

Autologous platelet concentrates for facial rejuvenation

Abstract

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Autologous platelet concentrates (APCs) are promising therapeutic agents in facial rejuvenation since they are a great source of cytokines, growth factors and other biologically active substances. Obtained from the patient's blood, they have the advantages of reducing immunological reactions, making the procedure safer, well tolerated, with minimal adverse effects and lower cost. Currently, they are used for facial rejuvenation both in combination with microneedling and in mesotherapy techniques, as well as to treat facial acne scars, melasma and wounds after laser ablative treatments. This review summarizes current knowledge on the use of APCs, ranging from basic concepts related to their composition and mechanisms of action to up-to-date information on their clinical efficacy. Methodology: MEDLINE (PubMed) was searched from inception through 2021 for English language publications on APCs for facial rejuvenation. Results: A total of 100 files were found. Based on the available literature, APCs for skin rejuvenation are safe and well tolerated. The most studied product is the first-generation material, platelet-rich plasma (PRP). Conclusions: The results are in general favorable, but the quality of the studies is low. The second and third generation products, platelet-rich fibrin (PRF) and injectable platelet-rich fibrin (i-PRF), respectively, are easier to be obtained and, at least *in vitro*, seem to induce greater collagen production than PRP, especially under lower relative centrifugation forces, but to date only a few clinical trials evaluating these products exist. More high-quality trials with appropriate follow-up are necessary to provide adequate evidence that may help to improve the treatment regimens with APCs. Many aspects should be considered when designing clinical trials to evaluate APCs, such as the patients' characteristics that best predict a favorable response, the optimal number of sessions and the interval between them, the characteristics of the studies and the development of better instruments to evaluate skin aging.

Keywords: Platelet-rich plasma. Platelet-rich fibrin. Facial rejuvenation. Autologous platelet concentrates.

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History and evolution of autologous platelet aggregates for facial rejuvenation

Autologous platelet concentrates are promising therapeutic agents in regenerative medicine since they are a great source of cytokines, growth factors and other biologically active substances. They are increasingly being used in distinct areas of Dentistry, such as periodontal surgery and orofacial harmonization and in Medicine, such as orthopedics, surgery, sports medicine and aesthetic dermatology. The use of autologous preparations has the advantages of reducing immunological reactions and disease transmission, making the procedure safer, well tolerated, with minimal adverse effects and lower cost, since the material is obtained from the patient after the collection of peripheral blood and its centrifugation.

The origin of the therapy comes from transfusiology, where platelet concentrates are used to treat thrombocytopenia.¹ In 1954, for the first time, the term "platelet-rich-plasma" (PRP) was employed by Kingsley² (1954), when referring to platelet concentrates for transfusion. The first clinical demonstration that autologous platelet concentrates promoted healing when used locally was reported by Knighton, et al.³ (1986). At that time, the preparation used was called "Platelet-derived wound healing factors" (PDWHF). The use of the term "platelet-rich plasma" (PRP) in the context of regenerative dentistry/medicine began with Marx, et al.⁴ (1998), when the product was used in maxillofacial surgery for bone reconstruction.

PRP has been used for facial rejuvenation, with modest improvement in facial appearance, skin texture and wrinkles.⁵ However, its preparation is difficult, as it requires double centrifugation.⁶ In addition, the anticoagulants required can impair healing by inhibiting the coagulation process.⁷ To overcome some of these limitations of PRP, platelet-rich fibrin (PRF),

a platelet concentrate called the "second generation", was developed by Choukroun, et al.⁸ (2001). PRF is obtained through a single centrifugation, without the need for anticoagulants, being, therefore, fully autologous. The resulting product contains different cell types (platelets, leukocytes, erythrocytes), an extracellular fibrin matrix and a range of bioactive molecules (predominantly growth factors). Depending on the collection tube and on the centrifugation protocol used, PRFs in liquid or solid gel forms can be obtained. Solid forms, obtained with the use of glass tubes, have been widely used in maxillofacial surgery⁷ and plastic surgery,⁹ with benefits for bone and soft tissue regeneration, infection control and patient satisfaction.

In 2014, a fluid, injectable form of PRF (called i-PRF) was developed by modifying the relative centrifugation force (RCF).¹⁰ By decreasing centrifugation speed and time and using plastic tubes (to reduce clotting time), fibrin clotting could be slower in the initial time periods, generating a product containing fibrinogen and thrombin that remains fluid for about 20 minutes after centrifugation, before the formation of fibrin. This makes it an appropriate material to be used in facial rejuvenation. Figure 1 summarizes the main differences among the distinct generations of autologous platelet concentrates.

Currently, autologous platelet concentrates are used for facial rejuvenation both in combination with microneedling (drug delivery), and in mesotherapy techniques.^{5,11,12}

In addition to being used for skin rejuvenation, platelet concentrates have also been used to treat facial acne scars,¹³ melasma,¹⁴ as well as wounds after laser ablative treatments,^{5,11} as they lead to more efficient and fast healing. The market for PRP presented an impressive growth from around \$ 45 million in 2009 to \$120 million in 2016. It is expected to exceed \$4.5 billion by 2024.¹⁵

This review summarizes current knowledge on the use of autologous platelet concentrates for facial

Generations of APCs	Number of Centrifugations	RCF	Centrifugation time	Collection tube	Anticoagulant	Reference
PRP	2	High	High	Glass	Yes	Kingsley ² (1954)
PRF	1	Medium	Medium	Glass/Plastic	No	Choukroun, et al. ⁸ (2001)
i-PRF	1	Low	Low	Plastic	No	Wang, et al. ¹⁰ (2014)

*APCs – autologous platelet concentrates; PRP – platelet-rich plasma ; PRF – platelet-rich fibrin ; i-PRF – injectable platelet-rich fibrin ; RCF – relative centrifugation force.

Figure 1- Differences between the distinct generations of autologous platelet concentrates

rejuvenation, ranging from basic concepts related to their composition and mechanisms of action to up-to-date information on their clinical efficacy.

