Chapter 10

# The Wonder Tool Platelet Rich Plasma in Cosmetic Dermatology, Trichology and Hair Transplant

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Additional information is available at the end of the chapter

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**Abstract**

Platelet-rich plasma or PRP therapy is a form of regenerative medicine where body's own cells, tissues or organs can be utilized by replacing, regenerating or engineering to restore or establish normal function. Various published articles demonstrating the role of PRP therapy in cosmetic procedures like scar revision, facial rejuvenation, stretch mark removal, androgenetic alopecia, alopecia areata and hair transplant were analyzed in depth to understand its efficacy based on facts and figures along with inputs from personal experience. PRP therapy is one of the most upcoming forms of regenerative medicine with the potential to improve the homeostasis of the treated cells and tissues, provided that harvesting standards are maintained.

**Keywords:** platelet-rich plasma, wound healing, platelet growth factors, vampire facelift, scar revision, hair transplant, hair fall

## 1. Introduction

Platelet-rich plasma or PRP therapy is a form of regenerative medicine where body's own cells, tissues or organs can be utilized by replacing, regenerating or engineering to restore or establish normal function. While the role of PRP therapy is already established in sports injuries, dental and oral surgery and pain relief, it also comes as a promising option in various procedures in cosmetic dermatology, trichology and more recently hair transplant.

## 2. Main body of the paper

Synonyms: Autologous platelet gel, plasma-rich growth factors, platelet-concentrated plasma, platelet-rich concentrate, platelet releasate [1, 2].

Definition: Platelet-rich plasma is volume of the plasma fraction of autologous blood with an above baseline platelet concentration (usually more than 1,000,000 platelets/μl), leading to 300–700% enrichment [3, 4].

## 3. What is PRP?

Whole blood consists of 93% red blood cells, 6% platelets and 1% white blood cells. In PRP, the proportion of these cells in blood is inverted, that is, the red cell layer is reduced to 5% and platelets and leucocytes are increased to about 94% to stimulate tissue regeneration [5].

The cellular response to injury occurs in four stages: hemostasis, inflammation, proliferation and remodeling. In each phase, there is an enhanced cellular or molecular activity involving the platelets. Platelets and plasma are responsible for hemostasis, leucocytes and activated platelets mediate the inflammation, and growth factors derived from platelet alpha granules influence regeneration of tissues. Leucocyte content of PRP influences the inflammatory process, and angiogenic and mitogenic growth factors aid tissue regeneration [6].

Various growth factors are secreted from the $\alpha$-granules of concentrated platelets activated by aggregation inducers [3]. These factors are known to regulate processes of cell migration, attachment, proliferation and differentiation and promote extracellular matrix (ECM) accumulation by binding to specific cell surface receptors [7].

Regenerative potential of PRP depends on the levels of released growth factors (GFs). PRP contains more than 20 GFs and other proteins, such as adhesion molecules, chemokines, etc., which interact, leading to inflammation, cell proliferation, differentiation and regeneration [3].

Activation of the platelets causes degranulation, leading to transformation of secretory proteins (e.g., Platelet derived growth factor (PDGF), Transforming growth factor-β (TGF-β) etc.) to a bioactive state by the addition of histones and carbohydrate side chains. Then, active proteins are secreted, which bind to transmembrane receptors of target cells, including mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells and epidermal cells. The agonist bound transmembrane receptors then activate an intracellular signal protein, leading to expression of a gene sequence which directs cellular proliferation, formation of matrix, collagen synthesis, etc., thereby provoking tissue repair and tissue regeneration (**Table 1**) [8].

The mean blood platelet level is approximately 200,000 ± 75,000/μl. Platelet concentration of more than 1 million/μl (about four to seven times the mean levels) is regarded as concentration of PRP that is therapeutically effective. A bell-shaped response curve which indicates a dose-dependent nature is associated with PRP. Lower or higher concentrations than 1.5 million platelets/μl inhibit the angiogenic potential in human endothelial cells. In vitro studies on dermal papilla cells also support that PRP should be used at the concentrations of 5–10 times the mean levels [2].

