Case Report

Evaluation of Ozonized Fibrin-Rich Plasma as a Therapy for Facial Rejuvenation: A Clinical Case Study

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Aim: To report the case of 5 patients who underwent facial skin treatment with ozonized fibrin-rich plasma (I-PRF).

Methods: This observational study of clinical instances involved 5 patients (4 women and 1 man), treated at the Unioeste dental clinic in Cascavel-PR. Treatment with ozonized I-PRF was performed in three sessions, focusing on areas such as the glabella, periorbital region, nasolabial fold, and chin. The protocol consisted of 5 consultations, including anamnesis, application of I-PRF, and final evaluations.

Results: The results showed a significant improvement in skin quality, reduction of expression lines, and decrease in hyperpigmentation, with individual variations. An improvement in facial muscle tone was also observed, demonstrating the overall rejuvenation effect. The treatment proved to be effective, providing a firmer texture and reducing skin imperfections.

Conclusions: The data suggest that ozonated I-PRF can be used as a promising and innovative therapy for facial rejuvenation, with sustainable and satisfactory aesthetic results.

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Introduction

The aging of the human body is a natural and organic process that results in significant changes to the skin. These include the appearance of wrinkles, reduced elasticity, and hyperpigmentation, resulting from a decrease in collagen synthesis. With advancing age, there is a progressive degradation of the structural components of the dermis, which compromises its elasticity and integrity [1].

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Facial treatments based on collagen biostimulators represent an effective alternative for patients seeking tissue recovery through non-surgical methods. These treatments promote the regeneration and synthesis of collagen in the skin, helping to improve the structure and elasticity of facial tissues without the need for surgical interventions [2]. A viable technique for delaying ageing is Fibrin Rich Plasma (I-PRF), which is used to stimulate tissue regeneration.

I-PRF (Injectable Fibrin-Rich Plasma) is a safe, second-generation autologous biomaterial obtained by low-speed centrifugation of blood collected in test tubes. Platelet-rich fibrin (PRF) contains growth factors such as lymphocytes and is characterized by the presence of type I collagen [3]. PRF used in injectable form can accelerate the process of cell renewal, stimulating growth factors that promote tissue repair [4]. In addition, I-PRF demonstrates the ability to promote angiogenesis, which increases tissue vascularization and collagen formation, facilitated by the activity of fibroblasts. This function is particularly significant since fibroblast activity tends to decrease with advancing age [5].

I-PRF is obtained from the patient's blood. In order to properly separate the blood by- products, the blood is subjected to low-speed centrifugation, a process which results in an increased concentration of platelets and leukocytes. These components are fundamental for the development of the vascular endothelium and play essential roles in the repair and healing of dermal tissues [6]. Because of these characteristics, I-PRF is considered a highly effective biomaterial for stimulating collagen production and restoring facial volume [7].

In order to enhance the clinical results obtained with the use of I-PRF, complementary therapies have been incorporated into aesthetic and restorative protocols. These include ozone therapy, a therapeutic approach involving the application of a gaseous mixture of oxygen and ozone [8]. In addition to aesthetic rejuvenation, this therapy is being used to treat various pathologies, promote preventive health, and improve quality of life [9].

Medical ozone, when present in the right concentration, is used to reduce the signs of skin ageing. Its bio-oxidative action improves cellular oxygenation, combats inflammatory processes, and stimulates local circulation. These effects promote greater red blood cell activity, which contributes to collagen synthesis and consequently reduces the production of free radicals in the skin [10].

I-PRF, when combined with medical ozone, has shown potential to promote facial rejuvenation, acting on the tone of facial cells and reducing sagging and hyperpigmentation [11]. This effect is attributed to the ability of ozonized i-PRF to accelerate healing and stimulate the production of collagen, elastin, and new

blood vessels, promoting skin regeneration and improving its overall appearance [12]. Therefore, this study aims to report the case of five patients who underwent facial skin treatment with ozonized fibrin-rich plasma.

Methodology

Type of study and sample

This study followed an observational design and consisted of the report of five clinical cases. It included four female and one male patient treated at the dental clinic of the State University of Western Paraná (Unioeste) in Cascavel, Paraná.

Ethical approval

The study was approved by the Research Ethics Committee (CEP) of the State University of Western Paraná (Unioeste) under CAAE number 80831924.8.0000.0.10107. All the participants signed the Free and Informed Consent Form (FICF) after being duly informed about the study's objectives and procedures.

Eligibility Criteria

Patients over the age of 25 with sagging skin, dark circles under the eyes, and small scars were included. Pregnant and breastfeeding patients, patients with systemic comorbidities, smokers, regular antibiotic use (14 days or more), and those undergoing treatment for neoplasms were excluded from the sample.

Clinical intervention

The cases presented were treated with ozonized I-PRF, carried out in three sessions with an interval of 30 days. The areas of application were: frontal, glabella region, periorbicular region bilaterally, bilateral nasolabial fold, and chin. In addition, areas with sagging skin and expression lines were also covered.

Sequence of Services

Initial consultation

Anamnesis

A questionnaire was used to obtain information on systemic and oral health, pre-existing conditions, allergies, and medications in use. Patients were instructed on the objectives, expectations, and pre- and post-treatment care.

Clinical examination

The general condition of the skin, including texture, elasticity and muscle tone, was assessed by inspection and palpation. Application areas were marked based on facial analysis and patient complaints.

Treatment planning

A plan was drawn up which included the areas to be treated, the number of sessions and the frequency of applications. The plan was discussed with the patients and their consent was recorded.

Registration and Documentation

Standardized photographs were taken before the start of treatment for future comparison.

