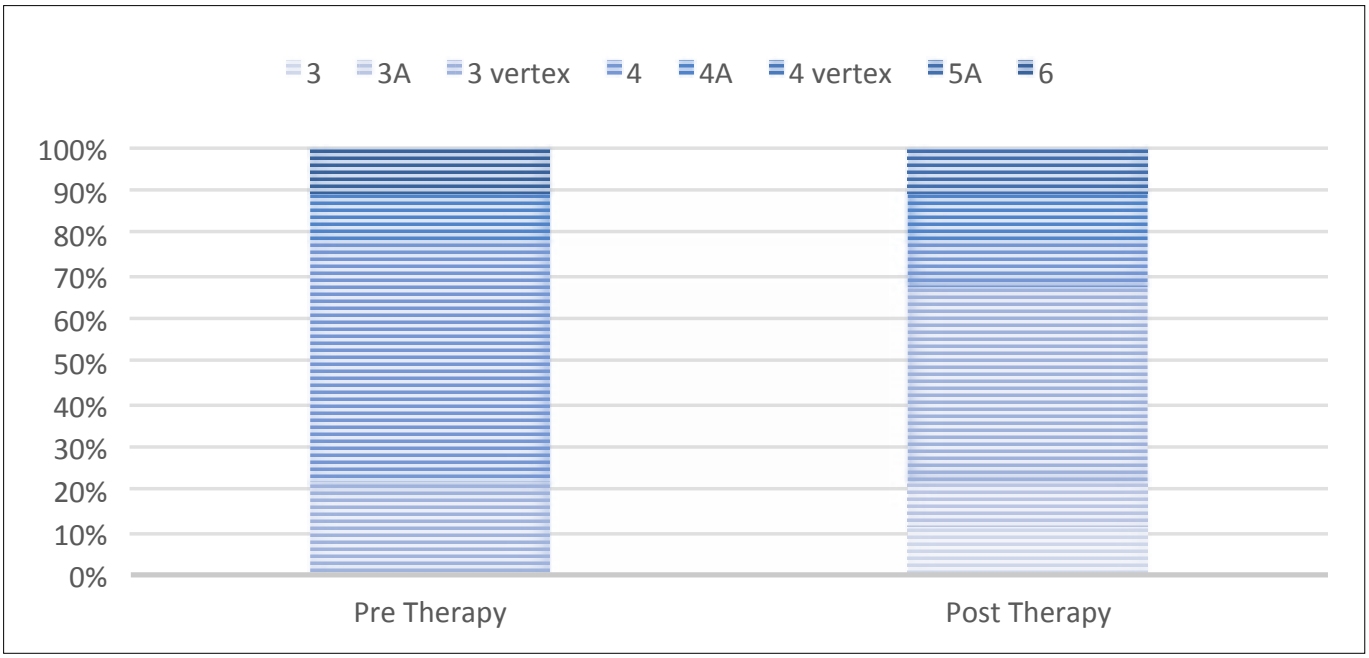


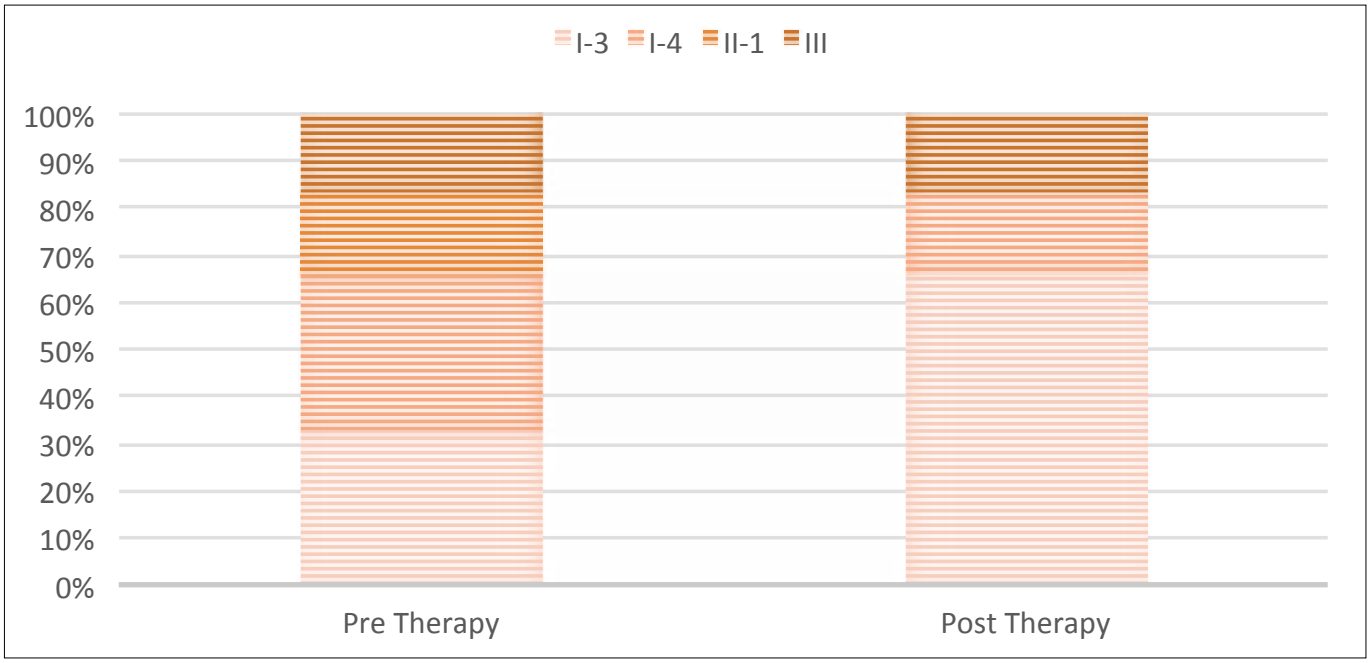
Figure 6: Female androgenetic alopecia classification table.

FEMALE PATIENTS	PRE THERAPY	POST THERAPY
1	II-1	I-4
2	I-3	I-3
3	III	III
4	I-4	I-3
5	I-4	I-3
6	I-3	I-3

Table 2b: Clinical modification (Ludwig scale) pre and post therapy in female patients.



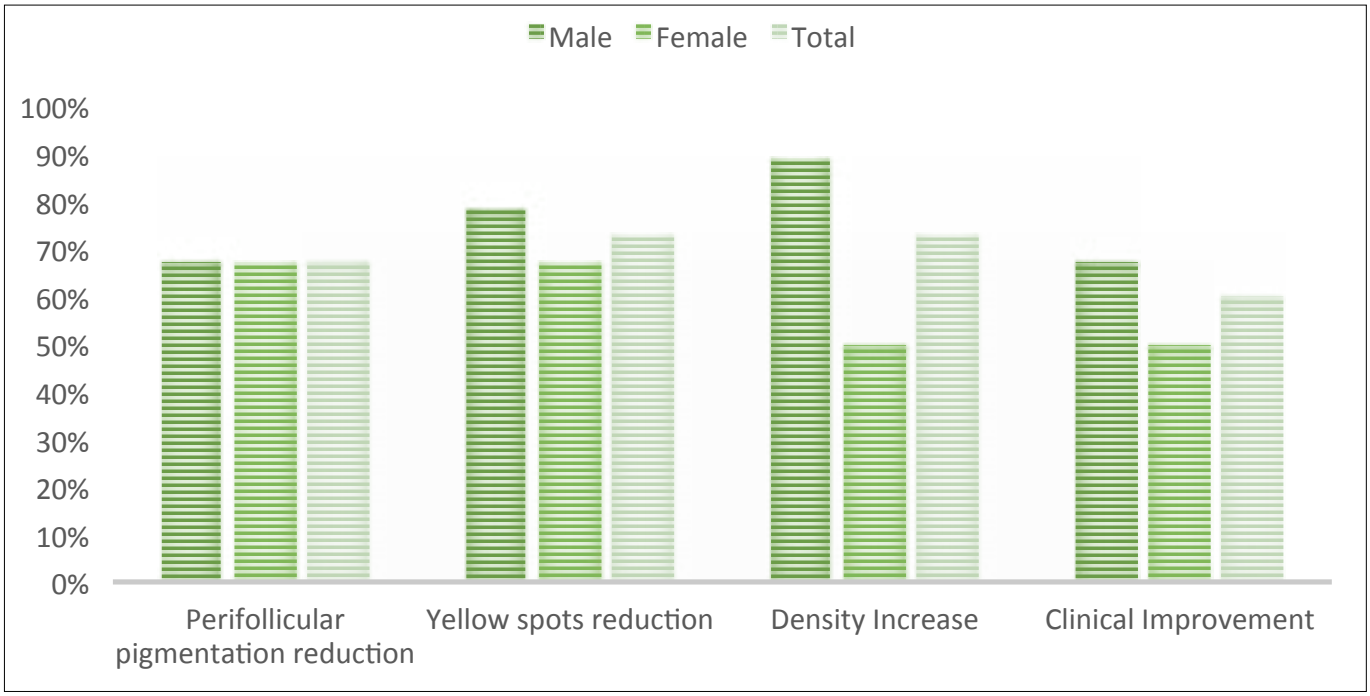
Graph 1a: Clinical modification (Norwood scale) pre and post therapy in male patients.



Graph 1b: *Clinical modification (Ludwig scale) pre and post therapy in female patients.*

	TRICHOSCOPY IMPROVEMENT			CLINICAL IMPROVEMENT
	PERIFOLLICULAR PIGMENTATION REDUCTION	YELLOW SPOTS REDUCTION	DENSITY INCREASE	
MALE	67%	78%	89%	67%
FEMALE	67%	67%	50%	50%
TOTAL	67%	73%	73%	60%

Table 3: *Trichoscopy and clinical improvement percentage in male, female and total patients.*



Graph 2: *Trichoscopy and Clinical Improvement in the population examined.*

QUESTION	AVERAGE SCORE
How do you feel your self-confidence in your relationship with others has changed?	4.2
During the therapy how painful was the part relating to the removal of adipose tissue?	2.0
How painful was the implant part in the scalp during the therapy?	1.7
How much do you feel the density of your hair has improved?	3.7
How much do you feel your hair loss has improved?	4.0

Table 4: *Questionnaire relating to the degree of satisfaction and appreciation of the therapeutic protocol and average scores.*

The treated patients were also subjected to a questionnaire (*Table 4*) relating to the treatment consisting of 5 questions in order to assess the degree of satisfaction and approval of the therapeutic protocol adopted through a score from 0 to 5 where zero indicates "not at all" and 5 "very much". The first question "how has your self-confidence evolved and how would you say your relationship with others has changed?" showed an average response of 4.2 with 87% of patients expressing a score equal to or greater than 4. The second question "how painful was the part relating to the removal of adipose tissue during the treatment?" showed an average response of 2 with 80% of patients expressing a score equal to or less than 2. The third question "during the treatment how painful was the part relating to the implantation of stem cells in the scalp?" showed an average response of 1.7 with 93% of patients giving a score equal to or less than 2. The fourth question "how much do you feel your hair density improved?" showed an average response of 3.7 with 60% of patients expressing a score equal to or greater than 4. The last question "How much would you say hour hair loss has been reduced?" showed an average response of 4 with 80% of patients expressing a score equal to or greater than 4.

Discussion

Regenerative medicine certainly represents the most recent approach to therapy for androgenetic alopecia. As can be seen from the results of this study, regenerative therapy through the use of stem cells derived from the adipose tissue has proved to be effective both considering the trichoscopic parameters and the clinical parameters. In the male population, as regards the trichoscopic characteristics, in 67% of cases there was a reduction of the perifollicular pigmentation, in 78% of cases a reduction in the presence of yellow spots and in 89% of cases an increase in density. hair; as regards the clinical characteristics, in 67% of cases there was an improvement in the degree of androgenetic alopecia according to the Norwood scale. In the female population, as regards the trichoscopic characteristics, in 67% of cases there was a reduction in perifollicular pigmentation and a reduction in the presence of yellow spots and in 50% of cases an increase in hair density; as regards the clinical characteristics, in 50% of cases there was an improvement in the degree of androgenetic alopecia according to the Ludwig scale. The percentages related to the improvement of the trichoscopic aspects were higher than those related to the clinical improvement: this could be indicative of the fact that the trichoscopic improvements are more rapid than a degree improvement in the classification scales of alopecia (Norwood or Ludwig) (*Figure 7* and *8*).

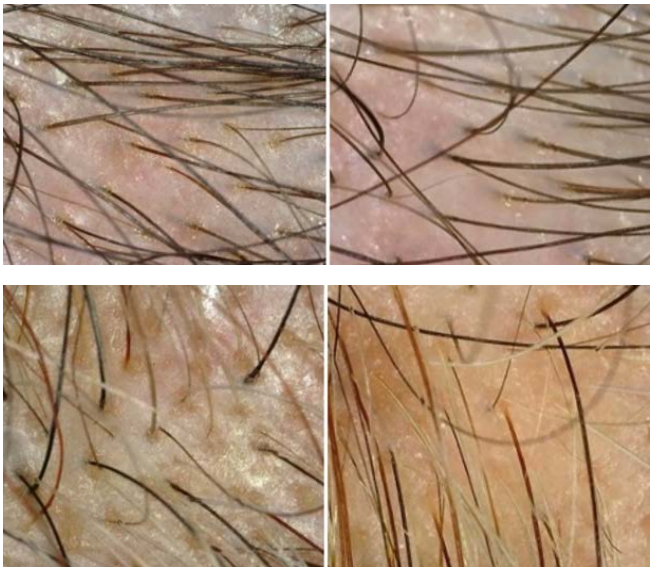


Figure 7 and 8: *Pre and post therapy trichoscopic images.*

The literature already contains data relating to the use of microcannulas, in combination with a mechanical digestion by means of an emulsification and a centrifuge procedure capable of facilitating the isolation in the infranatant component of SVF cells for the regenerative treatment[35]. Also in the literature, there is already data on how tissue regeneration through Autologous Fat Transfer can significantly improve hair growth in the area of scarring alopecia, emphasizing, once again, the enormous potential of this approach.
The role of this study is to confirm the validity of regenerative therapy through the use of stem cells derived from the adipose tissue associated with carboxytherapy in androgenetic alopecia. For several years now carboxytherapy has represented one of the most significant approaches in this type of pathology[36]: the combination with regenerative medicine, described in this study, aims to underline how the association of several methods is the winning weapon in fighting a pathology with a multifactorial etiology such as

Androgenetic Alopecia. From the scores relating to the satisfaction and satisfaction questionnaire completed by the patients, it is also clear how this type of therapeutic protocol is practically painless, has significantly improved the patients' self-confidence and relationship with others, hair density and how much it has reduced the hair loss.

Conclusions

From the analysis of the results obtained in this study, it is clear that the combination of regenerative therapy and carboxytherapy is a valid, promising technique and is part of a multidisciplinary approach more suited to the treatment of a pathology such as androgenetic alopecia (*Figure 9, 10* and *11*).

The future objectives are related to the increase in the number of the sample analyzed and the completion with the acquisition of post-treatment trichoscopic images in order to confirm the results obtained with this study.

Disclosure

Antonio Luca Amore and Emanuele Bartoletti have no conflict of interest. Alessandro Gennai is the scientific director of Seffiline Academy; Seffiline™ srl produces the device used to perform the study.

