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Original Article

Structural analysis of the tissue subjected to the technique of facial adipostructuring: A histological study

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ABSTRACT

Objectives: Aging affects dermal white adipose tissue (dWAT), causing an unbalanced facial distribution, with areas of fat loss and others with increased fat volume. Facial adipostructuring (FA) seeks to restore facial harmony without removing fat, using cannulas and active ingredients that reverse cellular senescence in adipose and dermal cells, stimulating collagen and elastin production. This study histologically evaluates the effects of FA on facial dermal and adipose structure.

Material and Methods: 30 biopsies from patients aged 35–65 years, treated with three sessions of FA in Bogotá during 2024, were analyzed. 22 and 27-gauge cannulas were used to redistribute adipose tissue and active ingredients of the Facestructure protocol. Samples were stained with hematoxylin-eosin and Masson's trichrome stain to observe changes in the dermis, epidermis, hypodermis, and collagen fibers (CFs). Observations were made using a Zeiss optical microscope and a 10MP Canon camera.

Results: Post-treatment biopsies showed clear differentiation between papillary and reticular dermis, an increase in dermal papillae, an increased thickness of the connective tissue septum, a higher density of CF, and keratinocyte proliferation in the epidermis. There was also evidence of an increased number of adipocytes per mm^2, with smaller diameters and better organization. These changes indicate tissue regeneration and overall structural improvement.

Conclusion: The FA technique promoted cell regeneration and tissue reorganization, increasing CF and thickening fatty spaces. It is concluded that mechanical stimulation with cannulas is essential to achieve these effects, surpassing mesotherapy alone. dWAT proves to be a key component in biostimulation and facial rejuvenation.

Keywords: Adipostructuring, Aging, Collagen, Dermal white adipose tissue, Facial histology

INTRODUCTION

In the aging process, adipose tissue (AT) undergoes structural changes in its shape and distribution in space in the superficial panniculopathic system known as dermal white AT (dWAT) or dermal AT, its distribution and morphological change at the facial level has been widely discussed and little understood; given that, hypothetically, it is said that all dWAT hypertrophies with aging, an assertion that does not coincide with the clinical reality of patients, because we can observe that in its facial distribution, there are areas that gain volume but there are others that lose it dramatically.[1,2] In this distribution, we observe panniculi such as temporal, supra parotid, goniac or posterior mandibular, frontal that atrophy throughout its extension and

we see panniculi such as nasolabial and jowls or anterior mandibular that hypertrophy dramatically, leaving the facial structure unbalanced in its entirety [Figure 1].

Clinically, the changes experienced by patients are evident with an increase in volume in the mandibular border and a drastic loss of volume at the level of the upper third, which evidently causes the patient to request the removal of fatty tissue without really knowing the consequences.[2,3]

The facial adipostructuring (FA) technique is designed to return tissues to their anatomical place of origin and algorithmically redistribute the weight of the facial structure without eliminating fatty tissue, maintaining a balance between the lipolytic and lipogenic activities of the tissue in the ATs. It is performed by means of cannular stimuli and active ingredients that have the ability to change the state of adipose senescence and take the energy to transform it, achieving not only the redistribution of tissues but also the production of collagen and elastin, in addition to improving the texture and quality of the skin.[3,4]

AT is a site of massive accumulation of senescent cells during aging.[5] Senescent cells accumulate in aged fat induced by a combination of replicative stresses, triggered by metabolic cytokines.[6] Their appearance in AT causes multiple dysfunctions, including defective adipogenesis, inflammation, aberrant adipocytokine production, and insulin resistance. These aged cells secrete senescence-associated secretory phenotype (SASPs), which include cytokines, chemokines, proteases, and growth factors, which are considered a sign of aging, ultimately deteriorating dermal tissue and giving the patient the appearance of rhytidosis and aged skin.

