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PLATELET-RICH PLASMA INJECTIONS FOR FACIAL REJUVENATION

Peter A. Everts, Pedro Contreiras Pinto and **Leonor Girão** discuss how the use of PurePRP® injections can lead to fewer wrinkles and firmer skin with an improved sub-epidermal low echogenic band density

COVER STORY

ABSTRACT
Since platelet-rich plasma (PRP) contains several growth factors, cell adhesion molecules, and various cytokines, it was hypothesized that it could play a role in facial rejuvenation with emphasis on fibroblast activation and type I collagen expression in human fibroblasts.
However, there are significant differences between PRP systems with regard to so-called plasma-based PRP and buffy coat PRP. The aim of this study was to assess the clinical effectiveness of three buffy coat Pure PRP injections in volunteers for facial rejuvenation, using specific and objective biometric devices for skin improvement analysis.
The study included 11 female volunteers and demonstrated a significant reduction in wrinkle count and volume and skin firmness. A decrease in sub-epidermal low echogenic band (SLEB) thickness was already noted at 2 months after the first injection, with an increase in SLEB density. This study suggests improvement in skin rejuvenation as demonstrated by biometric parameters

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KEYWORDS
Pure Platelet-Rich-Plasma, facial rejuvenation, anti-wrinkle, firmer skin, SLEB density

PLATELET-RICH PLASMA (PRP) HAS been used for a variety of indications for more than 25 years. Currently, close to 10,000 references can be found on PubMed, using the search term platelet-rich plasma. These various applications have given rise to considerable interest in the potential of PRP in facial rejuvenation and other aesthetic applications. It has been demonstrated that PRP contains several growth factors, cell adhesion molecules and various cytokines, and could, therefore, play a role in fibroblast activation and type I collagen expression in human fibroblasts.

Intrinsic and extrinsic factors are responsible for skin ageing, degeneration of connective tissue, and a decrease in hyaluronic acid polymers, with alterations in dermal extracellular matrix (ECM) proteins, presenting fewer fibroblasts[1]. Activation of dermal fibroblasts, collagen synthesis and remodelling of the ECM, are essential for skin rejuvenation.

The use of PRP for skin rejuvenation dates back to 2010 by Redaelli et al., and Sclafani, where the main objectives included facial and neck revitalization, and deep nasolabial fold improvement[2,3]. Currently, physicians can choose from more than 30 PRP, or PRP like, processing systems, producing different PRP compositions and cellular content (*Figure 1*). Optimal and consistent blood separation is only safeguarded by double-spin PRP centrifuges with dedicated disposable concentration devices when compared to the so-called plasma, single spin, PRP devices. Single-spin devices produce 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[4]. The literature revealed differences in outcomes when different PRP devices were used[5,6]. In this study, we employed the EmCyte PurePRP® (EmCyte Corporation, Fort Myers, FL, USA), double-spin system to generate a high concentration of platelets with minimal neutrophil contamination, to achieve a clinically significant effect.

Objectives of the study

The study objectives are to assess the clinical effectiveness of neutrophil poor PurePRP injections in highly selected subjects for facial rejuvenation, with emphasis on wrinkle reduction, skin firmness, and a higher sub-epidermal density. Scientifically measurable, non-invasive, biometric skin diagnostic techniques were used to determine therapy efficacy.

Materials and methods

PRP preparation and injection procedure

The EmCyte PurePRP® system technology, including the 544E Executive Eppendorf Centrifuge Series and the GS-60 PurePRP concentration device (*Figure 2*) (EmCyte Corporation, Fort Myers, FL, USA) was used at the point of care in the clinic, just before injection. Fifty millilitres of whole blood was drawn in a 60-mL syringe containing 10 mL of sodium citrate. In all volunteers, EmCyte's proprietary Protocol-A was carried out to produce Neutrophil PurePRP, in accordance with the instructions for use from EmCyte Corporation. The PurePRP is characterized as a double-spin buffy coat product, with a low erythrocyte concentration (<2.0 x 10^9 mL) and significantly reduced, pro-inflammatory, neutrophils. ▷

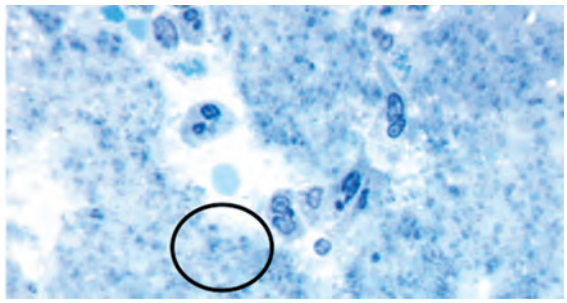


Figure 1 The black circle indicates a cluster of platelets. PRP is defined as small volume of plasma with a significant clinical PRP concentration