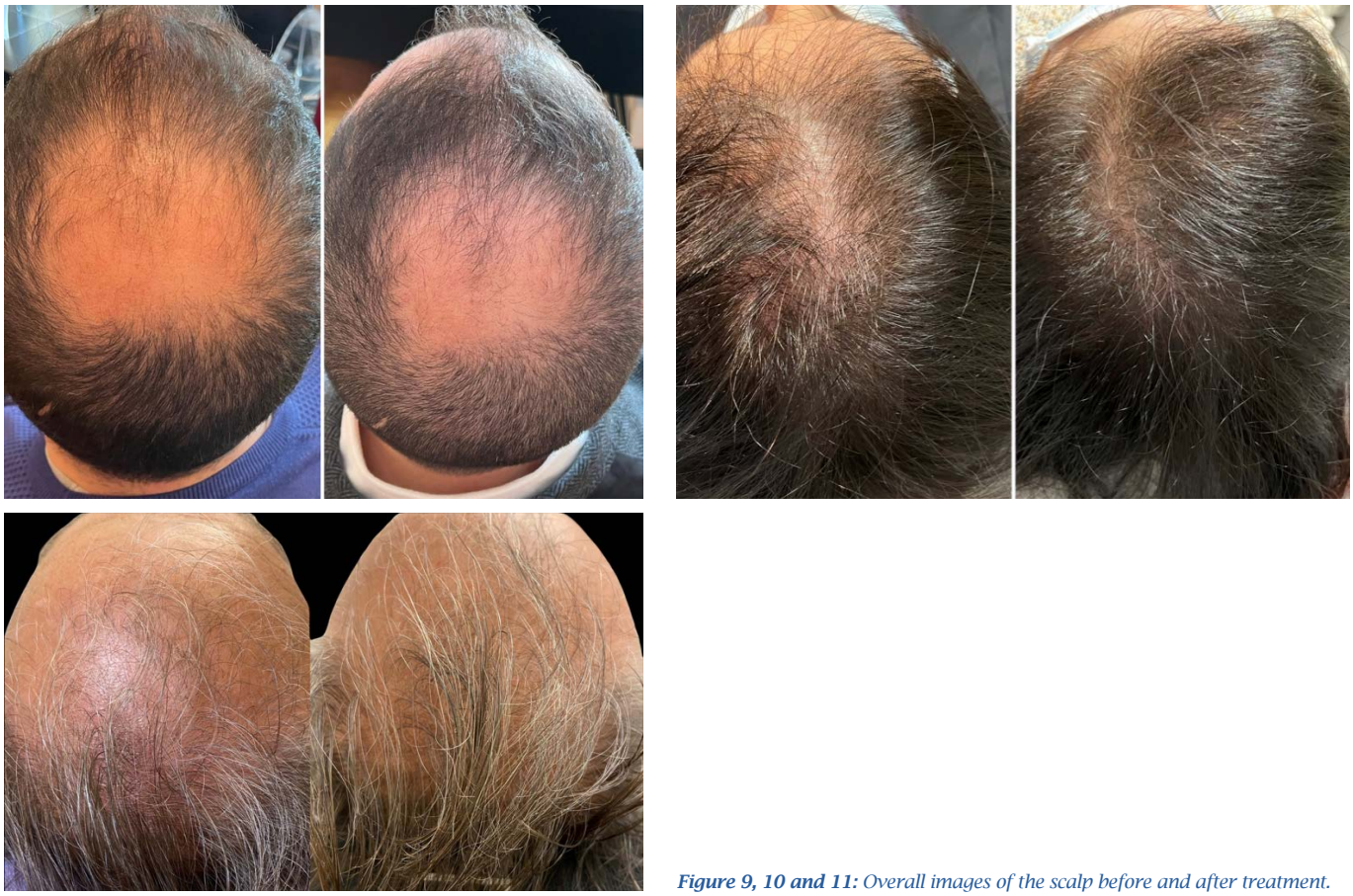


Figure 9, 10 and 11: *Overall images of the scalp before and after treatment.*

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aesthetic medicine

Original Article

The demand for medical-aesthetic care in Portugal. Study of this current reality

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Abstract

Background: there's a growing perception of the demand of the aesthetic medical treatments on both a national and international level. However, it is still difficult to characterize the current and objective reality of this medical field in Portugal.

Aim: the goal of this study is to portray the reality of the aesthetic medical care in Portugal nowadays and to understand its evolutionary pattern over the past nine years.

Methods: cross-sectional, descriptive, and observational study, based on data obtained from a questionnaire regarding the aesthetic medical care in Portugal. Only clinics in the Lisbon metropolitan area were included. A Statistical analysis was performed using SPSS®27.

Results: the sample consisted of 14 valid inquiries. The female sex was the most frequent in all the years that were analyzed (n=14). There is a growing trend (28%) of consultations at younger ages (<=40 years). Currently, 7 clinics have 11-30 weekly customers, 9 years ago 5 clinics had 1-10 weekly customers. There's an increasing trend (13%) of the number of 6-10 procedures per year per person and a 12% decrease of those who perform 1-5 procedures. Botulinum toxin is the most performed procedure (n=11). Filler materials represented the second most frequently performed surgery. (n=9).

Conclusions: the frequency of the consultations in a younger age group has been increasing. There is a higher number of aesthetic medicine consultations compared to previous years and a higher number of procedures performed annually per person. Botulinum toxin is the most performed medical-aesthetic procedure and filler materials are the second.

Key words
Esthetics, medicine, demand, Portugal

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Introduction

Aesthetic Medicine comprises all medical procedures, invasive or not, that aim to improve the patient's physical and cosmetic appearance[1].
Worldwide, Aesthetic Medicine is represented through the International Union of Aesthetic Medicine (UIME), an association founded in 1975. In 1999, the first society of Aesthetic Medicine was created in Portugal, but due to the reduced number of local doctors dedicated to this art, it ended up dissolving[2].Challenged by the Spanish Society of Aesthetic Medicine (SSAM)[3], the Portuguese Society of Aesthetic Medicine (PSAM) was formally constituted in 2015, fruit of the shared will of Aesthetic Doctors, Dermatologists and Plastic Surgeons[4]. PSAM is the Portuguese representative at UIME where it seeks to contribute to the development of Aesthetic and Anti-Aging Medicine in our country[4]. The technical basis of many medical-aesthetic procedures has progressively evolved until today in our country.
People are increasingly concerned about taking care of their shape, weight, appearance, diet, maintenance of beauty and youth. The care of aesthetics influences the care of health and physical fitness. Surgical and non-surgical procedures had a total increase of 7.4% in 2019, according to the International Society of Aesthetic Plastic Surgery, with medical-aesthetic procedures increasing by 7.6%[5].
Despite the growing perception of the demand for national and international medical-aesthetic care reflected in the number of physicians trained in this area, the volume of clinics, congresses, and publications, it is difficult to characterize the current and objective reality of this area in Portugal. This is due, on one hand, to the scarcity of studies and publications on the subject; and on the other hand, to the absence of a common clinical database due to the private nature of these services.

Aim

The aim of this work is therefore 1) to portray the reality of medical-aesthetic care in Portugal today and 2) to analyze its evolutionary pattern in the last 9 years.

Materials and method

This cross-sectional, descriptive, and observational study consisted of the analysis of questionnaires voluntarily filled in online from May 24th to June 7th, 2021. The survey population was of convenience, corresponding to the 34 aesthetic medicine clinics in the Lisbon metropolitan area. A medical representative of each clinic was responsible for completing the questionnaire. All the clinics surveyed had a minimum of 1 to a maximum of 12 aesthetic medicine practitioners and 31 of them had a website available, with location and contact information.
The questionnaire was structured into four parts: the first part included the characterization of the clinic (10 items); the second part included the characterization of the patients attending the clinic by collecting current data and data from 3, 6 and 9 years ago (4 items); the third part included the characterization of the medical-aesthetic procedures (16 items) and, finally, a fourth part related to customer dissatisfaction (2 items).
The questionnaire items were descriptively analyzed using the IBM SPSS Statistics software, version 27. Due to the small sample size, inference procedures were excluded, and the median was considered as the reference value in the analysis.

Results

The final sample consisted of 14 valid surveys. The refusal-to-answer rate was 59%, and no losses were recorded.

Information concerning the clinic itself

Regarding the headquarters of the clinic, 11 clinics out of 14 answered are in the municipality of Lisbon, with the remaining having their headquarters in other municipalities of the Lisbon metropolitan area. 3 of the clinics also have facilities in other cities. Regarding the year of foundation, 5 clinics were founded as of 2017, with the oldest founded in 1986 and the most recent in 2021. The median length of service was 11.5 years (range 0,35). As for the number of aesthetic medicine doctors per clinic, the average is 2. Only 4 clinics out of the 14 that answered are exclusive to aesthetic medicine. 60% of the remaining clinics have 2 or 3 other specialties. Plastic Surgery represents the most common additional specialty associated with Aesthetic Medicine, followed by Nutrition. About their dissemination, 12 clinics out of the 14 reported having a website, however, 8 clinics stated that their preferred way to reach the consumer is through a direct recommendation.

Information relating to patients

The most consuming gender was female in all the years that were analyzed (n=14). As shown in *figure 1*, regarding the most frequent age group in consultations, there was a higher prevalence in the age range 41-50 years 9, 6 and 3 years ago (n=6, n=6, n=7, respectively). However, currently the highest prevalence is in the age range 31-40 years (n=7) with an increasing trend of 28% of consultations in younger ages (<=40 years).

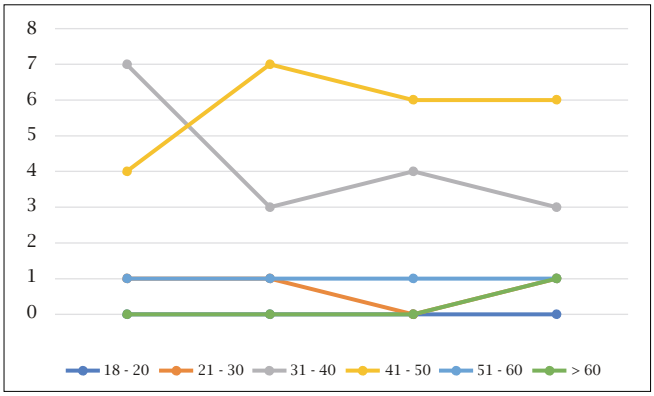

***Figure 1** - Most frequent age group in consultations (n).*

Currently around 7 clinics out of 14 answered have 11-30 clients per week. Also 3 years ago, 8 of the clinics had 11-30 clients per week. Six years ago, the frequency of 11-30 was the same as that of 1-10 clients in 4 clinics. Nine years ago, the highest frequency was from 1 to 10 clients per week (n=5) (*Figure2*).

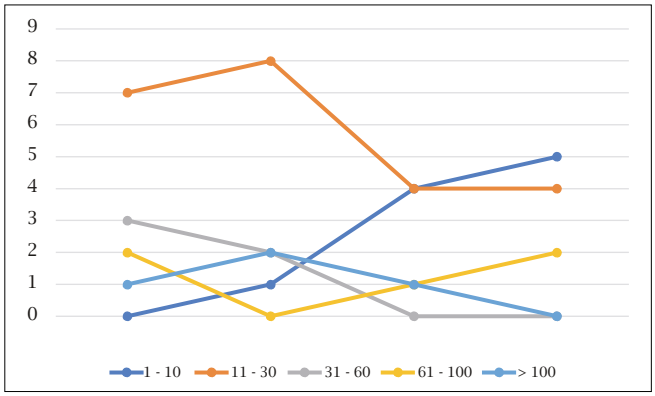

***Figure 2** - Number of weekly clients (n).*

Through the analysis of the number of clients per week and the number of specialties present in the clinic within our sample, we found that the high number of specialties (n=5 + aesthetic medicine) is not a factor for a higher number of clients. Clinics with 3 specialties other than aesthetic medicine show a tendency to have a higher number of weekly clients today.
There is a positive trend for the number of 6-10 procedures per person per year to increase by about 13%, accompanied by a 12% reduction in the number of 1-5 procedures.

Information relating to medical procedures

Aesthetic clinics have a median value of performing 5 types of aesthetic procedures, ranging from 3 to 13.
Botulinum toxin is the procedure with the highest demand in 12 clinics out of the 14 that answered. In second place, the filling materials also present a high demand according to 8 clinics. The level of demand for these procedures is only exceeded by mesotherapy by 1 of the clinics. The placement of tension and support wires, hair implants and nutrition consultations were also of high demand in 3 clinics.
Botulinum toxin is also the most performed procedure (n=11). Filler materials had the second highest number of procedures performed (n=9). The degree of performance of these procedures is only exceeded in 4 clinics by mesotherapy, tension and sustentation threads, hair implant and nutrition.
Clients have a median average amount spent per visit to the clinic of €425. Hair transplantation is the procedure with a median average spend of €3,000 and the one with the highest price fluctuation, where clients can pay a minimum of €1,700 to a maximum of €5,200. Botulinum toxin has a median average spend of €280 and fillers €290. Next, laser therapy and tension and support wires have a median average spend of €450 and €300 respectively. Carboxytherapy was the procedure with the lowest median value of €60, although it can vary between €40 and €400. Nutrition consultations have a median value of €80 and can vary between €50 and €180.
The existence of 3 specialties shows an increasing trend in the median spending- median value of a visit-customer.

Information concerning customer dissatisfaction

According to the data on patient dissatisfaction, 6 out of 14 answered reported having had complaints from patients in the last year, however none had any judicial follow-ups.

Discussion

Thirty-four Aesthetic Medicine clinics in Lisbon were surveyed, and we obtained only 14 valid responses. This translates into a refusal to answer rate of 59%. We may hypothesize that it is due to 1) the doctors' lack of availability (time) to answer the questionnaire and 2) the colleagues' preference to keep the questioned data confidential, considering the competitiveness of the profession. In any case, the reduced sample (N=14) obtained, underlines the difficulty in having access to a global perspective of the data in the Lisbon area, and that we extrapolate to be similar at a national level.
In order to study "The demand for medical-aesthetic care in Portugal", the study was restricted to the Lisbon metropolitan area to obtain a more homogeneous sample from a specific area of the country, which represents the reality of the country.
Regarding the results concerning the clinics, most of them (n=11) are based in the municipality of Lisbon, which reflects the greater supply and demand in terms of work, services, and commercial points in this location. 6 It is important to mention that all 34 clinics of the initial sample belonged to the Lisbon metropolitan area (that includes the city of Lisbon and other surrounding cities) and 28 of those clinics belonged in the municipality of Lisbon (as known as City of Lisbon).
When we analyze the year, the clinics were founded, we can see that there has been a pattern of increasing growth, especially since 2017, i.e. the last 4 years.
Regarding the number of doctors who practice aesthetic medicine per clinic, the average is 2, which can be 1) a small clinic or 2) a clinic that integrates different specialties. In fact, 60% of the clinics have 2 or 3 other specialties besides Aesthetic Medicine, with Plastic Surgery being the most frequent. This confirms our hypothesis that, on the one hand, it can be more profitable for a clinic to present different valences; on the other hand, the client who seeks medical-aesthetic care does so in a comprehensive way, not being restricted only to Aesthetic Medicine, but being allowed to branch out to Plastic Surgery and Nutrition as well, for example.
The fact that 12 clinics had a website does not invalidate the fact that respondents mentioned that the preferred way to reach the consumer was through direct recommendations (n=8). This data is interesting because, although there is a perception that social networks have gained increasing importance in the bridge between client-doctor and that they have a relatively high return on investment, 7,8, 14 the data from our sample leads us to believe that the traditional method of transmitting this type of information also continues to play an important role. Focusing on the information regarding

these patients we realize that the most consuming gender was, in all years analyzed, female. According to the International Society of Aesthetic Plastic Surgery, data from 2021 confirms that women (comprising roughly 90% of all the patients) continue to perform more aesthetic procedures than men[9,15]. Men accounted for only 8% of all procedures in 2017 to 2019[16]. However, it would be interesting to conduct a future study with a larger sampling and quantification of the number of male patients who attended the consultation, in order to objectify whether there is a real increase in demand by this group in our country. This is because, according to the American Society of Plastic Surgeons, in 2020 there was an increase in the performance, by the male class, of minimally invasive aesthetic procedures of 72% compared to the year 2018[10,17]. This increase is more relevant to filler materials, botulinum toxin and laser therapy with increases of 433%, 381% and 250%, respectively[10,16,17].