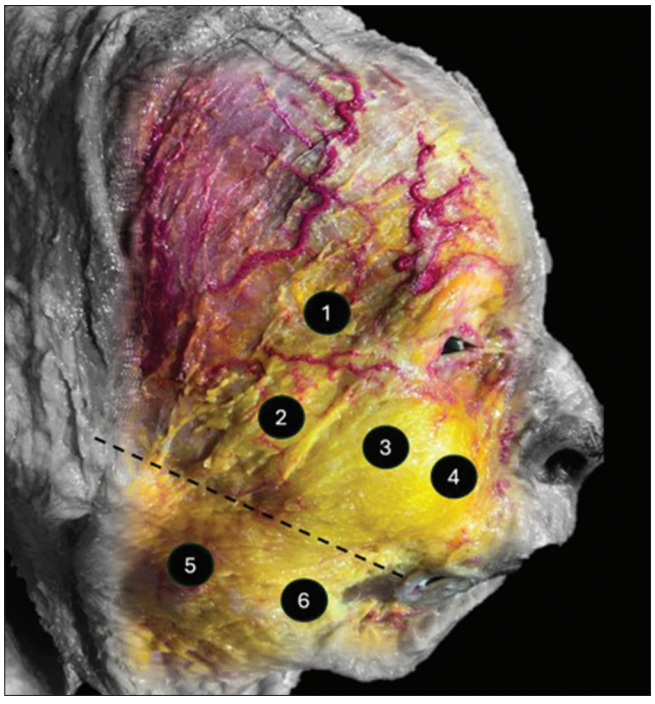

Figure 1: Distribution of the superficial panniculopathic system at the facial level. (1) P. Temporal, (2) Supraparotid, (3) Malar, (4) Nasolabial, (5) Gonia or posterior mandibular, (6) Anterior mandibular or Jowls. Dissection performed at the Latin American Center for Medical Training and Research (CLEMI), Bogotá, Colombia. Dashed lines: Camper plane (or wing-trago plane). P: Panicle

dWAT is then considered a very important element that provides structural support; its functions include wound healing, preservation of energy homeostasis, regulation of body temperature, lubrication of mechanical force, and tissue connection.[7] These adipocytes and dermal fibroblasts have common precursor cells and are found in the dermis; therefore, in recent years, scholars have proposed that dWAT may be an important tool in the emergence of an anti-senescent cell lineage that promotes the formation of new tissue.[8]

In this study, the improvement of dermal and AT with FA treatment was evaluated by biopsies in 30 patients undergoing this treatment.

MATERIAL AND METHODS

Thirty biopsies were processed from patients receiving three (3) FA technique sessions with cannulas No. 22 (for adipose panniculus) and No. 27 (for interseptal regions), using the Facestructure® Kit (MioFace Harmony™, Bogotá). The kit contains a drug cocktail administered in three steps (a drug cocktail for each session) during sessions spaced 25 days apart. The technique should be performed at least 3 times with a separation of 25 days to a month because the improvements are cumulative, opening the door to healthier tissue. Evaluations were conducted before and at 7, 30, and 90 days post-treatment. The patented kit includes 4 (four) vials of 5 (five) mL and is composed of Step 1 – Shaping Solution (Code: NS0C12681–22CO): Carnitine, Caffeine, Troxerutin, Melilotus Officinalis Extract, Asiatic pennywort or gotu kola Extract, Acetyl Hexapeptide-38; Step 2 – Firming Solution (Code: NS0C12666–22CO): Pyruvic Acid, Caffeine, other senolytics; and Step 3 – Renewal Solution (Code: NS0C12573–22CO): Carnitine, Methylsilanol Mannuronate, Sorbic Acid, Pyruvic Acid. In addition to step 3, there is a solution designed to treat the interseptal spaces that divide the fat compartments; it is called Interseptal Solution and contains:Dimethylaminoethanol Tartrate, Acetyl Hexapeptide-38, among other ingredients.

FA technique

The pursuit of facial harmony and rejuvenation has driven the development of various techniques in esthetic medicine. One of the most modern and promising is FA. This procedure seeks to restore the natural structure of the face, using a

person's own AT without extracting or lysing it, achieving long-lasting, natural, and minimally invasive results.

