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ORIGINAL CONTRIBUTION

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Autologous pure platelet-rich plasma injections for facial skin rejuvenation: Biometric instrumental evaluations and patient-reported outcomes to support antiaging effects

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Summary

Background: During skin aging, a degeneration of connective tissue and decrease in hyaluronic acid polymers occur. Since platelet-rich plasma (PRP) contains growth factors and various cytokines, it was hypothesized that it could play a role in fibroblast activation and type I collagen expression in human fibroblasts.

Objectives: This study was performed to assess the efficacy of autologous PRP injections for facial skin rejuvenation, measured by biometric instrumental evaluations and patient-reported outcomes.

Patients and Methods: Patients signed an informed consent form. The EmCyte PurePRP® system technology was used to produce neutrophil-poor PurePRP. The efficacy of the procedures was assessed by biometric parameters, and a patient outcome a self-assessment questionnaire on each visit and at 6-month follow-up.

Results: Eleven volunteers were included in the study, receiving 3 PurePRP® treatments. A significant decrease in brown spot counts and area ($P < 0.05$) was seen after 3 months. Wrinkle count and volume were significantly reduced ($P < 0.05$ for total wrinkle appearance). Skin firmness parameters were significantly improved. Skin redness was significantly improved after 169 days post-therapy for both the nasolabial and malar areas. A decrease in SLEB thickness was already noted at 2 months after the first injection, with an increase in SLEB density ($P < 0.05$ for both parameters), without affecting subcutaneous fat thickness. Self-assessment at 6-month follow-up revealed an average satisfaction score of >90%.

Conclusions: A series of 3 PurePRP injections at 6-month follow-up resulted in significant skin rejuvenation as demonstrated by biometric parameters and confirmed by patient self-assessment score.

KEYWORDS

antiaging, anti-wrinkle, biometric, pure platelet-rich-plasma, skin rejuvenation

1 | INTRODUCTION

During skin aging, a degeneration of connective tissue and decrease in hyaluronic acid polymers occur due to intrinsic and extrinsic factors. There are cellular changes, alterations in dermal extracellular matrix (ECM) proteins, and dermal atrophy, with solidified dermal-epidermal junctions and fewer fibroblasts.[1] Activation of dermal fibroblasts, remodeling of the ECM, and collagen synthesis is essential for aged skin rejuvenation. Since PRP contains several growth factors, cell adhesion molecules, and various cytokines, it was hypothesized that platelet-rich plasma (PRP) could play a role in fibroblast activation and type I collagen expression in human fibroblasts.

Platelet-rich plasma therapies and treatment protocols have evolved immensely over the past 20 years. Through laboratory, experimental, and clinical research, followed by meta-analyses, physicians, medical practitioners, and scientists have gained a better understanding of the effects of PRP on cellular physiology, especially with regard to the functions of some of the specific biological components in the platelet proteome,[2] affecting PRP treatment outcomes when used in regenerative medicine therapies. To optimize outcomes, based on patient-specific underlying conditions and tissue type, the practitioner needs to have a clear understanding of when to use which specific PRP treatment protocols in a variety of medical indications, to obtain the desired regenerative and tissue repair effects. These various applications have given rise to considerable interest in the potential of PRP in facial rejuvenation and other esthetic applications.

The functional design of existing PRP-processing systems, with the subsequent final PRP production, varies tremendously. Different platelet concentrations and biological compositions are obtained by PRP device-specific preparation protocols. Optimal blood separation is safeguarded by double-spin PRP centrifuges with dedicated disposable concentration devices. These double-spin PRP devices create a 3-layer buffy coat stratum, based on different centrifugal forces and specific gravities of individual blood components (Figure 1). Single-spin devices, or plasma PRP devices, prepare a product from the acellular plasma layer, excluding erythrocytes and leukocytes from the PRP preparation process, while collecting as many platelets as possible from the plasma layer.[3]

These differences in cellular compositions, and thus PRP characteristics, have recently been recognized in the literature.[4,5] The use of a poor-quality and inconsistent PRP product ultimately results in a lower treatment outcome. This was reported by Marques et al[6] for many studies. In this study, we used a PurePRP®, double-spin buffy coat product to obtain a specific concentration of platelets and other constituents, to achieve a clinically significant effect. We used several non-invasive skin diagnostic techniques to objectively assess the PurePRP facial injection effects.

The aim of this skin rejuvenation study was to assess the clinical effectiveness of 3 PRP injections in highly selected subjects, who also were asked to be compliant with the study constraints. Therapy efficacy was measured and calculated by specific, objective, biometric instrumental analysis protocols. Furthermore, we evaluated the patient-reported outcomes of this treatment.

2 | MATERIALS AND METHODS

2.1 | Patients

This single-center open-label study was conducted according to the principles of the Declaration of Helsinki, Good Clinical Practice Guidelines, and General Principles of Portuguese Law (46/2004). The study was reviewed and approved by the IRB. Eleven healthy female volunteers between 45 and 65 years old were enrolled in the study. All women signed an informed consent form before treatment with 3 facial PRP injections. All injections were given by the same physician (L.G.). Eleven patients were enrolled in the study.