Regarding the age range that was most frequently dealt with, we found a paradigm shift in the time period analyzed, where 9, 6 and 3 years ago the most prevalent age group was 41-50 years (n=6, n=6, n=7, respectively) and currently, 31-40 years (n=7). Additionally, there was an upward trend of 28% of consultations at younger ages (<=40 years). This change is already widely supported in the literature which argues that the demand for aesthetic medicine services in young people aged up to 35 years is increasing.11,17 This may be due to 1) the interest of clients in wanting to prevent early onset and evolution of aesthetic affections, 2) the emergence of new non-invasive aesthetic techniques or with fewer adverse effects, 3) the growing economic power of this consumer population, 4) the greater accessibility to medical-aesthetic care through websites and social networks.

Additionally, we found that the number of procedures performed annually per individual has been increasing, with the number of people who perform between 6 and 10 procedures/year increasing 13% and those who perform between 1 and 5 procedures/year decreasing 12%. This is a clear indicator of the growth in demand for medical-aesthetic care in the sample considered.

Regarding the volume of consultations, there has been an increase in the last 9 years. This means that, 9 years ago, the most frequent scenario was for a physician to have about 1-10 weekly clients. 6 years ago, the data we obtained was ambiguous, with two peaks equally distributed between 1-10 and 11-30 weekly clients. From 3 years ago to the present time, the most obtained response was between 11-30 weekly customers. These data confirm that the growing desire to look young and demonstrates the increase in demand for aesthetic treatments in developed countries[12].

As regards to the relationship between the number of clients per week and the number of specialties in the clinic, it was found in our sample that the presence of up to 3 specialties besides EM is associated with a higher number of weekly clients. Interestingly, a clinic that includes more than 5 specialties in addition to Aesthetic Medicine is not positively associated with an increase in the number of weekly clients. Having said this, we infer that a clinic with less valences which are however more interconnected with each other (e.g. Plastic Surgery, Aesthetic Medicine and Nutrition) may attract more Aesthetic Medicine clients than one with a larger number of valences which are more unrelated to each other (e.g. Aesthetic Medicine, Plastic Surgery, Pulmonology, General Practice and Cardiology). Regarding medical-aesthetic procedures, let us analyze the most sought-after procedures vs. the most performed ones. Regarding the most popular procedures, botulinum toxin was the most popular (n=12), followed by fillers (n=8), similar data to that published by the International Association for Physicians in Aesthetic Medicine[13]. Regarding the third most sought procedure, there is not one that stands out and it varies from clinic to clinic, which may be due to the existence of a technique in which the doctor specializes. Therefore, for example, the placement of tension and support wires, hair implants and nutrition consultations were performed in 3 clinics with very high and/or high demand. In other words, it could be the case that there is only one EM clinic dedicated to hair implants, which obviously has a direct impact on the type of demand.

Regarding the most performed procedures, we found a correspondence with the demand, with botulinum toxin being the most performed procedure (n=11), followed by fillers (n=9). The degree of performance varies only in 4 clinics, with greater emphasis on mesotherapy, tension and sustentation threads, hair implant and nutrition. Once again, we emphasize the focus on the method most practiced by the clinic or physician as an attributable factor of this difference.

About the amount invested in each procedure, we can see that there is a great variability between 1) the type of procedure and 2) between clinics and their location, regarding the same procedure. For example, a hair transplant has an average value of 3000€, and this treatment has a price variability between 1700 to 5200€ between different clinics.

When we look at the median value of average expenditure per consultation (425€), we can conclude that there is a high investment when compared, for example, to the national minimum wage. In other words, this means that the population seeking this type of care will be a differentiated population with a medium-high socioeconomic level.

When analyzing the data on patient dissatisfaction, we observed that 6 of the clinics reported having had complaints in the last year, however, none with any judicial follow-up. Considering the sample of this study, this is a considerable proportion of complaints. This may be due to 1) poor management of patient expectations or unrealistic expectations, 2) short experience of the physician or 3) a medical error. However, it would be interesting to study a larger sample and understand if this percentage remains similar or has discrepant values. Additionally, to see how it varies depending on a) the number of such procedures performed by the physician, b) the years of experience of the physician, c) the number of procedures already performed by the complaining client. The fact that no complaint was followed up in court leads us to think that a proactive attitude, preparing and explaining all the steps of the procedure and possible adverse reactions, as well as an openness to learning and sharing the error may be key for a good management of clinical practice.

The main limitations of the study are the low level of sampling and the fact that the sample corresponds only to the Lisbon metropolitan area. Other important

limitations are the evolutionary pattern limited to a few items and the retrospective investigation. In addition, the presentation of the questionnaire could be improved to make it simpler and more appealing, and some items in the questionnaire itself were not written in the clearest possible way, which may have influenced some responses. Thus, this study serves as an interesting starting point for the creation of other studies on the demand for medical-aesthetic care.

Conclusion

We can conclude that the practice of medical-aesthetic procedures is increasingly present in our country. There is a growth pattern regarding the incidence of new clinics in the last 9 years. There is a growing number of people who, without presenting any previous pathology, demand an improvement of their image. The frequency of younger age groups has been increasing. There is a growing number of people who attend aesthetic medicine appointments and an increasing number of procedures performed annually per person. As for procedures, the two most sought- after procedures coincide with those most performed: in the first place, botulinum toxin and in second place the filling materials. Complaints are a reality to be considered in clinical practice and their prevention and management is important.
In the future, further studies will be necessary to better characterize the profile of health care in the medical-aesthetic area in the country, with an adequate sample, of long duration and of good methodological quality to clarify the data. Further future collaboration is expected to improve in this area.

Acknlowedgments

None. The authors declare they did not have any financial or material support.

Conflict of interest

All authors declare they have no conflict of interest.

ANNEX

Questionnaire

The Demand for Medical Aesthetic Care in Portugal

This questionnaire is confidential and your data will only be processed by the authors of this study, students of the Postgraduate Diploma of Aesthetic Medicine at the Alcalá Faculty of Medicine, in order to obtain statistical information regarding the demand for medical and aesthetic care in Portugal. By completing this form you are consenting to the data being computerized so that we can process it statistically. Your participation does not involve any risk. Thank you for your cooperation.

CLINIC-RELATED

Clinic name:

_____

Location of the clinic (headquarters):

_____

_____

Year founded: _____

Does the clinic have facilities in another city/s? Yes ____ No ____

If you previously answered "Yes", where is it located? _____

Is the clinic exclusively for Aesthetic Medicine? Yes ____ No ____

If you previously selected "No", please indicate which specialties/validities are covered:

_____

Number of Aesthetic Medicine physicians in the practice:

_____

_____

Does the clinic have a website? Yes ____ No ____

Preferred means of reaching the consumer: _____

PATIENT-RELATED

Number of customers per week

	1-10	11-30	31-60	61-100	>100	N/A
Currently	O	O	O	O	O	O
3 years ago	O	O	O	O	O	O
6 years ago	O	O	O	O	O	O
9 years ago	O	O	O	O	O	O

Age group that most attends your appointments

	18-20	21-30	31-40	41-50	51-60	>60	N/A
Currently	O	O	O	O	O	O	O
3 years ago	O	O	O	O	O	O	O
6 years ago	O	O	O	O	O	O	O
9 years ago	O	O	O	O	O	O	O

Most consuming gender of consultations

	Female	Male	N/A
Currently	O	O	O
3 years ago	O	O	O
6 years ago	O	O	O
9 years ago	O	O	O

Number of aesthetic procedures performed on each patient per year

	1-5	6-10	11-15	>15	N/A
Currently	O	O	O	O	O
3 years ago	O	O	O	O	O
6 years ago	O	O	O	O	O
9 years ago	O	O	O	O	O

ON MEDICAL PROCEDURES

Select the following selected procedures in descending order of demand

	1º	2º	3º	4º	5º
Botulinum toxin	O	O	O	O	O
Fillers	O	O	O	O	O
Mesotherapy	O	O	O	O	O
PRP	O	O	O	O	O
Microneedling	O	O	O	O	O
Tension and support wires	O	O	O	O	O
Laser therapy	O	O	O	O	O
Carboxitherapy	O	O	O	O	O
Radiofrequency	O	O	O	O	O
Trichology/ Hair Transplant	O	O	O	O	O
Gynecoesthesia	O	O	O	O	O
Nutrition, Obesity or Hormone Modulation	O	O	O	O	O

Select the following selected procedures in descending order of performance

	1º	2º	3º	4º	5º
Botulinum toxin	O	O	O	O	O
Fillers	O	O	O	O	O
Mesotherapy	O	O	O	O	O
PRP	O	O	O	O	O
Microneedling	O	O	O	O	O
Tension and support wires	O	O	O	O	O
Laser therapy	O	O	O	O	O
Carboxitherapy	O	O	O	O	O
Radiofrequency	O	O	O	O	O
Trichology/ Hair Transplant	O	O	O	O	O
Gynecoesthesia	O	O	O	O	O
Nutrition, Obesity or Hormone Modulation	O	O	O	O	O

How much does the average patient spend per visit (for the total number of procedures performed)?

_____

_____

Please indicate the average price charged in your practice for a Botulinum Toxin treatment. If not performed, you should not answer.

_____

Please indicate the average price in your practice for a treatment with Fillers. If not performed, you should not answer. _____

Please indicate the average price charged in your clinic for a Mesotherapy treatment. If not performed, you should not answer.

_____

_____

Please indicate the average price practiced in your clinic for a PRP treatment. If not performed, you should not answer.

_____

_____

Please indicate the average price in your practice for a Microneedling treatment. If not performed, you should not answer.

_____

Please indicate the average price in your practice for a peel treatment. If not performed, you should not answer.

_____

_____

Please indicate the average price in your practice for a treatment with Tension and Support Wires. If not performed, you should not answer.

_____

_____

Please indicate the average price in your practice for a Lasertherapy treatment. If not performed, you should not answer.

_____

Please indicate the average price charged in your clinic for a Carboxitherapy treatment. If not performed, you should not answer.

_____

_____

Please indicate the average price in your practice for Radiofrequency treatment. If not performed, you should not answer.

_____

Please indicate the average price charged in your clinic for a Trichology/ Hair Transplant treatment. If not performed, you should not answer.

_____

_____

Please indicate the average price offered in your practice for a Gynecoesthesia treatment. If not performed, you should not answer.

_____

_____

Please indicate the average price in your practice for a Nutrition, Obesity or Hormone Modulation treatment. If not performed, you should not answer.

_____

_____

CONCERNING LEGAL MATTERS

How many complaints have you had in the last year?

_____

_____

Were any of them followed up in court? Yes No

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aesthetic medicine

Review

Clinical potential of Extracellular Vesicles in Regenerative and Aesthetic Medicine

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Running Head: EVs in aesthetic medicine

Abstract

Extracellular vesicles (EVs) represent a heterogeneous class of spherical particles released by cells that are known for the essential role they play in cell to cell communication in various processes, both physiological and pathological. Over the years, they have been considered an useful tool for the diagnosis and treatment of various diseases, mainly cancer. Lately, however, their use has also extended to other fields of medicine, since they could be administered through minimally or non-invasive approaches in clinical treatments. Among those novel application fields, we find the aesthetic medicine, where the use of EVs aims to support skin rejuvenation and to improve and correct skin-related cosmetic defects, including wrinkles, hair loss, and acne scars.

This review provides a general perspective and the latest findings supporting the efficacy of EVs application in Aesthetic Medicine.

Keywords

Extracellular vesicles, rejuvenation, acne vulgaris, hair loss, scars, aging

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Introduction

Aesthetic Medicine is a practice that deals with developing and using minimally or non-invasive approaches in order to improve the aesthetic appearance, health and well-being of patients. It was born from the union of the multiple types of knowledge related to different fields of both medicine and surgery[1].
The most popular treatments in aesthetic medicine are specifically aimed to improve the appearance of the skin and eliminate aesthetic defects due to age (wrinkles, hair loss), or skin trauma, or surgical operations (scars). Although the most commonly used techniques for skin therapy include hyaluronic acid fillers, autologous fat transplant, botulinum toxin injection, and lasers, over the past decade other approaches based on biological elements had spread to skin treatments, included administration of Platelet Rich Plasma (PRP)[2-5] and Extracellular Vesicles (EVs)[6-8].
This review focuses on EVs and gives an overview of the latest evidence and benefits of their administration for aesthetic improvement.

Main skin defects treated by Aesthetic and Regenerative Medicine

Biological aging is a multifactorial and irreversible process, which involves a wide range of mechanisms that compromise normal cell functions, resulting in structural and functional alterations of organs and tissues[9,10]; on an aesthetic level, it manifests itself with skin morphological changes resulting in wrinkles, due to bone, muscle and fat alterations[11] (*Fig. 1A*). Aging is also manifested with hair thinning or loss, caused by the dysregulation of the hair follicles-stem cells and miniaturization of the hair follicles [12,13] (*Fig.1B*).
Skin lesions are a further process we are subjected to during life: they can occur as a result of physical trauma, post-surgical operations, acne, burns, etc. and can result in integral tissue repair, or in the formation of scars, the generation of which is mediated through the production of a granulation tissue and the differentiation of myofibroblasts, responsible for the deposition of collagen in wound sites and the consequent formation of scars[14]. Depending on the severity of the damage, wound healing can result in the formation of different types of scars: the simplest have a small, almost invisible line; others are abnormal tissue alterations, such as atrophic scars, scar contractures, hypertrophic scars, and keloid scars[15,16].
Among these skin lesions, the most common seem to be those induced by Acne vulgaris , a chronic inflammatory skin disease that usually occurs during puberty and lasts throughout adolescence. Face, back and chest are most affected and its onset is given by a series of multiple factors, which contribute to i) keratinization, sebum accumulation, and bacterial colonization of the skin pores; ii) formation of the whitehead which expands more and more due to the increasing accumulation of sebum and keratinization, resulting in the iii) formation of the blackhead; iv) follicular rupture which triggers an inflammatory state; v) stimulation of the wound healing process that often results in skin lesions, the most common of which are atrophic or hypertrophic scars[17] (*Fig.1C*).