Figure 2 (Left) The EmCyte Executive Series Centrifuge II, managing a 2-spin procedure to produce PurePRP® from a unit of whole blood. (Right) The PurePRP concentrating and accessory devices for pure platelet rich plasma preparation

▷ The final PurePRP volume was standardized to 7mL of PurePRP in all subjects. To compensate for the anticoagulant effects of sodium citrate, 0.05 mL of 10% calcium chloride was mixed with 1mL of PurePRP prior to facial injection. A local anesthetic (EMLA®, Astra Zenica, Cambridge, United Kingdom) was applied in all facial areas for at least 30 min before the injection. Small aliquots of PurePRP, 1mL insulin syringes, were administered intradermally and subcutaneously, using a 13mm long 27G or 4mm long 32G needle (depending on the thickness of the skin to be treated). All subjects underwent 3 sessions of PurePRP treatment at 1month intervals, with a follow-up period after 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 min to promote skin penetration. No ice packs were used after the procedure. Daily applications of sunscreen protection were recommended.

> “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 min to promote skin penetration.”

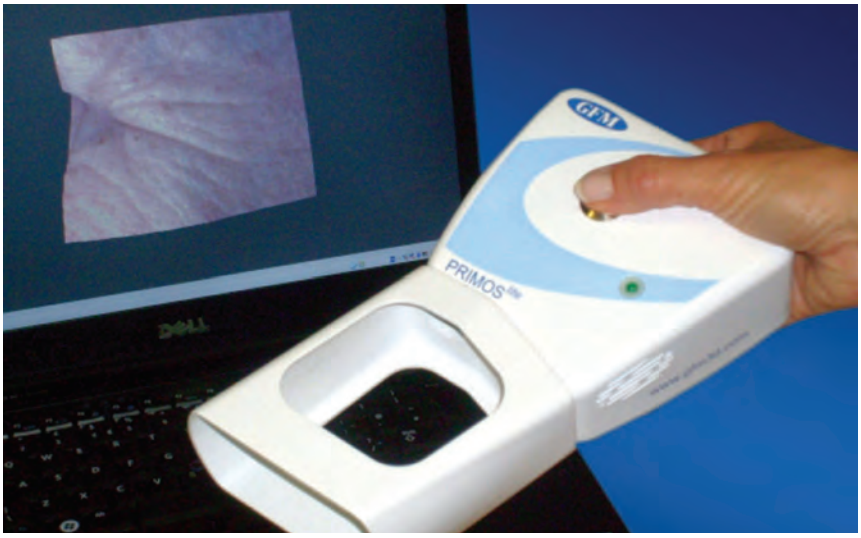


Figure 3 The Primos 3D device for assessment of the skin roughness and wrinkle reduction

Patients

This 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 signed informed consent forms before treatment with three facial PRP injections. The subjects ranged in age between 45 and 65 years old and had Fitzpatrick's classification between II and IV[6]. All injections were given by the same physician (L.G.).

Exclusion criteria were as follow:

- Individuals who performed an anti-ageing or aesthetic treatment prior to the study: Botox or Botox-like products, peelings, plastic surgery, resurfacing with Laser, IPL, threads, radiofrequency treatments, hyaluronic acid treatment, platelet-rich plasma treatment, or any other specific treatments prone to change the skin aspect during the last 18 months
- 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.)
- Systemic disorders: cardiovascular, pulmonary, digestive, neurologic, psychiatric, genital, urinary, endocrine
- Hematological or hemorrhagic diseases
- Thrombocytopenia moderate, or severe (greater than 100.000 platelets count)
- 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 intense 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 months before the beginning of the study
- Treatment with topical corticoids on the experimental area within 16 days 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 was 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 months ▷

Figure 4 The Cutometer MPA 580 device for measuring the skin elasticity and the firming effects

"All subjects completed the study, receiving three 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 PurePRP treatment procedures were all consistently performed without any complications or adverse effects."

- 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 a malignancy within 6 months prior to enrollment or who are currently under treatment for asthma or diabetes.

