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Case report

Biostimulating effect of ozone therapy on facial rejuvenation, a multilayered approach with combined therapies: a case report and literature review.

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Keywords

biostimulating effect, ozone therapy, neocollagenesis, skin densification.

Abstract

Backgorund: The search for aesthetic improvement of the skin and searching for effective methodologies that can generate good safe results in a short period of time is a constant. Ozone therapy is also indicated for improving skin aesthetics, adjusting tissue oxidative levels, increasing collagen production and even skin volumizing.
Aims: Biostimulators promote neocollagenesis and skin densification restoring tissue vitality and generating a great impact on the patient's emotional health. This paper aims to carry out a case report that demonstrated positive results of the biostimulating effect of ozone therapy, used alone and in association with other techniques, supporting the technique in the literature review.
Patients/ Methods: A case report that demonstrated positive results of the biostimulating effect of ozone therapy, used alone and in association with other techniques as botulinum toxin and jett plasma.
Results: The application of ozone therapy showed positive results for biostimulating activities in the reported cases demonstrating to be a viable clinical technique.
Conclusions: Biostimulating effect of ozone therapy is a promising aesthetic therapeutic modality with fast and safe results, the versatility in association with other techniques reinforces the clinical importance of ozone therapy as an aesthetic therapeutic option.
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Introduction

The search for aesthetic improvement of the skin and for attenuating the signs of age are present in today's society, that becomes averagely older every day, but which is greatly influenced by high aesthetic standards disseminated in various media and social networks.
Searching for effective methodologies that can generate good results in a short period of time in a sustainable and reproducible way make the non-surgical and minimally invasive aesthetics market have an expressive growth in the coming years.[1]

Among the most popular aesthetic treatments sought in clinics, we can highlight botulinum toxin, to help control dynamic wrinkles and attenuate static wrinkles, fillers, with the purpose of volumetric skin rescue, and biostimulators that promote neocollagenesis and skin densification.
In isolation or in combined therapies, these techniques can soften the signs of time and promote facial rejuvenation, restoring skin vitality and generating a great impact on the patient's emotional health.

Ozone therapy is also indicated for improving skin aesthetics, adjusting tissue oxidative levels, increasing collagen production and even skin volumizing by favoring adipogenesis, making it an interesting, safe and affordable alternative for facial rejuvenation.[2-4]
This paper aims to carry out a case report that demonstrated positive results of the biostimulating effect of ozone therapy, used alone and in association with other techniques as botulinum toxin and jett plasma, supporting the technique in the literature review, discussing the clinical approach, describing the application protocol and evaluating the results obtained.

Ozone in aesthetic medicine, background

Theoretical basis

Ozone therapy is a methodology that uses a mixture of O2/O3 gases to promote physiological responses, the oxidant activity promotes different physiological response, meeting the precept of hormesis, that is, its physiological response is directly related to the concentration of application. At low dosages the modulation of subclinical stress activates mechanisms that promote mechanisms of repair, development and radical control, while at high dosages it installs processes of inhibition, degradation, induction of apoptosis and installation of necrosis.[4-7]

The gas can be administered directly to the treated tissue or it can be dissolved in vehicles, such as water and saline solution. Ozone can also be used indirectly through activations and preparation of actives, such as ozonized oil, which does not have ozone, but secondary products of the reaction with the unsaturated compounds of the oil exposed to the ozone attack, these new products acquire therapeutic properties. It is also used as a process activator to enhance results, as in the case of PRP activation, which after ozone treatment increases the release of growth factors from the alpha granules of platelets.[8-10]

There is the option of working with ozone therapy through systemic routes, such as rectal insufflation, vaginal insufflation, intravascular administration of ozonized saline or auto-hemotherapy. These routes are potentiators of local results by promoting physiological adjustments and homeostasis, but not are mandatory in the direct cutaneous approach. In the reported clinical cases we used only the local approach to assess the biostimulating capacity of ozone therapy without any systemic support.