Methodology

MEDLINE (PubMed) was searched on August 25, 2021 for English language publications on autologous platelet concentrates for facial rejuvenation. Search terms were: [*facial rejuvenation AND (platelet rich plasma OR platelet rich fibrin OR injectable platelet rich fibrin OR iPRF OR PRF OR PRP)*]. A total of 100 files were found. Titles, abstracts and full-texts were independently screened by two reviewers (MB and FM). One file was excluded because it was an editorial. Other 28 files were articles unrelated to facial rejuvenation and were also excluded.

Results and discussion

Composition and mechanisms of action of autologous platelet concentrates

To understand the mechanism of action of platelet concentrates in facial rejuvenation, it is necessary to know the platelets. These cells are cytoplasmic

fragments of megakaryocytes, formed in the bone marrow, approximately 2 μm in diameter. Platelets contain, in their α granules, protein growth factors with a capital role in hemostasis and wound healing: CTGF (conjunctive tissue growing factor), EGF (epidermal growing factor), FGF-2 and -9 (fibroblast growing factor), IGF-1 (insulin growing factor), PDGF $\alpha\alpha$ (platelet-derived growing factor), PDGF $\alpha\beta$, PDGF $\beta\beta$, TGF α (transforming growing factor), TGF $\beta 1$, TGF $\beta 2$ and VEGF (vascular endothelial growing factor). After platelet exogenous or endogenous activation, these α granules fuse with the cell membrane, in a process called degranulation (Figure 2). These growing factors are then secreted, bind to transmembrane receptors on target cells (undifferentiated mesenchymal cells, osteoblasts, fibroblasts, endothelial cells and epidermal cells), activating an intracellular signaling protein that causes the expression of a protein, which, in turn, triggers effects such as cell proliferation, angiogenesis, synthesis of collagen and extracellular matrix components, and reduced apoptosis.^{6,16-19} Active secretion of these growth factors by platelets begins 10 minutes after activation, with more than 95% of pre-synthesized growth factors being secreted within 1 hour.²⁰

With skin aging, fragmented collagen fibrils accumulate, which impairs the growth of new

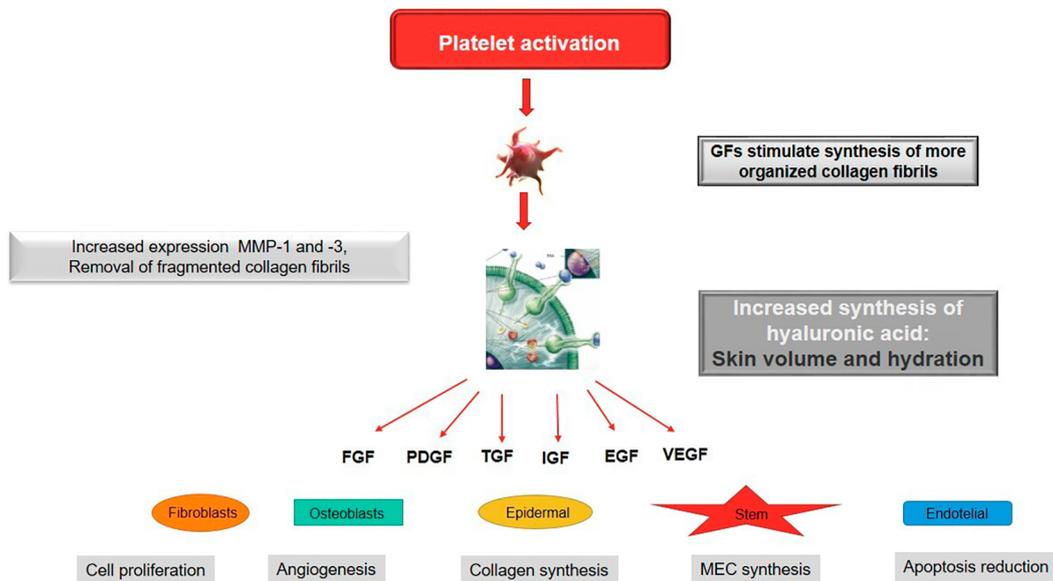


Figure 2- Mechanisms of action of autologous platelet concentrates in facial rejuvenation. After platelets activation, their α granules fuse with the cell membrane, in a process called degranulation. Their growth factors are then secreted, bind to transmembrane receptors on target cells (mesenchymal stem cells, osteoblasts, fibroblasts, endothelial and epidermal cells), activating an intracellular signaling protein that causes the expression of a protein, which, in turn, triggers effects such as cell proliferation, angiogenesis, synthesis of collagen and extracellular matrix components, and reduced apoptosis. With skin aging, fragmented collagen fibrils accumulate, which impairs the growth of new collagen fibers and disrupts the extracellular matrix. Activated platelet aggregates increase the expression of matrix metalloproteases (MMP-1 and -3), stimulating the removal of fragments of collagen fibrils. In addition, they contain several growth factors that stimulate fibroblasts to synthesize new, more organized collagen fibers, besides increasing the synthesis of hyaluronic acid, which binds to water, increasing the skin volume and hydration

collagen fibers and disrupts the extracellular matrix.²¹ Activated platelet aggregates increase the expression of matrix metalloproteases (MMP-1 and -3), stimulating the removal of fragments of collagen fibrils. In addition, they contain several growth factors that stimulate fibroblasts to synthesize new, more organized collagen fibers,^{22,23} besides increasing the synthesis of hyaluronic acid, which binds to water, increasing the skin volume and hydration²⁴ (Figure 2).