FA is based on a detailed understanding of the facial fat compartments, which function as anatomical cushions. Their replacement and redistribution allow for the re-creation of youthful facial harmony, restoring lost volume and correcting irregularities. AT, due to its richness in growth factors and regenerative capacity, is ideal for this purpose. Knowing that maintaining its integrity is essential.

Clinical technique

The FA technique includes several stages, which require precision and anatomical knowledge.

Evaluation and planning

Before the procedure, a detailed clinical assessment of the face is performed, considering the anatomy, degree of aging, skin quality, and patient expectations. A facial map is designed to identify the areas to be treated, clearly locating the location of each facial fat pad and its natural insertion [Figure 2].Subsequently, a system of vectors is drawn on each panniculus that must be directed toward the natural insertion of the panniculus to avoid reverse displacements, as observed in Figure 3, always respecting the initial insertion of the panniculus, with No. 22 cannulas that are used to perform the carving in the adipose panniculums, due to the necessary rigidity that this cannula experience`s to perform the carvings, necessary in each of these structures with two basic movements which are the advance and retreat of the cannula only to where the limits of the panniculum are and additionally a torque movement which means rotation on its own axis [Figure 4].After carving, the active ingredient is placed, which are senolytics, thus called those elements capable of removing cells from states of senescence, which

Figure 2: Anatomical location of the facial panniculum system and its limits based on the recognition of the structures in order (1) Temporal panniculum, (2) Supraparotid panniculum, (3) Malar panniculum, (4) Nasolabial panniculum, (5) Gonial panniculum, (6) Anterior mandibular panniculum or Jowl, (7) Posterior submandibular panniculum, (8) Submental panniculum. Dissection performed at the Latin American Center for Medical Training and Research (CLEMI), Bogotá, Colombia.

Figure 3: Design of the vector system in each of the panniculums, labeled PE, which stands for entry points to each of the structures and the vector system, respecting the natural insertions of fatty tissue and its arrangement. Dissection performed at the Latin American Center for Medical Training and Research (CLEMI) in Bogotá, Colombia. PE: entry point to each panicle. The dotted lines delimit the area of each panniculus or adipose compartment.

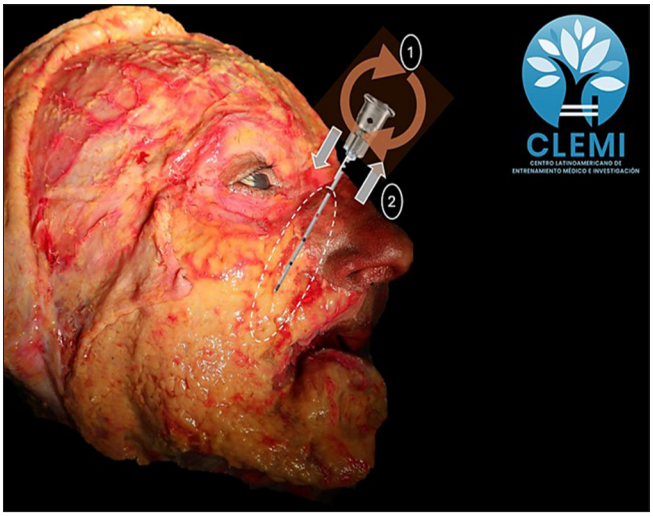

Figure 4: Cannular handling with a graphic representation of the movements performed on the fatty tissue. (1) Torque movement, (2) Forward and backward movement; both should be performed simultaneously. Dissection performed at the Latin American Center for Medical Training and Research (CLEMI) in Bogotá, Colombia. Dashed oval: Demarcates the planned cannula entry point and operative field. Arrow: Represents the trajectory and direction of cannula movement.

is significant in the aging process, producing chronic low-grade inflammation known as inflammaging, the active ingredients are basically Asiatic pennywort or gotu kola, caffeine in low percentages, organic silicon, dimethyl amino ethanol (DMAE), rutin, among others, preferably in cocktails with the exception that the formulation cannot be repeated in progressive sessions, that is, we must always change the formulation since fatty tissue has metabolic memory, which implies that if we repeat the formulations, we probably will not have the same responses; in this way, the condition of AT is further understood.