Second Consultation - Production of Ozonized I-PRF

Blood collection:

For blood collection, a tourniquet and alcohol *swab* were used to aseptically clean the site. A trained nurse collected 10 mL of venous blood by puncturing the cephalic vein using a 21-G vacuum scalpel and a dry tube free of additives (Figure 1). The blood was processed in a specialized centrifuge (KASVI 500) (Figure 2) at 3,300 RPM for 3 minutes, separating it into two fractions: red blood cells and I- PRF (Figure 3).

Obtaining ozonized I-PRF

4 mL of medical ozone at a concentration of 60 mcg/mL was produced using an ozone generator (Med Plus, Philozon, Balneário Camboriú, SC, Brazil) (Figure 2). The previously centrifuged plasma was then collected using a 10 ml syringe with a 0.30 x 0.70 mm needle. A 1:1 ratio of ozone and I-PRP was used. The

two syringes were then connected to a three-way mixing tap, allowing the two products to be combined (Figure 4).

Application of Ozonized I-PRF

After facial asepsis with 70% alcohol or 2% chlorhexidine solution, the product was applied to the target areas in the form of papules using a sterile needle.

Third consultation

Thirty days after the first application, the ozonized I-PRF was reapplied, following the same protocols.

Fourth appointment

Sixty days after the first application, ozonized I-PRF was administered, following the same protocols.

Last Consultation - Final Evaluation

A final evaluation was carried out 60 days after the last application, with standardized photographs and a thorough inspection of the skin. The reduction in expression lines, periorbicular pigmentary changes, wrinkles, sagging, texture, elasticity, and muscle tone were assessed.

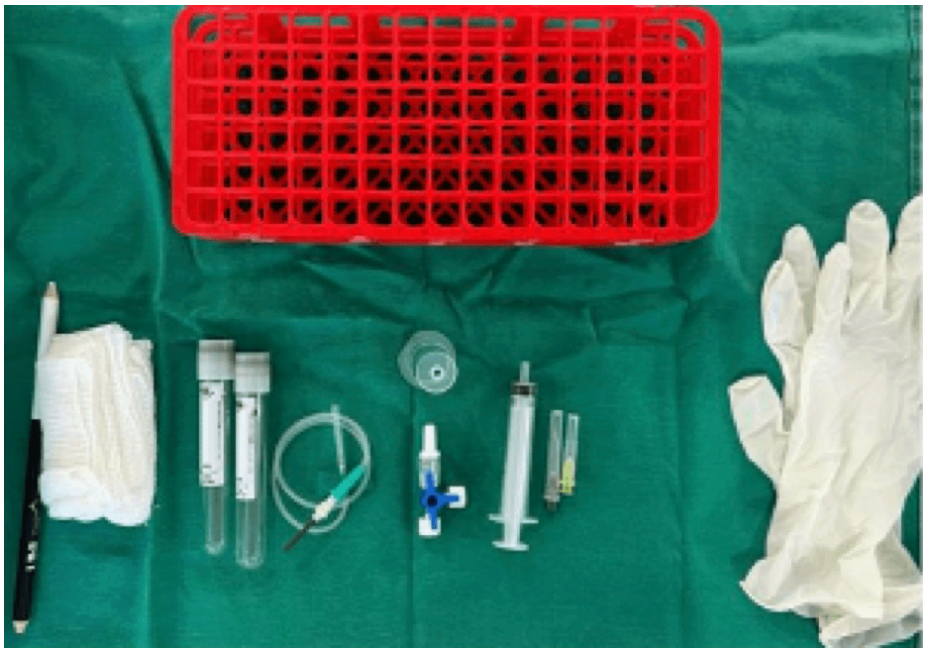

Figure 1. Clinical Procedure Table

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Figure 2. Centrifuge Device (Kasvi) and Medical Ozone Generator (Med Plus, Philozon, Balneário Camboriú, SC, Brazil)

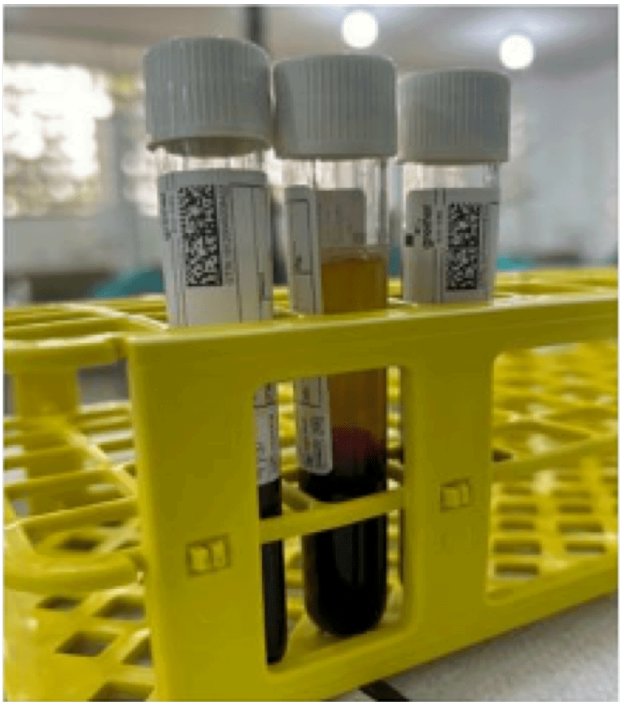

Figure 3. Tubes Containing I-PRF

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Figure 4. Ozonized I-PRF

Case Reports

1st Patient

Patient V.A.B., 25, student, has no comorbidities and does not take medication regularly. The patient reported dissatisfaction with the uneven texture of her skin, describing it as rough to the touch and lacking in radiance. She also mentioned the presence of spots of varying shades, possibly associated with sun exposure, acne scars, or hormonal factors. Her main goal was to even out the skin, improve its luminosity, and smooth out imperfections. A photo session was carried out during the patient's anamnesis phase to follow up the treatment sequentially. Another photo session was held at the end of the treatment to record the results obtained (Figure 5-8).

