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Case Report

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

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Introduction: Facial rejuvenation through ozonized injectable platelet-rich fibrin (I-PRF) represents an innovative approach that combines regenerative and bio-oxidative properties.
Objectives: To report and describe the outcomes of five patients treated with ozonized injectable platelet-rich fibrin (I-PRF).
Case report: Five patients (four women, one man; age range: 25-70 years) presented with complaints of skin laxity, uneven texture, hyperpigmentation, expression lines, or scars. No participants had systemic contraindications to treatment. The therapeutic protocol involved three monthly sessions of ozonized I-PRF injections in multiple facial areas (glabella, periorbital region, nasolabial fold, and chin). At the 60-day follow-up after the final session, patients demonstrated visible improvements in skin quality, with a reduction of expression lines, increased elasticity, enhanced muscle tone, and decreased pigmentation. No adverse effects or complications were observed.
Conclusion: This case report series suggests that the combination of autologous fibrin matrices with ozone may enhance regenerative outcomes, offering a promising alternative in aesthetic practice.

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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].

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

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skin, helping to improve the structure and elasticity of facial tissues without the need for surgical interventions [2]. A viable technique for delaying aging 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. To properly separate the blood by-products, the blood is subjected to low-speed centrifugation, a process that 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].

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 aging. 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 the 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 cases of five patients who underwent facial skin treatment with ozonized fibrin-rich plasma.

Case Report

Type of Study and Sample

This case report followed an observational design and consisted of a series of five clinical cases, conducted in accordance with the CARE (CAse REport guidelines) to ensure transparency of clinical data. The sample comprised four female and one male patient who received treatment at the dental clinic of the Universidade Estadual do Oeste do Paraná (UNIOESTE), located in Cascavel, Paraná, Brazil.

Ethical Approval

The present study was approved by the Research Ethics Committee (CEP) of the Universidade Estadual do Oeste do Paraná (UNIOESTE) under CAAE number 80831924.8.0000.0.10107. All participants provided written informed consent by signing the informed consent form after being thoroughly informed about the objectives, procedures, potential risks, and expected benefits of the study. Ethical principles for research involving human subjects were strictly observed throughout all stages of the study.

Eligibility Criteria

The inclusion criteria comprised patients over 25 years of age presenting with sagging skin, infraorbital dark circles, or small cutaneous scars. Exclusion criteria included pregnancy or breastfeeding, presence of systemic comorbidities, smoking habit, continuous antibiotic use for 14 days or more, and current treatment for neoplastic diseases. These criteria were applied to ensure both the safety of participants and the homogeneity of the sample regarding the clinical condition.

Clinical Intervention

All reported cases underwent treatment with ozonized injectable platelet-rich fibrin. The therapeutic protocol consisted of three application sessions performed at 30-day intervals. The anatomical regions treated included the frontal area, glabellar region, bilateral periorbital region, bilateral nasolabial folds, and chin. In addition, areas presenting sagging skin and expression lines were specifically targeted. The intervention was conducted following a standardized protocol to promote reproducibility and comparability among cases, while aiming to improve skin quality and overall facial aesthetics.

Baseline Assessment

At the first visit, a structured questionnaire was used to collect systemic and oral health information, pre-existing conditions, allergies, and current medications. Patients were counseled on treatment objectives, expected outcomes, and pre- and post-care recommendations. A clinical examination assessed skin texture, elasticity, and muscle tone, and treatment sites were defined based on facial analysis and patient concerns. An individualized plan, including session number and frequency, was established, reviewed with patients, and consent was documented. Standardized baseline photographs were obtained for comparison.

Preparation and application of ozonized I-PRF

Blood collection was performed under aseptic conditions, with 10 mL of venous blood centrifuged (KASVI 500, 3,300 RPM, 3 min) to obtain I-PRF. Medical ozone (4 mL, 60 mcg/mL) was produced with an ozone generator (Med Plus, Philozon, Brazil) and combined with I-PRF in a 1:1 ratio using a three-way stopcock. After facial antisepsis with 70% alcohol or 2% chlorhexidine, the ozonized I-PRF was administered via microinjections in papular form to the predetermined areas.

Treatment protocol

Three sessions were performed at 30-day intervals, following the same standardized procedure to ensure reproducibility.