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| Platelet growth factor | Biological actions |
|---|---|
| PDGF αα,αβ,ββ | Mitogenic factor for mesenchymal cells, proliferation of fibroblasts/smooth muscle cells, secretion of collagenase and synthesis of collagen, macrophage proliferation and chemotaxis of neutrophils |
| TGF (alpha-beta) | Stimulates mesenchymal cells proliferation; regulates mitogenesis of endothelial cells and fibroblasts; secretion of collagenase and synthesis of collagen, regulates mitogenic effects of other growth factors, stimulates angiogenesis, inhibition of proliferation of macrophage and lymphocyte |
| VEGF | Stimulates angiogenesis, increases vessel permeability and stimulates mitogenesis of endothelial cells |
| EGF | Stimulates angiogenesis, regulates secretion of collagenase and stimulates epithelial and mesenchymal mitogenesis |
| FGF | Promotion of growth and differentiation of fibroblasts and mesenchymal cells |
| CTGF | Promotes neoangiogenesis, regeneration of cartilage, fibrosis and platelet adhesion |
| IGF-1 | Chemotactic for fibroblasts stimulates protein synthesis, in combination with PDGF, and enhances rate and quality of wound healing |
| HGF | Mediates regeneration |
| FGF-9 | Aids generation of new follicles |

**Table 1.** Growth factors present in alpha granules of platelets and their biological actions [2, 36, 37].

The platelets actively secrete growth factors within 10 min after activation, and more than 95% of the presynthesized growth factors are secreted within 1 h [8].

Therefore, PRP should be used within 10 min of activation. The viability of the concentrated platelets remains for up to 8 h, and it stays sterile if placed on a sterile surgical table [2].

The platelets remain viable for 7–10 days and continue releasing the growth factors in tissue during this period [6].

## 4. Method

Centrifugation separates the blood components, depending on their specific gravities, that is, RBCs are the heaviest, followed by WBCs, whereas platelets are the lightest. The first centrifugation is slow, so that the spinning down of platelets is avoided and isolation of plasma occurs easily. Platelets are mostly concentrated right on the top of buffy coat layer. Subsequent centrifugation is faster, so that platelets are spun down, leading to separation as a pellet at the bottom of the tube from the platelet-poor plasma (PPP) above. The final concentration of platelets depends on volume reduction of the PPP. About three-fourth of the supernatant is discarded, and the platelet-rich pellet is resuspended in rest of the plasma. The suspension formed is used as PRP. Double-spin method is preferred over the single-spin method, as the therapeutic concentration of platelets is not achieved by using the latter [2].

### 4.1. Manual method

#### *4.1.1. PRP method*

1. Whole blood is collected by venipuncture in acid citrate dextrose (ACD) tubes [8].
2. Blood is not chilled at any time before or during platelet separation.
3. Blood is centrifuged using a 'soft' spin.
4. Supernatant plasma containing platelets is transferred into another sterile tube (without anticoagulant).
5. Centrifuge the second tube at a higher speed (hard spin) to get a platelet concentrate.
6. The lower one-third is PRP, and upper two-thirds are called platelet-poor plasma. Platelet pellets are formed at the bottom of the tube.
7. PPP is removed, and the platelet pellet is suspended in a minimum quantity of plasma (2–4 ml) by gently shaking the tube.

#### *4.1.2. Buffy coat method*

1. Whole blood is stored at 20–24°C before centrifugation.
2. After storage, blood is centrifuged at a 'high' speed.
3. Three layers are formed because of the density: The bottom layer consists of RBCs, the middle layer consists of platelets and WBCs and the top is PPP layer.
4. Supernatant plasma is removed from the top of the container.
5. The buffy-coat layer is transferred to another sterile tube.

#### *4.1.3. Automated method*

There are various automated devices and kits available in the market.