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Figure 5: Baseline frontal view before the first application of ozonized I-PRF.

Figure 6: Baseline lateral view before treatment.

Figure 7: Frontal view 60 days after the third application.

Figure 8: Lateral view at final evaluation, demonstrating improved skin texture, brightness, and reduced hyperpigmentation.

Figure 5-8. Sequential photographic documentation of patient V.A.B. throughout the treatment period.

2nd Patient

Female patient C.A.C.B, 51 years old, works in the sun, has no comorbidities, and does not take medication regularly. In the patient's initial facial examination, carried out before treatment began, she expressed dissatisfaction with the texture of her skin, as well as reporting the presence of sagging and expression lines. The patient also mentioned that she doesn't eat a diet restricted to fats and carbohydrates, but that she keeps adequately hydrated. A photo session was carried out during the patient's anamnesis phase to follow up the treatment sequentially (Figure 9-18). Another photo session was held at the end of the treatment to record the results obtained.

Figure 9: Baseline frontal view before the first application

Figure 10: Baseline oblique view.

Figure 11:Frontal view after the second application.

Figure 12: Final frontal view 60 days after the last application.

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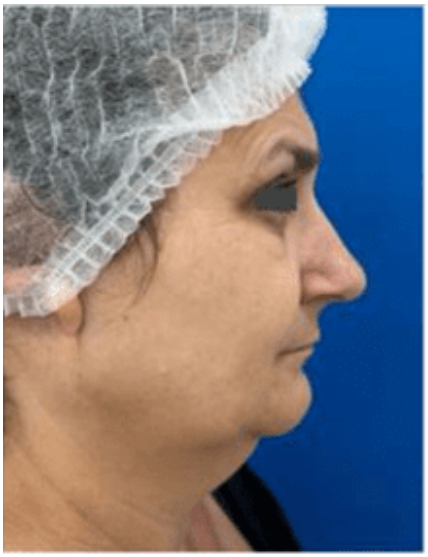

Figure 13: Lateral post-treatment views

Figure 14:oblique post-treatment views.

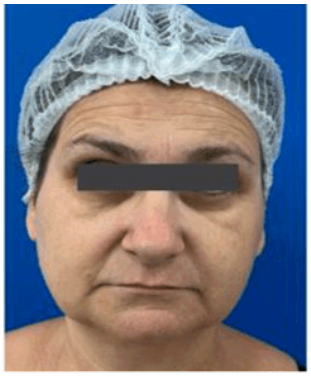

Figure 15: Frontal post-treatment views

Figure 16: Reduction of expression lines

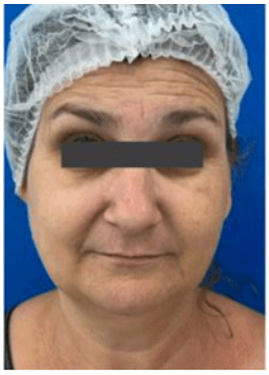

Figure 17: Frontal view 60 days after the third application, demonstrating improved skin texture

Figure 18: Lateral view at final evaluation, demonstrating improved skin texture, reduction of expression lines, and increased firmness

Figure 9-18. Sequential photographic documentation of patient C.A.C.B. during the treatment protocol.

3rd Patient

Male patient G.N., years old, has no comorbidities and does not take medication regularly. In the patient's initial facial examination, carried out before the start of treatment, he expressed dissatisfaction with the texture of his skin, and also reported being bothered by fine lines, and expression in the infra-orbital region. The patient reported eating a balanced diet and exercising regularly. A photo session was carried out during the patient's anamnesis phase to follow up the treatment sequentially (Figure 19-26). Another photo session was held at the end of the treatment to record the results obtained.

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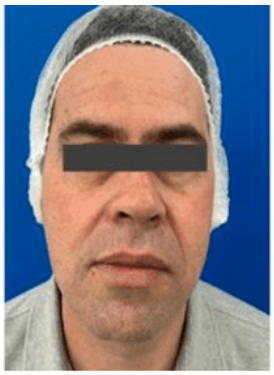

Figure 19: Pre-treatment frontal views.

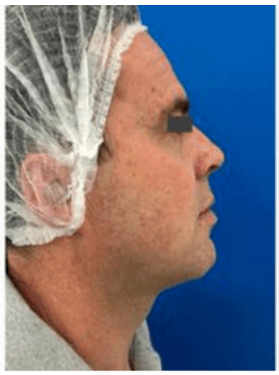

Figure 20: Pre-treatment oblique views.