Local insufflation demonstrated viability to increases collagen production by activation of fibroblast growth factors (bFGF). This process is highly viable in cutaneous aging as the neocollagenesis capacity drops significantly and cell reactivation can promote dermal densification. The application of bFGF has also demonstrated *in vivo* studies the ability to promote cell proliferation and collagen synthesis, *in vitro* studies have shown that it has a potent capacity for cell migration and proliferation, in addition to inducing angiogenesis in the treated region.[11]
The technique also demonstrated a positive effect in increasing FGF2 expression and modulating inflammatory processes, favoring wound recovery. The infiltration demonstrated the ability to increase cell differentiation, modulated by FGF2, with a significant increase in collagen-1 production, increased collagen deposition in the tissue and accelerating recovery time. Another important modulation is the reduction of the neutrophil activity period, increase in the presence and functionality of myofibroblasts, and also an increase in the microcirculatory density of the treated region. This increase of FGF2 was observed both in the dermis and in the epidermis of the treated region, stimulating the re-epithelialization process. [12]

Ozone therapy also promotes an increase the local antioxidant process. The subclinical oxidative stress induced by low doses of therapy promotes the activation of erythroid-derived nuclear factor 2 (Nrf2), promoting an increase in the production of endogenous antioxidant protection system as the reduced glutathione (GSH), carbon monoxide, and bilirubin; increased levels of enzymes that detoxify oxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione transferase (GSTr), NADPH-quinone oxidoreductase (NQO1), heme oxygenase-1 (HO-1) , end-shock protein 70 (HSP70).[13]

The activation of Nrf2 promotes modulation of the inflammatory process, in addition to the modulation of redox homeostasis, it adds this control with the reduction of pro-inflammatory signals and cytokines such as Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interferon -Gamma (IFN-γ), Tumor Necrosis Factor-Alpha (TNF)-α), Interleukin 17a (IL-17a), Transforming Growth Factor-β (TGF-β), Interleukin-1β (IL-1β), and Interleukin -6 (IL-6). This modulation happens due the activation of Nrf2 decreases the activity of Nuclear Factor Kappa-B (NF-κB), responsible for the production of inflammatory signals.[14-16]

The low-dose ozone concept with its moderate oxidative stress represents an ideal hormesis strategy. In living organisms, single or repetitive administration of otherwise potentially dangerous or toxic substances in small doses increases their homeodynamics (homeodynamic space), their self-regulatory capacity or, alternatively, moderate oxidative stress stimulates the protective mechanisms of cells and organs and is biologically useful. [7]

This modulation is important to maintain skin volume, patients exposed to antiretroviral medication for HIV treatment may present early facial lipoatrophy, the modulation of this oxidative stress proved to be a powerful ally of the biostimulating effect, with satisfactory clinical results in the combined therapies of PRP activated with ozone. [17]

This atrophic process also occurs with aging, the reduction in the fat pads of the face, tissue mobilization, loss of support, volume reduction and ptosis in all horizontal thirds of the face, with an accumulation of remaining fat in the middle fifth, in special at jowl line.[18-21]

The changes in facial contours resulting in to aging and “quadralization” of the face. This facial squaring happens because the in youth the face is shaped like an inverted trapezoid, with aging it tends to become a square shape.[22]

In addition to the control of oxidative stress, infiltration of ozone in low concentration (5-10mcg) can induce a direct response of the subcutaneous tissue as adipogenesis. Another functionality observed is that ozone also has the ability to favor cell differentiation of mesenchymal cells, the concomitant activation of Nrf2 stabilizes the hypodermic tissue, decreasing its reabsorption even in cases of surgical fat grafts.[4,23,24]

Ozone therapy has demonstrated several benefits in promoting accelerated tissue repair, including the release of growth factors such as fibroblasts, platelet derivatives, β transformer and vascular endothelial, bactericidal action, anti-inflammatory action, increased granulation tissue and re-epithelialization, greater vascularization, reduction of local pain, decreased edema, stimulation of tissue contraction and reduction in the extension and depth of wounds.[25,26]

The reduction of reactive oxygen species (ROS) and modulation of the inflammatory process also promote repair processes based on recruit lymphocytes until the treated tissue site, to act in effective tissue repair, regulate blood vessel formation (angiogenesis) and optimal blood perfusion in the healing area, increase immunity through phagocytes that induce bacterial damage.[25,27]