Inclusion criteria were as follows: female patients with voluntary participation, age between 40 and 60 years, phototype according to Fitzpatrick's classification between II and IV, and all skin types showing signs of photoaging, chronologic aging, or smoking habits. Exclusion criteria are indicated in Table 1. Furthermore, the volunteers had to agree to several study constraints, as specified in Table 2. The subjects were requested to note every day any reaction observed, and sensation of discomfort felt on the individual observation sheet they were given at the beginning of the study. This examination was performed visually under standard "daylight" source, before, during, and then after treatment.

2.2 | Biometric instrumental assessments

The efficacy of the procedures was assessed by clinical and biometric instrumental parameters in all subjects according to the study

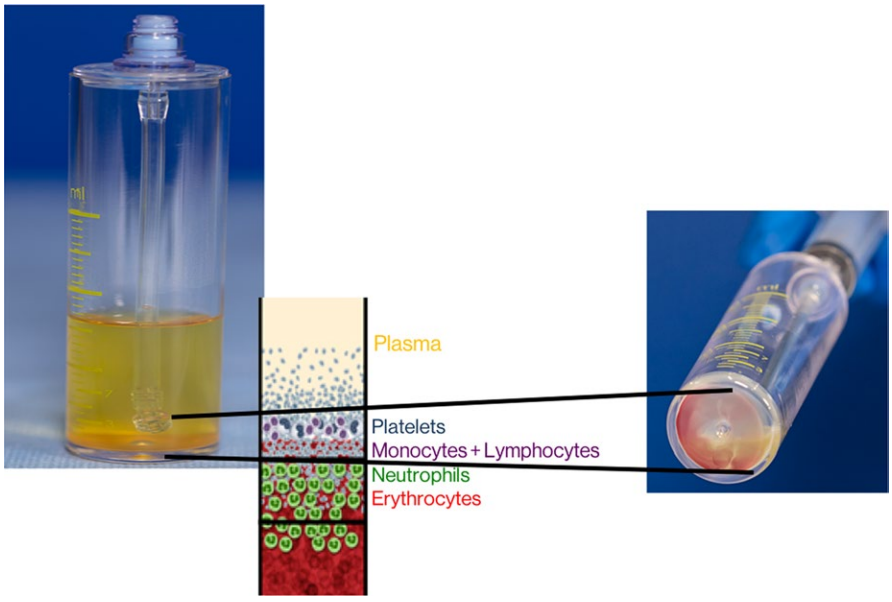

FIGURE 1 EmCyte PurePRP® Concentrating Device, showing the buffy coat layer after a double-spin centrifugation procedure from a vertical position and tilted position

TABLE 1 Exclusion criteria

Individuals who performed an antiaging or esthetic treatment prior to the study: Botox or Botox-like products, peelings, plastic surgery, resurfacing with Laser, IPL, threats, radiofrequency treatments, hyaluronic acid treatment, Plasma-Rich Platelets treatment, or any other specific treatments prone to change the skin aspect during the last 18 mo
Cutaneous marks on the experimental area which could interfere with the assessment of skin reactions (pigmentation problems, scar elements, over-developed pilosity, ephelides, and naevi in too great quantity, sunburn, beauty spots, freckles, etc.)
Eczematous reaction still visible, scar, or pigmentary sequelae of previous tests on the experimental area
Pregnancy and breastfeeding
Intention to pregnancy in the next 3 mo after the start of the study
Forecast of vaccination during the study period or last vaccination within 3 wk before the study
Systemic disorders: cardiovascular, pulmonary, digestive, neurologic, psychiatric, genital, urinary, endocrine
Hematological or hemorrhagic diseases
Thrombocytopenia moderate or severe (<than 100 000 platelets/μL)
Allergy to colophony or nickel
Allergy or reactivity to drugs, food or cosmetic products previously observed, including perfumes or cologne products
Skin hyper-reactivity
Intensive sun exposure within the month before the study
Forecast of intensive sun or UVA exposure (UV lamps) during the test period
Intensive or regular practice of one or several sports whose temporary interruption creates difficulties
Treatment with Vitamin A acid or its derivatives within 3 mo before the beginning of the study
Treatment with topical corticoids on the experimental area within 16 d before the study
Treatment with antibiotics, anti-allergic, anti-inflammatory (systemic or topical corticosteroid therapy) treatment with patent medicines containing vitamin A acid or its derivatives during the study (if therapeutic requirement: exclusion foreseen)
Individuals with a history of any dermatological disease or condition, including but not limited to active atopic dermatitis, psoriasis, eczema, active seasonal allergies, collagen diseases, or skin cancer within the past 6 mo
Individuals who have undergone a bilateral mastectomy with lymph node removal, a unilateral mastectomy with lymph node removal within the last year, or a bilateral axillary lymph node removal
Individuals with a history of immune deficiency or auto-immune disease treated for malignancy within 6 mo prior to enrollment or who are currently under treatment for asthma or diabetes
Treatment with salicylic acid or any anticoagulant drugs during the study
Treatment with PUVA or UVB within 1 mo before the study

schedule as shown in Table 3. The efficacy of the treatment was also evaluated by the volunteer's assessment schedule.