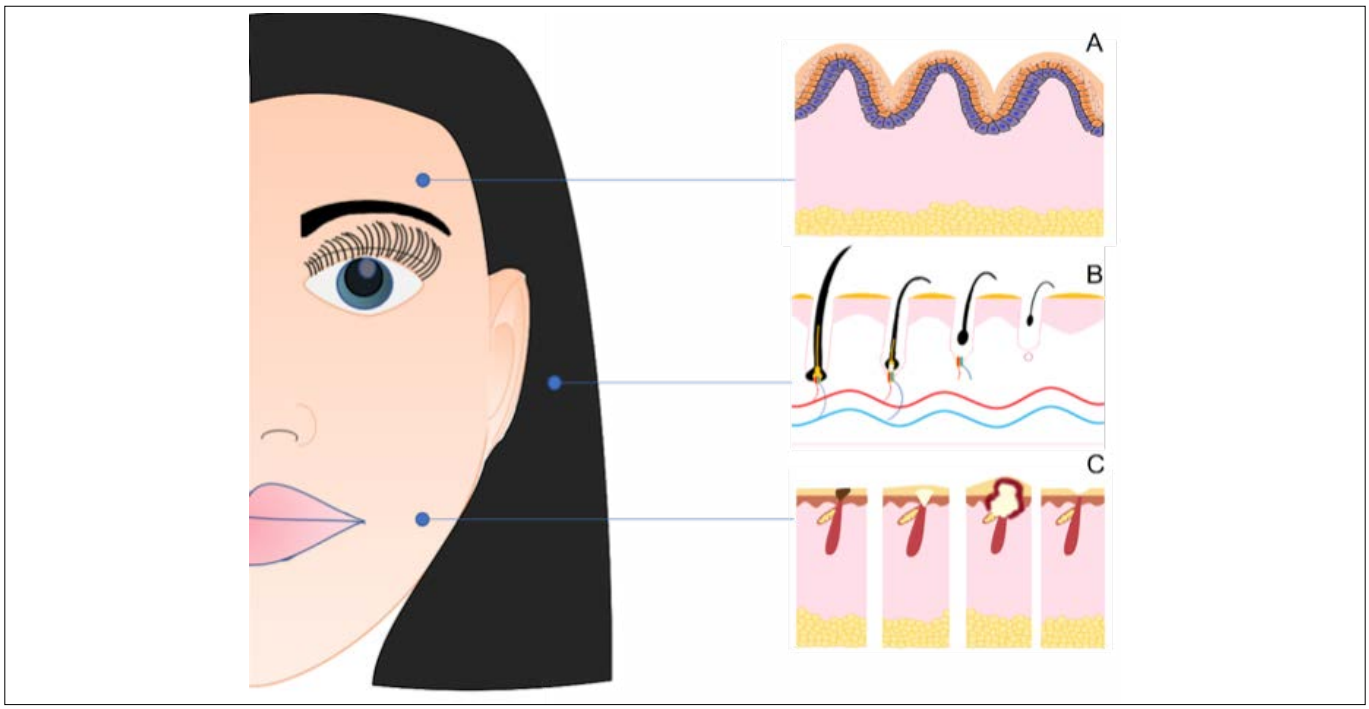

Figure 1 *- Examples of some of the most common aesthetic skin defects. A) represents how aged skin looks like: even if the segmentation of the skin layers does not change, the aged skin shows a thinning of the dermis, epidermis and elastic fibers; there is also degradation of collagen fibers, decrease of the junctions between dermis and epidermis, reduction in the vascularization of tissues and subcutaneous fat, causing a reduction in the elasticity and tone of the skin and leading to wrinkles appearing. In B) it's schematically reported how hair loss occurs: hair growth cycle is divided into 3 phases that are repeated cyclically: anagen (or growth phase), catagen (or regression phase) and telogen (or resting phase). Hair loss occurs when the period of the anagen phase reduces and the telogen phase increases, resulting in a miniaturization of the hair follicle and thinning of the hair. C) portrays how skin appears following the genesis of an acne scar. Pustule that forms gradually from a microcomedo leads to an inflammatory phase, which causes the rupture of the pustule and the attraction to the wound site of the various factors (both growth factors and cells) involved in tissue repair. The formation of the different types of scars occurs because of an overproduction of collagen at the wound site.*

Treatment	Application	Effects	Ref
Dermabrasion	- Facial Rejuvenation - Acne Scars - Scar revision - Facial Rhytids	Re-epithelialization within 7-10 days since dermabrasion. Post-treatment erythema, that usually resolves over time.	18-20
Laser Resurfacing	- Keloids - Hypertrophic Scars - Facial Rhytids - Facial Rejuvenation	IIncreasing in collagen production, decreasing in irregularities and increasing in skin firming; prolonged redness due to dispersed thermal lesions.	20,21
Chemical Peels	- Photoaging - Wrinkles - Acne	Production of collagen type 1 and 4 and elastin fibers, which contribute to the formation of new layers of epidermis; post-treatment is often characterized by inflammation that resolves over time. In the case of acne, reduction of sebum, comedones, pustules or papules.	22-24
Facial Fillers	- Acne Scars - Rhytids - Facial Sculpting / Contouring / Augmentation - Facial Rejuvenation	Depending on the fillers used, there is a different mechanism of action, which however lead to collagen synthesis as a final result, ensuring an improvement in the contours of aged or scarred skin. Side effects including erythema, edema, redness, and bruising can occur in the days post-treatment.	25-28
Micrograft	- Androgenetic alopecia	Micrografts have been shown to improve hair regrowth and density, and hair follicle development. However, further studies are needed to establish it as a safe and side-effect-free therapy.	29-31

***Table 1** - Most popular treatments in aesthetic medicine for skin rejuvenation, scars, wrinkle and androgenetic alopecia treatments; they ensure a smoother and more sculpted skin, improving its texture and contouring.*

Physical signs related to aging or skin lesions can affect a person's self-esteem, having an impact on personal relationships and social dynamics, as they cause discomfort, insecurity, and sometimes psychological problems; thus, people become more and more inclined to appeal to aesthetic treatments to improve or even correct aesthetic defects.
The most common treatments routinely used in aesthetic medicine are summarized in *Table 1*.
More recent approaches are based on regenerative medicine, a branch of medicine given by the set of biomedical approaches and clinical therapies based on the use of stem cells, scaffolds and biological molecules, capable of regenerating, repairing, or replacing parts of tissues or organs that have undergone structural and/or functional damage[32]. Among the different techniques of regenerative medicine, the attention of aesthetic medicine has turned to platelet derivatives, focusing more attentively on Platelet Rich Plasma (PRP), whose therapeutic efficacy is to be ascribed to the in loco administration of a wide range of growth factors and proteins, stored in platelets, able to support tissue repair and the regeneration processes. The administration of autologous PRP improves the skin elasticity and texture, and protects from aging and photoaging, due to the increase in elastin and collagen I expression, and in the proliferation of dermal fibroblasts, supporting the effectiveness of PRP in skin rejuvenation[2,33,34]. PRP has also shown significant effects in the treatment of hair loss since it can stimulate follicular and perifollicular angiogenesis, which is crucial for hair growth; PRP-based therapy has shown promising results in both women and men[35-37], with positive results becoming more evident 3 months after treatment, compared to the following 6-12 months[35].

Finally, the combined therapies based on PRP and Microneedling, or PRP and Fractional CO_2 laser, have been found to be useful for the treatment of acne scars: Microneedling and Fractional CO_2 laser are tecniques that remodel scars, improving their appearance and texture; the combination with PRP shows an improvement in wound healing and post-inflammatory hyperpigmentation, due to the high amount of growth factors it contains[38,39].
The administration of Extracellular vesicles is a further strategy lately taken into consideration to be used in aesthetic and regenerative medicine.

Extracellular Vesicles in Aesthetic and Regenerative Medicine

Extracellular Vesicles (EVs) are spherical particles secreted by all cells and enclosed in a phospholipid bilayer, ranging in size between 40 and 2000 nm; they mediate the intercellular communication in physiological and pathological processes and are generally classified according to their biogenesis and

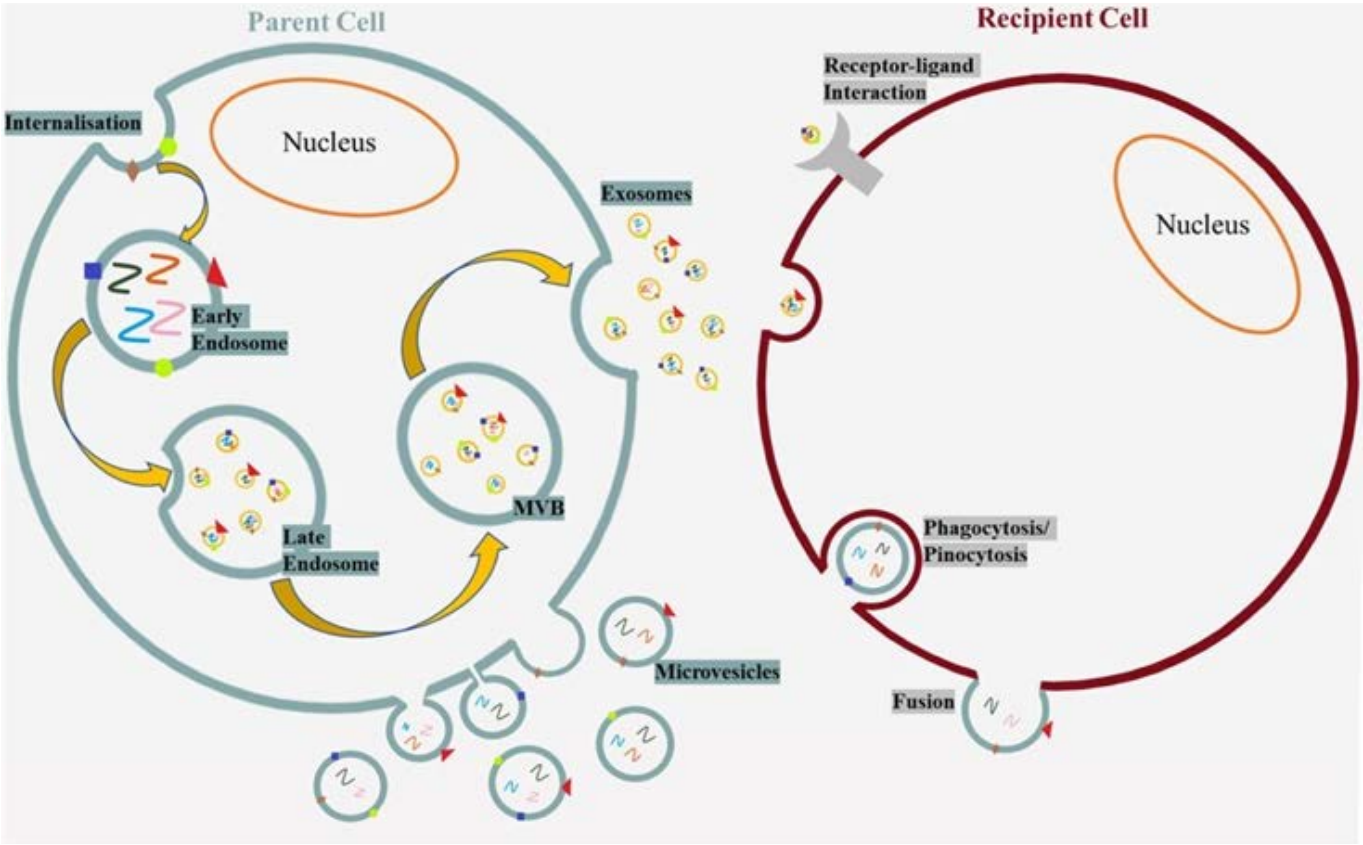

***Figure 2** - Biogenesis of Exosomes and Microvesicles, and EVs-mediated communication. Exosomes derive from the endolysosomal pathways, which involves the formation of "early endosomes" by inward budding of the cell plasma membrane; a further invagination of the endosomal membrane results in the formation of a "late endosome" leading to the generation and the release of intraluminal vesicles (ILVs) within the endosome and the formation of the so-called multivesicular bodies (MVB); the latter will fuse with lysosomes (in this case the contents in MVBs undergo degradation, not shown) or will merge with the cell membrane, releasing the ILVs, that make up the exosomes once outside the cells. The microvesicles, on the other hand, are released into the extracellular space by outward budding of the cell plasma membrane towards the extracellular space. Both exosomes and microvesicles, to carry out their role in communication, once released from the donor cell, interact with the recipient cells through various processes: phagocytosis/pinocytosis, fusion, and ligand/receptor or clathrin-mediated interaction. When this phase is finished, they can release their cargo inside the host cell, going on to perform their functions.*

size into exosomes (40-120 nm), microvesicles (50-1000 nm) and apoptotic bodies (500-2000 nm)[40] (*Fig. 2*).
While the generation of exosomes involves the endolysosomal pathways, microvesicles are formed by the outward budding of the cell membrane. Lastly, apoptotic bodies are formed from the cell surface by cytoskeletal rearrangement, through the extroflection of the membrane of apoptotic cells[40,41]; they are less frequently involved in intercellular communications, which is why, generally, only exosomes and microvesicles are referred to[42,43].
The discovery of EVs can be traced back to the first studies on blood coagulation: initially, they were observed in 1946 by Chargaff and West in plasma, as procoagulant particles of platelet origin. Later, in 1967, Wolf isolated and characterized this material from blood samples defining it as "platelet dust"[44,45]. These studies were followed by others, which led to the isolation of EVs from a variety of cell types and biological fluids and the understanding that they are not, as was initially thought, the cells' waste material, but they can rather be considered as structures used by the cell as a communication mechanism (*Figure 2*), in physiological and pathological processes[45]. No less important is their involvement in the maintenance of homeostasis, angiogenesis, coagulation, inflammation and related response, and in the management of cellular waste[44,46-49]. Therefore, given their importance in cell biology, they are important targets for the development of new non-invasive and low-toxicity therapies.
EVs-based therapies are gaining more and more attention in different fields of medicine EVs, such as regenerative medicine. From several years now, stem cell-derived EVs (SC-EVs) have been studied for their potential application in the treatment of several tissue damages, such as organ-specific injuries, brain disorders, diabetes. Mesenchymal Stem Cells (MSCs) and Adipose Stem Cells (ASCs) are the most used sources of EVs; several studies have shown how EVs derived from the latter are involved, alone or in conjunction with other bioactive molecules or biomaterials, in a variety of processes related to tissue regeneration[50-52]. In this context, among the most novel studies, Yang et al. showed how the administration of human umbilical cord-derived exosomes (hUCMSC) encapsulated in a hydrogel to an induced skin wound in a diabetic rat leads to an upregulation of growth factors – vascular endothelial growth factor (VEGF) and transforming growth factor beta-1 (TGFβ-1) -, and therefore to an acceleration in wound healing[53]. Zhang et al. instead revealed that uMSC-derived exosomes combined with a hydrogel and applied to the site of a femoral fracture in a mouse model are able to improve bone healing by stimulating angiogenesis. The same group demonstrated

in vitro that these exosomes are able to increase the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1α (HIF-1α), and contribute to the proliferation, migration and formation of tubes in endothelial cells[54]. More recently the same author has shown in his studies how the pre-treatment with ASCs-derived EVs are effective against hepatic damage from ischemia-reperfusion, as the EVs are able to induce an increase in superoxide dismutase and a reduction in inflammatory mediators such as IL-1β and TNF-α, protecting against mitochondrial damage, apoptosis and hepatocytes from injury[55]. No less important are the studies of Chen et al. on lesions of peripheral nerves, with which they demonstrated *in vitro* that ASCs- derived EVs, internalized by Schwann cells, can promote the proliferation, migration and myelination of axons and the secretion of trophic factors, accelerating the regeneration of peripheral nerves[56].
More recently, moreover, the EVs use has also extended to the novel field of aesthetic medicine. In the skin, indeed, EVs-mediated communication helps restoring normal cellular homeostasis, playing a key role in the four stages that make up the wound healing process[57,58]; in addition, several studies have shown that EVs help restoring damages due to skin degeneration, caused by processes such as aging, skin diseases, hair loss and the generation of scars[6,7,59]. The most used sources of EVs to this purpose are represented by stem cells (particularly mesenchymal stem cells (MSCs)), plants, and platelet derivatives[6,8,60-62].

EVs and Aging

Several *in vitro* and animal model studies have demonstrated the great potential of EVs in the treatment of aging and photo-aging. The administration of MSCs-derived EVs is among the most studied applications. For example, in a study conducted on mouse models, Wang et al. have shown how engineered EVs derived from the human umbilical cord MSCs (hucMSCs) can promote the proliferation and migration of human dermal fibroblasts (HDFs) and increase the expression of ECM proteins, including collagen, elastin, and fibronectin[63]. On the other hand, Zhang showed that the combined administration to derma of EVs deriving from hucMSCs with Sponge Spicules increases the absorption of the EVs themselves in the mouse skin, improving the appearance of wrinkles, UV-induced photo-aging, and the expression of constituents of the ECM[64]. Zhao et al. investigated the effect of subcutaneous administration of the human placenta MSCs (hPMSCs)-derived EVs incorporated into a chitosan hydrogel on naturally aging mice; the chitosan hydrogel allowed a slow and prolonged release of the EVs, contributing a prolonged therapeutic effect over time. The authors demonstrated an improvement in the skin of aged mice, assuming that the effect is due to a reduction in the expression of MMPs and an increase in the expression of TIMP, as well as to a regeneration of the ECM in aged dermal fibroblasts[65].
In addition to MSCs, other sources of stem cells have been investigated as EV as a resource for the treatment of aged skin. For example, Oh et al. studied the *in vitro* effect of EVs derived from induced pluripotent human stem cells (iPSCs) on HDF naturally aged or UV-treated to induce photo-aging. Their results showed that iPSCs-derived EVs administrated to senescent HDFs increased the collagen production and, at the same time, decreased the production of senescence-associated β-Galactosidase and MMP1, MMP3 (MMPs, that have been found to be upregulated in aged fibroblasts, quicken the degradation of skin matrix, impairing the skin's innate regenerative ability)[66]. Similarly, Choi et al. demonstrated that EVs derived from the human adipose stem cells (HASC) ameliorates *in vitro* induced photo-aging on HDF by increasing levels of elastin, collagen I, II, III, and V, TIMP-1 (which inhibits MMPs), and Transforming Growth Factor-β (TGF-β) involved in the synthesis of the ECM[67].
Finally, Hu et al. demonstrated how exosomes derived from 3D HDF models provide an up-regulation of collagen I and TGF-β levels and a down-regulation of MMP-1 when administered in nude mice in which photo-aging had been induced with UV rays[68].