First generation autologous platelet concentrates: PRP

PRP is an autologous plasma preparation with high concentrations of platelets derived from whole blood,¹⁶ containing more than 800 bioactive molecules.^{25,26} The normal concentration of platelets in the blood ranges from 150,000 to 450,000/ μ L. PRP, by definition, should contain more than 1,000,000 platelets/ μ L to promote increased tissue healing.²⁰ PRP preparations generally have a 4- to 8-fold higher platelet concentration than peripheral blood.²⁷ A linear relationship between the concentrations of growth factors and platelets in PRP has been reported.²⁸ Although there is still no consensus on the most effective PRP preparation, platelet concentrations higher than 6-fold those of peripheral blood may inhibit healing.²⁹ At last instance, the regenerative effect of PRP depends not only on its platelet concentration, but also on the number/type of leukocytes entrapped in the fibrin matrix, and the release of bioactive molecules at the site of injury.³⁰

PRP contains leukocytes, with catabolic and pro-inflammatory activity, in combination with plasma and growth factors, with anabolic function. These constituents must be in balance so that there is adequate tissue healing and growth, for which the PRP preparation process is fundamental. The two main methods of preparation are the "PRP method" and the "buffy coat" method.⁶ The latter typically produces PRP with higher platelet concentrations.³¹ There are several commercial kits for preparation of PRP. The composition of the PRP obtained from the different commercial kits varies remarkably. The purpose of PRP preparation methods is to concentrate platelets and to reduce red blood cells. However, the leukocyte levels cannot be neglected. Typically, the kits that employ the "buffy coat" method produce a concentrate containing higher amounts of platelets and red blood cells, but the content of leukocytes is also increased.³¹ Variation in the content of cells

and growth factors also depends on the RCF and time of centrifugation employed. Longer and more forceful centrifugation cycles may push platelets down, discharge growth factors and disrupt cellular integrity.³² Typically, the bottom layer of red blood cells (RBCs) is discarded, but variable proportions of plasma and buffy coat lead to distinct platelet preparations. These preparations were classified according to the inclusion of the buffy coat (presence of leucocytes) and the use of anticoagulants (formation of fibrin matrix) into 4 categories: 1 – pure platelet-rich plasma (P-PRP); 2 – leucocyte-rich platelet-rich plasma (L-PRP); 3 – pure platelet-rich fibrin (P-PRF); and 4- leucocyte-rich platelet-rich fibrin (L-PRF).³³ The last two categories are activated fibrin-based matrices, not a liquid platelet suspension. They are called "second generation" PRP and will be discussed later. Figure 3 shows the main findings of laboratorial studies evaluating different preparations of autologous platelet aggregates.

It is important to avoid contamination with erythrocytes when collecting PRP, as they contain reactive oxygen species, which produce unwanted inflammatory reactions at the site, probably resulting in pain and edema for the patient.³⁴ There has been some discussion in the literature about whether the efficacy of the PRP is affected by the inclusion of leukocytes. Despite they might act as antimicrobial agents,³⁵ they may also release catabolic cytokines, leading to inflammation and fibrosis, which is more pronounced in the case of neutrophils.³⁶ When PRP is employed in soft tissues, there is no need of exogenous activation (with CaCl_2 or thrombin), since collagen is a natural activator of PRP. When PRP is activated, fibrinogen is transformed in fibrin, creating a fibrin membrane or cloth.³⁷

Interestingly, the pH of the platelet concentrate influences its regenerative potential. Preincubation of lysed platelet concentrate at close to pH 5.0 increases its content of available PDGF and its capacity to stimulate fibroblast proliferation. On the other hand, incubation at pH 7.1 increases TGF- β production, which increases collagen production.³⁸ However, the applicability of this concept to facial rejuvenation has not been evaluated so far.

The clinical efficacy of PRP depends on the release of bioactive molecules. Therefore, the composition of the PRP is crucial for the clinical effectiveness of the procedures. The main limitations of the

Type of autologous platelet aggregate	Aim	Main outcomes	Reference
PRP	Expression of G1 cell cycle regulatory proteins, type I collagen, matrix metalloproteinase-1 (MMP-1), and MMP-2 in human skin fibroblasts (HSF).	PRP increased the expression of G1 cell cycle regulators, type I collagen and MMP-1, accelerating the wound healing process.	Cho, Kim and Lee ²² (2012)
PRF	Influence of the RCF on leukocytes, platelets and growth factor release within fluid PRF matrices.	Reducing RCF according with protocol-II (177 g) led to significantly higher platelets and leukocytes numbers compared to protocol I (710 g). Protocol-III (44 g) significantly increased platelets and leukocytes compared to II and I. Protocol II produced significantly higher levels of TGF-β1 and VEGF compared to -I.	Choukroun and Ghanaati ⁶⁴ (2018)
PRF matrices (PRF, A-PRF and A-PRF+)	Growth factor release was measured over 10 days using ELISA for PRF matrices prepared using different relative centrifugation forces (RCF) and centrifugation times.	There was a higher release of growth factors from A-PRF+ when compared with the other matrices. Platelets were more homogeneously distributed within the A-PRF and A-PRF+ matrices, while in PRF they were located mainly in the lower portion.	El Bagdadi, et al. ⁶⁶ (2019)
i-PRF vs. PRP vs. control	Viability, migration, spreading, proliferation, mRNA levels of PDGF, TGF-β, type 1 collagen and fibronectin.	Platelet concentrates were nontoxic to dermal skin fibroblasts; i-PRF induced greater migration and proliferation than PRP, as well as significantly higher mRNA levels of TGF-β, type 1 collagen and fibronectin.	Wang, et al. ⁶⁷ (2019)
PRP	Effects of PRP on extracellular matrix remodeling through evaluation of human skin fibroblasts proliferation and migration, expression of human procollagen I alpha 1, elastin, MMP-1 and MMP-2, phosphorylation of c-Jun N-terminal kinase (JNK) and JNK levels.	PRP increased expression of type I collagen, elastin, MMP-1, and MMP-2 and reduced the phosphorylation of JNK, thereby accelerating wound healing.	Cho, et al. ⁶⁸ (2019)

*PRF = Platelet-rich fibrin; PRP = Platelet-rich plasma; RCF = Relative centrifugation force