Biometric evaluations consisted of using different instrumental devices. The VISIA-CR system (Canfield, Parsippany, NJ, USA) was used to quantify the development of the antiaging effects by in a full-aligned facial image. To evaluate qualitatively and quantitatively skin profile changes, to assess wrinkle count, depth, and volume, the Optical In Vivo Primos 3D Skin Device (GFMesstechnik GmbH, Berlin, Germany) was applied to the periocular area (left or right). Skin biomechanical evaluations to measure elasticity of the upper skin layers using negative pressure were performed with a Cutometer dual MPA 580 (Courage & Khazaka, Cologne, Germany) using a 2-mm probe. Skin color measurements for luminance were obtained in the malar area with the tri-stimulus color analyzer, Minolta Chromameter CR-400 (Minolta, Osaka, Japan). The Dermascan-C ultrasound device with a modified 20-MHz probe (Cortex Technology, Hadsund, Denmark) was used to measure thickness and density of the subepidermal low echogenic band (SLEB), with calculations of subcutaneous fat thickness.

TABLE 2 Study constraints

No application of products on the experimental area (except the suggested ones), particular any antiaging cosmetic products
No change in hygiene habits
No application of any cosmetic moisturizing products on the face or any makeup on face and lips, on the day of biometric evaluations and PurePRP injections
No drugs that interfere with the study outcomes (anticoagulant, immunosuppression, and salicylic acid drugs)
No change in the way of life or in the physical activity
No dietary activities, or any treatment that significantly impacts body weight
No exfoliating treatment on the experimental areas
Description of any treatment undertaken during the study and all eventual deviations

All biometric instrumental protocol evaluations were performed in a fully controlled room and after an initial acclimatization process of at least 30 minutes in a fully controlled and acclimatized room (controlled temperature: T = 21 ± 2°C; controlled relative humidity: RH = 55 ± 10%).To avoid circadian changes, 2 evaluation periods were defined (morning: 9 AM-1.30 PM; afternoon: 1.30 PM-6 PM). All the evaluations were performed during the same period of the day chosen by the volunteers.

2.3 | Patient self-assessment

All subjects were requested to complete a self-assessment questionnaire on each visit and at 6-month follow-up. The questions of the assessments are charted in a spider-web graph to represent the multivariate data in the form of a two-dimensional chart, including the 8 quantitative variables after each injection and at D169 (Table 4).

2.4 | Pure platelet-rich plasma injection procedure

Subject preparation, injection, and aftercare.

A local anesthetic (EMLA®, Astra Zenica, Cambridge, UK) was applied in all facial areas for at least 30 minutes before the injection.

TABLE 3 Study treatment schedule

Assessments	Screening/admission	D0		D28		D56		D84	D169
Informed written consent	X		1st Pure PRP procedure immediately after D0		2nd Pure PRP procedure immediately after D28		3rd Pure PRP procedure immediately after D56		
Demographic data	X								
Medical history	X								
VISIA-CR		X		X		X		X	X
Primos 3D		X		X		X		X	X
Cutometry		X		X		X		X	X
Color evaluation		X		X		X		X	X
Ultrasound evaluation		X		X		X		X	X
Specific questionnaire				X		X		X	X
Skin examination and questioning		X		X		X		X	X
Adverse events monitoring	X	X							

TABLE 4 Self-assessment questionnaire

Q1. Do you notice an improvement in your wrinkles aspect
Q2. Do you notice your facial complexion more even
Q3. Do you notice your skin firmer
Q4. Do you feel your skin more moisturized
Q5. Do you feel your skin has healthier aspect
Q6. Do you feel your skin softer
Q7. Do you notice your skin more radiant
Q8. Do you notice an improvement on your skin's visible youth fullness

Prior to the injections, excess local anesthetic was removed, and the skin was disinfected with chlorhexidine. Small aliquots of PurePRP in 1-mL insulin syringes were administered intradermally and subcutaneously, using a 13-mm-long 27G or 4-mm-long 32G needle (depending on the thickness of the skin to be treated).

Volunteers underwent 3 sessions of PurePRP treatment at 1-month intervals, with a follow-up period after of 6 months. At the end of each injection procedure, platelet-poor plasma, a byproduct of the PurePRP preparation procedure, was applied to the skin at all injection sites, and the treated area was then covered with polypropylene film for 10 minutes to promote skin penetration. After film removal and skin cleaning, a moisturizing cosmetic product was used (Toleriane Ultra Creme, La Roche-Posay, La Roche-Posay, France). No ice packs were used after the procedure. Daily applications of sunscreen protection were recommended.