#### *4.1.4. Our experience*

We use YCell Bio kit and REMI centrifuge for preparation of PRP.2 Vials, each containing 13.5 ml of whole blood mixed with 1.5 ml of ACD-A solution and centrifuged for 4 min at 3000 RPM. A total of 5–6 ml of buffy coat along with PRP is harvested from these two vials and injected as required. It gives five to seven times concentration of the baseline platelet count.

## 5. Classification

Ehrenfest et al. proposed a classification in 2009 according to which platelet concentrates can be classified into four main families depending on their cell content and fibrin architecture [9].

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1. Pure platelet-rich plasma (P-PRP) or leucocyte-poor platelet-rich plasma-preparations which are leucocyte poor and have a low-density fibrin network on activation. They can be used as liquid solutions or as an activated gel. Therefore, it can be injected (as used in sports medicine) or can be placed during gelling on a wound or suture (similar to fibrin glue). Following the first slow spin centrifugation, only the superficial buffy coat layer is aspirated out and taken for second centrifugation [2]. Example: PRGF (plasma rich in growth factors or preparations rich in growth factors).
2. Leucocyte- and platelet-rich plasma (L-PRP) products-preparations with leucocytes and with formation of low-density fibrin network on activation. Largest number of commercial or experimental systems belongs to this group. During preparation of L-PRP, PPP, entire buffy coat layer and upper 1–2 mm of red blood cell layer are pipetted out after the first centrifugation [2]. Like P-PRP, they can be in liquid or gel form.
3. Pure platelet-rich fibrin (P-PRF) or leucocyte-poor platelet-rich fibrin-preparations which are leucocyte poor and with a fibrin network which is of high density. They exist only in a strongly activated gel form. To make P-PRF, P-PRP is mixed with an activator and a specific separator gel is used. After incubating for some time, a stable platelet-rich fibrin matrix (PRFM) clot is formed [2]. It cannot be injected or used like traditional fibrin glue but as it has a strong fibrin matrix, it can be handled like a real solid material and used for various other applications.
4. Leucocyte- and platelet-rich fibrin (L-PRF) or second-generation PRP product-preparations with leucocytes and a high-density fibrin network. Blood is centrifuged immediately after collection without any anticoagulant, thrombin or $CaCl_2$. Natural coagulation process leads to formation of three layers—lowest RBC layer, middle L-PRF layer and topmost plasma layer which is acellular. The PRF clot is pressed between two gauzes to form a strong membrane [2]. Like P-PRF, it exists in strongly activated gel form only.

## 6. Factors which influence yield of PRP

### 6.1. Blood withdrawal technique

In most of the protocols, large bore needles (>22) are used for withdrawal of the blood to avoid unintentional activation of platelets [10].

Waters and Roberts, in their study, found that there was decrease in platelet counts with longer draw time [11].

### 6.2. Centrifugal force

Separation of blood's cellular constituents is achieved by the process of differential centrifugation. In differential centrifugation, acceleration force is adjusted, leading to sedimentation of certain cellular constituents, whereas other constituents are left in suspension. Relative centrifugal field or RCF is the force which is required for separation of two phases. RCF is

expressed as multiples of the earth's gravitational field (g). On accelerating g, speedy sedimentation is achieved. 'g' is the actual force being exerted on the spinning rotor's contents, which leads to separation of the aqueous solutions [8].

Revolutions per minute (rpm) is calculated using the equation [12].

$$g = (1.118 \times 10^{-5}) R S^2 \quad (1)$$

where 'g' is the RCF, $R$ is radius of the rotor from center of rotor to the sample (cm) and $S$ is speed of the centrifuge (revolutions per minute).

The RCF calculation is dependent on radius of the centrifuge rotor used [8].

### 6.3. Temperature

AABB manual recommends temperature of 21–24°C for blood centrifugation for the purpose of obtaining PRP [13]. Macey et al. reported that cooling may retard the platelet activation, and therefore, it may be essential to obtain PRP with viable platelets [14].

### 6.4. Anticoagulants

An ideal anticoagulant should preserve best functionality, integrity and morphology of platelets [8].

Anticoagulants with citrate and dextrose of sodium citrate are recommended for the PRP preparation [15].