Application technique

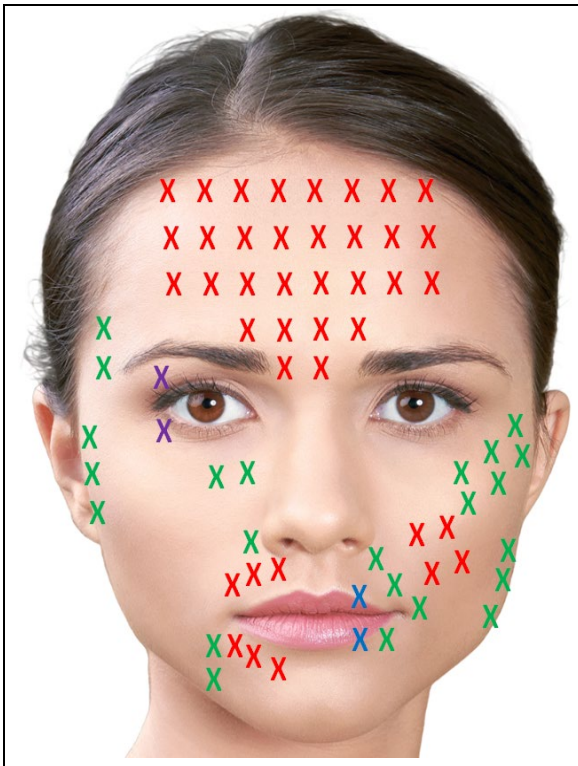
Intrinsic and extrinsic aging impacts in skin regularity, homeostasis and loss of functions, the multiple aggressions accumulated throughout life by exposure to irregular diet, pollution, ultraviolet radiation, stress, ROS attacks and chronic subclinical inflammatory processes, promoting skin thinning in all layers.[18-20]

The epidermis reduces its barrier function, causing dehydration and a lower rate of protection against external agents. The dermis significantly reduces its collagen production and support, promoting epidermal malnutrition, less water retention and firming function of the epidermis and skin appendages. There is an irregularity between the support fibers, which are glycated and with a high incidence of elastosis.[18,28]

The atrophy of adipocyte tissue associated with bone resorption intensifies the loss of support for superficial tissues and worsens ptosis conditions, promoting an inversion in the facial support, generating the facial squaring in the aging process.[20,22]

Ozone therapy has already shown clinical feasibility for biostimulating purposes.[29] When applied at low concentrations (5 µg/mL – 10 µg/mL) it can promote the activation of collagen production, stimulation of keratinocytes and adipogenesis, it shows clinical viability to stimulate different facial tissues.

A concentration of 5 µg was standardized to induce the biostimulating effect of ozone, the regions were subdivided according to the facial topography for dermal fillers injections. The volume, distance between applications and depth infiltration varied according to the therapeutic target and need for hypodermic stimulation, as shown in figure 1.



1 – Frontal, glabellar, cutaneous upper and lower lip, cheek: 1 mL every 1 cm of distance with a depth of 2-3 mm (X).

2 – Upper and lower lip: 2-3 mL, in the midline of the vermilion border, 2-3 mm depth with free flow through the lip and posterior accommodation massage (X).

3 – Temporal, zygomatic, malar, canine fossa, preauricular, região mandibular posterior, nasolabial sulco, labiomentual sulco, mentonian: 2 mL every 2 cm of distance with a depth of 4-6 mm (X).

4 – Upper and lower eyelid: needle insertion into the mobile eyelid, delivery of 1 mL, depth of 1 mm with free flow through the eyelid and accommodation massage without pressing the eyepiece (X).

Figure 1 - Standardized application scheme of ozone therapy for facial biostimulating.

3 – Case report

Ozone therapy is a clinical methodology accepted in Brazil and regulated by Ordinance no.702 published in March 2018 by the Brazilian Ministry of Health, not being considered an experimental treatment or without legal support.

All patients signed the informed consent.

Its clinical indication with a biostimulating effect has already been presented in the literature, however with a fluctuation between application concentrations, volumes and depths used, based on the standardization presented, patients were treated during a cycle of 5 service sessions, with a weekly interval between sessions, a 30G needle was used for ozone infiltration.

As an exclusion factor for treatment, patients with G6PD enzyme deficiency, anemic history, recent heart attack, decompensated chronic diseases, history of cancer, active lesions in the treatment region, dietary restrictions, recent major surgical procedure.

The group consisted only of female patients, aged between 39 and 72 years, who were looking for facial rejuvenation with a biostimulating effect and did not present any of the exclusion factors, all patients assigned the ethical concern. They were instructed to use OzonCare® facial harmonizing serum and sunscreen FPS 30 twice a day throughout the treatment, no nutraceuticals or other systemic stimuli were used to better assess the biostimulating effect of the technique without complementary supports. Ozone therapy was conducted according to the standardization presented in all patients, including those who had combined therapy.

The clinical protocol is composed of initial cleaning, OzonCare® cleaning foam, exfoliation with Fler® glycoactive gel activated with abrasion using the round until reaching skin redness, the product was removed with ozonized water at 60 µg, ozone was applied according to standardization presented, the OzonCare® facial harmonizing serum was used to compress the gas, using manual facelift maneuvers until the reduction of cutaneous emphysema, the session was ended with the application of Fler´s® sunscreen FPS 30.