The fourth step is the treatment of the so-called interceptal spaces or interseptum, which are nothing more than the dividing lines between each panniculus that come from deep ligaments and are part of the dermal structure called retinaculum cutis, on which no type of movements should be made because they are highly innervated areas and necessarily merit a different treatment where only an active principle based on choline stimulants and collagen promoters such as methylsilanol and DMAE is entered and left with a 27 × 40 cannula and with smooth and precise entries as we see in Figure 5.

Participants and histological study

Participants aged 35–65 years, both genders, were randomly selected from a prior clinical trial conducted at the Latin American Center for Medical Training and Research (CLEMI) in Bogotá, with ethical approval from the institution.[9] The study ran from January to October 2024. This histological descriptive study followed a longitudinal design.

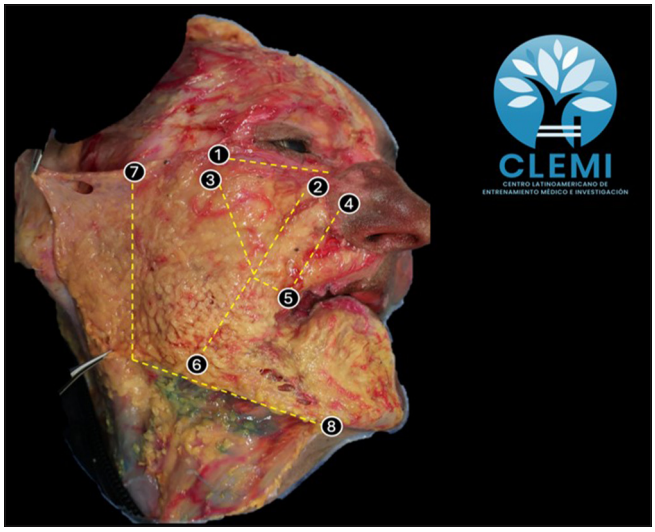

Figure 5: Indication of the interceptal spaces that must be treated in the facial adipostructuring technique (1) Infraorbital, (2) Malar/nasolabial, (3) Malar/Supraparotid, (4) Nasolabial, (5) Nasolabial/anterior mandibular, (6) Anterior mandibular/goniac, (7) Supraparotid/Pretragal, (8) Submandibular. Dissection performed at the Latin American Center for Medical Training and Research, CLEMI, Bogotá, Colombia.

Biopsies were taken twice: At the beginning of the study, before treating each patient, and at the end of the protocol, 90 days after the first FA session. Biopsies were collected from the nasolabial panniculus through a 3 mm lateral insertion near the nasal wing using fine lancets and local anesthesia. Samples were fixed in 10% formalin and stained using Masson's trichrome for collagen fibers (CF) and hematoxylin-eosin (HE) for dermis, epidermis, and hypodermis.

Histological evaluations were performed using an Axiostar Plus binocular microscope, ZEISS® (Technical Scientific Instrumentation, SL, Spain), and photographic records were made with a Canon PowerShot A640 camera (© Canon Inc., Tokyo, Japan) of 10 megapixels.

RESULTS

The clinical changes represent the replacement of facial tissues 3-month post-treatment, with evident rejuvenation (as shown in the [Figures 6-9]), which was corroborated by the following histological study, in which samples were taken from 30 patients treated with the FA technique.All post-treatment biopsies (100%) demonstrated:

Clear differentiation between papillary and reticular dermis (RD), increased dermal papillae and epidermal support, thickened connective tissue (CT) septa, increased collagen fiber density and keratinocyte proliferation, and higher adipocyte count/mm^2 with reduced size and better organization. These results strongly suggest tissue regeneration and improved structural integrity. Some of these changes are illustrated below ([Figures 10-14] illustrate microscopic evidence).