Ethylene diamine tetra acetic acid (EDTA) is not preferred because it can damage the platelet membrane [8].

ACD binds calcium and prevents the clotting cascade initiation by the coagulation proteins. Citrate also makes blood more acidic than is physiological. As some growth factors are influenced by the tissue pH, some protocols recommend that PRP should be buffered back to a physiologic range before injection [16].

### 6.5. Activation of PRP

PRP is exogenously activated by thrombin, calcium chloride or mechanical trauma. Collagen is a natural activator; thus, when PRP is used in soft tissue, there is no need to be exogenously activated [10].

On activation of PRP, a fibrin network begins to form and solidification of the plasma occurs, leading to fibrin clot or membrane formation. If PRP is activated too strongly, the fibrin network formed will be a bivalent, unstable network. If it is activated in a more physiologic manner, there is formation of a tetramolecular stable network that enhances cells and growth factor enmeshment. It is undesirable to have overly viscous PRP when injecting into the soft tissue [16].

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### 6.6. Utility of inhibitor of platelet aggregation

Anticoagulants do not interrupt platelet aggregation. This has been overlooked in preparing conventional PRP. Aggregated platelets stick to the syringe wall and do not get easily detached from them. The primary aggregation of platelets is reversible, so the platelets come off from the wall and float in the plasma again after several hours. Waiting for such a long time is not feasible in the daily practice. So platelet aggregation inhibitor (PGE1) can be used to prevent this aggregation [17].

### 6.7. Contraindications

Absolute contraindications:

1. Platelet dysfunction syndrome.
2. Critical thrombocytopenia.
3. Hemodynamic instability.
4. Septicemia.
5. Local infection at the site.
6. Patient unwilling to accept risks.

Relative contraindications:

1. Consistent use of NSAIDs within 48 h of procedure.
2. Corticosteroid injection at treatment site within 1 month.
3. Systemic use of corticosteroids within 2 weeks.
4. Use of tobacco.
5. Recent fever/illness.
6. Cancer—especially hematopoietic or bone.
7. Hemoglobin < 10 g/dl.
8. Platelet count < $10^5$/μl.

### 6.8. Complications

1. Pain in the injected area, headache, heaviness of head.
2. Swelling and redness.
3. Infection-PRP is antimicrobial and is effective against most bacteria except *Klebsiella*.

4. *Enterococcus* and *Pseudomonas*.
5. Allergic reaction—urticarial rash.
6. Skin discoloration, bruising.
7. Bleeding.
8. Cross labeling of samples—leading to serious side effects, for example, severe hypersensitivity reaction [18].

## 7. Indications of PRP in cosmetic dermatology, trichology and hair transplant

### 7.1. Trichology

*7.1.1. Androgenetic alopecia (AGA)*

Growth factors from platelets act on stem cells present in the bulge area of the follicles, stimulating the new follicular development and promotion of neovascularization. They activate the proliferation and transdifferentiation of hair and stem cells and produce new follicular units. Basic fibroblast growth factor promotes the proliferation of papilla cells in vitro and, therefore, plays a key role in hair shaft elongation. Activated PRP promotes the proliferation and prevents apoptosis of dermal papillary cells [19].

Singhal et al. found out that by the end of 3 months, all 10 androgenetic alopecia (AGA) patients treated with PRP had a good hair growth with reduction in number of hair pulled out by average 65%. New hair growth was observed in six patients as early as 7 days and in four patients in 15 days. Three patients developed mild headache after the procedure. There was no inflammation or infection [20].

In an another study on PRP in 11 AGA patients, the hair pull test after four sessions (once in 2 weeks) of PRP became negative in nine patients. Moderate improvement in hair volume and coverage was reported [19].

Greco and Brandt in their study, involving five patients who were given PRP therapy and five patients in non-PRP group (10 AGA patients in total), concluded that PRP used as mesotherapy in AGA patients leads to a significant increase in hair diameter and hair density [21].