EVs and Hair growth

EVs have been evaluated for their involvement in the different phases of hair production, particularly since Wnt molecules, involved in hair follicle morphogenesis and growth, were found in the EVs cargo[69]. In this regard, Rajendran et al. have shown that MSCs-derived EVs can induce an *in vitro* increase in IGF-1 and VEGF levels in the cells of the dermal papilla (DP) and in the expression levels of the anti-apoptotic factor Bcl-2, which activates the pathway of MapK and Erk cell signaling; in vivo, on the other hand, the intradermal injection of MSCs-derived EVs into mice is able to induce the hair growth by increasing the levels of Wnt3 and Wnt5[70]. Similarly, they showed that also EVs derived from dermal fibroblasts can activate in vitro Wnt/β-catenin signaling pathways and induce proliferation of DP cells, demonstrating their pro-proliferative capacity in hair follicle cultures[71]. Moreover, the same group studied macrophages as an alternative source of EVs, capable of stimulating DP cells, confirming the results already obtained *in vitro* with the previously described studies, demonstrating that the Wnt contained in the EVs derived from macrophages can stimulate the Wnt/β-catenin signaling pathways and increase Bcl-2 levels, thus suggesting the therapeutic potential of these sources of EVs in the treatment of hair loss[72].
If, on the one hand, the effect on hair growth has been evaluated by the direct administration of the EVs, on the other hand studies have been conducted using microgels for the administration of EVs: Chen et al., for example, focused their attention on the administration of EVs derived from human DP cell cultures, encapsulated in oxidized sodium alginate (OSA) and its related effects. OSA ensures the prolonged release of EVs, which up-regulate the expression levels of Wnt3, b-catenin, and MMP-3, resulting in the promotion of hair growth[73].
Finally, Hu et al. isolated EVs from DP cells from 3D cultures and tested them *in vitro* and on mouse models to evaluate hair follicle production versus the only DP cell administration. They demonstrated that EVs isolated from 3D cultures induce greater hair growth, compared to control EVs isolated from 2D cultures, also because of the higher levels of β-catenin expression and the down-regulation of inhibitors of the Wnt factors,

resulting from their administration. These results are because EVs from 3D cultures contained higher levels of miR-218-5p, which is essential for the development of hair follicles and, consequently, for hair growth[74].

EVs and Scars

The role of EVs in wound healing processes is well known since they are important for communication in all healing phases; the main sources of EVs in this context are represented by neutrophils, macrophages, platelets, and MSCs. Therefore, EVs have been considered as potential non-toxic therapeutic agents for the treatment of skin wounds, and in particular for the treatment of scars[57,75].

Zhu et al. described how the administration of EVs derived from human adipose stem cells (hASCs) prevented the differentiation of myofibroblasts and the synthesis of collagen I, and thus the formation of scars in rabbits in which a wound had been produced[76].

Furthermore, in vivo, the application of a gel consisting of exosomes derived from hASC after laser treatment, showed a remarkable improvement in the healing of acne scars and accelerated healing, with a decrease of post-treatment side effects, such as edema, pain, dryness, or erythema, compared to treatments carried out with the administration of a gel without exosomes[77].

Despite some of the studies that demonstrate the therapeutic potential of EVs in tissue repair, it has also been shown that their efficacy is sometimes limited due to the reduced half-life and to the rapid clearance of these particles[78-81], forcing to multiple administrations for treatment. An alternative to this problem is given by the administration, in situ or subcutaneously, of hydrogels in which the EVs are encapsulated, ensuring a prolonged release over time and consequently greater efficacy[82].

Plant derived EVs

Even if most sources of EVs are human-derived, in recent decades the isolation and use of plant-derived EVs has become increasingly widespread, as they have different therapeutic properties. Despite their discovery dates back to 1967[83], only more recently plant-derived EVs are gaining medical relevance. In plants the EVs play two fundamental roles: i) defense against pathogens; ii) regulation of communication and nutrient exchange in plant-microbial symbiosis processes[84,85]. Studies conducted in animal models have shown that the EVs of grapes, strawberries, broccoli, grapefruit, ginger, carrot and orange, have mainly antioxidant and anti-inflammatory activities for different types of human pathologies[86,87]. Furthermore, studies conducted on citrus fruits have shown that the EVs derived from them have anti-neoplastic activities in different types of tumors, although the mechanisms of action are still uncertain[88-90].

In the dermatological field there is still a limited number of studies concerning plant-derived EVs for therapeutic purposes. Şahin et al. demonstrated *in vitro* how the exosomes derived from wheat contribute positively to the healing processes of skin wounds: the tests showed how exosomes of wheat increase the expression and production of collagen I, and the proliferation and migration of fibroblasts in wound healing. They also have a pro-angiogenic effect, due to the increase in the formation of tubular structures *in vitro*[91]. Moreover, Cho demonstrated *in vitro* how EVs derived from ginseng roots have positive effects on senescent fibroblasts of the human dermis, as they are capable of downregulating the activity of β-gal associated with senescence[92].

Conclusion

This review aimed to provide a general overview of the latest findings on EVs supporting the potential application as new potential therapeutic agents in the field of aesthetic medicine, and particularly in rejuvenation, photo-aging, scarring and hair loss. Although it has been established that EVs are promising, non-invasive tools for the treatment of aesthetic defects, as they show good therapeutic efficacy, further studies are needed to clarify their mechanisms of action and improve the degree of purification and isolation.

Above all, it is needed to clarify whether their administration can lead, in the long term, to the onset of any side effects or adverse events, which can affect the patient's health.

Conflict of interest disclosure

The Authors declare that they have no conflict of interest.

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aesthetic medicine

Review

Systematic review of the literature on the properties, quality and reliability of calcium hydroxyapatite: results of an Italian experts' meeting

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Abstract

Background: the aging process of the face is linked to several factors and is evident in all layers of the facial massif and especially in the superficial tissues.
Aging affects the three-dimensional structure of all facial tissues: bones, muscles, ligaments, adipose tissue and skin. In the last years there has been a tendency to personalize the treatments to make them minimally invasive for every individual patient. Calcium hydroxyapatite (Radiesse®) is a device that can be used on the entire face and in some body areas, leading to improved outcomes and is more safe than other devices. A consensus of Italian experts wanted to carry out a systematic review of the literature to confirm the hypothesis.
Methods: According to the recommendations of PRISMA30 (Preferred Reporting Items for Systematic Review and Meta-Analysis), MEDLINE, EMBASE and COCHRANE databases were explored (up to February 4th, 2021) and the PICO framework (problem = skin aging; intervention = calcium hydroxylapatite; comparator = any comparator; outcome = efficacy/effectiveness reflected in filling and fibroblast stimulation and safety) was used to design searches and define the eligibility of studies/papers for inclusion.
the opinion of The Italian experts is reported based on their clinical experience and is used to describe similarities or differences versus the international publications selected.
Results: At the end of the selection process (*Tab. 1*), the quality assessment was performed on 29 articles, 15 reviews and 14 original articles.
Conclusion: following the results of a systematic review and the experience of Italian clinicians, Radiesse® can be considered the first choice to improve skin quality
In fact, it demonstrated to have documented, proven outcomes as a skin biostimulator, for the contouring redefinition of the face and for a lifting effect.

Keywords

Calcium hydroxyapatite, Radiesse®, Dermal filler, Facial aging, Facial rejuvenation, Collagen stimulation

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Introduction

The Ageing processes can be schematically divided into two main aspects: tissues laxity and tissues loss of volumes. These aspects impact both facial tissues and the body in general. Regarding the first aspect, alterations in the activity of fibroblasts and the quality of collagen fibers lead to a progressive loss of tissues tension and elasticity. Skin becomes 0.3% thinner every year[1], as well as the epidermis, showing changes in the shape and size of basal cells[2], the dermis loosens over time[3], and aged fibroblasts show a decrease in the proliferation and synthesis of type I collagen, when compared with young ones[4]. The Loss of volumes involve both bone resorption and the atrophy of fat pads. The Repetitive action of facial muscles, together with these two aspects, contributes to the formation of new rhytides and to the deepening of pre-existing wrinkles.

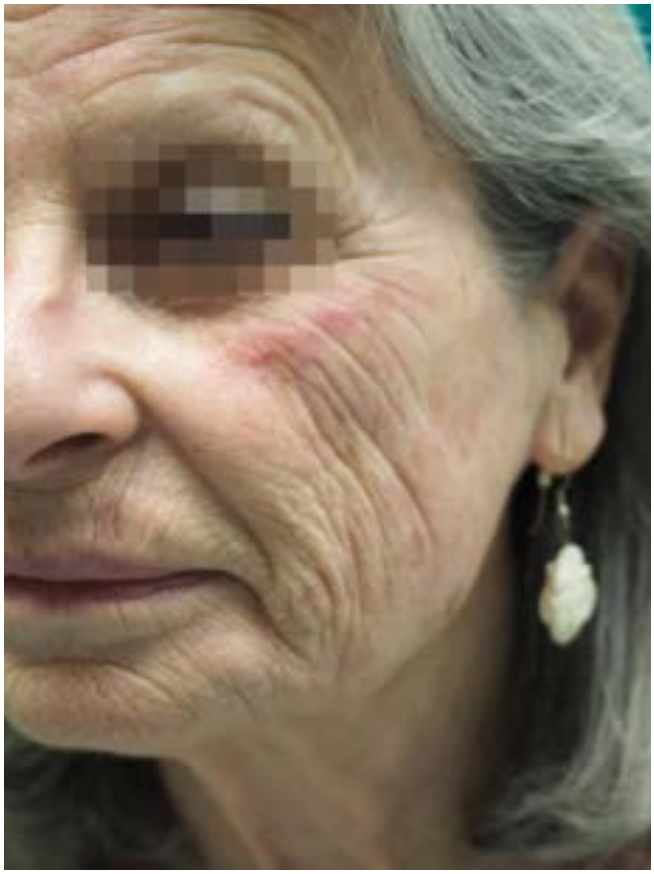

Figure 1: *The skin aging. 72- year-old woman with a significant face ageing. She presents a notable loss in the zygomatic malar area, a visible lack of face contouring and a clear skin atrophy.*

An effective rejuvenation procedure should target all the aspects mentioned above to obtain an effective result: skin quality, tension and tightening should be improved along with the restoration of soft tissue volumes.
In the past decades, dermal fillers have become more and more commonly used, as a valid alternative to surgery, in the correction of facial soft tissue volume and skin quality[3,5]. The ability of soft tissue fillers to act both as volumizers and skin bio-stimulators, together with the practicality of these treatments in an outpatient context has led to an increased interest among patients seeking facial rejuvenation[5,6]. While the initial approach targeted the correction of single wrinkles or defects, modern practices follow a 3-dimensional approach[5].
A wide variety of products has been developed through time and has been approved by the FDA, ranging from hyaluronic acid to poly-L-lactic acid, calcium hydroxylapatite and polymethylmethacrylate[2,3,6,7].
The ideal soft-tissue filler should be safe, biocompatible, stable after implantation, nonmigratory, and resistant to phagocytosis. It should persist and maintain its volume through time. It should induce minimal foreign body reactions, including the formation of granuloma. Moreover, it should be nonteratogenic, noncarcinogenic, noninfectious, and nonallergenic[2,3,6,8].
Radiesse® (Merz, Frankfurt, Germany) is a synthetic, GEL filler composed of 30% calcium hydroxylapatite (CaHA) and 70% carrier gel. It was first approved in 2006 for the treatment of facial wrinkles and folds, as well as HIV-associated facial atrophy and then later approved, in 2009, for more aesthetic indications.
It is composed of smooth regular synthetic microspheres composed of 30% calcium hydroxylapatite (ranging 25-45 µm in diameter) suspended in a gel of glycerin and carboxymethylcellulose[3,6]. Differently from other dermal fillers, Radiesse® acts not only by filling target tissues but also by stimulating the activity of fibroblasts, promoting endogenous collagen type I fibers synthesis and the activity of fibroblasts and periosteal cells[3-6]. While the filling effect lasts around 3 months, the inflammatory reaction and bio-stimulating effect can last up to 24 months[3-6]. The 2015 approval of CaHA with integral 0.3% lidocaine hydrochloride allowed to increase the patients' comfort during treatment, without affecting the rheological properties of the product[5]. Radiesse® on-label treatment areas include, naso-labial folds, marionette lines, the periorbital region, the medial and lateral cheek, mid face, mandibular angle, chin, jawline, and rejuvenation of the dorsal face of the hands[3,8]. In this article Radiesse®'s degree of effectiveness and safety has been analyzed by performing a systematic qualitative review of the literature.

Materials and methods

Search strategy and eligibility criteria

According to the recommendations of PRISMA30 (Preferred Reporting Items for Systematic Review and Meta-Analysis), MEDLINE, EMBASE and COCHRANE databases were explored (up to February 4th, 2021) and the PICO framework (problem = skin aging; intervention = calcium hydroxylapatite; comparator = any comparator; outcome = efficacy/effectiveness reflected in filling and fibroblast stimulation and safety) was used to design searches and define the eligibility of the studies for their inclusion in the piece (*Fig 2*).
We retrieved systematic literature reviews, clinical trials, observational studies, and studies using animal models, in vitro or ex-vivo experiments.
The records were excluded in case of: (i) no relevant information reported about the efficacy/effectiveness

and safety of calcium hydroxylapatite; (ii) case series (for trials or observational studies only papers with at least 40-50 individuals); (iii) languages other than English; (iv) letters to the editor, commentaries, abstract-only, case reports, and not published trials.
A second selection was further performed by fully reading the manuscripts of the previously selected references and each step of this review process was graphically represented using a PRISMA diagram. Articles grouped in the "poor content" exclusion group are articles which showed irrelevant or obvious information or articles where the content and scientific method was not considered fully clear and coherent by the Authors.
The review selection was independently performed by two reviewers (X and Y), whereas potential discrepancies were solved through a discussion with a third party expert (Z). A critical review of the selected literature was finally performed.