Figure 3- Laboratorial studies evaluating autologous platelet aggregates

PRP research are the imprecise reporting of PRP composition, activation, and dosing, as well as the use of subjective outcome measures. In a systematic review, Frauttschi, et al.³⁹ (2017) noticed lack of important information in clinical studies evaluating the efficacy of PRP in aesthetic surgery. Most of the studies disregarded either the baseline platelet concentration in the patient's whole blood or the final platelet concentration in the PRP. This aspect is crucial, since the normal platelet concentrations varies between 150,000-450,000/μL. This 3-fold difference already has an impact in the platelet concentration in the resulting PRP, regardless the technique used for preparation. Like other pharmaceutical drugs, a dose-response relationship has been reported between the platelet concentration and proliferation of fibroblasts, mesenchymal stem cells, and synthesis of type I collagen.⁴⁰ Thus, information regarding the baseline platelet concentration in the whole blood and final platelet concentration in the PRP preparation is

crucial.³⁹ The use of anticoagulants is not reported in nearly half of the studies.³⁹ The type of anticoagulant can have an impact on platelet yield and function.³² Another important information involves exogenous activation of PRP. Although there is no consensus on the detriments/benefits of this step, most of the studies (71%) still were found to activate PRP in the time of application. The role of PRP leucocyte concentration is controversial and this information is inconsistently reported. Only 29% of the studies provided this variable.³⁹ With these inconsistencies in mind, and considering the PAW classification system,⁴¹ Frauttschi, et al.³⁹ (2017) proposed the FIT PAAW classification system. This system is composed of 7 items, each of them containing important information that must be described in clinical studies that evaluate the efficacy of autologous platelet aggregates: (1) **F**orce of centrifugation; (2) **I**teration or sequence of centrifugation; (3) **T**ime of centrifugation; (4) **P**latelet concentration (baseline of patient whole blood and

final PRP preparation); (5) **Anticoagulant** use; (6) **Activator** use; (7) **White blood cells**.

Figure 4 summarizes the evidence for the use of PRP for facial rejuvenation. Twenty-three studies were found. Regarding the periorbital area, (including crow's feet, dark circles and infra-orbital wrinkles), seven studies were found.^{17,42-47} PRP was used as a standalone treatment in most of the cases, or used after CO₂ laser.⁴⁵ In most of the cases, PRP was applied in two or three sessions, with two/four-week intervals. The studies in general reported favorable results. However, most of the studies employed subjective outcome measures.

In all studies that evaluated PRP for treatment of nasolabial folds, significant improvement was reported^{42,43,46,48}, both by self-assessment or evaluation by physicians, as well as by biometric evaluation. In one of the studies, PRP was injected only once;⁴⁶ however, treatment used to be performed in two or three sessions, with a one-month interval. Patients were followed up to six months.⁴⁸

Regarding the use of PRP for the treatment of the cheeks and malar area, eight studies were found.^{43,45-47,49-52} In general, the beneficial results reported were less evident than those noticed for the nasolabial folds. Hersant, et al.⁵⁰ (2021) did not find any beneficial effect of PRP when used alone, but only when employed together with hyaluronic acid (HA). The association between PRP and HA, applied in two steps (mesotherapy and dermaroller) was also proven to be efficient in the study by Hersant et al.⁵¹ (2017). Alam, et al.⁴⁹ (2018) reported that patients and dermatologists rated PRP nominally but not significantly better than saline. Lee, et al.⁵² (2019) reported that WSRS scores improved in one patient, GAIS improved in 14 patients and FACE-Q indicated significant increases in patients' satisfaction with facial appearance after 4 mL of PRP were injected in six standardized points of the face. Hui, et al.⁴⁵ (2017) found a synergistic effect between PRP and CO₂ laser for facial rejuvenation.

Eight studies evaluated the whole face⁵³⁻⁶⁰ after treatment with PRP. In some of them, PRP was combined with other treatments/actives, such as erbium fractionated laser;⁵³ HA gel⁵⁸, dermaroller microneedling⁵⁶ or lipofiller.⁶⁰ In all cases, the association was reported to provide beneficial results. PRP provided significantly better improvement and less side effects when compared with readymade

growth factors.⁵⁷ A split-face study showed that three PRP injections at multiple sites administered with 15-day intervals improved skin quality by ameliorating wrinkles, texture and pores.⁵⁵ In a recent split-face study, monthly intradermal injections (mesotherapy) of lyophilized PRP were compared to saline solution for two months. Although lyophilized PRP presented 10 times the platelet baseline value and growth factors in adequate levels, only saline solution significantly increased dermis thickness.

A recent systematic review evaluated the safety and effectiveness of PRP for skin aging. In total, 24 studies, with 480 patients, were included. As monotherapy, PRP induced modest improvement in facial skin texture, appearance, and lines, besides improving pigmentation and fine lines, based on physician assessment. The degree of satisfaction of patients was high, although the degree of improvement was in general lower than 50% and the duration of the effect was uncertain. The degree of evidence is limited by heterogeneity in PRP preparation and administration, and lack of standardization in outcome measures. Moreover, only half of the studies employed "blind" examiners, which might have led to overestimation of effectiveness. The authors concluded that more high-quality trials with appropriate follow-up are necessary to provide appropriate evidence that may help to improve the treatment regimens. Several aspects should be considered when future clinical trials evaluating PRP are to be designed, such as the patients' characteristics (age, gender, history of sun exposure, ethnicity) that best predict a favorable response; the optimal number of sessions and the interval between them; the characteristics of the studies (quantification of the main parameters of PRP growth factors, longitudinal evaluation, examiners blinding); development of better instruments to evaluate skin aging.⁵

A recent review, including six articles, reported that PRP has been used effectively not only as an adjuvant therapy, but also as a standalone treatment for melasma. Among the growth factors present in PRP, TGF- β plays a central role in the treatment of melasma, since it reduces signal transduction of microphthalmia-induced transcription factor, thereby decreasing tyrosinase and tyrosinase-related proteins. Moreover, PRP also induces collagen synthesis, improving the quality and texture of the