Final evaluation

Sixty days after the last application, outcomes were assessed through standardized photographs and clinical examination, focusing on changes in expression lines, periorbital pigmentation, wrinkles, sagging, skin texture, elasticity, and muscle tone. This systematic evaluation enabled an objective comparison with baseline findings and an appraisal of treatment efficacy.

Case Reports

Case 1 – Patient V.A.B. (female, 25 years old)

The patient presented with complaints of irregular skin texture, decreased luminosity, and hyperpigmented lesions. She reported no known comorbidities and was not on any regular medications. These cutaneous alterations were considered likely associated with cumulative sun exposure, residual acne scarring, or hormonal factors. The primary therapeutic objective was to enhance skin radiance and minimize superficial imperfections, addressing not only aesthetic concerns but also promoting a positive impact on the patient's self-esteem and overall well-being. To assess the efficacy of the intervention, standardized baseline and post-treatment photographs were obtained (Figure 1), providing an objective basis for the evaluation of clinical outcomes.

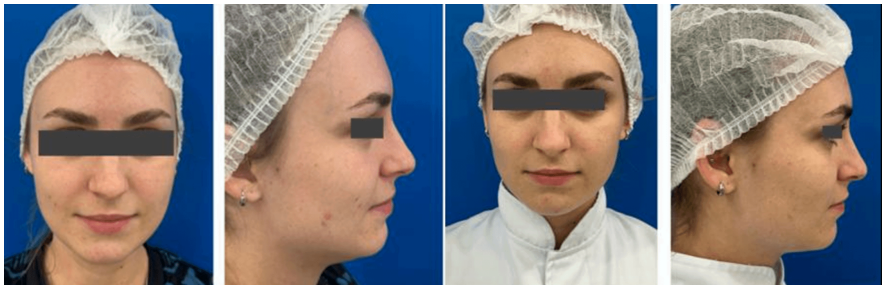

Figure 1. Sequential clinical photographic documentation of patient V.A.B. illustrating treatment progression. (a) Baseline frontal and lateral views, depicting initial irregular skin texture and hyperpigmented lesions. (b) Frontal and lateral views at the final evaluation, demonstrating partial improvement in skin radiance and a reduction of superficial imperfections.

Case 2 – Patient C.A.C.B (female, 51 years old)

The patient reported dissatisfaction with skin laxity, fine expression lines, and altered texture. She informed that she works outdoors, has no comorbidities, and does not use regular medications. The therapeutic objective was to improve skin firmness, reduce visible lines, and restore a more uniform texture, thereby enhancing facial aesthetics and overall self-confidence. Standardized photographs were obtained at baseline and after completion of the treatment protocol (Figure 2).

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Figure 2. Sequential clinical photographic documentation of patient C.A.C.B. (a) Baseline frontal and lateral views, showing sagging and expression lines. (b) Frontal and lateral views at the final evaluation, showing enhanced texture and improvement in skin firmness and a reduction of expression lines.

Case 3 – Patient G.N. (male, 35 years old)

The patient reported dissatisfaction with skin texture, the presence of fine lines, and infraorbital wrinkles. He informed that he has no comorbidities, does not take regular medications, and maintains a balanced diet and regular physical activity. The main therapeutic objective was to improve skin texture, reduce fine lines, and enhance the appearance of the periorbital region, aiming to promote a more youthful and refreshed appearance while supporting self-esteem. Standardized photographs were obtained before and after treatment to objectively assess clinical progression (Figure 3).

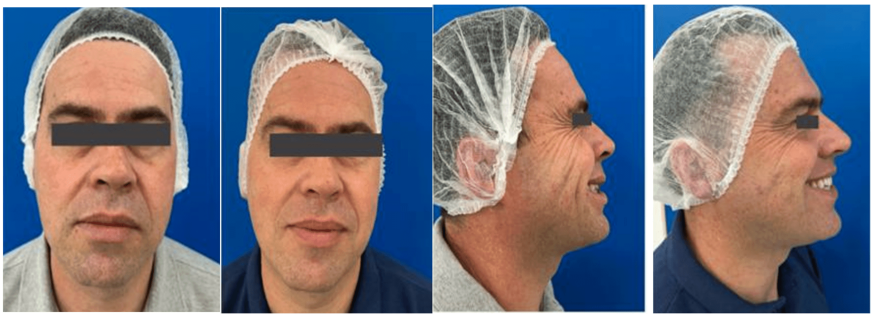

Figure 3. Sequential clinical photographic documentation of patient G.N. (a) Frontal view at baseline and final evaluation, showing noticeable improvement in the infraorbital region and overall facial appearance. (b) Lateral view at baseline and final evaluation, demonstrating a reduction in fine lines and improved skin texture.