2.5 | PurePRP preparation

The EmCyte PurePRP® system technology (EmCyte Corporation, Fort Myers, FL, USA) was used at point of care in the clinic, just before injection. Fifty milliliters of whole blood were pre-donated in a 60-mL syringe containing sodium citrate. Processing was in accordance with the instructions for use from EmCyte Corporation. In all volunteers, EmCyte's proprietary Protocol-A was carried out to produce PurePRP using the GS-60 platelet concentrating devices and the 544E Executive Eppendorf centrifuge. The PurePRP is characterized as a double-spin buffy coat product, with a low erythrocyte concentration and significantly reduced, pro-inflammatory, neutrophils. The final PurePRP volume for the facial injections was standardized to 7 mL of PurePRP in all subjects.

To compensate for the anticoagulant effects of sodium citrate, 0.05 mL of 10% calcium chloride was mixed with 1 mL of PurePRP prior to facial injection.

2.6 | Adverse event monitoring

Adverse effects including erythema, edema, bruising, and altered pigmentation were assessed by questioning the volunteers and observing skin responses at admission and during all follow-up visits.

2.7 | Statistical analysis

All data were analyzed using IBM SPSS Statistics-20 (Armonk, NY, USA). The biometric instrumental efficacy data are expressed as numbered data. All continuous data comparisons were submitted to the Student *T* test or the Wilcoxon signed-rank test. A 5% level of significance was used. The subjective data of efficacy were submitted to binomial testing. $P < 0.05$ was considered significant.

3 | RESULTS

All 11 enrolled female volunteers completed the study, receiving 3 PurePRP injections. The average age was 51 years (range 47-60 years old), and 82% of the women were classified as Fitzpatrick skin type III. The treatment procedures and PurePRP preparations were all consistently performed without any complications. No skin reactions were noted after each procedure. None of the volunteers experienced any discomfort during the study, or during the follow-up period. A well-tolerated burning sensation was reported after the injections. Minor ecchymosis, which resolved within 3 days, was noted with no signs of inflammatory or allergic reactions. None of the subjects reported adverse events during the entire study period.

There was a significant decrease ($P = 0.029$) in brown spot counts after 6 months and a 26.3% reduction ($P = 0.004$) in total brown spot area compared to conditions on admission (Figure 2). No significant changes in red spot counts and red spot area were observed. Mean wrinkle count ($P = 0.000$) and wrinkle volume ($P = 0.049$) were significantly reduced, compared to the count and measurement before the first PurePRP injection, respectively, 66.2 and 2 (Figure 3). Evaluation of the true wrinkle count and volume % changes in all subjects after 169 days revealed a relative transformation in relation to D0 of −37.2% and −11.5%, respectively ($P < 0.05$) (Figure 4). Figure 5 displays a significant % increase in skin firmness parameters in the malar area, compared to baseline. Also, changes in the nasolabial area demonstrated a significant improvement after 56, 84, and 169 days ($P = 0.000$). Skin redness for both nasolabial and malar area was significantly reduced, 29% and 44.3%, respectively, but only after 169 days post-PurePRP procedures (Table 5). On the other hand, the luminance of the malar area improved significantly ($P = 0.016$) after the first PRP injection and continued to improve following the PurePRP procedures, until 3 months after the last injection ($P = 0.006$). A decrease in SLEB thickness ($P = 0.021$) was already eminent at 2 months after the first injection and continued to decrease at D169 ($P = 0.033$), with a simultaneous increase in SLEB density ($P = 0.042$) at 6-month follow-up (Figure 6). The increase in density, pixels/mm^2, did not affect subcutaneous fat thickness ($P = 0.224$).

All subjects respected the study constraints and completed the self-assessment outcome scores. At 6-month follow-up, the average satisfaction score was >90%, as shown in Figure 7.

4 | DISCUSSION

It is well known that during aging, epidermal and dermal changes in the skin are naturally occurring phenomena, with degradation of the ECM.[7] Also, the cessation of collagen fiber and elastin synthesis, with degradation of proteoglycans, results in loss of skin elasticity.[8] Furthermore, skin aging is characterized by flattened dermal-epidermal junctions, dermal atrophy, and the presence of less fibroblasts. The remodeling of ECM and the activation of dermal fibroblasts are essential for rejuvenation of aged skin. It has been reported that the activity of PRP in facial skin rejuvenation induces the synthesis of new collagen by dermal fibroblasts via different molecular mechanisms. Particularly, PRP increases mRNA expression of type I collagen and metalloprotease-1. As a consequence, enhanced dermal elasticity stimulates the removal of photo-damaged ECM components.[9] Choi et al[10] confirmed these data, where they also showed an increase in type I collagen expression in human fibroblasts treated with PRP.