Figure 6: (a) Before (Baseline) and (b) after (90-day post-treatment) facial adipostructuring (FA) applied in a patient with facial aging. Note the great improvement in facial tissues after 3 sessions of FA.

Figure 7: (a) Before and (b) after of facial adipostructuring treatment in a patient with facial aging. In just 3 treatment sessions, the replacement of sagging tissues was induced, relocating the facial fat compartments to a more superior position.

Figure 8: (a) Before and (b) after of facial adipostructuring. A greater definition of the facial oval and a notable improvement in the area of dark circles and nasolabial folds are observed.

Figure 10 shows optical microscopy images of a section of human skin stained with hematoxylin and eosin at ×4 magnification. The first histological preparation on the left [Figure 10a] illustrates the initial state of the skin before FA treatment, where little differentiation is observed between papillary dermis (PD) and RD as well as other characteristics of the dermis in aging patients such as collapse of CF, decreased dermal thickness, and wavy epidermis (representative of deflation or sagging of the tissue). On the right [Figure 10b], after FA treatment, substantial tissue improvements are observed following the implementation of a complete FA protocol.

Figure 9: (a) Before and (b) after three treatment sessions, this patient's severe facial aging was reversed without the use of biofiller materials, neurotoxins, or tensioning threads. Only by biostimulating her own autologous repair through senolytic active ingredients and proper mechanical stimulation of the facial fatty compartments including the fat on the eyes, nose, and lips areas.

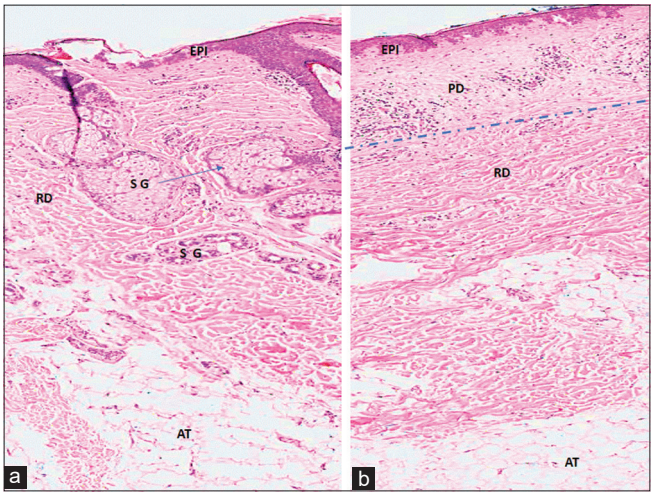

Figure 10: Optical microscopy images of a section of human skin stained with Hematoxylin and eosin (×4). (a) Before facial adipostructuring treatment, tissue disorganization was evident with limited collagen fibers. (b) After facial adipostructuring treatment, it shows rectification of the epidermis and greater definition between the Reticular dermis (RD) and papillary dermis (PD). Fiber bundles were observed in the DR, densely packed and organized collagen. AT: Adipose tissue, SG: Sweat glands, EPI: Epidermis.

The epidermis (EPI) shows a significant increase in dermal papillae, increasing nutrition and support for the epidermis, which may be related to a process of tissue renewal and regeneration. Furthermore, in the image after treatment, there is a clear differentiation between PD and RD, highlighted in the image as the dotted line. In PD, the loose CT exhibits a predominance of fibroblasts and low

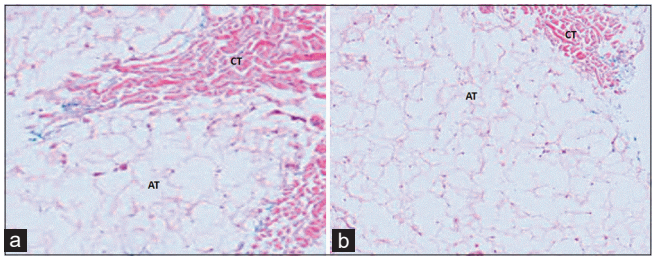

Figure 11: (a) Fat tissue biopsy before facial adipostructuring (FA) with hematoxylin-eosin (HE) staining showing rounded adipose cells of standard size. 100X. (b) Biopsy after the FA procedure, same staining showing an increased number of adipocytes/mm^2. 200X. AT: Adipose tissue, CT: Conective tissue.