Case 4 – Patient C.H. (female, 50 years old)

The patient reported a prior diagnosis of Hashimoto's thyroiditis, managed with continuous hormonal therapy. She also reported a 30 kg weight loss associated with significant facial sagging, likely due to reduced subcutaneous adipose tissue. She maintained a balanced diet, adequate hydration, and regular physical exercise. Her primary concerns were facial laxity and loss of skin firmness. The treatment objective was to restore tissue support, improve skin texture, and

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enhance facial contour, contributing positively to aesthetic satisfaction and overall self-confidence. Baseline and post-treatment photographs were obtained for documentation of clinical outcomes (Figure 4).

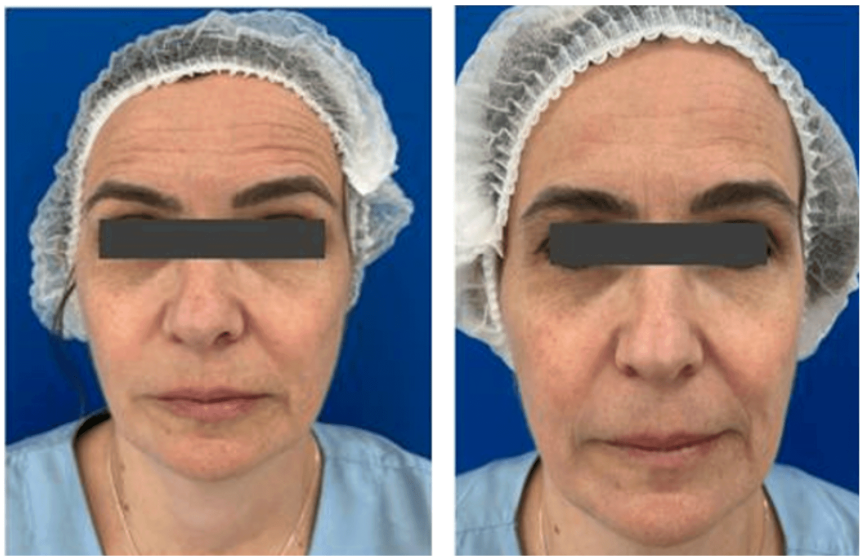

Figure 4. Sequential clinical photographic documentation of patient C.H. at baseline and at the final evaluation, showing enhancement of facial volume and overall skin texture.

Case 5 – Patient R.C. (female, 70 years old)

The patient reported dissatisfaction with uneven skin texture and hyperpigmented areas, which persisted despite routine skincare. She informed that she has no comorbidities and does not take any regular medications. The main therapeutic objective was to achieve a smoother, more homogeneous complexion, reduce pigmentation irregularities, and improve overall skin quality, promoting increased self-confidence and subjective well-being. Standardized baseline and post-treatment photographs were obtained for objective assessment (Figure 5).

Figure 5. Sequential clinical photographic documentation of patient R.C. (a) Baseline frontal and lateral views, showing irregular texture and hyperpigmented areas. (b) Frontal and lateral views at the final evaluation, demonstrating enhanced overall skin quality and evenness.

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Discussion

The present case series aimed to evaluate facial aesthetic outcomes following a standardized treatment protocol with ozonized injectable platelet-rich fibrin (I-PRF). All patients underwent three treatment sessions at 30-day intervals, with evaluations conducted 60 days after the final application. Clinical assessments included visual inspection, palpation of the skin, and analysis of standardized photographs obtained at each treatment interval. In all five cases, a marked improvement in skin quality and facial rejuvenation was observed. Clinically, this was characterized by smoother, more even, and hydrated skin, along with a reduction in fine lines and expression marks, increased elasticity, and enhanced radiance. Patients reported subjective benefits, including a sensation of greater firmness and overall facial revitalization. Importantly, no adverse effects or signs of irritation were reported during the follow-up period, indicating that the intervention was well tolerated.

These findings align with the regenerative properties of platelet-rich fibrin (PRF), which serves as the biological foundation of the applied protocol. PRF is rich in growth factors, platelets, fibrin, leukocytes, and stem cells, collectively promoting tissue repair, collagen synthesis, and cellular regeneration [13]. It is derived from autologous blood without chemical additives through a centrifugation process that concentrates its biological components. When applied to the skin, PRF stimulates collagen production and accelerates cellular regeneration, improving skin texture and elasticity [13].