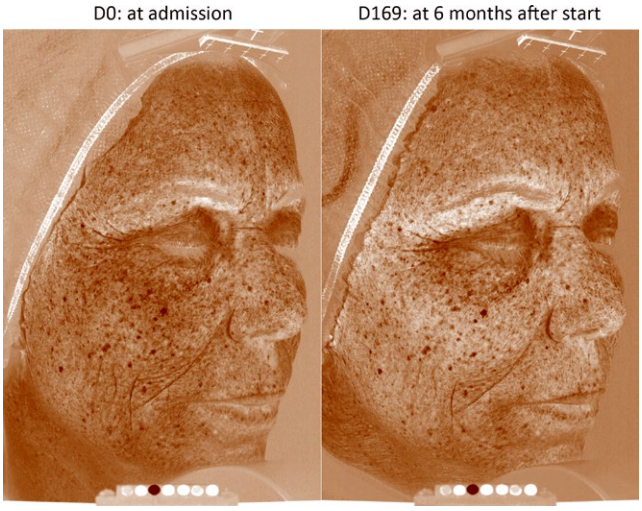

FIGURE 2 Brown spot images and count/area evolution in subject number 7

In recent years, PRP has been used in many medical areas, including indications to support acute and chronic wound healing. Research in wound healing models has provided interesting information with regard to the pathophysiology of photoaging, indicating that there are several parallel mechanisms between pathways involved in wound healing and those necessary for skin rejuvenation. Biological and biochemical processes are involved in wound formation which are similar to the needed changes to reverse the effects of intrinsic and extrinsic skin aging.[11] A diversity of platelet growth factors (PGFs) with their specific characteristics (Table 6) is located in the platelet alpha granules and play pivotal roles in regenerative processes. Following the mechanisms in wound healing, PRP acts on skin aging through collagen remodeling, stimulating a thickening of the superficial layer of the skin, and simultaneously improving cell regeneration.[12] Tissue repair, as in skin rejuvenation, and surgical wound healing are well orchestrated, and there is a complex series of events involving cell-cell and cell-matrix interactions, in which PGFs serve as messengers to regulate various regenerative processes.[13]

The effectiveness of PRP injections and therefore bio-cellular activity is determined by which type of PRP is being used, plasma PRP or buffy coat PRP. Specific PGFs in combination with available platelet proteins, cytokines, and chemokines regulate fundamental cellular activities, including mitogenesis, angiogenesis, chemotaxis, formation of the ECM, and ultimately control the activity of PGFs.[14] These adjunctive and obligatory effects (Table 7) should be part of the total biological activity of PRP.

Commercially available whole blood separation systems are designed for the preparation of PRP; however, they produce major differences in cellular composition.[3] A buffy coat PRP product is capable of enhancing cell proliferation and differentiation, cell migration, and ECM buildup, producing a concentration of platelets meeting the definition outlined by Marx of >1 million cells/μL.[12,15] "PRP-like" products (single-spin plasma PRP devices) have a low to no platelet concentration in the final product and consist mainly of plasma. These devices will demonstrate a less significant to no effect when compared to a buffy coat (double-spin) PRP product, which is