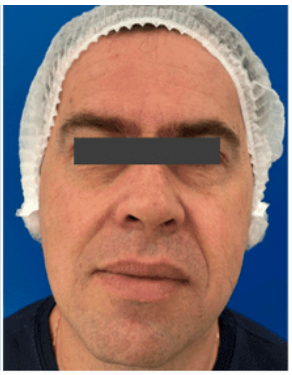

Figure 21: Mid-treatment image showing gradual improvement, frontal views

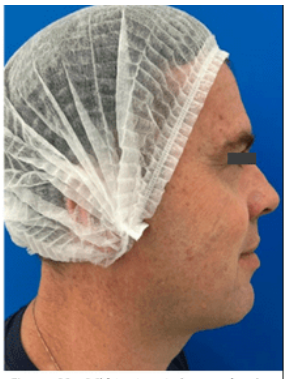

Figure 22: Mid-treatment image showing gradual improvement, lateral views

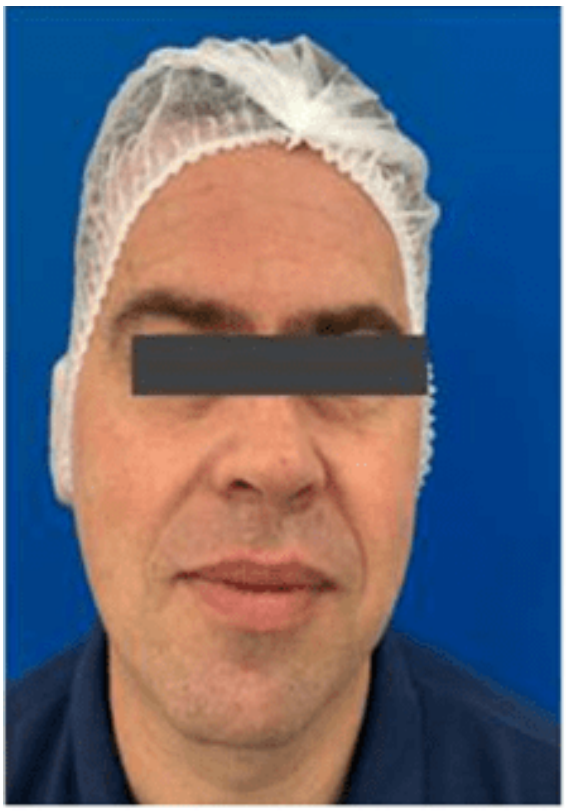

Figure 23: Final evaluation image demonstrating improved skin tone.

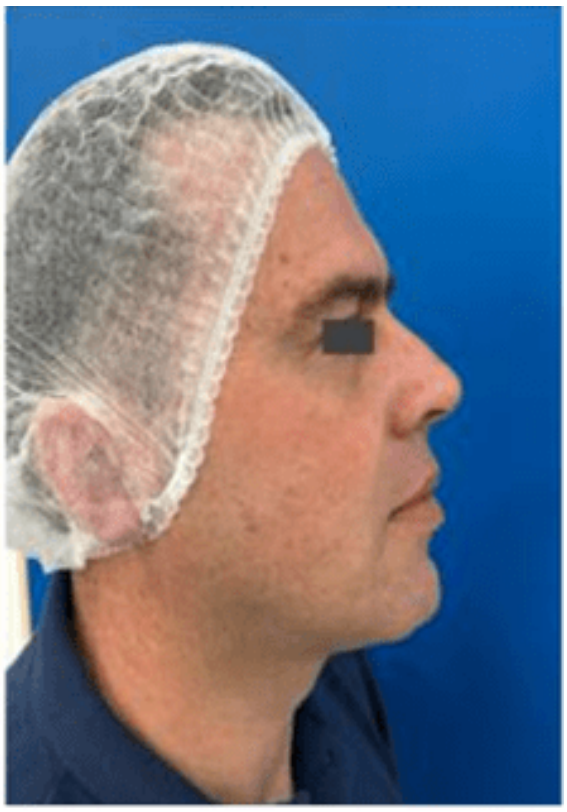

Figure 24: Final evaluation images demonstrating reduction in infraorbital lines

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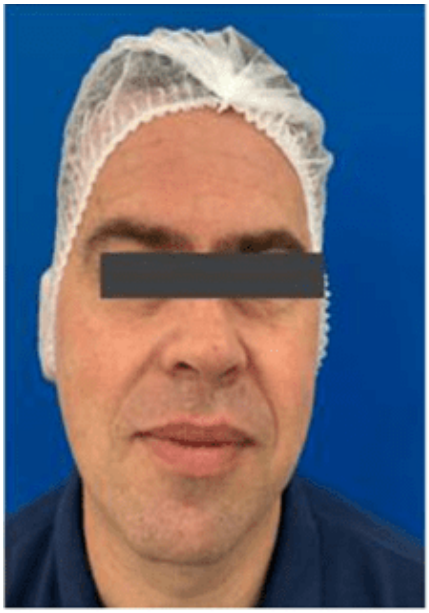

Figure 23: Final evaluation image demonstrating improved skin tone.

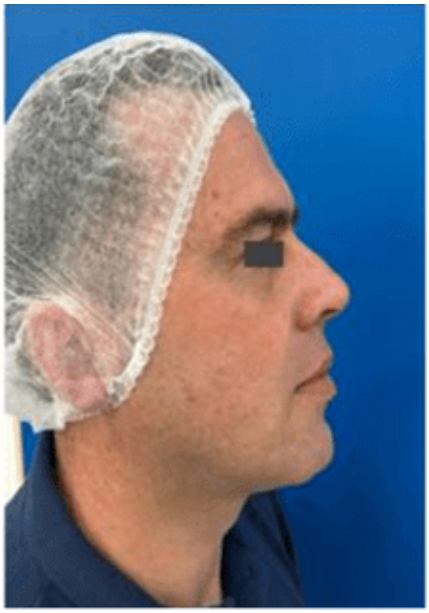

Figure 24: Final evaluation images demonstrating reduction in infraorbital lines

Figure 19-26. Sequential photographs of patient G.N. illustrating the clinical progression

4th Patient

Female patient C.H., 50 years old, diagnosed with Hashimoto's disease (autoimmune thyroid), on continuous medication to regulate hormones. The patient exercises regularly, maintains adequate hydration, and follows a balanced diet. During the anamnesis, the patient reported a weight loss of 30 kg, associated with a perception of sagging in the facial region as a consequence of this significant change in body weight. The patient reported that this change in facial structure became more evident after losing weight, which may be related to the loss of subcutaneous adipose tissue and the consequent reduction in skin support in the region. A photo session was carried out during the patient's anamnesis phase to follow up the treatment sequentially (Figure 27-34). Another photo session was held at the end of the treatment to record the results obtained.