Biometric instrumental assessments

The effectiveness of the PurePRP facial rejuvenation injections were assessed by several biometric instrumental devices. The Optical In-Vivo Primos 3D Skin Device (GFMesstechnik GmbH, Berlin, Germany) (*Figure 3*) to evaluate qualitatively and quantitatively skin profile changes, to assess wrinkle count, depth, and volume was applied to the periocular area (left or right). Quality surface imaging and dense 3D geometry mesh in a single capture were performed, measuring fine wrinkle improvements in skin surface structures. Skin biomechanical evaluations to measure the elasticity of the upper skin layers using negative pressure were performed with a Cutometer dual MPA 580 (Courage & Khazaka, Cologne, Germany) using a 2mm probe (*Figure 4*). The parameters used were R0 to measure skin distensibility, representing the passive behavior of the skin to force. This parameter is correlated to skin firmness. The R2 parameter measures overall elasticity, representing the ability of the skin to return to its basal state. The Dermascan-C ultrasound device with a modified 20Mhz probe (Cortex Technology, Hadsund, Denmark) was used to measure thickness and density of the sub-epidermal low echogenic band (SLEB), with calculations of subcutaneous fat thickness.

All biometric instrumental protocol evaluations were performed in a fully controlled room and after an initial acclimatization process of at least 30 minutes.

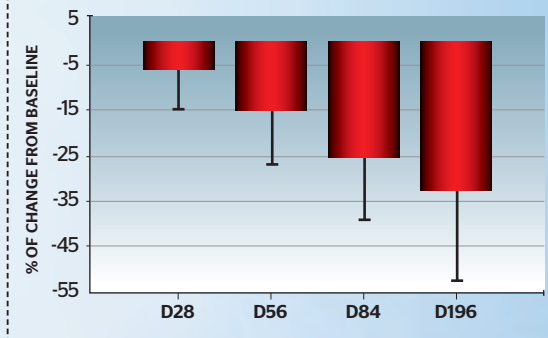
Statistical analysis

All data were analyzed using IBM SPSS Statistics-20 (Armonk, New York, 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 rank sign test. A 5% level of significance was used. The subjective data of efficacy was submitted to binomial testing, $p<0.05$ was considered significant.

Results

All subjects completed the study, receiving three 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 PurePRP treatment procedures were all consistently performed without any complications or adverse effects. No skin reactions were noted after each procedure. None of the volunteers experienced any discomfort during the study, or during the follow-up period. Minor ecchymosis, which resolved within 3 days, was noted with no signs of inflammatory or allergic reactions. 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 (*Table 1*). Evaluation of the true wrinkle count and volume percentage changes in all subjects after 169 days revealed a relative transformation in relation to D0 of -37.2 and -11.5%, respectively ($p<0.05$). A significant percentage increase in skin firmness parameters in the malar area, compared to baseline was noted (*Figure 5*). Also, changes in the nasolabial area demonstrated a significant improvement after 56, 84, and 169 days ($p=0.000$). 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 months follow-up (*Figure 6*). The increase in density, ▷

Figure 5 Change in skin firmness in the malar area



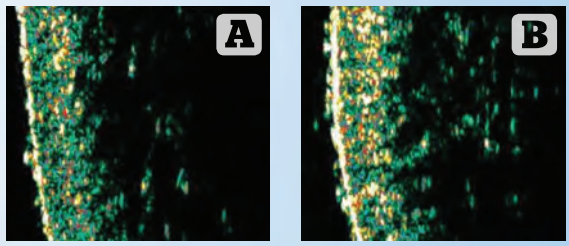
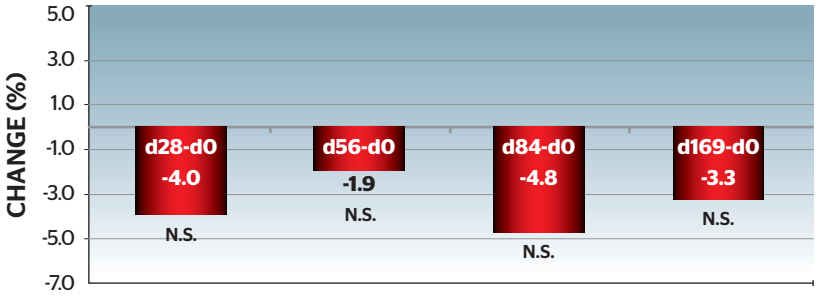


Figure 6 Ultrasonography images assessing the sub-epidermal low echogenic band of the malar area of subject number 6. (A) Day 0, (B) Day 169.

> The bio-cellular activity of PRP injections, and thus effectiveness, is determined by which type of PRP is being used, buffy coat PRP, or plasma PRP.