fibrillar density; in RD, bundles of densely packed and organized CF (FP) Hair follicle, Sebaceous glands (G Se), Sweat glands (G Su), and Adipose Tissue (AT) are observed. Figure 11 shows human facial AT before and after FA, stained with EH. Before treatment [Figure 11a], rounded fat cells with standard diameters are observed in the AT. After treatment [Figure 11b], there is an increase in the number of adipocytes/mm^2, with a smaller diameter, homogeneous, and regularly organized. CT septa are observed in pink.

Likewise, Figure 12 shows optical micrographs of human facial skin before and after the FA technique, stained with EH at a magnification of ×4. In these images, before the AF treatment [Figure 12a] the septum (Sep) of connective tissue that separates the superficial fat pads into lobes and lobules, it is observed to be thinner throughout its entire extension. In the image after FA treatment [Figure 12b], an increase in the CT septum (Sep) that separates the superficial ATs is observed. Furthermore, better organization of the Dermis (De), Hair Follicle (FP), G Se, G Su, and AT is observed. Figure 13 also contrasts changes before and after FA, particularly in the quality of dermal fibers. Figure 13a, before FA treatment, shows a preparation corresponding to the initial conditions of a section of human skin, stained with Masson's trichrome, observed at ×10 magnification. Before FA, a lower density of CF is observed.

Figure 13b, after FA treatment, observes that in the (EPI) epithelium, there is greater cellularity at the level of the basal layer, indicative of an increase in the proliferation of keratinocytes with a significant increase in dermal papillae, increasing the nutrition and support of the epidermis, which may be related to a renewal process and tissue regeneration. Other constituent elements are also observed in better disposition, such as (FP) Hair Follicle, G Se, and (FC) Collagen Fibers, which showed an increase. Fibers have a cord or ribbon shape, densely packed with fibroblasts (Fb) between the fibers, predominantly in the PD. This indicates an improvement in fiber quality. Finally, Figure 14 presents optical microscopy images of a section of human skin stained with Hematoxylin-Eosin, at 4X magnification.Figure show tissue before adipostructuring treatment [Figure 14a] and after

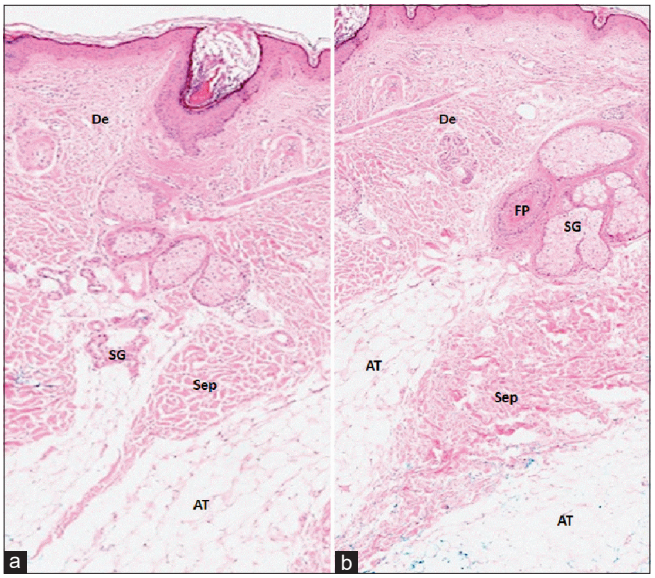

Figure 12: Optical microscopy images of a section of human skin stained with hematoxylin and eosin, at ×4 magnification. (a) Before facial adipostructuring (FA) treatment, the septum is thinned throughout. (b) The septum after FA treatment, with clear thickening and increase in connective tissue. Hair Follicle (FP), Dermis (De), Sweat Glands (G Su)connective tissue septum (Sep) and Adipose tissue (AT).