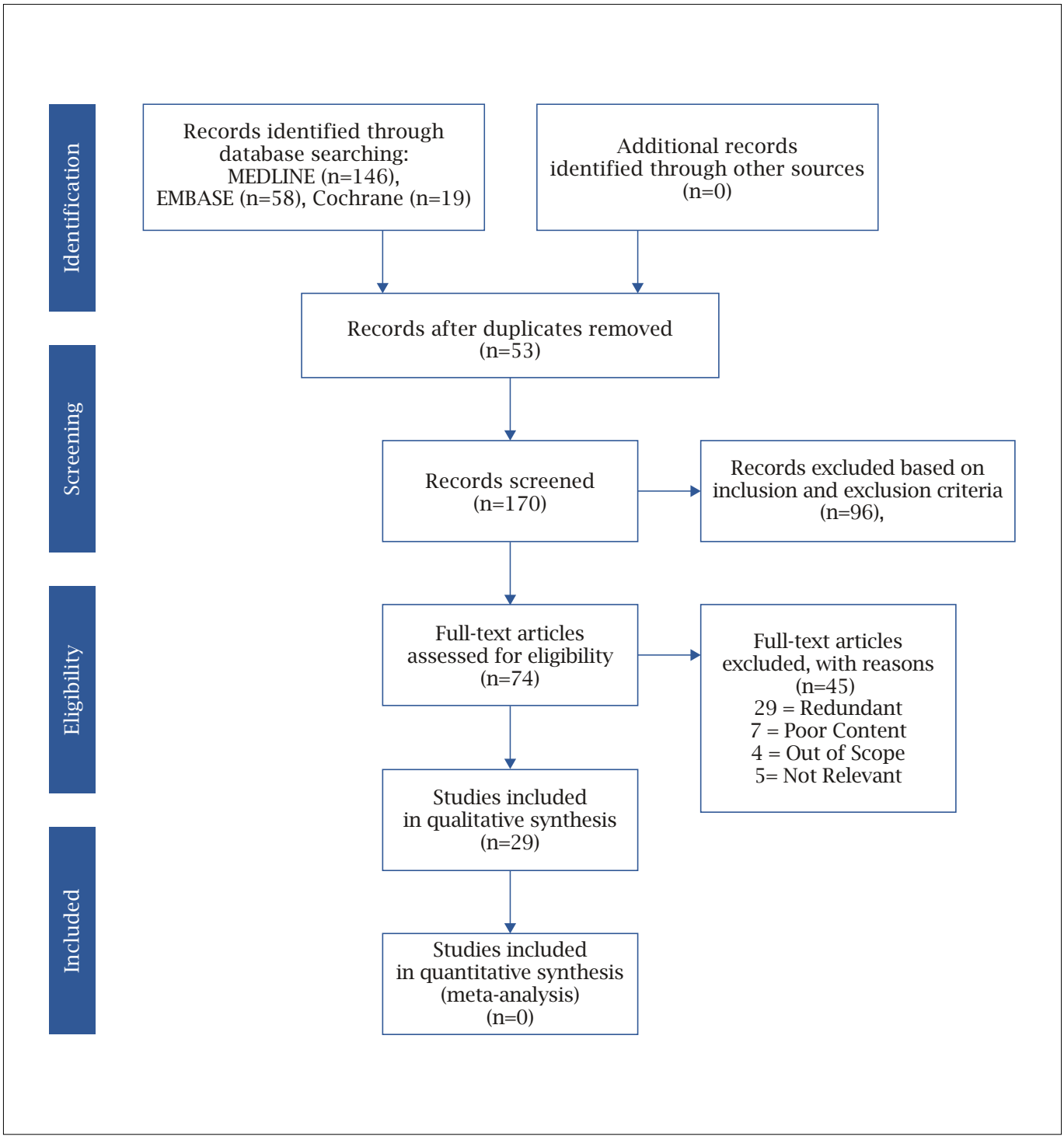

Figure 2: *PRISMA 2009 Flow Diagram.*

Results

At the end of the selection process (*Table 1*) the quality assessment was performed on 29 articles, 15 reviews and 14 original articles.

The main characteristics of the manuscripts are shown in *table 1*. We classified each study to expand the following topics: product with biomechanical properties and its formulations, on label areas and treatment, off label areas and treatment, effectiveness, and safety.

Author	Year	Type of publication	Topic
P.P. Rovatti et al.	2020	Original article	Off label treatments, effectiveness
R. Bartsch et al	2020	Original article	Off label treatments, effectiveness
D.J. Goldberg et al.	2018	Original article	On label treatments, off label treatments, effectiveness, safety
M.H. Graivier et al.	2018	Original article	On label treatments, effectiveness, safety
Z. Paul Lorenc et al.	2018	Original article	Material properties
K. Goldie et al.	2018	Review	Off label treatments, effectiveness
J.A. Kadouch	2017	Review	Safety
K. Frank et al.	2017	Original article	On label treatments, safety, effectiveness
H.M. Rayess	2017	Review	Safety
Y.A. Yutskovskaya et a.	2017	Original article	Off label treatments, effectiveness, material properties
R. Fathi et al.	2016	Review	On label treatments, effectiveness
Xiao-hua Shi	2016	Review	Safety
S. Fabi et al.	2016	Original article	Off label treatments, effectiveness
C. Courderot-Masuyer	2016	Original article	Material properties, effectiveness
A. Breithaupt et al.	2015	Original article	Material properties, effectiveness
Natalie Huang Attenello et al.	2015	Review	Material properties
J. Jagdeo et al.	2015	Review	On label treatments, material properties, effectiveness
Christine S Ahn et al.	2014	Review	Material properties
J. Emer et al.	2013	Review	On label treatments, off label treatments, effectiveness, safety
J.M. Dallara et al.	2013	Original article	On label treatments, effectiveness
T.M. Greco et al.	2012	Review	On label treatments, material properties, effectiveness
L.S. Bass et al.	2010	Original article	On label treatments, effectiveness, safety
O. Hevia	2009	Review	Off label treatments, effectiveness, safety
J. Kim	2008	Original article	Off label treatments
T.L. Tzikas	2008	Review	On label treatments, off label treatments, material properties, safety
M.H. Graivier et al.	2007	Review	On label treatments, effectiveness
M.S. Ahn	2007	Original article	On label treatments, off label treatments, effectiveness
Kevin W. Broder et al.	2006	Original article	Material properties, effectiveness
D.A. Jansen	2005	Original article	On label treatments, effectiveness

Table 1 *- Features of each study, divided per topic: effectiveness, material properties, on label treatment, off label treatment and safety.*

Matherial Properties

Radiesse® is a synthetic semisolid filler composed of 30% calcium hydroxylapatite (CaHA) and 70% Carboxymethylcellulose (CMC) carrier gel. Its composition differentiates it from other FDA approved fillers and explains its properties and effectiveness in both filling and bio-stimulating tissues. The filling effect lasts around 1-3 months and is mainly due to the carrier gel. The remaining effect is mediated by CaHA microspheres which elicit an inflammatory reaction, characterized by giant cells and the migration of macrophages, the activation of fibroblasts and collagen deposition[3,5,6].
Being CaHa a mineral component of the bone, it should not activate any immune response. The tissue reaction is clinically evident in terms of a gradual and improving filling and liftign of the tissue that lasts up to 14 months[26]. The filler is completely reabsorbable and marketed as 1,5 ml vials.
CaHA has a higher G1 and viscosity than other commercially available HA fillers. Its rheological properties make it effective in providing a lift, support, and volume to soft tissues, and making it highly suitable for many areas of the face except the lips and glabella.
In vitro studies have shown that CaHA, beyond promoting the deposition of new collagen fibers, allows fibroblasts to develop higher contractile forces when compared with non-treated fibroblasts[4] Radiesse®) and lidocaine.Patient discomfort and pain could be challenging during treatments with injectable and CaHA, especially when a full-face correction had to be performed.
In 2009 Radiesse® was approved in its premixed formulation with lidocaine 0,3% (one 1.5 ml syringe), easing the treatment and making it more practical. The premixed formulation has shown effectiveness in increasing patient comfort and decrease the pain perceived by the patient during the procedure[10].

The Italian clinical experience on CaHA is mainly focused on the use of the formulation without premixed lidocaine.
Anyhow, it is preferred to proceed with a tailored approach focused on the clinical experience and the obtained results.
Here are the recommendations suggested by the group of experts:

- 1.5 ml CaHa without dilution to increase volume (angle and edge mandibular, nasolabial folds, cheek bone, temple, chin)

- 1.5 ml diluted CaHa 1:1 – pre-diluted 1:3 - with saline solution in addition to 0.5 ml of lidocaine, depending on the treatment areas and the objectives that need to be reached.

- 3 different depths of infiltrations:
· supraperiosteal (pure)
· in the subcutaneous tissue with 1:1 dilution
· in the subdermal layer with 1:3 dilution

On label Areas

Radiesse® was firstly approved in 2006 for the treatment of facial wrinkles and folds, as well as HIV-associated facial atrophy[17,23,26]. In 2009 it was approved also for more cosmetic applications, and more recently, in 2015, it has been approved for dorsal hand augmentation[9].

Midface (ZIGOMATIC AREA) and Cheeks

Radiesse® is highly effective when employed for the midface and cheeks[2,9,23,26,27]. No over correction is needed, the proportion between the material employed and the result obtained is 1:1. The injection can be made with either a needle or cannula.The Material should be distributed evenly to avoid the formation of palpable nodules. The highly lifting effect given by CaHA, when injected on a deep plane in the upper cheeks, may be sufficient to contextually correct nasolabial folds[9].
It is then advisable, when approaching a full-face treatment, to start with this area, since, due to the lifting forces created, defects of the lower third or of the nasolabial folds may be indirectly corrected with little or no need of correcting them directly.

ZIGOMATIC AREA

The decision of Italian experts to treat THE ZIGOMATIC AREA is based on THE FOLLOWING USE:

1.5 ML CaHA pure (these indications are suggested with Radiesse® premixed with lidocaine)

CANNULA 25 G 50 mm and/or needle 27 G 20 mm

INJECTION PLAN: supraperiosteal and/or subcutaneous

TREATMENT OBJECTIVE: volume

CHEECKS

The decision of Italian experts to treat cheeks is based on the following use:

1.5 ml di CaHA diluted 1:1 with saline solution

CANNULA 25G 50 mm

INJECTION PLAN: subcutaneous

TREATMENT OBJECTIVE: filling

1.5 ml di CaHA diluted 1:3 with saline solution

INJECTION PLAN: subdermal

TREATMENT OBJECTIVE: restructuring

Jawline and chin

Radiesse® is widely used for jawline and chin augmentation, both for aesthetic reasons and for the correction of asymmetries or hypognatism[2,9,10,23].
When treating these areas, the product should be

injected on a deep supraperiosteal plan. The product can be injected both with a needle and with a cannula, depending on the distribution technique and the treated area[2,9,10,26].

JAWLINE
The decision of Italian experts to treat the jawline is based on the following use: 1.5 ml di CaHA pure NEEDLE 27 G 20 mm CANNULA 25 G 50 mm INJECTION PLAN: subcutaneous and/or supraperiosteal TREATMENT OBJECTIVE: contouring redefinition

Nasolabial folds, oral commissure and marionette lines

Correction of nasolabial folds, oral commissure, and marionette lines with Radiesse® can be made with either needle or cannula[2,9,10,20,23,26,27,28].
The product should be injected in the subdermal plan and distributed evenly with a slight fanning pattern to avoid nodules formation.
Several publications have focused on the treatment of the nasolabial folds[2,3,4,8,9,21,28]: interestingly patients have shown to be more satisfied with the CaHA outcome compared with HA outcome in treating nasolabial folds[21,28] both in terms of duration of aesthetic outcome and satisfaction with the result.

NASOLABIAL FOLDS, ORAL COMMISSURE AND MARIONETTE LINES
The usage of CaHA in these areas is suggested in minimal quantities (not more then 0,22 cc each side): 1.5 ml di CaHA pure CANNULA 25 g 50 mm and/or needle 27 G 20 mm INJECTION PLAN: subdermal TREATMENT OBJECTIVE: line lifting

Hands

Radiesse® has been approved for hand rejuvenation in 2015. The advantage of CaHA in treating the hand dorsum lies in its rheological properties that make it durable through time, despite the high degree of mechanical stress and mobility of the hands11. When treating hands, Radiesse® should be diluted 1:1 or 2:1 with lidocaine 1%, depending on the degree of correction needed; a dilution inferior to 2:1 is not recommended14. The Injection should be made with a cannula rather than needle, and with a proximal to distal fanning technique[29]. Alternative treatments are HA, poly-L-lactic acid or fat graft injections. The advantages shown by CaHA, in comparison with HA, resides in a longer lasting result, a lower risk of migration and no risk of the Tyndall effect[11]. When compared with fat graft, the Radiesse® treatment is more comfortable and practical, being feasible on an outpatient practice and the result is predictable11. Differently from poly-L-Lactic acid, Radiesse® is FDA approved[11].

HANDS
The decision of Italian experts to treat the HANDS is based on the following use: 1.5 ml di CaHA diluted 1:1 CANNULA 25 G 50 mm INJECTION PLAN: subdermal TREATMENT OBJECTIVE: Rejuvenation of hands and camouflage of the veins.

Off Label Areas

The use of Radiesse® in off label areas has increased through time, due to the widening of its employment and due to the growing experience of physicians. Along with the previously described on label areas and treatments, Radiesse® is currently employed, alone or combined with other mini-invasive procedures, to correct skin quality, improving the skin tone and tightening. As previously described, CaHA has a strong action and effect both in filling volumes and in stimulating fibroblasts and the deposition of new collagen. It is this second aspect that has driven practitioners gradually towards new skin quality-oriented techniques based on CaHA injections.

Forehead and temple

Forehead and temple augmentation have been disregarded for a long time but have gained more attention in recent years.
Forehead augmentation can be achieved effectively with CaHA injected deep on a supraperiosteal plan; when compared with HA, Radiesse® gives more predictable and effective results and a lower risk of vascular compromise in this area[12]. Tumescent anesthesia performed before treatment in this region allows to lower the level of the patient's discomfort and pain and the risk of a vascular compromise[22].

TEMPLES
The decision of Italian experts to treat the TEMPLES is based on the following use: 1.5 ml di CaHA diluted 1:1 CANNULA: 25G 50 mm and/or 27 G 20 mm INJECTION PLAN: subdermal, supraperiosteal TREATMENT OBJECTIVE: filling

FRONT
The decision of Italian experts to treat the front; the suggestion of small quantities (maximum 0.2 ml each part) 1.5 ml di CaHA pure CANNULA 25 G 50 mm INJECTION PLAN: supraperiosteal TREATMENT OBJECTIVE: increase of volume

Infraorbital region

The Infraorbital region is one of the most delicate areas to treat, when it comes to dermal fillers.
CaHA can be used alone or in combination with HA fillers. It has been employed to treat tear trough defects or eye circles, injecting the material on a deep supraperiosteal plan, and employing a different dilution with lidocaine, depending on the treated subunit[2,16].

INFRAORBITAL REGION
This infraorbital region, in the Italian experience, should be treated with high caution, only by clinicians who have experience with the product. Max 0,1 cc each side. 1,5 ml of pure CaHA CANNULA 25 G 50 mm INJECTION PLAN: supraperiosteal TREATMENT OBJECTIVE: depth reduction of lacrimal sulcus

Neck and decolletage

Radiesse® has recently shown great effectiveness in skin rejuvenation[18,19,24]. The bio-stimulating fibroblast activating properties have encouraged the use of diluted or hyper-diluted CaHA in the lower face and neck areas, to improve skin quality, obtaining satisfying results[18,19,24].
The Immunohistochemical examination of tissues treated with CaHA has shown a significant increase in collagen I production, neo angiogenesis and neo elastogenesis when compared with the baseline[19]. Radiesse® has also proven to be effective in the correction of acne scars[9,20].

NECK AND DECOLLETAGE
Italian experts' decision to treat these areas is based on the following use 1.5 ml CaHA hyperdiluted 1:3 CANNULA: 27 G 38 mm (décolletage) 25 G 50 mm INJECTION PLAN: subdermal TREATMENT OBJECTIVE: bio restructuration

Skin rejuvenation and tightening: body

More and more studies are focusing on CaHA bio-stimulating properties for skin quality and tightening, applied to body districts other than the face, including the buttock area, upper arms, neck and décolletage and abdomen[1,24,25]. A recent study has shown the efficacy of combining concomitant micro-focused ultrasound (MFU) and calcium hydroxylapatite injections, followed by a tissue stabilized guided subcision, for the improvement of skin laxity and skin dimpling in the buttock and thigh area1. A combined aesthetic approach using MFU, CaHA and botulinum toxin in correcting skin laxity, quality and tone has been described in another study, reporting encouraging results also in the treatment of the abdominal area and upper arms[24].