Figure 7 Subcutaneous fat thickness percentage change during the study



Mean values of all the subjects (n=11). Also shown is the statistical comparison against D0 (*: p<0,05; N.S.: Non-significant)

▷ pixels/mm², did not affect subcutaneous fat thickness as shown in *Figure 7* (p=0.224).

Discussion

Commercially available PRP systems are designed for the preparation of a small volume of plasma, which might contain a substantial concentration of platelets, leukocytes and erythrocytes. These systems produce major differences in cellular composition[7]. 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/microliter, or 1 billion per mL[8]. 'PRP-like' products (single-spin plasma PRP devices) have a low to no platelet concentration in

Table 1 Results from the wrinkle count score before and after treatment

Ref.	Name/ Surname	D0	D28	D56	D84	D169	D28- D0	D56- D0	D84- D0	D169- D0	%D28	%D56	%D84	%D169
1	SANT.MA	146	108	98	92	70	-38	-48	-54	-76	-26	-32.9	-37	-52.1
2	RATA.VI	279	209	206	206	159	-70	-73	-73	-120	-25.1	-26.2	-26.2	-43
3	RAMO.MA	203	153	150	155	169	-50	-53	-48	-34	-24.6	-26.1	-23.6	-16.7
4	BAPT.MA	135	88	81	80	98	-47	-54	-55	-37	-34.8	-40	-40.7	-27.4
5	MADR.MA	278	176	193	182	183	-102	-85	-96	-95	-36.7	-30.6	-34.5	-34.2
6	MEND.AN	285	204	197	195	148	-81	-88	-90	-137	-28.4	-30.9	-31.6	-48.1
7	MEND.CE	42	17	20	27	27	-25	-22	-15	-15	-59.5	-52.4	-35.7	-35.7
8	HENR.MA	107	66	71	74	66	-41	-36	-33	-41	-38.3	-33.6	-30.8	-38.3
9	ANJO.MA	99	67	70	57	73	-32	-29	-42	-26	-32.3	-29.3	-42.4	-26.3
10	REIS.MA	247	160	163	147	152	-87	-84	-100	-95	-35.2	-34	-40.5	-38.5
11	CORR.LI	105	74	76	73	53	-31	-29	-32	-52	-29.5	-27.6	-30.5	-49.5
Mean		175.1	120.2	120.5	117.1	108.9	-54.9	-54.6	-58	-66.2	-33.7	-33	-34	-37.2
SD		86.6	63.6	63.4	61.7	54.4	25.9	24.5	28.3	40.8	9.8	7.6	6.1	10.8
Median		146	108	98	92	98	-47	-53	-54	-52	-32.3	-30.9	-34.5	-38.3
Minimum		42	17	20	27	27	-102	-88	-100	-137	-59.5	-52.4	-42.4	-52.1
Maximum		285	209	206	206	183	-25	-22	-15	-15	-24.6	-26.1	-23.6	-16.7

Table 2 Overview of platelet growth factors present in platelet-rich plasma and their effects on skin rejuvenation

PLATELET GROWTH FACTOR	BIOLOGICAL ACTIVITIES IMPORTANT TO FACIAL REJUVENATION
Platelet Derived Growth Factor, PDGF(a-b)	Mitogenetic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/ smooth muscle cells; regulates collagenase secretion and collagen synthesis
Transforming Growth Factor TGF (α–β)	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.
Vascular endothelial growth factor, VEGF	Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells.
Epidermal Growth Factor, EGF	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis.
Fibroblast Growth Factor, FGF	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenetic for mesenchymal cells, and keratinocyte proliferation.
Connective tissue growth factor CTGF	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion
Insulin-like growth factor-1 IGF-1	Chemotactic for fibroblasts and stimulates protein synthesis.

the final product and consist mainly of plasma.

These devices do not have the ability to increase the baseline platelet count to 5–7 times the native concentration and will demonstrate a less significant to no effect when compared to a buffy coat (double spin) PRP product, which is rich in platelets and specific leukocytes capable of tissue regeneration[9]. This PRP should be referred to as Clinical-PRP.

It is well known that during ageing, epidermal and dermal changes in the skin are naturally occurring phenomena, with degradation of the ECM[10]. Also, the cessation of collagen fiber and elastin synthesis, with degradation of proteoglycans, results in loss of skin elasticity[11]. 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[12].

Based on the specificity for facial rejuvenation, including cell proliferation, angiogenesis, and cell migration aiming at remodeling of the ECM. Wound healing models have provided interesting information with regard to the pathophysiology of photo-ageing, 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 required changes to reverse the effects of intrinsic and extrinsic skin ageing[13].