Study design	Area treated	Intervention	Main outcome	Reference
Split-face RCT (n=40 patients)	Periorbital	PRP vs. plasma gel: 2 sessions with 4-week interval.	Both modalities yielded a significant improvement of periorbital wrinkles after the 2 nd session, with significantly better results on the plasma gel injected side; however, the improvement was not maintained for the following 3 months. Objective assessment did not show any improvement in periorbital hyperpigmentation.	Diab, et al. ⁴⁴ (2021)
Pilot RCT (n=10)	Neck	PRP + fractional radiofrequency microneedling vs. fractional radiofrequency microneedling: 3 sessions, one month apart.	Both modalities showed a statistically significant improvement in dermis thickness and cervicomentalar angle (measured by OCT), GAIS and patient satisfaction score.	Gawdat, et al. ⁷⁵ (2021)
Prospective, uncontrolled study (n=30)	Periorbital and nasolabial fold	PRP: 2 sessions with 3-month interval.	Significant improvement in dark circles and nasolabial folds, as evaluated by the therapeutic physician (photography).	Banihashemi, et al. ⁴² (2021)
Phase II non-randomized, split-face pilot study (n=19)	Face	Monthly intradermal injections (mesotherapy) of lyophilized PRP vs. saline solution during 2 months.	Although lyophilized PRP presented 10 times the platelet baseline value and growth factors in adequate levels, only saline solution significantly increased dermis thickness.	Silva, et al. ⁵⁴ (2021)
Randomized controlled prospective study (n=93)	Facial cheeks	Patients underwent a series of 3 treatment sessions with either PRP, hyaluronic acid or a mixture of both (Cellular Matrix) injected into facial cheeks.	Treatment with Cellular Matrix significantly improved overall facial appearance compared with treatment with PRP or hyaluronic acid alone, with significantly improved skin elasticity as evaluated by biophysical measurements.	Hersant, et al. ⁵⁰ (2021)
Prospective, uncontrolled study (n=11)	Malar (1 mL each side), nasolabial fold (0.5 mL each side) and upper lip skin above the vermilion border (1 mL)	Patients received monthly intradermal injections of i-PRF over 3 months. Efficacy assessed by objective skin analysis (VISIA®) and FACE-Q at baseline and at 3 months.	Significant improvement in skin surface spots and pores was seen at 3 months. Skin texture, wrinkles, ultraviolet spots showed numerical improvement. FACE-Q scales that measure satisfaction with appearance revealed significant improvement from baseline.	Hassan, Quinlan and Ghanem ⁶⁹ (2020)
Placebo-controlled split-face trial (n=30)	Mid-cheek and nasolabial fold	PRF matrix was injected in the mid-cheek and nasolabial fold on one side of the face and saline on the contralateral side. Primary outcome measure was the difference between pre- and posttreatment total VISIA® scores at 6 and 12 weeks.	PRF matrix can improve skin quality compared to placebo, but texture was the only skin parameter that was significantly improved, which is consistent with the role of PRF matrix as a filler agent. The results seem to persist for at least 6 weeks.	Hu, Bassiri-Tehrani and Abraham ⁷⁰ (2021)
Prospective, placebo controlled study (n=30)	Face	3 PRP injections (1 mL) at multiple sites administered with 15-day intervals between injections. Saline injected on contralateral side. Effects were evaluated using VISIA® and skin computed tomography.	PRP improved skin quality by ameliorating wrinkles, texture and pores.	Du, et al. ⁵⁵ (2020)
Prospective cohort (n=158)	Face	Erbium fractional laser irradiation combined with autologous PRP and PPP (3 sessions). Results evaluated by patients and experienced physicians.	Erbium fractional laser irradiation combined with PRP and PPP application is an effective and safe approach for improving facial skin aging and has minimal side effects.	Cai, et al. ⁵³ (2020)
Prospective, uncontrolled study (n=32)	Lower face (nasolabial folds, oral commissures, marionette lines, mandible and prejowl sulcus)	4 sessions of i-PRF with 2 to 3-week intervals (Cleopatra technique). Results were assessed by photographs (initial-prior to 2 nd session as well as initial-after completion of treatment) examined by blinded ratters.	Mean percentages of true answers of the blinded ratters attested the success of the treatment.	Nacopoulos and Vesala ⁷⁰ (2020)
Prospective clinical trial (n=25)	Perioral wrinkles	After a single session of fractional CO ₂ laser skin resurfacing plus intradermal injection of PRP, 5 drops of PRP mixed with "medical device" were topically applied twice a day for 12 weeks vs. hyaluronic acid.	PRP significantly improves moisture, amount of collagen fibers and skin elasticity	Araco ⁷⁶ (2019)
Split-face RCT (n=27)	Cheek rhytids of Glogau class II or greater	Each participant received 3 mL of intradermal injections of PRP to one cheek and saline to the contralateral one. Primary outcomes were photoaging scores rated by 2 masked dermatologists. Secondary outcomes included participant self-assessment scores of improvement and satisfaction.	Fine and coarse texture improved with single PRP application, as evaluated by the masked participants. Participants and dermatologists rated PRP nominally but not significantly better than saline.	Alam, et al. ⁴⁹ (2018)
Prospective single-center open-label (n=11)	Nasolabial and malar areas	Volunteers received 3 sessions of PurePRP (EmCyte system) at 1-month intervals, with a follow-up period after 6 months. Efficacy was assessed by clinical and biometric instrumental evaluation (Visia CR, Optical In Vivo Primos 3D Skin Device, Cutometer dual MPA 580, Minolta Chromameter CR-400, Dermascan-D ultrasound) and by self-assessment (questionnaire)	A series of 3 PurePRP injections resulted in significant skin rejuvenation (decrease in brown spot counts and area, wrinkle count and volume, improve in skin firmness and redness) at 6-month follow-up as demonstrated by biometric parameters and patient self-assessment score	Everts, Pinto and Girao ⁴⁸ (2019)
Prospective Split-face (n=24)	Face	24 volunteers with photoaging were randomly divided into 3 groups according to treatment (1 session every 2 weeks for 6 sessions) performed on each side of the face (microneedling by dermarolling alone or combined with PRP or TCA 15% peeling). Photography and punch biopsies were performed before and after 3 months of treatment for clinical, histometrical and histological evaluation.	Combined treatment showed significant improvement compared to dermaroller alone. Significant increase in epidermal thickness was observed, especially in the treatment with TCA. Organized collagen bundles with newly formed collagen were observed in all three groups. Improvement of dermal structures was more evident after combination of dermaroller with PRP, which apparently is more beneficial for facial rejuvenation.	El-Domyati, Abdel-Wahab and Hossam ⁵⁶ (2018)