ARMS - KNEES - ABDOMEN
peri-umbelical injection -1.5 ml CaHA hyperdiluted 1:3 - arms - 1:2 - abdomen, arms, knees CANNULA: 25G 50 mm INJECTION PLAN: subdermal TREATMENT OBJECTIVE: bio restructuration

Clinical cases from real life italian experience

FULL FACE

Figure 3 *- Treatment objective: retonification. Treatment: 1,5 ml Radiesse - dilution 1:1. Cannula 27G 38 mm. 3 syringes of 1,5 ml Radiesse +0,3 lidocaine + 1,2 saline solution. Total volume used 6 ml in 3 rounds. MELFA FABRIZIO.*

Figure 4 *- CHEEKS - Treatment objective: rejunetation of skin face. Treatment schedule: PRE: 2 vials 1,5 ml. POST 1 month: left side (commissure, chin and mandibular border up to jowl 1,5 ml pure + zygomatic region 0,5 ml pure and 1 ml iperdiluted in the chin. 3) post 3 months from the first treatment. a total of 1 vial /1,5 ml; 0,5 ml pure in zigomatic region and 1,0 ml iperdiluted in the chin. 4) post 6 months: same treatment – pure 0,5 ml in the cheekbone. ROVATTI PIERPAOLO.*

Figure 5 *- CHEEKS - Treatment objective: Rejuvenation. Treatment: 1,5 ml Radiesse® - iperdiluted. Total volume used 3 ml (0,5 ml/each CHEEK). ROVATTI PIERPAOLO.*

Figure 6 *- MALAR AREA - Treatment objective: redifinition. Needle e 25G - dermo injection in the adipose superficial derma Cannula da 25 G. Treatment: 1,5 ml Radiesse® +0,5 ml lidocaine. Total volume used 2 ml (1 ml / side). RENZI MASSIMO.*

Figure 7 *- CHIN - Treatment objective: redifinition. Treatment: 1,5 ml Radiesse® - dilution 1:4. Needle 27G bolo technique. 1 central. 2 lateral. 1 syringe of 1,5 ml Radiesse + 0,5 ml lidocaine . Total volume used 2 ml. RENZI MASSIMO.*

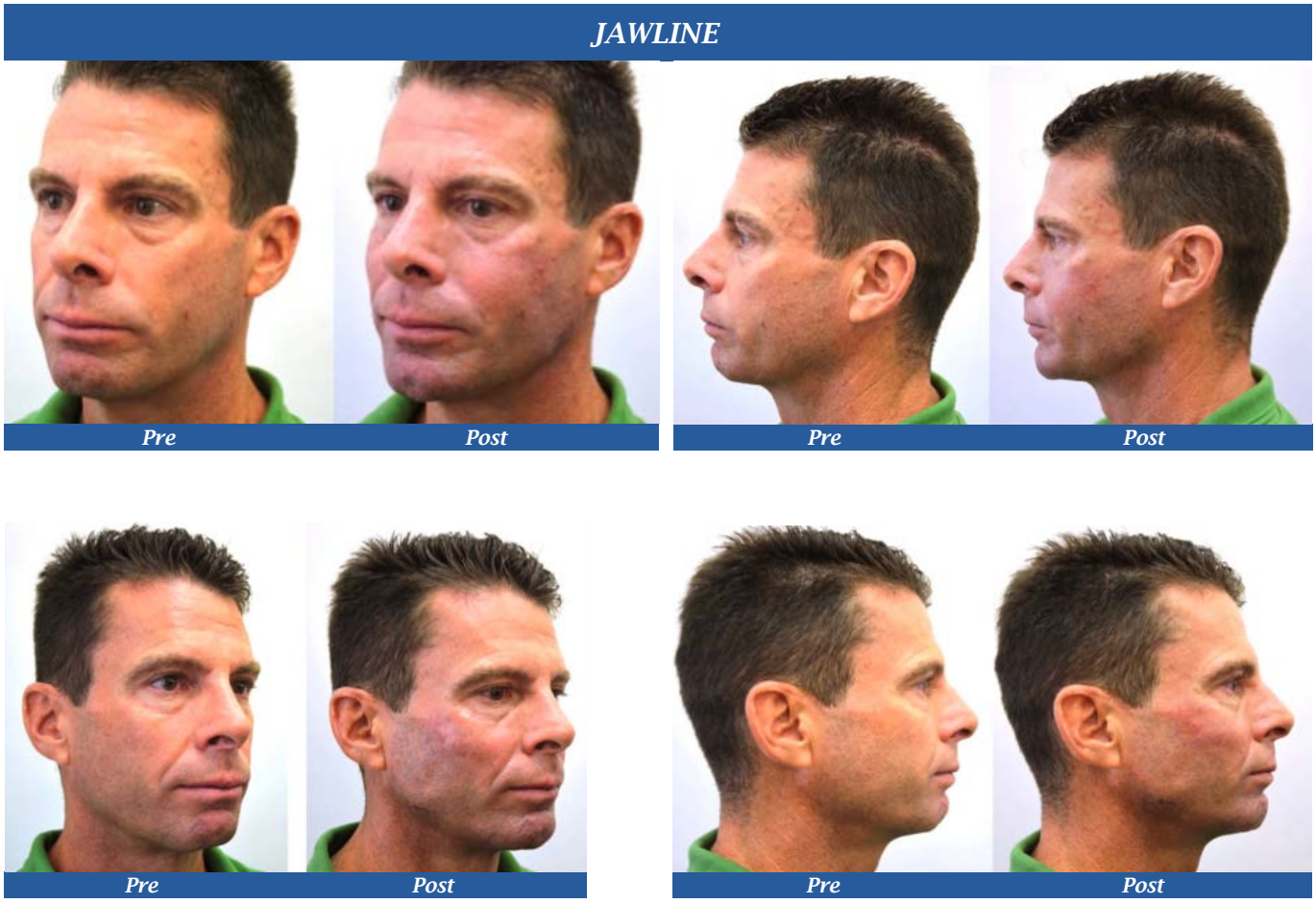

Figure 8 *- JAWLINE - Treatment objective: redifinition. Treatment: 1,5 ml Radiesse® +lidocaine – dilution 1:4. Needle 27G. 1 syringe of 1,5 ml Radiesse + 0,5 ml lidocaine. Total volume used 1,2 ml (0,6 ml /side). RENZI MASSIMO.*

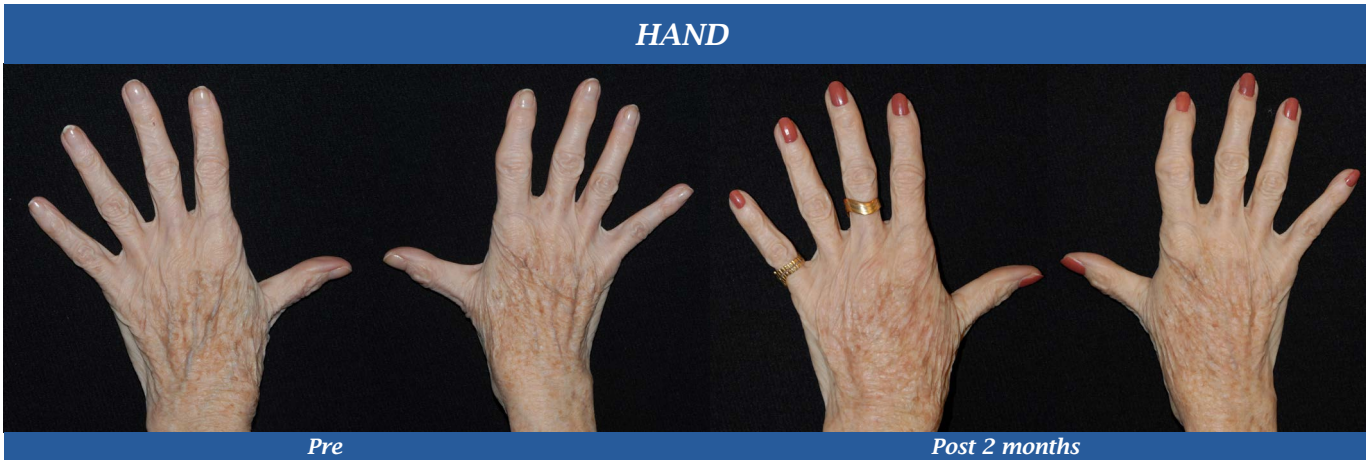

Figure 9 *- HANDS - Treatment objective: rejuvenating of back of the hand and camuflage of veins. Treatment: 1,5 ml Radiesse®. 1 syringe of 1,5 ml Radiesse +0,5 lidocaine + 2 ml saline solution. Total volume used 4 cc (2 cc/each hand). Cannula 25 G 6 cm. After one month second treatment with 0,8 ml Radiesse + 0,2 of Lidocain + 1 ml of saline socution (1 cc / hand). BARTOLETTI EMANUELE.*

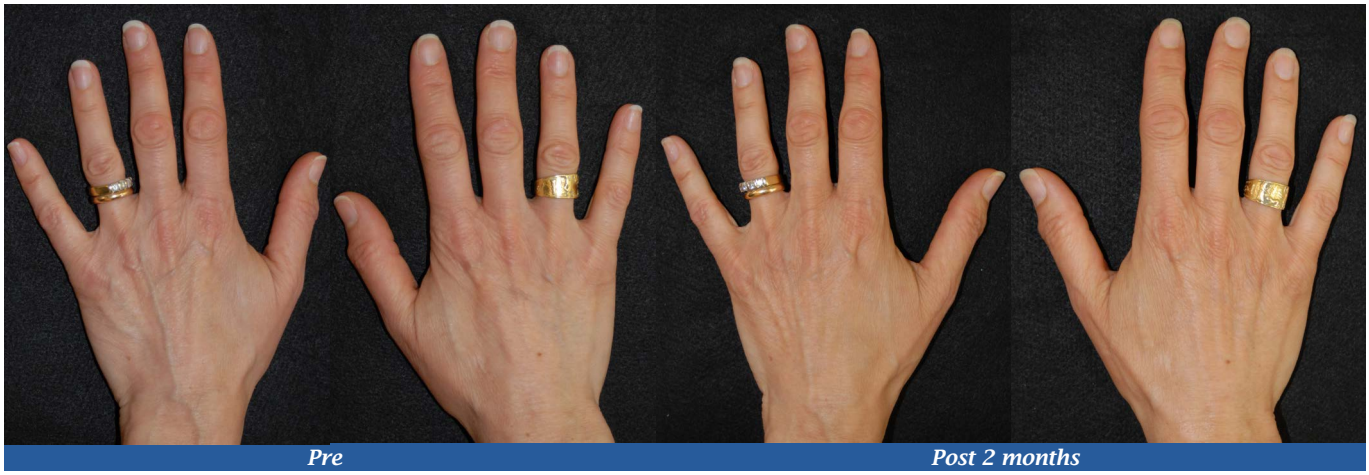

Figure 10 *- HANDS - Treatment objective: rejuvenating of back of the hand and camuflage of veins. Treatment: 1,5 ml Radiesse®. Cannula 25 G 6 cm. 1 syringes of 1,5 ml Radiesse +0,5 lidocaine + 2 ml saline solution. Total volume used 4 ml (2 ml/each hand). BARTOLETTI EMANUELE.*

Safety, Disadvantages and Limits

CaHA has proven to be a safe product, when injected, diluted and used accurately. Potential complications can be divided into injection-related events, undesirable results from suboptimal technique, and true complications[9,23].

Common injection-related events include bruising, swelling, erythema and pain, and are common to all other injectable treatments[9,12,23,27,28,13].

Suboptimal technique may lead to papules and nodules formation which may eventually spontaneously resolve[9,15,23,27]. True complications include uncommon events like granulomas[9,15,23,27], which may develop around the product and, differently from product papules and nodules, which can be treated with corticosteroid or 5-fluorouracil injections[9,15]. In cases of inflammatory nodules, whether arising early or late, an infection and antibiotic treatment should always be considered[13,15]. Vascular complications are rare and are due to intravascular injection of the product and include tissue necrosis and, more rarely, blindness. The product should also be injected in small quantities and extra care should be paid when treating highly vascularized areas[9]. CaHA is not intended for superficial injection, or injection into highly mobile areas such as the lips or above the orbital rim, where accumulation of material from muscle contraction might result in transient nodule formation. Moreover, it is contraindicated in the glabellar region for the correction of frown lines[10].

The types of complications observed following the injection of Radiesse® are reported in *table 2*.

Author	Year	Complication
A. Breithaupt et al.	2015	-
J. M. Dallara et al.	2013	-
X. Shi et al.	2016	-
M. Rayess	2018	Infection 0,008% Pain 0,007% Necrosis 0,003% Blindness 0,0001%
J.A. Kadouch	2017	Overall AEs 3%: - Nodules 96% - Persistent swelling 2% - Persistent erythema 1% - Overcorrection 1% Per area: - Lip 9,4% - Tear trough 1,8% - Perioral area 1,6% - Cheeks 0,8% - NL folds 0,3%.
D.A. Jansen et al.	2005	Lip nodules (mucosa) 12,4% Lip nodules (radial lip lines) 3,7%,
T.L. Tzikas et al.	2008	Lip nodules 5,9%-2% NL folds nodule 0,002%
L.S. Bass et al.	2010	No adverse events reported
K. Frank	2017	Mild erythema 0,2% Mild discoloration 0,2% Severe discoloration 0,2% Mild swelling general 0,7% Moderate swelling general 0,7% Severe swelling local 0.9% Severe erythema 0.9% Severe pain 1,1% Severe swelling general 2,3%

***Table 2** - Complications rate after CaHA treatment.*

Discussion and/or conclusion

With the dramatic increase in popularity of non-surgical techniques, it is very important to approach patients seeking improvement and/or rejuvenation in a soft way that is respectful of their anatomy and age.
Doctors must be driven by safety for the patient and by an aesthetic approach, respectful of proportions.
Correction of skin and facial aging is the main reason for consultations in aesthetic medicine. This demand from patients, which has become a social phenomenon, has increased thanks to the considerable progress made in non-surgical and less invasive therapies such as the use of botulinum toxin, fillers and lasers.
In this article, we provide an analysis on the use of calcium hydroxyapatite: how important and rewarding it can be for the doctor and satisfying for the patient, as an alternative to the hyaluronic acid fillers, in the full face treatment and in other areas such as the neck, décolleté and hands.
To support our clinical opinion, the positive results on all treated patients (in our long experience in our clinics) and the wide number of published data, we carried out an important and substantial systematic review of the literature, concluding that the use of Radiesse® can be considered a first choice treatment, better than the existing alternatives, Infact, in addition to the improvement in volumes and the lifting effect, it determines a clear improvement in the quality of the skin on the treated areas, including the body. CaHa is a non-hyaluronic filler, it is safe, boasts a wide experience in face and body usage with reliable and natural results.
Flexibility of dilution can increase the requirements of clinicians and patients to obtain high level results.

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aesthetic medicine

Letter to the Editor

Microplastics in everyday cosmetics

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Abstract

This study offers a brief review of the presence of primary microplastics in cosmetics and how to recognize them, the legislative measures taken against them to date and the alternative to the use of microplastics in cosmetics.

Microplastics are particles smaller than 5 millimetres that can be released into the external environment as "primary" directly in their microscopic size. Primary microplastics can be added to cosmetic products and their presence can be assessed through the INCI (the International Nomenclature of Cosmetic Ingredients).

Although microplastics in cosmetic products do not account for a high percentage of microplastic pollution, they do pose a threat to the marine environment as they are not retained by water purification systems, become accessible to the marine wildlife, are transferred along the food chain and also adversely affect our bodies.

Whilst several countries have adopted measures and bans - already operational or in the process of being implemented - to abolish primary microplastics in cosmetic products, there is currently no European ban. As a matter of fact, new measures will only be introduced from 2022.

Furthermore, in Italy, the Budget Act of 2018 banned the marketing of rinse-off products with exfoliating or cleansing actions containing microplastics from January 1rst 2020. Unfortunately, the spread and danger of these pollutants remain a global issue.

An alternative to microplastics are bioplastics such as polylactic acid, polyhydroxyalkanoates and cellulose acetate, whose global production is growing and diversifying.