The bio-cellular activity of PRP injections, and thus effectiveness, is determined by which type of PRP is being used, buffy coat PRP, or plasma PRP. Specific PGFs (*Table 2*) 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]. The authors, therefore, used 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 a buffy coat PRP product[15,16]. The neutrophil poor PurePRP® protocol can be best described as an anticoagulated volume of plasma containing concentrated platelets, monocytes, lymphocytes, a fraction of red blood cells, and cell adhesion molecules. This protocol was used in all subjects, producing an almost absolute neutrophil cell depletion, with a high concentration of mononuclear monocytes and lymphocytes (unpublished data)[17].

> "Specific PGFs (*Table 2*) 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."

The authors' biometric instrumental study revealed that significant biological facial skin stimulation and tissue repair are possible in patients with ageing skin after three 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 (*Figure 8*). PGFs mediate inter- and ▷

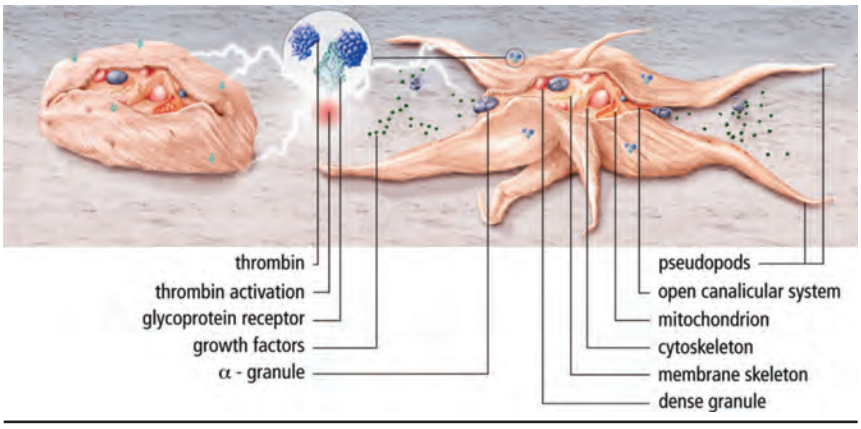


Figure 8 Representation of a resting platelet and an activated platelet with the subsequent release of the platelet granules content to the extra cellular matrix

▷ 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[13]. Furthermore, PFGs and signaling cells interact with fibroblasts, endothelial cells and stem cells, mediating cell proliferation and migration, and production of ECM proteins[18].

> "According to the results, the PurePRP procedures were easy to perform, and all subjects tolerated them well, with no complications or reports of adverse events."

The biometric parameters showed undoubtedly the effect of the three PurePRP injections leading to noteworthy facial skin rejuvenation. The authors' data are in agreement with a recent paper from Cameli and associates, using a comparable study protocol[19]. In the authors' study, 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 after the first PurePRP injection, in contrast to the observation by Yuksel and associates, where skin firmness-sagging only improved after three PRP injections[20]. 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.

The SLEB area undergoes significant changes as the skin ages, as it becomes wider, with loss of density. In order to have an objective measure of a decrease in SLEB thickness, with a concomitant increase in density, the authors' 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.'

To conclude, the authors' realized that the sample size was relatively small, so they, therefore, analyzed the relative transformation of biometric instrumentation data in relation to the skin condition prior to the first PurePRP injection. Furthermore, they forcefully monitored the exclusion criteria, with strict study constraints throughout the study duration. According to the results, the PurePRP procedures were easy to perform, and all subjects tolerated them well, with no complications or reports of adverse events. The authors' found the PurePRP injections safe to perform, generating effective facial skin rejuvenation, with high levels of patient satisfaction.

Key points

- PurePRP is a safe autologous biologic for facial rejuvenation
- The bio-cellular activity of PRP injections and effectiveness is determined by the type of PRP used
- The Biometric Instrumental study objectively indicated the positive effects of Clinical PurePRP on decreasing wrinkles, firmer skin, and improved SLEB
- The decrease in SLEB thickness and increase in density is attributed to an increase in collagen production following 3 Pure PRP injections

▶ ***Declaration of interest*** *No honoraria were paid. EmCyte Corporation provided funding for distribution of the kits and costs for the laboratory analysis*

▶ ***Figures 1 & 8*** *© Peter A. Everts;* ***2*** *© EmCyte Corporation;* ***3*** *© Canfield Scientific;* ***4*** *© Courage+Khazaka electronic GmbH;* ***6*** *© PhD Trials Center, used with permission.*

▶ ***Tables 1-2*** *© Peter A. Everts*

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