Figure 4- Clinical trials on the use of autologous platelet aggregates for facial rejuvenation

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Prospective clinical study (n=31)	Cheeks	4 mL of PRP were injected into 6 standardized points on each side of the face. Outcomes were assessed by independent physicians using WSRS, GAIS and FACE-Q. Median follow-up was 5.7 weeks	WSRS scores improved in 1 patient; GAIS improved in 14 patients; FACE-Q indicated significant increases in patients satisfaction with facial appearance.	Lee, et al. ⁵² (2019)
RCT (n=103 test and n=128 control patients)	Forehead, cheeks and chin	103 patients with facial aging skin underwent nanofat and intradermal PRF injection (treatment group) and 128 patients underwent hyaluronic acid (HA) injection (control). Outcomes were evaluated by assessing pictures taken before and after treatment, and after 1, 12 and 24 months using the VISIA Skin Image Analyzer	Facial skin texture was improved to a greater extent after nanofat + PRF treatment compared with HA. The first also had a higher satisfaction rate. Neither treatment caused complications, such as anaphylaxis, paresthesia or infection during follow-up. Nanofat-PRF injection is safe, effective and long-lasting method for skin rejuvenation.	Liang, et al. ⁷² (2018)
RCT (n=62 test and n=77 control patients)	Facial soft tissue depression areas	62 test patients with soft tissue depression and signs of aging underwent combined nanofat, PRF and autologous fat structural transplantation vs. 77 controls (autologous fat transplantation).	Facial soft depressions and skin texture were improved to a greater extent after nanofat+PRF transplants, which led to overall satisfaction rate above 90%. Transplants combining nanofat, PRF and autologous structural fat granules are safe, highly effective and a long-lasting method for remodeling the facial contours and promoting rejuvenation.	Wei, et al. ⁷³ (2017)
Open label prospective study (n=31)	Cheek	2 mL of PRP was mixed with 2 mL hyaluronic acid (noncross-linked, 1550 kDa). Meso-therapy was performed at 0, 1 and 2 months by injecting 4 mL of the mixture per cheek, in 2 steps. 1 st step: deep intradermal injections of PRP-HA; 2 nd step: spreading 1 mL of the mixture per cheek followed by intradermal punctures with a 1-mm SkinRoller. Outcomes were assessed before injection and at 1, 3 and 6 months (Cutometer MPA 580 and FACE-Q).	FACE-Q scores and biophysical measurements revealed significant improvement at 6 months compared with baseline.	Hersant, et al. ⁵¹ (2017)
Split-face prospective study (n=20)	Face	Patients were randomly assigned to treatment (6 sessions at 2-weeks interval) by readymade growth factors (area A) or PRP (area B). Evaluation was made by GAIS (skin turgor and vitality) and OCT (epidermal and dermal thickness)	Patients receiving growth factors had significantly higher burning sensation and lower degree of satisfaction. Improvement was more sustained in patients receiving PRP.	Gawdat, et al. ⁵⁷ (2017)
Pilot prospective study (n=12)	Forehead, cheeks, nasolabial folds and crow's feet area	3 sessions of PRP injection (4 mL) at 1-month intervals. Outcomes were assessed before and 1 month after completion of the treatment by Cutometer, Visioface, Visioscan, corneometry, water loss and transepidermal water loss. PRP was evaluated by flow cytometry	Both patient and clinical evaluation revealed improvement of skin texture. Skin smoothness, gross elasticity, capacitance and barrier function were improved. Flow cytometry showed reproducibility in PRP samples and low content in proinflammatory cells	Cameli, et al. ⁴³ (2017)
Prospective single-dose open-label non-randomized split-face (n=20)	Upper site of right infra-auricular area	Punch biopsy was taken from the right infra-auricular area before treatment and PRP was injected to the upper site of this area (1.5-2.0 mm deep), while saline was injected to the left infra-auricular area. 28 days after treatment, punch biopsy was performed on the PRP and saline injected sites. Mean optical densities (MODs) of collagen were measured.	The MODs of collagen were 539±93.2, 787±134.15, 1,019±178 in the pre-treatment, control (saline) and PRP groups, respectively. Single treatment with PRP increased MOD of collagen in 89% which was significantly higher when compared to saline (46%; attributed to needling)	Abuaf, et al. ⁷⁷ (2016)
Case-series (n=94)	Face	PRP was mixed to 0.5 mL 3.5% HA gel and 0.5 mL procaine and infiltrated 5-6 mm deep into dermis and hypodermis. In "intense" periods, consecutive treatments were performed 3-4 weeks apart. In "maintenance" periods, patients received up to 5 sessions at 8-10-week intervals. The mean number of injections was 3.6±2.0; range 1-8). Patients rated satisfaction with skin pigmentation, texture, and sagging. Overall results were rated by 3 physicians.	Significant improvement was noticed regarding skin texture, firmness/sagging and general appearance by the physicians and patients. The degree of satisfaction and improvement was directly correlated with the number of PRP injections.	Ulusal ⁵⁸ (2017)
Prospective open-label (n=20)	Forehead, cheeks, nasolabial folds and crow's feet area	Single intradermal injection of PRP. Evaluation carried out by a period of 8 weeks using SHnT, WSRS, as well as physician assessment and patient satisfaction scale	Mean WSRS reduced from 2.9±0.9 to 2.1±0.8 after 8 weeks, with better results for younger patients with mild-moderate wrinkles of nasolabial folds.	Elnehrawy, et al. ⁴⁶ (2017)
Randomized split-face (n=13)	Forehead, cheeks and periopercular layer	Ultra-pulsed fractional CO ₂ laser was applied. PRP was injected into one side (2.2 mL) and saline into the other. Clinical efficacy was assessed by satisfaction scores, dermatologists' evaluation and VISIA analysis system	After 3 months, PRP increased subjective scores of facial wrinkles, skin elasticity and texture and decreased duration of edema, crusting and erythema when compared to control. PRP combined with CO ₂ laser had a synergistic effect on facial rejuvenation and shortened the duration of side-effects.	Hui, et al. ⁴⁵ (2017)