Keywords

Microplastics, cosmetics, cosmetic Europe, INCI, bioplastics

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Introduction

The impact of mankind on natural ecosystems has today significantly changed 75% of the terrestrial environment and about 66% of the marine one and put about 1 million animal and plant species at risk of extinction.
Some consequences of human action on the planet and ecosystems could be: a) an increase of the breeding sites of disease vectors, b) transfers of pathogens between different species, c) environmental contamination with infectious disease agents, meaning human-induced genetic changes of disease vectors or pathogens (such as the resistance of mosquitoes to some pesticides). From this it is clear how essential it is to build a world in which mankind can live in harmony with nature. This year, due to the Covid-19 pandemic[1], the obligation of social isolation and the impossibility of carrying out all those activities that every day and frantically, for necessity or for pleasure, each of us performed, allowed nature to take back its spaces reminding us how important it is for the future to re-establish a correct relationship between man and nature, rationalize the correct use of resources, promote a sustainable production and promote responsible consumption models.

Microplastics

Plastic is now part of our daily life: it wraps food, it is part of the fibers of our clothes, it makes up appliances, shoes, tires, cosmetic packaging and much more. However, there are less visible plastics, called "microscopic" which are released into the environment without the realistic possibility of total recovery.
Microplastics[2] are small fragments of very solid and non-recyclable plastic, composed of a series of polymers, which have a diameter ranging from 330 micrometers to 5 millimeters. *Microplastics* are classified[3] into: a) *PRIMARY*: released directly in their microscopic dimensions (e.g. synthetic fibers of clothes, pre-production pellets, cosmetics). b) *SECONDARY*: released by the degradation and fragmentation of large plastic waste.
What many consumers are unaware of is the frequency with which they use these dangerous materials, found in everyday products, such as cosmetics for routine use and make-up, which is directly assimilated by our body, which could lead to a dangerous alteration of the balance of our body. They pollute the fundamental realities of our life, since they are released and introduced into a vast number of environments[4]. These particles, in fact, are not retained by the purification systems and end up directly in the water networks, in public waters, in seas, lakes and rivers. Once in the sea, these particles are ingested by the fauna, increasing the risk that fish and mollusks contaminated by plastic may end up on our tables with the obvious danger to our health and the entire ecosystem. In this way, microplastics generate a type of pollution so incalculable and irreversible that the 2016 Report of the United Nations Environment Agency (UNEP)[5] , includes the pollution of microplastics in the oceans, among the six most dangerous emerging environmental threats in the world[6]. Unfortunately, even cosmetics are not immune from this contamination. In the last twenty years, microplastics have been widely used in personal care products. In the nineties, in fact, manufacturers for make-up and cosmetics began to insert "microspheres" in skin cleansers, toothpastes, face-body creams / scrubs, shaving creams, deodorants, shampoos and conditioners, cosmetics, sunscreens, with the functionality of exfoliating agents or additives, thus increasing the possibility of direct contact between our body and these particles and increasing marine pollution since these plastic powders are not retained by the purification systems and end up directly in the water networks. The reason why these pollutants for our planet have long been used is the concomitance of numerous factors, such as: a) low cost for companies, b) extreme stability in any type of formulation, c) the chemical inertness of the polymer preventing it from reacting with other ingredients, d) the low microbial load, e) the fact that they are not allergenic. Although cosmetics are not, even remotely, the main source of microplastics in the sea, due to their frequent and large-scale use they represent a non-negligible source of marine pollution: it is estimated that up to 24 tons of derived plastic "dust" from the use of cosmetics pours into European seas every day, for a total of 8600 tons per year.
In 2015 Cosmetic Europe[6] (*European Trade Association for the Cosmetic Personal Care Industry*) recommended its members to stop, by 2020, the use of used synthetic, solid, plastic particles (microspheres). to exfoliate and purify, which are not biodegradable in the aquatic environment. In 2016, a survey by Cosmetic Europe, covering the year 2015, assessed the effectiveness of the voluntary actions implemented by companies in the sector.
The Cosmetic Europe survey found a rapid and substantial reduction of 82% in the use of plastic microspheres for exfoliating and cleansing purposes in cosmetic and personal care products. In 2017, UNEP (*United Nations General Assembly*) launched the "Clean Seas"[7] 2017 campaign with the aim of engaging governments, private and public sectors to eliminate the main sources of marine litter, such as microplastics in cosmetics and the excessive use of disposable plastics, by 2022. Furthermore, while in 2017 the use of microplastics was already banned in many countries, this was not yet the case in Italy where a bill banning their use by 2020 was still under discussion. The Clean Seas campaign entitled "*What's in your bathroom?*"[8] was aimed at raising awareness among consumers about the damage caused by the plastic materials present in personal care products and about the changes that could be made to reduce the consequences deriving from their use.
In fact, direct contact with these microplastics, being the result of a chemical concentration, is highly harmful and dangerous for our organism and especially for our skin which is the organ most exposed and subject to receiving and internalizing the elements coming from the outside world. In fact, our skin has no defensive barriers and being the largest organ in our body, it tends to absorb and deeply assimilate the substances it's exposed to. That is why it is important to try to

avoid this type of contact. The microplastics that have been most frequently used as cosmetic ingredients are a) Polyethylene (PE), b) Polymethylmethacrylate (PMMA), c) Nyilon, d) Polyethylene terephthalate (PET), e) Polypropylene (PP).
In 2018, the cosmetic industry, following the voluntary actions taken by cosmetic companies and the recommendations of Cosmetic Europe itself, reduced the use of plastic microspheres in rinse-off cosmetics by 97.6%. In the same 2018, Cosmetic Europe activated ECHA[9] (*European Agency for Chemical Substances*) for the restriction proposal, under REACH[10] (*Registration, Evalutation, Authorization of Chemicals - registration, evaluation, authorization and restriction of chemical substances*), of the use of microplastic particles added to products of all kinds. In 2019, ECHA published its proposal and opened a public consultation pending ECHA's Scientific Committees for Risk Assessment (*RAC: Committee for Risk Assessment*)[11] and Socio-Economic Analysis (*SEAC: Committee for socio-economic analysis*)[12] took into consideration the information received about the proposed restriction and assessed the costs and benefits of the restriction and of a transitional provision. In 2020, *ECHA's RAC* and *SEAC* approved the limitation of the use of intentionally added microplastics by recommending a lower limit threshold and also proposing a ban on the use of microplastics in synthetic turf sports fields for six years.

INCI

It therefore becomes clear how important it is for consumers to be aware of this "hidden" form of pollution, with which they are unknowingly very involved.
It is important to carefully read the label of the ingredients of the product you intend to purchase and not be misled by the sometimes ambiguous communication of some products (perhaps on sale among "natural" products), which overlook the presence of polyethylene, and otherwise enhance other natural natural, which are present in smaller quantities.
To take care of our skin it is essential to choose cosmetic products in a conscious and judicious way, or try to choose products that respect the environment and are free of harmful substances that damage our health.
When buying a product, it is important to make an ethical choice, both to safeguard our "direct" health and to try to preserve our planet as much as possible.
It is therefore of extreme importance to read the list of ingredients of the cosmetic product: INCI[13]. INCI is the acronym for "*International Nomenclature of Cosmetic Ingredients*" and is a code that is used to indicate the ingredients of a cosmetic product, listed in descending order, taking into account their concentration. According to what is imposed by the legislation on cosmetics, the label of each product must contain the list of ingredients contained in the cosmetic preceded by the word "ingredients": the list follows an order based on the percentage amount of ingredient found in the product, where the highest percentage reaches up to a maximum concentration greater than or equal to 1%, while those in percentages lower than 1% can be indicated in no particular order. The INCI code, unique for all EU countries and also used in other countries such as the USA, Russia, Brazil, Canada, South Africa, was introduced on January 1, 1997 by the European Commission, and updated on May 8, 2019, with the 'goal of providing greater consumer protection. This common nomenclature makes it possible to identify substances with the same name in all Member States and allows consumers to specifically know the composition of the products purchased not only in Italy, but in any European country, and to identify some substances that they were advised to avoid.
INCI has a language used to name the individual ingredients: a) the pure substances that have been included in the formulation of the product, without undergoing any chemical alterations, are listed keeping the Latin name. This, for example, is the case with pure vegetable oils and natural ingredients: the Latin names refer to botanical ingredients or those present in the pharmacopoeia. If there is an asterisk * next to the name, it means that the ingredient comes from organic farming. The name in Latin is always followed by the name of the part used, whether it is fruit, root or leaf, and by the type of product, whether oil or extract; b) substances that are the result of a chemical synthesis are written in English; c) dyes are indicated in no particular order after the other ingredients, in accordance with the international list called "*Color Index*" which sees the presence of the initials CI followed by a numerical series consisting of 5 digits attributed progressively and according to the chromophore group. Depending on their number in the Color Index, the dyes are divided into four groups: - CI 10,000-74,999: synthetic organic dyes - CI 75.000-75.999: natural organic dyes - CI 76.000-76.999: oxidation bases and nitro dyes - CI 77.000-77.999: inorganic pigments; d) odorant and flavoring compounds and their raw materials must be indicated with the terms "perfume" or "parfum" and "aroma". Ingredients that are not prohibited, but not recommended, include: a) *SLES and SLS or Sodium Lauryl Sulfate (SLS) and Sodium Laureth Sulfate (SLES),* b) *PEGs or Poly Ethylene Glycol c) SILICONES* d) PARABENS e) PETROLATES d) ALUMINUM SALTS e) PLASTIC POLYMERS.

Alternative use to microplastics in cosmetics

A cosmetic product, although natural, cannot be exempt from minimal and acceptable chemical transformations, since otherwise it would be unstable, easy to deteriorate and full of bacteria, molds and spores.
Synthetic substances are necessary to purify, thicken, emulsify and preserve, but there are eco-sustainable and biodegradable synthetic molecules that can be used alternatively.
Bioplastics[14] represent a small part of the plastics family and have had a significant growth rate in recent years. The term refers to all the plastics of renewable origin and the biodegradable and compostable ones present on the market today.
According to European Bioplastics, bioplastic is a type of plastic that can either be biodegradable, bio-based

("bio-based") or possess both characteristics. More precisely: a) it can derive from biomass and not be biodegradable (for example: bio-PE, bio-PP, bio-PET) b) it can derive from non-renewable raw materials and be biodegradable (for example: PBAT, PCL, PBS) c) it can derive from biomass and be biodegradable (for example: PLA, PHA, PHB, starch-based plastics).
There are three different methods exploited to produce bio-based plastics: 1. Using natural polymers that can be modified but which to a large extent remain intact (e.g. starch plastics), 2. Producing monomers by fermentation or conventional chemical processes and polymerizing these base units at a later time (eg PLA)[15] 3. Producing polymers directly in microorganisms or in genetically modified crops (eg PHA)[16]. Among these methods, the first is the most widely used, the second seems to be the one destined to acquire greater importance in the next few years, while the third is still far from mass production.

Italian legislation on microplastics

Italy began working in January 2015 to eliminate microplastics from rinse-off cosmetics.
In fact, the proposed law[17] "Rules on the ecological certification of cosmetic products" was presented to the Chamber of Deputies.
Following an amendment presented to the 2018 budget law, the marketing of rinsing cosmetic products with exfoliating or cleansing action containing microplastics was prohibited starting from January 1, 2020. Law 27 December 2017, n. 205 (Budget Law 2018)[18] art. 1: a) 546: from 1 January 2020 it is forbidden to market cosmetic rinse-off products with an exfoliating or cleansing action containing microplastics b) 547: the purposes referred to in paragraph 546, refer to: 1) microplastics: solid particles in plastic, insoluble in water, of a size equal to or less than 5 millimeters, intentionally added to the cosmetic products referred to in paragraph 546, 2) plastic: molded, extruded or physically manipulated polymers in different solid forms, which, during use and subsequent disposal, maintain the shapes defined in the intended applications. c) 548: the violation of the prohibition referred to in paragraph 546 is punishable with the financial administrative sanction of the payment of a sum from 2,500 euros to 25,000 euros, increased up to four times the maximum if the violation concerns large quantities of cosmetic products referred to in paragraph 546 or a value of the goods exceeding 20 percent of the offender's turnover.
In the event of recidivism, the suspension of production activity is in place for a period of no less than twelve months. The sanctions are applied in accordance with the law of the 24th of November 1981, n. 689. Without prejudice to the provisions of article 13 of the aforementioned law n. 689 of 1981, the administrative police bodies provide, ex officio or upon denunciation, to ascertain the violations. The relationship envisaged by article 17 of the aforementioned law no. 689 of 1981 is presented to the chamber of commerce, industry, crafts and agriculture of the province in which the violation was ascertained.

Conclusions

It is important to highlight that although plastic microgranules had multiple uses, the cosmetic industry, concomitantly with the various European national legislations, has been committed to reducing this type of pollutant by strongly believing in the creation of ecological products. However, since the amendment to the 2018 budget law does not include all cosmetic products, but only those with an exfoliating and cleansing action, it is essential to continue to raise awareness and promote conscious consumption, at least until the regulation of the fragments contained in the bottles of all products is in place.
Cosmetic companies have often distinguished themselves for their ability to adapt, transforming limitations into new commercial opportunities thanks to marketing and significant investments in research and development.
It is essential, for these restrictions to lead to results, that the world's major cosmetic markets, as well as individual nations, join in this important change. The use of some beauty products that contain microplastics may appear to be an apparently harmless gesture, however they strongly contribute to the pollution of the marine environment.

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Courses and Congresses 2022

24 - 26 February - Malaga (Spain)
37th National Congress of SEME
Spanish Society of Aesthetic Medicine
Palacio de Ferias y Congresos de Malaga
President: P. Vega
Email: seme2022@pacifico-meetings.com
Web: www.seme2022.org

22 - 23 April - Brussels (Belgium)
National Congress of the Belgian Society of Aesthetic Medicine
Hotel Du Congres - Radisson Collection Hotel
President: J. Hebrant
Web: sbmebveg.be

13 - 15 May - Rome (Italy)
43rd SIME Congress
Italian Society of Aesthetic Medicine
Rome Cavalieri Congress Center
President: E. Bartoletti
E-mail: congresso@lamedicinaestetica.it
Web: www.lamedicinaestetica.it

3 - 5 June - Paris (France)
IMCAS World Congress 2022
Palais des Congrès
President: B. Ascher
Web: www.imcas.com/en/attend/imcas-world-congress-2022

17 - 18 June - Opatija (Croatia)
3rd Croatian Congress of Aesthetic and Antiaging Medicine
Croatian Association of Aesthetic Medicine - HUEM
Hotel Milenij
President: E. Bunar
Web: www.huem.eu/congress

23 - 25 June - Mexico City (Mexico)
23rd World Congress of Aesthetic Medicine - UIME
Mexican Scientific Society of Aesthetic Medicine
Pepsi Center, WTC Mexico City
President: B. Miller
Email: inscripciones@congressmcme.com
Web: https://congressmcme.com/2022/

8 - 10 September - Pretoria (South Africa)
17th Aesthetic Medicine Congress of South Africa
AMCSA 2022
CSIR International Convention Centre
President: A. Clark
Email: info@aesthmed.co.za
Web: https://aesthmed.co.za/

9 - 10 September - Paris (France)
42nd Congress of Aesthetic Medicine and Dermatological Surgery
French Society of Aesthetic Medicine
Palais des Congrès de Paris
President: JJ. Legrand
Email: info@sfme.org
Web: www.sfme.org

29 September - 1 October - Lima (Peru)
3rd Scientific Congress of Aesthetic Medicine
Scientific Association of Aesthetic Medicine of Peru - ASOCIME
Hotel Sol de Oro
President: I. Ogata
Email: informes3@grupomilenium.pe
Web: https://www.facebook.com/Asocime/

21 - 22 October - Toronto (Canada)
CAAM 19th Annual Conference
Canadian Academy Aesthetic Medicine
The Westin Harbour Castle
President: J. Carroll
Email: info@caam.ca
Web: https://www.caam.ca/conference-education-

11 - 12 November - Long Beach (California - USA)
18th AAAMC
American Academy of Aesthetic Medicine Congress
Hilton Long Beach Hotel
President: M. Delune
Email: enquiries@aaamed.org
Web: http://www.aaamed.org/congress/

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