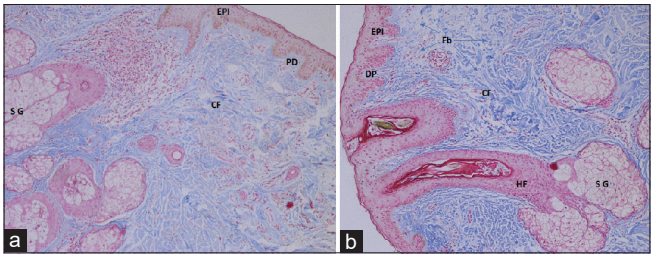

Figure 13: Optical microscopy images of a section of human skin stained with Masson's trichrome at ×10 magnification. (a) Appearance before the facial adipostructuring (FA) procedure. (b) After the FA procedure: Collagen fibers are observed, showing their increase. The fibers present a cord or ribbon shape, thin and densely packed with fibroblasts between the fibers, predominantly in the papillary dermis. Epidermis (EPI), Hair follicle (HF), Sebaceous glands (SG Se), Papillary dermis (PD), Collagen fibers (CF), Fibroblasts (Fb).

adipostructuring treatment [Figure 14b]. An improvement in the arrangement of the constituent tissue elements and a typical increase in the interseptum (Sep) are observed again, a CT septum that separates the superficial ATs into lobes and lobules, after treatment with FA.

DISCUSSION

The main objective of this research is to provide a morphological characterization of dWAT through an

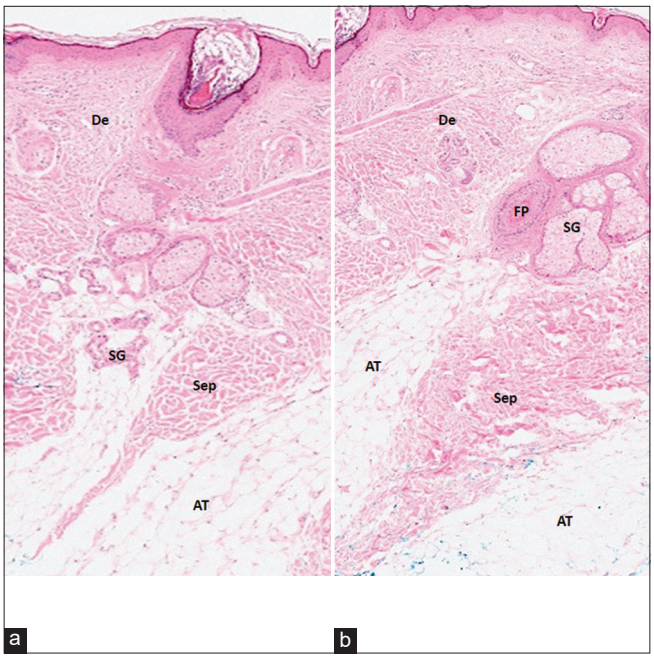

Figure 14: Optical microscopy images of a human skin section. (a) Before adipostructuring treatment, (b) After adipostructuring treatment stained with Hematoxylin-Eosin, at ×4 magnification. Sep - Connective tissue septum that separates the superficial adipose tissues into lobes and lobules. In the image after treatment, an increase in the septum is observed. De: Dermis, FP: Hair follicle, G Se: Sebaceous glands, G Su: Sweat glands, AT: Adipose tissue.

exploratory approach, as reported in the studies of Boschi *et al*. in 2024.[8] White AT dWAT depots in the facial framework have become focal points of extensive research due to their significant implications in aging processes and tissue regeneration.[4] In particular, dWAT is predominantly associated with pilosebaceous units, hair follicles, as we could verify in our study, coinciding with results already published in the literature.[7] Human dWAT exhibits a distinctive cone-shaped geometry known as dermal cones, which surrounds the interseptal spaces throughout its extension; these structures are responsible for separating and compartmentalizing fat in addition to forming an important part of the reticular extension to the skin.[2] Therefore, its stimulation with cannulas and senolytic active ingredients through FA improves the condition of the interseptal spaces, increasing their thickness as we could observe in this study.