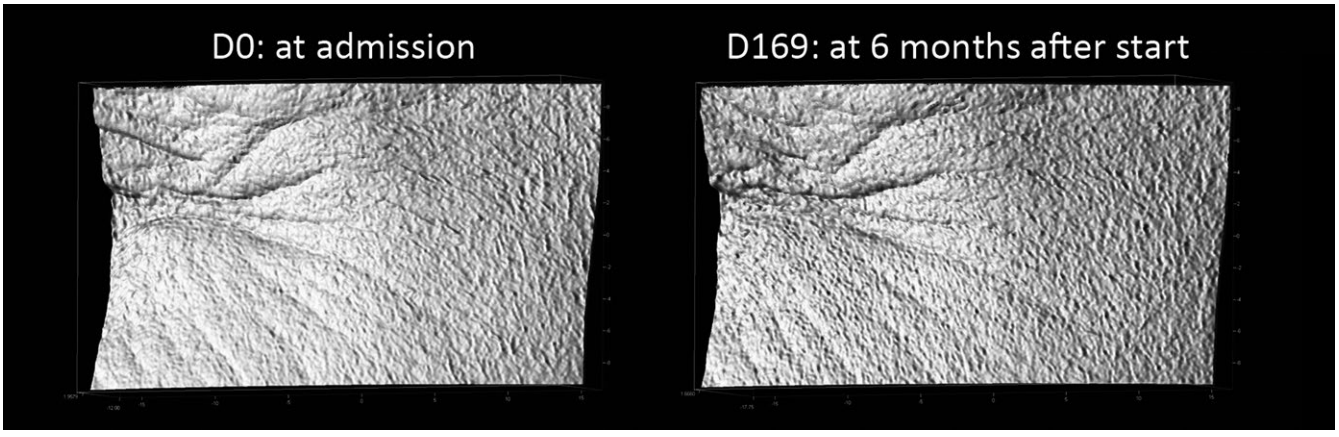

FIGURE 3 Changes in wrinkle aspect in periocular area of subject number 11

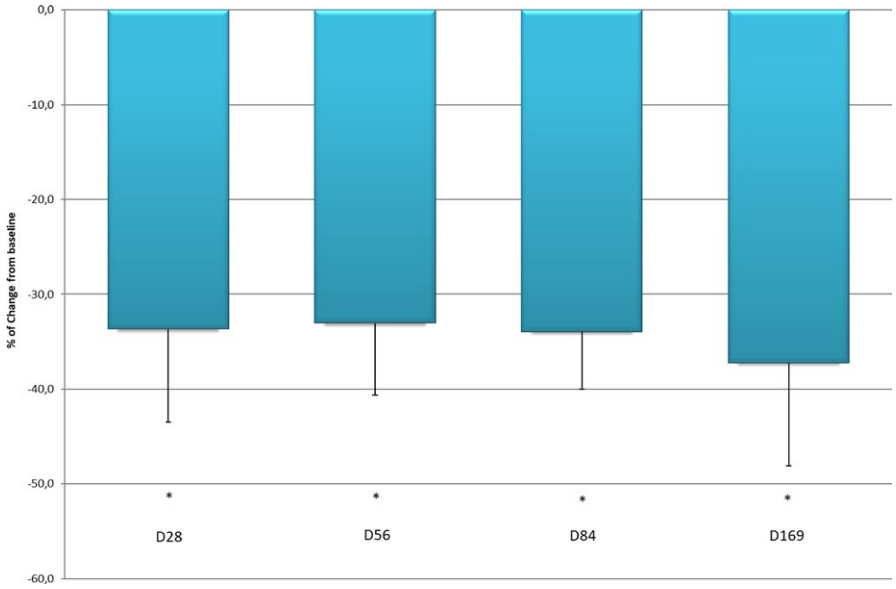

FIGURE 4 Wrinkle count % change after the first PurePRP® injection

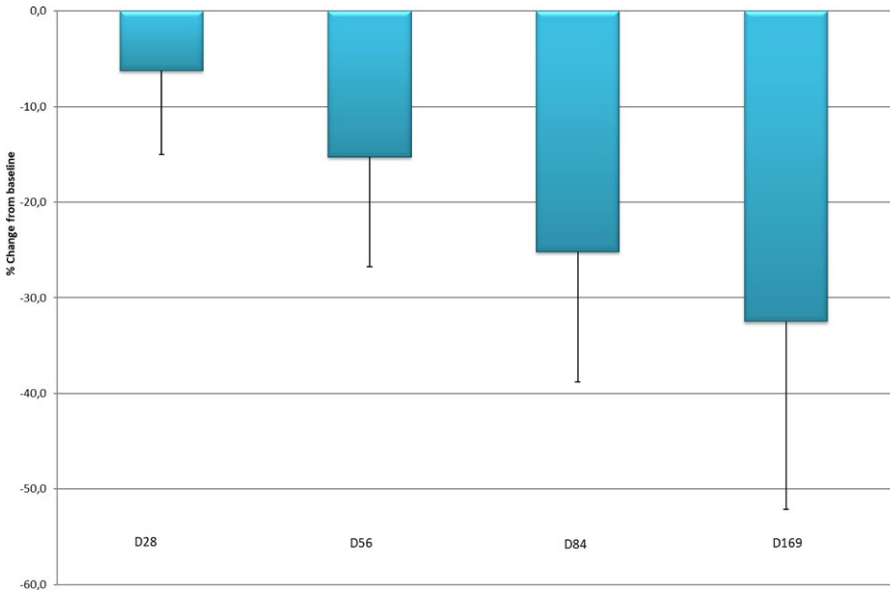

FIGURE 5 Change in skin firmness in the malar area

rich in platelets and leukocytes capable of tissue regeneration such as monocytes. Moreover, most PRP technology systems are often based on antiquated devices and preparation protocols, using outdated science, a lack of solid research to show proof of concept of a newly developed product, and a too simplified view on the true regenerative capacities of all the PRP constituents. The consequential effects are that some PRP treatments show little, or even no therapeutic effects, and even deleterious effects have been reported, albeit these systems have not been adapted to meet current research findings.

Platelet-rich plasma should therefore be characterized as a small volume of plasma, with a substantial concentration of platelets, specific leukocytes, and minimal red blood cell contamination, since skin rejuvenation results from cell proliferation, angiogenesis, and cell migration aimed at remodeling the ECM.[16] Magalon et al[17] recently categorized the many PRP devices in classifications based on their specific characteristics and biological composition, such as the purity of PRP with regard to platelet concentration, leukocyte composition, and erythrocyte concentration, to provide support in selecting a system that meets the specific needs for a given indication.

In this study, we used a 4th generation PRP system, where the design characteristics are centered on optimizing the efficacy of patients' treatment outcomes based on the capability of preparing various bio-cellular PRP protocol formulations. The EmCyte PurePRP® system can be categorized as giving a buffy coat PRP product.[18,19] Following a density gradient double-spin centrifugation protocol of whole blood, a buffy coat PurePRP product is created. The PurePRP® can be best described as an anticoagulated volume of plasma containing concentrated platelets, white blood cells, a fraction of red blood cells, including fibrin and cell adhesion molecules.