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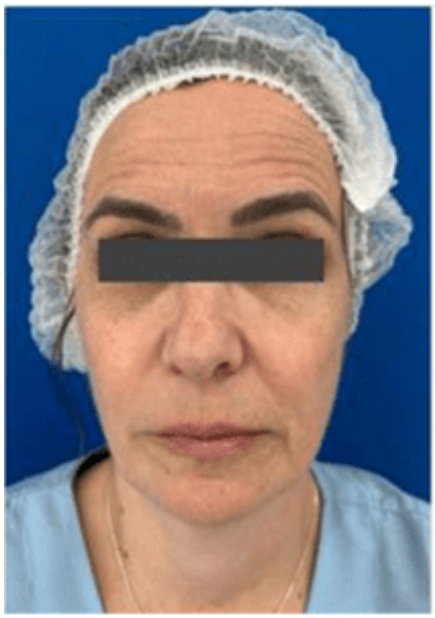

Figure 27: Baseline frontal views.

Figure 28: Baseline frontal views.

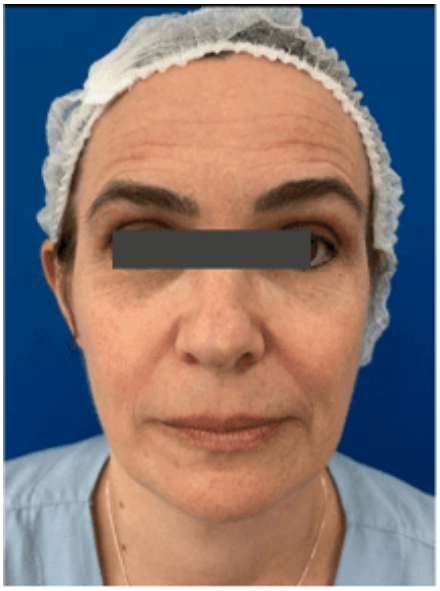

Figure 29: Image frontal taken after the second session

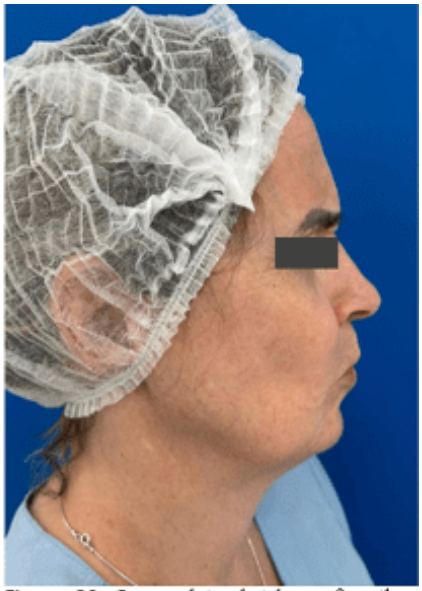

Figure 30: Image lateral taken after the second session

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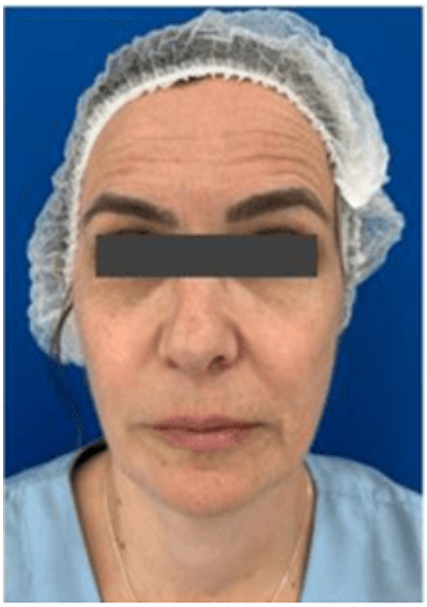

Figure 31: Final session outcome images showing reduced sagging.

Figure 32: Final session outcome images showing improved elasticity.

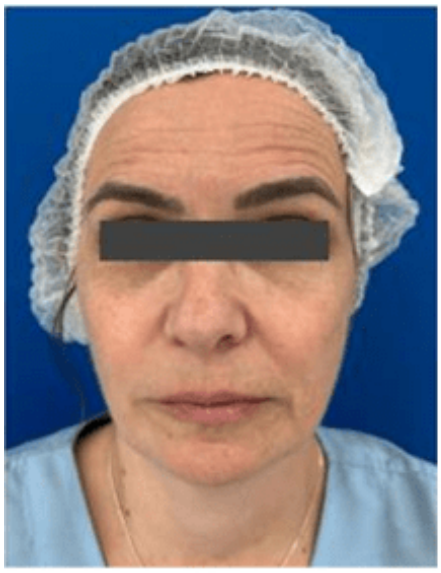

Figure 33: Comparative views highlighting facial contour definition and rejuvenation.

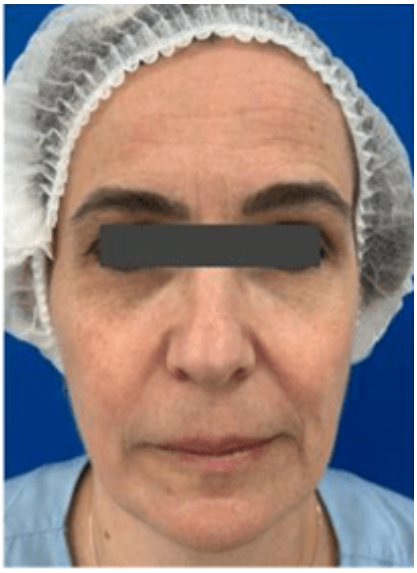

Figure 34: Comparative views highlighting facial contour definition and rejuvenation.