The dWAT has been recently recognized as an important organ for non-metabolic and metabolic health in the regeneration and even rejuvenation of the skin,[7] coinciding with the results obtained in AT treated with FA, which showed improvement and also increased the thickness of the dermis and epidermis, resulting in healthier, more turgid skin and obviously in better conditions than the histological preparations observed before treatment. Dermal adipocytes can protrude into the upper dermis and produce spatial "fat bridges" between the skin surface. Confocal microscopy allows the evaluation of the epidermis and upper dermis as observed in this study, basically with HE staining, where the extension of fatty tissue into the dermal layers is observed, which explains the very favorable response of the skin tissue in general.[10]

Histologically, this favorable response restructuring of the cutaneous and AT after treatment with FA had already been documented in previous investigations, such as that of Donderis *et al*.[4] study in which before treatment with AF, the epidermis presented mild-to-moderate hypotrophy. At the level of the papillary and RD, areas of mild-to-moderate intercellular edema, focal solar elastosis, slight dilation of the blood vessels, and mild focal inflammatory infiltrate were observed. A decrease in CF and elastic fibers was also observed. Post-treatment with FA: The epidermis showed thickening of the previously hypotrophic areas and a moderate increase in type I and type III CF and elastic fibers.

Clinically, this histologically observed improvement translated into an optimization of the facial contour with a notable repositioning of the tissues, improvement of the quality and elasticity of the skin with greater hydration and firmness, especially in patients with greater skin sagging, reduction of wrinkles, and expression lines. In patients with lipomatosis and lipodystrophy, the FA technique resulted in a significant remodeling of facial fat deposits, resulting in a noticeable rejuvenation of the treated areas.[9]

Regarding the proliferation of collagen as a structural protein of the tissue, with the FA technique, a considerable increase in the number and quality of CF was corroborated without signs of fibrosis or abnormal scarring, but as an organized set of fibers in response to the improvement of the fatty tissue, coinciding with the results obtained in other studies because dWAT was additionally identified as a contributor to the biostimulation response that exists in the tissues.[11] It should be noted that dWAT has a remarkable capacity for adaptation due to a large number of internal signals and external ones that cause it to respond by repositioning itself due to the mechanical stimulus.[12] It is of utmost importance to note that the same active ingredients have been applied but in the form of mesotherapy, without achieving the results that we obtain with mechanical cannular stimulation.

CONCLUSION

The FA technique has been shown to promote cell regeneration, the repair of skin layers, as well as an increase in the number of CF and the thickness of the interseptal spaces, which definitively favors the reversal of facial aging, with beneficial results for the patient and improvements that open new horizons in facial harmonization. The use of the FA technique

has demonstrated a significant increase in the quality of CF, improving fatty tissue. This coincides with other studies.

Studies that identify dWAT as a key contributor to tissue biostimulation. dWAT demonstrates a remarkable capacity to adapt to mechanical stimuli, which has not been achieved with mesotherapy application of the same active ingredients.

FA represents a revolution in esthetic medicine, using the patient's own AT to restore beauty, symmetry, and youthfulness to the face. Its advantages in terms of safety, naturalness, and regeneration position it as a preferred option for many patients and physicians. However, its success depends on proper assessment, the experience of the practitioner, and the integration of realistic expectations. With the advancement of medical technology, the future of FA will be even more promising, allowing for a harmonious appearance and improving the quality of life of people seeking safe and effective facial rejuvenation.

Authors' contributions: Gladys J Velazco de Maldonado, Dubraska V Suárez-Vega, Eduvigis Adelina Solórzano, Víctor Mercado and Marta Amin : Concepts, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. Dubraska V Suárez-Vega is the Guarantor for the manuscript.

Ethical approval: The research/study was approved by the Institutional Review Board at LIBCEM Integrated Laboratory of Molecular and Cellular Biology (LIBCEM), University of the Andes. Mérida, Venezuela, number 001, dated January 10, 2024.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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