TABLE 5 Evolution of the a* (AU) values and change in red color (a*) parameters for malar and nasolabial area

	Time	n	Mean	SD	*P*-value
	D0	11	14.6	2.3	
	D28	11	13.3	1.5	**0.014**
Malar	D56	11	14.2	1.2	0.477
	D84	11	13.8	2.7	0.073
a*	D169	11	7.9	1.6	**0.000**
	D0	11	14.0	2.7	
	D28	11	13.7	2.2	0.306
Nasogenial	D56	11	13.6	2.1	0.445
	D84	11	13.4	2.2	0.306
	D169	11	9.8	1.8	**0.003**

In the malar and nasolabial areas at D169 the treatment reduced the red color

parameter significantly, P = 0.000 and P = 0.003, respectively (in bold).

Our biometric instrumental study revealed that significant biological facial skin stimulation and tissue repair are possible in patients with aging skin after 3 PurePRP injections. After injection into the dermis and subcutaneous layers, endogenous platelet activation occurs by the subject's own coagulation factors, such as thrombin and collagen, leading to platelet clot formation, aggregation, and ultimately platelet degranulation. During this degranulation period, the platelets release their dense alpha granules, PGFs, biologically active proteins, histamine, serotonin, and other substances into the dermis and subcutaneous extracellular milieu. PGFs interact and attach to platelet tyrosine kinase receptors (TKR) on the cell surface, present in the tissue layers, thus not directly on the cell nucleus.[13]

Successively, they activate inactive messenger proteins, which in turn, via cell-signaling, exert their effects on the cell nucleus, where genes that control cell division are triggered, inducing transcription of messenger RNA, producing a biological response that starts tissue rejuvenation cascades. Via this interaction, PGFs mediate inter- and intracellular signaling pathways that control cell proliferation and differentiation. Unlike in hormone therapy, the cell PGFs are synthesized by fibroblasts, keratinocytes, platelets, lymphocytes, and mast cells.[11] Furthermore, PFGs and signaling cells interact with fibroblasts, endothelial cells and stem cells, mediating cell proliferation and migration, and production of ECM proteins.

The EmCyte PurePRP® Protocol A, following the preparation instructions of the manufacturer, was used in all subjects, producing an almost absolute neutrophil cell depletion, with a high concentration

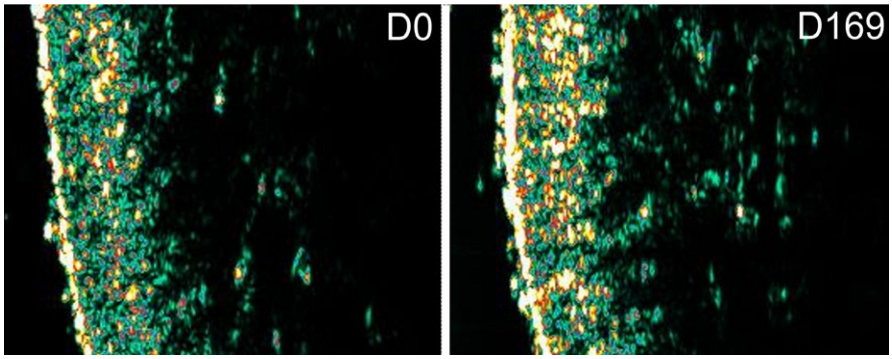

FIGURE 6 Ultrasonography images assessing the subepidermal low echogenic band of the malar area of subject number 6

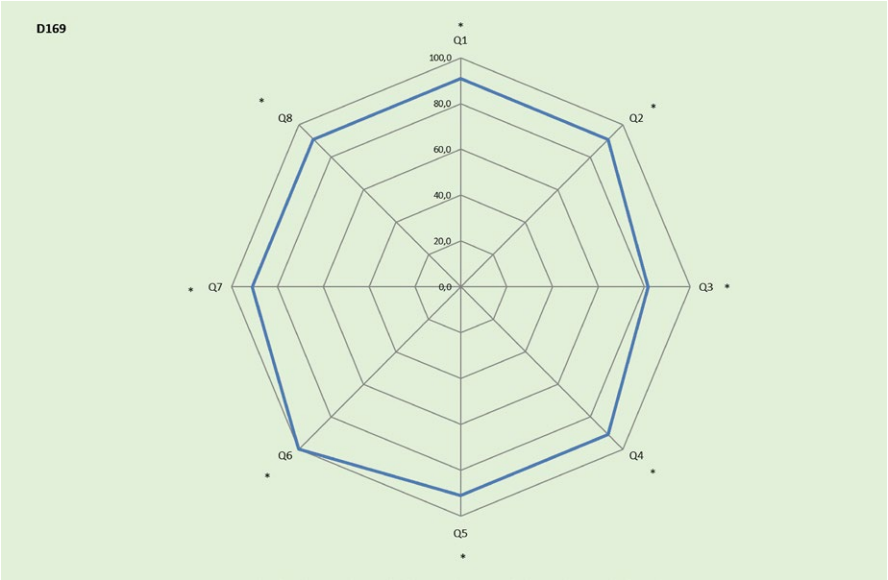

FIGURE 7 The mean values from the patient-reported outcomes, at D169, in the two-dimensional spider-web graphic