After the session, the patients presented mild facial swelling, which completely reduced within 48 h. Anesthetic bases were not used to perform the technique because the patients had a good resistance threshold, however, in more sensitive patients, it is recommended to seek resources to reduce application discomfort.

The final photo was taken at least 7 days after the last visit, with the exception of the patient who also applied botulinum toxin, in which the photo was taken 15 days after the last ozone therapy session.

Case 1

Female patient, 39 years old, denies smoking and drinking, has no comorbidity and does not use any medication, has no food restrictions, practice physical activity regularly and takes supplements with whey protein, has a regular menstrual cycle and does not use oral contraceptives and has children.

Patient went to a private clinic complaining of facial aging. She had already had other cosmetic treatments such as peeling, radio frequency, phototherapy and botulinum toxin application for more than 6 months ago, but at the moment she is only using cosmetics with vitamin C and sunscreen.
The treatment gas was prepared according to the manufacturer's instructions (Philozon Medplus® MX Ozone Generator. Brazil, ANVISA Registration No. 80472910001).

At the initial evaluation she did not present advanced aging, being classified as Glogau 2, dynamic wrinkles in frontal and lower eyelid, a soft nasolabial fold, a soft malar and lower eyelid with lift loss.

After treatment the patient presents with natural results, the quality of the skin and luminosity increase, the frontal and eyes orbicular wrinkles practically disappeared. A dermal turgor improvement is noticed and a filling at nasogenian fold and infraorbital region. Patient reports that she was satisfied. The treatment results are in figure 2.

Figure 2 – Digital photography for the comparison of case report 1 outcomes of 5 sessions.

Case 2

Female patient, 52 years old, denies smoking and drinking, has no comorbidity and does not use any medication, has no food restrictions, do not practice physical activity regularly and does not drink much water, in menopause, no hormone replacement and has children.

Patient went to a private clinic complaining of facial aging and wrinkles, had never been submitted to any kind of aesthetic treatment or the routine use of any homecare product, sometimes applies sunscreen.

The treatment gas was prepared according to the manufacturer's instructions (Philozon Medplus® MX Ozone Generator. Brazil, ANVISA Registration No. 80472910001).

At the initial evaluation she presented some skin aging spots, being classified as Glogau 3, static wrinkles in the frontal and lower eyelid, a soft nasolabial and median labiomental fold, an important malar and lower eyelid loss of lift with darkened periorbicularis and infraorbital bags, significant loss of dermal turgor.

After treatment the patient presents a natural result, the skin´s quality and luminosity increase, it is noticed a dermal turgor improvement and face lifting, the frontal wrinkles practically disappeared, periorbicularis presented a filling and whitening effect, an important filling at nasogenian and labiomental fold. Patient report that she was satisfied, but she would like to carry out another treatment cycle to improve even more the periorbicular and labiomental area. The treatment results are in figure 3.

Figure 3 – Digital photography for the comparison of case report 2 outcomes of 5 sessions.

3.3 – Case report 3

Female patient, 58 years old, denies smoking and drinking, controlled hypertensive patient uses daily atenolol 50 mg and hydrochlorothiazide 25 mg, has food restrictions for salty foods, do not practice physical activity regularly, in menopause, no hormone replacement and have kids.

Patient went to a private clinic complaining of facial aging, tired appearance and loss of volume, never been submitted to any kind of aesthetic treatment, the routine use moisturizer with hyaluronic and apply sunscreen daily.

The treatment gas was prepared according to the manufacturer's instructions (Tonederm Oxy® Ozone Generator. Brazil, ANVISA Registration No. 10411520027). One week after the last ozone therapy session, the patient applied the toxin in the upper third of the face and in the lower eyelid. At the initial evaluation she presents some skin aging spots, being classified as Glogau 3, some dyschromia at frontal, significant loss of lift in the temporal area, static wrinkles in the glabella and lower eyelid, a soft nasolabial and labiomental fold, an important malar and lower eyelid loss of lift with deep periorbicularis, significant loss of dermal turgor and cheek volume reduction.

After treatment the patient presents a natural result, the skin´s quality and luminosity increase, it is noticed a dermal turgor improvement and face lifting, frontal dyschromias decreased, periorbicularis presented a filling and whitening effect, a filling effect at nasogenian and labiomental fold, an important biostimulating effect on the cheek and a decrease in the tired appearance. Patient report that she very surprise with results, that exceeded expectations we can check the treatment result at figure 4.