Injectable PRF (I-PRF) is a variation prepared with a lower-speed centrifugation protocol, resulting in a higher concentration of stem cells and growth factors in the liquid phase, allowing efficient application to soft tissues such as the skin [13]. Previous studies have reported that PRF, I-PRF, and platelet-rich plasma (PRP) are safe and well-tolerated in aesthetic procedures, promoting collagen stimulation and cutaneous rejuvenation [13]. Clinical evidence further supports these findings. Mikhael and El-Sawy [14] reported that autologous I-PRF led to an average improvement of over 50% in facial rejuvenation, accompanied by high patient satisfaction. Similarly, Redaelli [15] observed significant improvements in facial and neck skin texture in a case series of 23 participants after three sessions of I-PRF, noting its potential to stimulate cellular regeneration and enhance skin quality. Elaine et al. [16] also reported notable improvements in skin quality following I-PRF application. Collectively, these studies support I-PRF as an effective therapeutic option for facial aesthetics and pigmentary disorders [14][15][16].

The combination of I-PRF with medical ozone introduces additional regenerative potential. Ozone therapy has been widely employed in facial aesthetics to address signs of aging, including skin laxity, fine lines, and hyperpigmentation, contributing to cutaneous rejuvenation [17]. The biological effects of ozone on collagen production and tissue regeneration involve multiple mechanisms, including stimulation of collagen synthesis and cellular biostimulation [17][18]. Valacchi et al. [19] demonstrated that medical ozone-oxygen mixtures can activate redox transducers, inducing growth factor synthesis, accelerating the cell cycle, and promoting tissue regeneration.

Ozonized I-PRF has been proposed as a potent collagen biostimulator due to ozone's capacity to enhance growth factor concentration, extracellular matrix

remodeling, and cellular regeneration. Archangelo et al. [20] reported that ozonation improves the biochemical properties of I-PRF, yielding a material enriched in bioactive proteins and growth factors that are critical for dermal revitalization and extracellular matrix organization. Elaine et al. [16] documented clinical improvements in wrinkle depth, expression lines, skin brightness, and elasticity following combined ozone-I-PRF therapy. These outcomes are attributed to the antioxidant and regenerative properties of medical ozone, which promotes clearance of senescent cells and stimulates collagen synthesis when applied with liquid fibrin [16].

Although no adverse effects were observed in the current case series, injectable treatments, including ozonized I-PRF, may carry risks such as allergic reactions, infections, hematomas, asymmetries, or unsatisfactory aesthetic outcomes [21]. Therefore, careful evaluation of all therapeutic options and potential risks is essential before treatment. Despite promising results, the literature on I-PRF combined with medical ozone remains limited and in early development. Few in-depth studies have investigated this combination for facial rejuvenation, highlighting the need for further research to elucidate mechanisms, refine protocols, and minimize potential adverse effects [16][17][18][19][20].

This study has several limitations that should be acknowledged. As an observational case series with a small sample size, the findings cannot establish causality, and the possibility of a placebo effect cannot be excluded. Furthermore, outcomes were assessed subjectively through clinical observation and patient reports without the use of validated quantitative scales or blinded assessors, introducing potential bias. The absence of a control group further limits attribution of the observed effects solely to the intervention. Future research employing randomized controlled trial designs with objective, standardized assessment tools is necessary to validate these preliminary findings and confirm the efficacy of ozonized I-PRF in facial rejuvenation.

Conclusion

The findings of this case series suggest that treatment with ozonized I-PRF may be a promising option for improving skin quality, facial rejuvenation, and patient-perceived outcomes. Improvements were observed in skin texture, hydration, elasticity, and the reduction of fine lines, with high patient satisfaction and no adverse effects. However, given the uncontrolled design, small sample size, and reliance on subjective assessments, these results should be interpreted cautiously. Further controlled studies are warranted to corroborate these preliminary observations, elucidate underlying mechanisms, and optimize therapeutic protocols.

Abbreviations

- I-PRF: Injectable Platelet-Rich Fibrin
- 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

Funding

No specific funding was received for this work.

Potential Competing Interests

No potential competing interests to declare.

Ethics

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

Data Availability

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

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.

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Declarations

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