Figure 4- Clinical trials on the use of autologous platelet aggregates for facial rejuvenation

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Prospective (n=13)	Preauricular areas	Fragments of skin were removed before and 3 months after injection of fat plus PRP, fat (stromal vascular fraction – SVF) or adipose-derived stem cells and analyzed by optical and electron microscopy	Fat plus PRP led to more pronounced inflammatory infiltrate and greater vascular reactivity, increase in vascular permeability. The addition of PRP did not improve the regenerative effect. The use of PRP was not advantageous for skin rejuvenation over the use of SVF-enriched fat or expanded adipose-derived stem cells. Due to increased vascular reactivity, the combination of fat plus PRP might be useful in situations when an intense angiogenesis is desirable, such as tissular ischemia	Rigotti, et al. ⁷⁸ (2016)
Prospective open-label (n=10)	Forehead, malar, jaw and crow's feet	PRP was applied thrice at 2-week-intervals by a dermaroller and injected into the wrinkles of crow's feet. Participants graded general appearance, wrinkle state, skin firmness-sagging and degree of pigmentation of their face before and 3 months after the last PRP procedure. 3 dermatologists also evaluated.	There was significant difference in general appearance, wrinkle state, skin firmness-sagging before and after 3 PRP applications according to the patients' evaluations. However, according to the dermatologists' assessment, the only significant difference perceived was in skin firmness-sagging.	Yuksel, et al. ⁴⁷ (2014)
Retrospective study (n=82)	Face	Recovery time and aesthetic outcome after treatment with fat grafting only (GI), fat grafting and PRP (GII), minimal access cranial suspension (MACS)-lift and fat grafting plus PRP (GIV) (GIII) and MACS-lift, fat grafting. Assessment was made by 10 plastic surgeons by a questionnaire and photographs	Addition of PRP to fat grafting led to a significant reduction in the number of days needed to recover before returning to work/restart social activities (GI took 18.9 days vs. GII took 13.2 days, p=0.019). The aesthetic outcome of GII/GIV was significantly better than GI/GIII. The addition of PRP to facial lipofilling reduces recovery time and improves the overall aesthetic outcome of a MACS-lift.	Willemsen, et al. ⁶⁰ (2014)
Prospective, randomized, split-face trial (n=20)	Infraorbital wrinkles and skin tone	10 patients received a PRP injection in one side of the face and the other was treated with PPP; the remaining 10 were treated with PRP vs. saline (3 sessions at 4-week intervals). Evaluations performed at baseline and 3 months after the final treatment, including self-assessment questionnaire, subjective satisfaction scale and clinical assessment by 3 dermatologists (photographs). Erythema and melanin indices were evaluated spectrophotometrically.	Infraorbital skin treated with PRP presented significant improvement of tone and wrinkles compared with saline or PPP treated skin. After PRP treatment, the erythema and melanin indices significantly decreased	Kang, et al. ⁷⁹ (2014)
Prospective open-label (n=23)	Face and neck	3 monthly injections (4 mL) of PRP (Regen Lab Kit). Evaluation performed by patients and physicians through questionnaires, dermoscope and photographs	No serious and persistent side-effects were observed. Overall, the results were satisfactory.	Redaelli, Romano and Marciano ⁵⁹ (2010)
Prospective open-label (n=15)	Nasolabial fold	PRF matrix (Selphyl system) was injected into the dermis and immediate subdermis below the nasolabial folds. Patients were photographed before and up to 12 weeks after single injection. Evaluation performed using GAIS and WAS	All patients were treated to maximal correction, with mean reduction in WAS score of 2.1±0.6, that 0.7±0.6 after 1 week but rose to 1.1±0.7 at 12 weeks after treatment. Fibrosis, restricted movement, irregularity hardness or lumpiness was not noticed by any patient. PRF matrix provides significant long-term reduction of deep nasolabial fold.	Sclafani ⁷⁴ (2010)

*PRP = Platelet-rich plasma; PPP = Platelet-poor plasma; PRF = Platelet-rich fibrin; OCT = optical coherence tomography; GAIS = Global Aesthetic Improvement Scale; FACE-Q = subjective patient-reported outcome assessment; TCA = trichloroacetic acid; WSRS = Wrinkle Severity Rating Scale; HA = Hyaluronic acid; SHnT = Skin Homogeneity and Texture Scale; WAS = Wrinkle Assessment Scale.

Figure 4- Clinical trials on the use of autologous platelet aggregates for facial rejuvenation

skin.¹⁴

One of the most frequent complaints of the patients undergoing treatment with PRP is pain during application, especially when the treatment is performed by multiple injections. It has been reported that covering the area of application of PRP with a cooled (20°C) hydrogel dressing for 20 minutes before and after PRP injection reduces pain and edema by needle picking and accelerates patient recovery and overall appearance of the skin straight after the procedure.⁶¹ Moreover, a thermosensitive formulation able to embed PRP and growth factors that stays liquid when the temperature is lower than 20°C, but becomes a gel when the temperature exceeds 35°C (when the product touches the skin), was developed. This thermosensitive gel formulation was named "medical device" and allows storage

of platelets and growth factors for seven days, maintaining their full activity.