TABLE 6 Description of platelet growth factors, their sources, and potential biological activities

Platelet growth factor	Growth factor sources	Biological activities
Platelet-derived growth factor, PDGF(a-b)	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenetic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis
Transforming growth factor, TGF (α-β)	Platelets, extracellular matrix of bone, cartilage matrix, activated TH_1 cells and natural killer cells, macrophages/monocytes and neutrophils	Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells
Epidermal growth factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis
Fibroblast growth factor, FGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenetic for mesenchymal cells, chondrocytes, and osteoblasts
Connective tissue growth factor, CTGF	Platelets through endocytosis from extracellular environment in bone marrow.	Promotes angiogenesis, cartilage regeneration, fibrosis, and platelet adhesion
Insulin-like growth factor-1, IGF-1	Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells,osteoblasts, bone matrix	Chemotactic for fibroblasts and stimulates protein synthesis. Enhances bone formation by proliferation and differentiation of osteoblasts

of mononuclear monocytes and lymphocytes (unpublished data).[20] On the basis of recent studies, we identified the plasticity of monocytes and macrophages. The monocytes present in the PurePRP injectate, and the subsequent injection, will differentiate to tissue macrophages phenotypes M1 and M2 in the dermal layers. M1 macrophages contribute to a pro-inflammatory response, while M2 macrophages impart anti-inflammatory properties, with the stimulation of pro-reparative tissue processes.[21] The application of this specific PurePRP formulation exerted significant skin rejuvenation effects, which might have attributed not only to the effects of PGFs but also to a high concentration of monocytes.

The biometric parameters showed undoubtedly the effect of the 3 PurePRP injections leading to noteworthy facial skin rejuvenation. Our data are in accordance with recent reports from Cameli and coworkers, using a similar study protocol, and Diaz.[22,23] Brown spot count and brown spot area were significantly decreased in all treated subjects. In particular, the wrinkle count started to decrease significantly after the first PurePRP injection and continued to decrease until 3 months after the last injection. This effectiveness of a single PRP intradermal injection in reducing wrinkles was also reported by Elnehrawy et al.[1] A significant improvement was noted regarding general appearance. Skin firmness improved significantly already after the first PurePRP injection, in contrast to the observation by Yuksel and associates, where skin firmness-sagging only improved after 3 PRP injections.[12] In their study, a single-spin plasma PRP was used, with an average volume of 1.5 mL. The platelet-poor plasma was used on a gauze-sponge to cover the entire face after PRP injection. Curiously, no changes in red skin color were seen after any of the PurePRP injections. A significant decrease in red coloration was only seen 3 months after the third PurePRP injection, indicating that an inflammatory response occurred after each PurePRP procedure. Patients were very satisfied with the luminance and brightness resulting from the skin treatments. Although skin luminance is not easy to assess with physical parameters, the instrumental biometric data indicated a clear improvement in the brightness of the skin, which was confirmed by the patient. The SLEB area undergoes significant changes as the skin ages, as it becomes wider, with loss of density.

TABLE 7 Description of the protein, chemokine, and cytokine biological activities in PRP

Proteins-chemokines-cytokines	Biological activities
Adhesive proteins	Cell contact interactions
	Extra cellular matrix composition
Proteases and anti-proteases	Angiogenesis
	Vascular remodeling
	Cellular regulation
	Cellular behavior
Mitogenic factors	Increases angiogenesis
	Cell proliferation
	Chemotaxis
Chemokines and cytokines	Cellular interaction
	Vascular remodeling
	Bone formation
Membrane glycoproteins	Platelet aggregation
	Platelet adhesion
	Inflammation
	Platelet and leukocyte interaction
Granules	Capillary permeability
	Vascular local regulation

In order to have an objective measure of a decrease in SLEB thickness, with a concomitant increase in density, we used skin ultrasonography to monitor the changes in the SLEB, the area between the epidermis and dermis. The increase in density is attributed to an increase in collagen production, creating a "filler effect."

We realize that the sample size was relatively small, so we therefore analyzed the relative transformation of biometric instrumentation data in relation to the skin condition prior to the first PurePRP injection. Furthermore, we forcefully monitored our exclusion criteria, with strict study constraints throughout the study duration.

According to our results, the PurePRP procedures were easy to perform, and all subjects tolerated them well, with no complications or reports of adverse events. We found the PurePRP injections safe to perform, generating effective facial skin rejuvenation, with high levels of patient satisfaction, as demonstrated by the 8 questions on the self-assessment form, which was completed by all subjects. A weakness of this study was that we did not have the possibility to assess baseline and PurePRP platelet and white blood cell counts. This will be included in our follow-up studies.

5 | CONCLUSIONS

In our study, we evaluated and documented the clinical effects of a standardized 3-dermal injection protocol using PurePRP on facial skin rejuvenation. Instrumental assessments showed that PurePRP applications had a significant, reproducible, positive impact on biological facial rejuvenation, which was confirmed by patient's evaluations. The protocol used can be considered an effective and safe method.

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