Figure 27-34. Clinical images of patient C.H. documenting treatment effects over time.

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5th Patient

Female patient R.C, 70 years old, has no comorbidities and does not take medication regularly. In the patient's initial facial examination, carried out before treatment began, she expressed dissatisfaction with the uneven texture and some pigmentation on her skin. She reported that, despite daily care, she was unable to achieve the desired uniformity. Her goal was to achieve a more lush and even complexion. After a detailed assessment, a personalized protocol was recommended to treat her complaints. A photo session was carried out during the patient's anamnesis phase to follow up the treatment sequentially (figure 35-43). Another photo session was held at the end of the treatment to record the results obtained.

Figure 35: Pre-treatment image frontal indicating uneven skin texture and pigmentation

Figure 36: Pre-treatment image lateral indicating uneven skin texture and pigmentation

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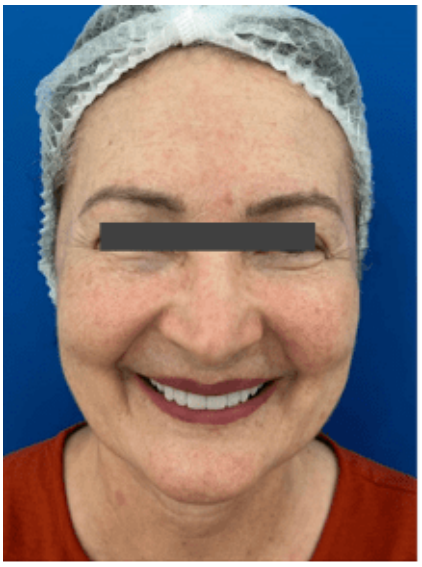

Figure 37: Intermediate progress image.

Figure 38: Intermediate progress image.

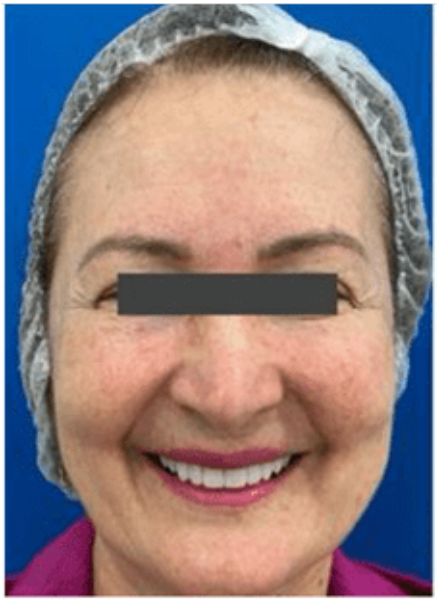

Figure 39: Final evaluation demonstrating enhanced skin brightness and smoothness

Figure 40: Final evaluation demonstrating enhanced skin brightness and smoothness

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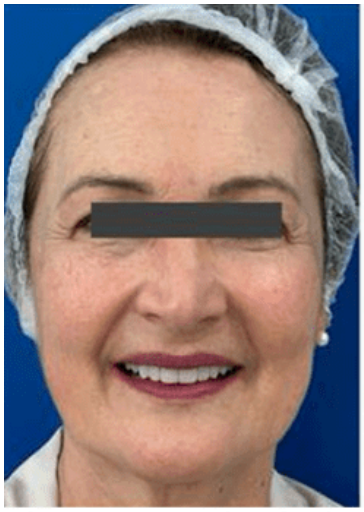

Figure 41: : Pre-treatment image frontal

Figure 42: : Pre-treatment image lateral

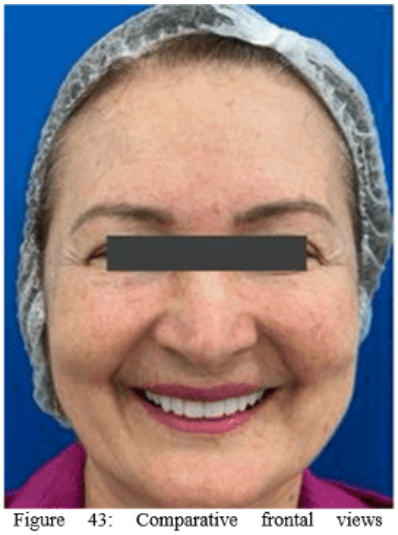

Figure 43: Comparative frontal views confirming overall rejuvenation.

Figure 35-43. Sequence of clinical photographs of patient R.C. during I-PRF therapy.

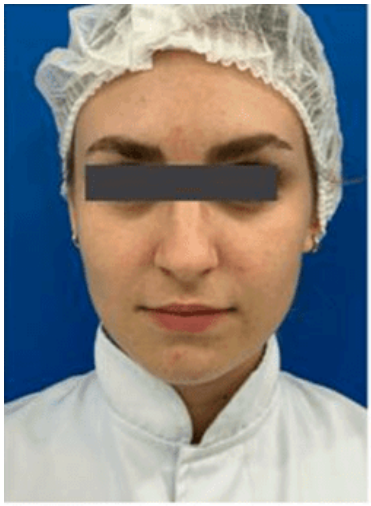

Figure 44: Final evaluation images of patient V.A.B.

Figure 45: Final evaluation images of patient V.A.B.

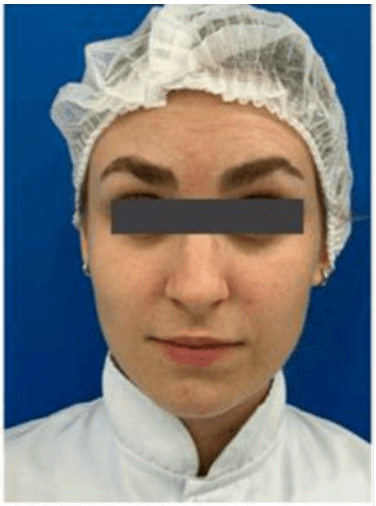

Figure 46: Final evaluation images of patient V.A.B.