Although PRP has been reported to be used for the treatment of infraorbital hyperpigmentation and also for treatment of post-inflammatory hyperpigmentation, especially seen after peeling or laser applications⁶², this is controversial, since there are reports showing increased pigmentation when it is applied over the pigmented skin lesions that were present before the application.⁶³ This is the reason why it has been reported that PRP should not be used to treat post-inflammatory hyperpigmentation.⁶³

PRP preparation is difficult due to the requirement of double centrifugation.⁵ In addition, the need to use anticoagulant might impair the healing process, due to the inhibition of the coagulation process.⁷ To overcome these limitations, platelet-rich fibrin

(PRF), an autologous platelet aggregate of "second generation," was developed by Choukroun, et al.⁸ (2001).

Second and third generation autologous platelet concentrates: PRF and i-PRF

PRF is obtained after a single centrifugation, without the need of using anticoagulants.⁸ The resulting product contains different cell types (platelets, leucocytes, erythrocytes), an extracellular fibrin matrix and several bioactive molecules (primarily growth factors). Depending on the type of tube used for collecting the blood and on the protocol for centrifugation, PRFs in the form of liquid or solid gel can be obtained. The solid forms obtained using glass tubes have been used in plastic and bucomaxillofacial surgeries.^{7,9}

In 2014, a fluid (injectable) form of PRF (called i-PRF; "third generation") was developed, by modification of the RCF.¹⁰ Reducing the RCF and the time of centrifugation and using plastic tubes (to reduce the coagulation time), the time required for the coagulation of the fibrin could be slower in the initial periods, generating a product containing fibrinogen and thrombin, which remains fluid for around 20 minutes after centrifugation, before the fibrin matrix is formed. This makes the product proper to be employed for facial rejuvenation.

By employing lower RCFs (enough to separate erythrocytes from platelets), the characteristics of the PRF are improved. The numbers of platelets and leucocytes and the concentrations of growth factors in the fibrin matrix are increased. Moreover, platelets and cytokines are entrapped in the fibrin matrix after the injection, leading to a slow and gradual release of growth factors along time.^{64,65} In the study by Choukroun and Ghanaati⁶⁴, plasma was centrifuged using RCFs of 710 *g*, 177 *g* and 44 *g* for 8 min. A higher concentration of platelets and leucocytes was found in the iPRF when the RCF of 44 *g* was employed, while higher concentrations of growth factors (VEGF and TGF- β 1) were found with 177 *g*. In another study, it was evaluated the pattern of platelets distribution and the release of growth factors (EGF, VEGF and TGF- β 1) along time from three PRF matrixes, produced from distinct RCFs and times of centrifugation: PFR (708 *g*, 12 min), A-PRF (advanced; 208 *g*; 14 min), A-PRF+ (advanced+; 208 *g*; 8 min). A-PRF+ led to a higher release of growth factors when compared with the

other matrices. In addition, platelets had a more homogeneous distribution in the A-PRF and A-PRF+ matrices, while in the PRF matrix, they were located mainly in the lower portion⁶⁶ (Figure 3).

Experiments with human dermal skin fibroblasts showed greater cell migration and proliferation, as well as higher levels of m-RNA for type I collagen, TGF- β and fibronectin, besides a higher capacity to induce the synthesis of collagen matrix in the presence of i-PRF when compared with PRP⁶⁷ (Figure 3). PRP reduces the phosphorylation of JNK, thereby accelerating wound healing.⁶⁸

Figure 4 shows clinical trials on the use of autologous platelet aggregates for facial rejuvenation. Only six clinical trials evaluated the use of PRF and i-PRF for facial rejuvenation⁶⁹⁻⁷⁴, while most of the studies evaluated PRP.^{42-60, 75-79} Regarding the studies evaluating PRF or iPRF, two of them found beneficial results when PRF was combined with nanofat.⁷²⁻⁷³ When used alone, PRF matrix provided significant long-term reduction of deep nasolabial fold.^{70,74} It has been reported, using i-PRF with low RCF (combination of 60 *g* for 3 min and 208 *g* for 5 min), good results for the rejuvenation of the lower third of the face (nasolabial fold and labial commissure) after an intradermal application.⁷¹ In another study, the effect of three monthly intradermal injections of i-PRF (low RCF 60 *g*, 3 min) in three facial regions was evaluated: malar area, nasolabial fold, and region above the vermilion of the upper lip. An improvement in skin texture, pores, wrinkles, as well as patient satisfaction was observed after three months.⁶⁹ However, additional studies are needed to establish the centrifugation protocol that leads to the best clinical effects. In addition, more high-quality trials with appropriate follow-up are necessary to provide appropriate evidence that may help to improve the treatment regimens.

Conclusion

Autologous platelet aggregates for skin rejuvenation are safe and well tolerated. PRP, the first-generation product, is more studied in the literature, with several clinical trials and case series, whose results have been compiled in a systematic review.⁵ The results, in general, are favorable, but the quality of the studies is low and additional studies are

required. The second and third generation products, PRF and i-PRF, respectively, are easier to be obtained and, at least *in vitro*, seem to induce greater collagen production than PRP,⁶⁷ especially under lower RCFs.⁶⁴ However, only a few clinical trials evaluating these products are available to date.

More high-quality trials with appropriate follow-up are necessary to provide appropriate evidence that may help to improve the treatment regimens with autologous platelet aggregates. Several aspects should be considered when future clinical trials evaluating PRP are to be designed, such as the patients' characteristics that best predict a favorable response, the optimal number of sessions and the interval between them, the characteristics of the studies and the development of better instruments to evaluate skin aging.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Buzalaf, Marília Afonso Rabelo: Conceptualization (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Resources (Equal); Software (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing – original draft (Equal); Writing – review & editing (Equal). **Levy, Flavia:** Conceptualization (Equal); Data curation (Equal); Formal analysis (Equal); Funding acquisition (Equal); Validation (Equal); Visualization (Equal); Writing – original draft (Equal); Writing – review & editing (Equal).

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