Figure 4 – Digital photography for the comparison of case report 3 outcomes of 5 sessions and one session of toxin botulinic.

Case 4

Female patient, 72 years old, denies smoking and drinking, has no comorbidity and use hormone replacement with Climene® and Calcitran®D3 600 mg for calcium supplement daily, has no food restrictions, practice soft physical activity regularly, in menopause and have kids.

Patient went to a private clinic complaining of facial aging, wrinkles and volume loss, she has already had other cosmetic treatments such as peeling, radio frequency, phototherapy, fillers, biostimulators and botulinum toxin application for more than 6 months ago, but at the moment she´s using cosmetics with vitamin C and growth factor blend twice a day, moisturizer with hyaluronic at night and sunscreen.

Before ozone infiltration e used the plasma jet equipment Plasmed® Ibramed (ANVISA Registration No. 10360319016) with the straight pointer at intensity 3 to improve endothelial cell proliferation. The treatment gas was prepared according to the manufacturer's instructions (Philozon Medplus® MX Ozone Generator. Brazil, ANVISA Registration No. 80472910001).

At the initial evaluation she presents significant skin aging spots, being classified as Glogau 4, static wrinkles in the frontal, glabella and lower eyelid, a deep nasolabial and labiomental fold, an important malar and lower eyelid loss of lift and infraorbital bags, significant loss of dermal turgor, temporal and cheek volume reduction.

After treatment the patient presents natural results, the skin´s quality and luminosity increase, it is noticed a dermal turgor improvement and face lifting, the frontal, glabella e lower eyelid, evident wrinkles decrease, periorbicularis presented a filling with reduction of infraorbital bags, an important filling at nasogenian and labiomental fold. Patient reports that she was satisfied, but she would like to carry out another treatment cycle to improve even more the labiomental area. The treatment results are in figure 5.

Figure 5 – Digital photography for the comparison of case report 4 outcomes of 5 combined sessions with jett plasma.

Discussion

The application of ozone therapy in low concentration (5µg) showed positive results for biostimulating activities in the reported cases, it is worth noting that it is a technique that does not generate irritation, inflammatory processes, risks of vascular obstruction and allergies when applied correctly, with several clinical indications for aesthetic purposes.[31]

The modulation of inflammatory processes, adjustment of local redox, adjustment of increment of microcirculation, increase of growth factors and influence on cellular activity in a direct and indirect way presented in the literature demonstrate a real favoring the activity of fibroblasts and keratinocytes, promoting skin regularity, lighting and hyperchromias decrease, the skin turgor is suggestive of neocollagenesis and filling volume demonstrating adipogenesis.

In comparison with other traditional methodologies for biostimulations that use actives such as calcium hydroxyapatite and poly-l-lactic acid, it is interesting due to its affordability, safety points and the fact that its clinical effectiveness can be observed in the first month of treatment, with significant clinical impact from the third session, the other methodologies also imply multiple sessions, but with a monthly interval, generating results from the second month of treatment, with a significant clinical response around the fourth month.

Longer treatment cycles can be suggested, considering the safety issue, there would be no restrictions for the increase in sessions, however this study did not consider whether there would be a significant difference in the result with a greater number of treatment sessions.

Its versatility in association with other techniques reinforces the clinical importance of ozone therapy as a therapeutic option, botulinum toxin has a better response in the control of dynamic wrinkles than static ones, a skin that has shallower static wrinkles will increase the impression of the therapeutic effect of botulinum toxin in the control of roughness, all the cases presented showed a decrease in the depth of the wrinkles, being viable as a preliminary tissue conditioning procedure in patients with Glocau 3 onwards.

The synergism of results can be observed in case report 4, demonstrating that other treatments can be associated simultaneously with ozone therapy. The plasma jet technique was indicated in association because endothelial cell proliferation enhanced by low dose of plasma through fibroblast growth factor-2 release is observed, the technique is promising in promoting skin improvement.[32,22,34,35]

The patient did not want to have marks on her face and, using low dose of plasma emission, we optimized results as there was also the request of the patient who did not want to put herself in social isolation due to the recovery time of the injuries of the high intensity treatment, generating the client's satisfaction about the result and the therapeutic conduct.

More studies need to be carried out to understand the clinical feasibility of the biostimulating effect in patients with different clinical profiles, elucidating the best associations and determining a correlation between the expected result and the total number of treatment sessions.

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Conflict of interest

There is no conflict of interest

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