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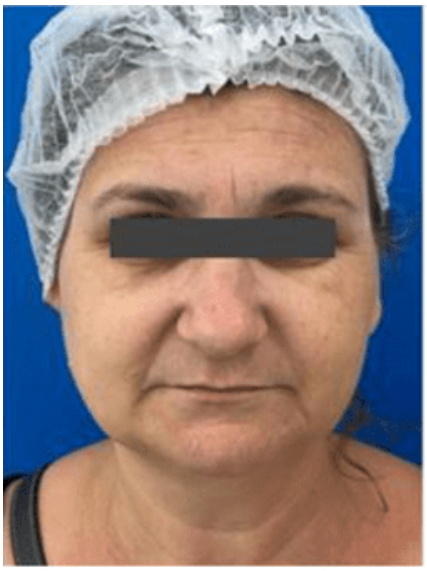

Figure 47: Final outcome of patient C.A.C.B.

Figure 48: Final outcome of patient C.A.C.B.

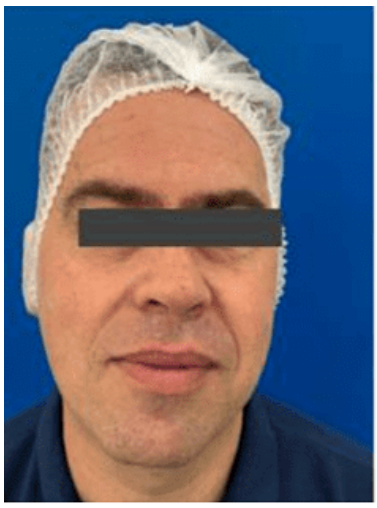

Figure 49: Final images of patients G.N.

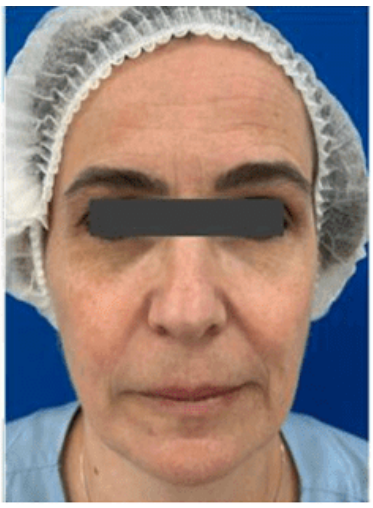

Figure 50: Final images of patients C.H.

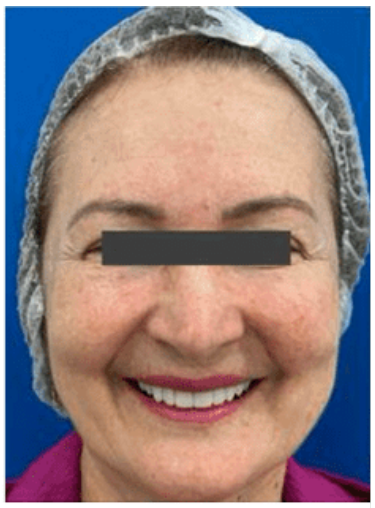

Figure 51: Final photographic outcome of patient R.C., showing global improvement in facial aesthetics.

Figure 44-51. Post-treatment follow-up and clinical documentation of all five patients.

Results

The patients underwent three treatment sessions with ozonized I-PRF, carried out at 30-day intervals. The evaluation of the five participants took place 60 days after the last application, including analysis of the images recorded during each evaluation period, visual clinical examination, and palpation of the skin.

In all cases, a substantial improvement in skin quality and facial rejuvenation was observed, characterized by a smoother, more even, and hydrated appearance. In addition, a reduction in fine lines and expression marks was identified, accompanied by an increase in skin elasticity and radiance. Subjective analysis revealed that patients reported a feeling of greater firmness and facial revitalization. No participants showed any adverse effects or signs of skin irritation during the follow- up period.

Discussion

This study aimed to evaluate facial aesthetic outcomes following the implementation of a treatment protocol involving ozonized injectable platelet-rich fibrin (I-PRF). The effectiveness of the protocol was assessed subjectively through photographic analysis, visual clinical examination, palpation of the skin, and evaluation of images recorded at each treatment interval. Improvements were noted in various facial aspects, including hydration, brightness, texture, and reduction of minor pigmentation, suggesting that ozonized I-PRF may serve as a promising therapy for enhancing facial aesthetics.

To understand the biological mechanisms underpinning these clinical outcomes, it is essential to consider the regenerative properties of platelet-rich fibrin (PRF), which serves as the foundation of the applied protocol. PRF plays a critical role in skin regeneration and rejuvenation due to its composition rich in growth factors and bioactive substances such as platelets, fibrin, leukocytes, and stem cells, which collectively promote tissue repair and cellular regeneration. PRF is derived from autologous blood samples without chemical additives, using a centrifugation process that concentrates its biological components. When applied to the skin, PRF stimulates collagen production and accelerates cell regeneration, improving skin texture and elasticity.

Injectable PRF (I-PRF) is a variation of PRF, prepared using a similar method but with a lower-speed centrifugation process. This results in a higher concentration of stem cells and growth factors in the liquid phase, enabling more efficient application to soft tissues such as the skin. As noted by Buzalaf [13], PRF, I-PRF, and platelet-rich plasma (PRP) are safe and well-tolerated approaches in aesthetic procedures, promoting collagen stimulation and cutaneous rejuvenation.

Dashore et al. [14] provided detailed guidelines for the preparation and clinical use of PRF in dermatology, emphasizing its utility both as a monotherapy and in combination with other therapies for skin rejuvenation and ulcer healing. Moreover, these authors reported that concentrated PRF (C-PRF)

significantly enhances growth factor release and Type I collagen synthesis, establishing it as an effective strategy for tissue regeneration.

In a study conducted by Mikhael and El-Esaway [15], the use of autologous I-PRF resulted in an average improvement of over 50% in facial rejuvenation, accompanied by high patient satisfaction based on self-assessment. These findings are consistent with those reported by Redaelli [16], who observed significant improvements in facial and neck skin texture in a case series of 23 participants following three sessions of I-PRF, despite the absence of a control group. Redaelli emphasized the treatment's potential to stimulate cellular regeneration and enhance skin quality—effects frequently sought in facial rejuvenation therapies. These results reinforce the findings of the present case report, further supporting the use of I-PRF as a promising alternative in regenerative aesthetics, with a positive impact on skin texture and patient satisfaction.

Supporting the current case report, the study by Hassan et al. [17] investigated the efficacy of I-PRF in facial rejuvenation. This study involved 11 healthy patients who underwent intradermal I-PRF injections over a three-month period. Results demonstrated a highly favorable response to treatment, particularly in the significant reduction of facial hyperpigmentation, suggesting the effectiveness of I-PRF in enhancing skin appearance. These outcomes align with those of Elaine et al. [18], who also reported significant improvements in skin quality following I-PRF application. Collectively, these findings contribute to the growing body of evidence supporting I-PRF as an effective therapeutic option for facial aesthetics and pigmentary disorders.

Ozone therapy has been widely used in facial aesthetics as an effective alternative for treating signs of aging and improving overall skin quality. It is particularly beneficial for addressing skin laxity, fine lines, and hyperpigmentation, contributing to cutaneous rejuvenation [19]. The positive effects of ozone therapy on collagen production and skin regeneration involve multiple biological mechanisms. The technique stimulates collagen synthesis, which is essential for improving skin firmness and texture. Lacerda et al. [20] emphasized that ozone therapy promotes cellular bio-stimulation, ensuring safe and effective results with high patient satisfaction. Additionally, Valacchi et al. [21] explained that the medical ozone-oxygen gas mixture can induce the synthesis of growth factors via redox transducer activation, accelerating the cell cycle and promoting tissue regeneration.

Ozonized I-PRF has emerged as a highly promising collagen bio-stimulator due to ozone's ability to increase the concentration of key growth factors involved in cellular regeneration and extracellular

matrix remodeling. Archangelo et al. [22] argued that ozonation enhances the biochemical properties of I-PRF, making it a more effective collagen-inducing biomaterial compared to non-ozonized formulations. These enhancements result from modifications in the plasma's composition and structure, yielding a material enriched with growth factors and bioactive proteins that are essential in dermal revitalization, cellular regeneration, and extracellular matrix organization.

Elaine et al. [18] reported clinical results from the application of I-PRF combined with medical ozone, which closely mirrors the present case. Following three sessions of ozonized I-PRF, improvements were observed in wrinkle depth, expression lines, skin brightness, and elasticity. These outcomes are attributed to the therapeutic properties of medical ozone, which induces an antioxidant response that promotes the removal of senescent cells and tissue regeneration. When combined with liquid fibrin, ozone further stimulates collagen production—a key protein for maintaining skin firmness and structural integrity [23].

Although no adverse effects were observed in the clinical reports reviewed, it is important to note that, like any injectable treatment, the use of collagen biostimulators—including ozonized I-PRF—may lead to complications such as allergic reactions, infections, hematomas, asymmetries, and unsatisfactory aesthetic outcomes [24]. Therefore, all therapeutic options and potential risks should be carefully evaluated and discussed with a qualified healthcare provider before opting for ozonized I-PRF treatment.

Despite the promising results reported, the literature concerning the combination of I-PRF with medical ozone remains limited and in its early stages of development. Few in-depth studies have investigated this association for facial rejuvenation, underscoring the need for further research to better elucidate the mechanisms of action, refine therapeutic protocols, and minimize potential adverse effects.

Conclusion

The findings of this study indicate that ozonized I-PRF therapy represents a promising approach for facial rejuvenation, offering significant improvements in skin quality, including the reduction of expression lines, decreased hyperpigmentation, and enhanced muscular tone. These results reinforce its potential as a safe and effective therapeutic alternative, contributing to sustained aesthetic benefits in regenerative facial treatments.

Abbreviations

- I-PRF: Injectable Fibrin-Rich Plasma

- PRF: Platelet-Rich Fibrin
- PRP: Platelet-Rich Plasma
- C-PRF: Concentrated Platelet-Rich Fibrin
- CEP: Research Ethics Committee
- FICF: Free and Informed Consent Form
- RPM: Revolutions Per Minute

Statements and Declarations

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Author contributions

NAC: Conceptualization, Investigation, Writing-original draft. MPS: Investigation, Data collection, Writing-original draft. MRVC: Investigation, Patient monitoring, Writing-review & editing. JKU: Validation, Supervision, Writing-review & editing. PON: Methodology, Validation, Writing-review & editing. VC: Conceptualization, Supervision, Project administration, Writing-review & editing. All authors read and approved the submitted version.

Ethical approval

The study was approved by the Research Ethics Committee (CEP) of the State University of Western Paraná (Unioeste) under CAAE number 80831924.8.0000.0.10107.

Consent to participate

Informed consent to participate in the study was obtained from all participants.

Consent to publication

Informed consent to publication was obtained from all participants, including specific consent for the use of facial photographs for scientific publication purposes.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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