Various modes of PRP therapy for AGA are as follows [2]:

1. Interfollicular PRP injection—an amount of 0.05–0.1 ml/cm$^2$, in a retrograde fashion from deep to superficial, at distance of a centimeter, throughout the treated site.
2. PRP mesotherapy—microneedling with a roller of 1–1.5 mm long needles followed by interfollicular PRP injections (or using mesogun) over the treated area, and later, PRP is sprayed on top of the scalp and left overnight. It is usually repeated at an interval of 1–3 months.
3. PRP as an adjunct to hair transplantation:

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**a.** The follicular grafts are dipped into PRP for about 15 min, before implantation to increase their rate of survival following implantation [22].

**b.** PRP is injected into the recipient area of scalp before or just after graft implantation [23].

**c.** PRP is injected at and around the donor strip excision line, in follicular unit transplantation (FUT), to decrease bleeding, stimulation of wound healing and reduction of scarring.

*7.1.2. Our experience*

At our institute, we studied the effects of PRP therapy in 30 male patients with grade I to IV androgenetic alopecia. Three sessions of PRP at an interval of 1 month were given. Along with PRP, multivitamin supplements, peptide-based topical serum and high protein diet were also advised. Growth of new hair was observed to start at about 4–6 weeks after first session in 60% of the patients. By 8 weeks, increase in hair diameter was noted in 80% of the patients. There was significant improvement with perceptible difference in diameter and density of hair at 3 months after the completion of PRP sessions (**Figure 1**).

There were nine cases of female pattern hair loss in whom, we observed that more number of sessions were required for perceptible improvement to occur, that is, mean of six PRP sessions was required. New hair growth was seen as early as 6–8 weeks. On video-microscopy, moderate increase in density and diameter of hair was noted at about 6–10 months. Marked improvement in skin texture was observed. We also noted gradual diminution of the perifollicular halo with successive PRP therapy. Reduction in active hair fall was noticed as early as 4–6 weeks (**Figure 2**).

*7.1.3. Our experience in FUE with PRP therapy*

We conducted a randomized control study of 40 patients with androgenetic alopecia undergoing hair transplant, and 20 patients each were allocated to PRP and the control group. In the PRP group, after harvesting and slitting, 0.2–0.3 ml PRP was injected at 1 cm gap to the depth of dermis and subcutis in freshly done slits, whereas the non-PRP group received normal saline instead of PRP. There was more than 75% growth in all patients in PRP group after

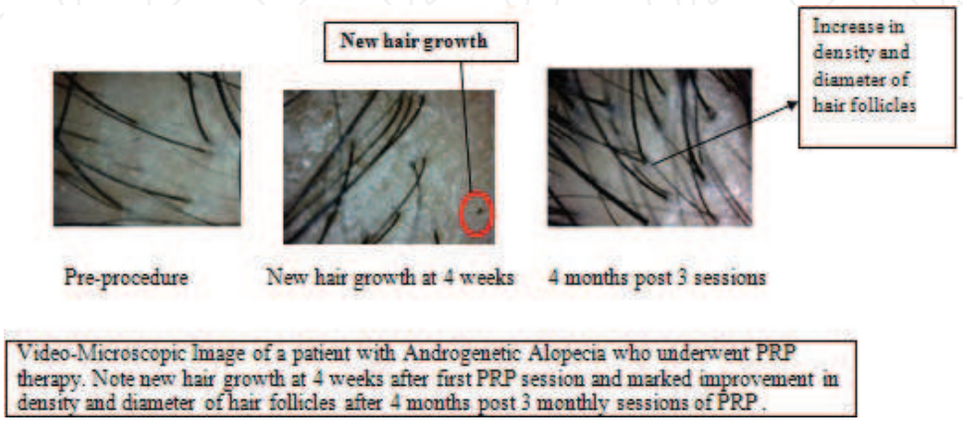


**Figure 1.** Video-microscopic image of a patient with androgenetic alopecia who underwent PRP therapy. Note new hair growth at 4 weeks after first PRP session and marked improvement in density and diameter of hair follicles after 4 months post 3 monthly sessions of PRP.

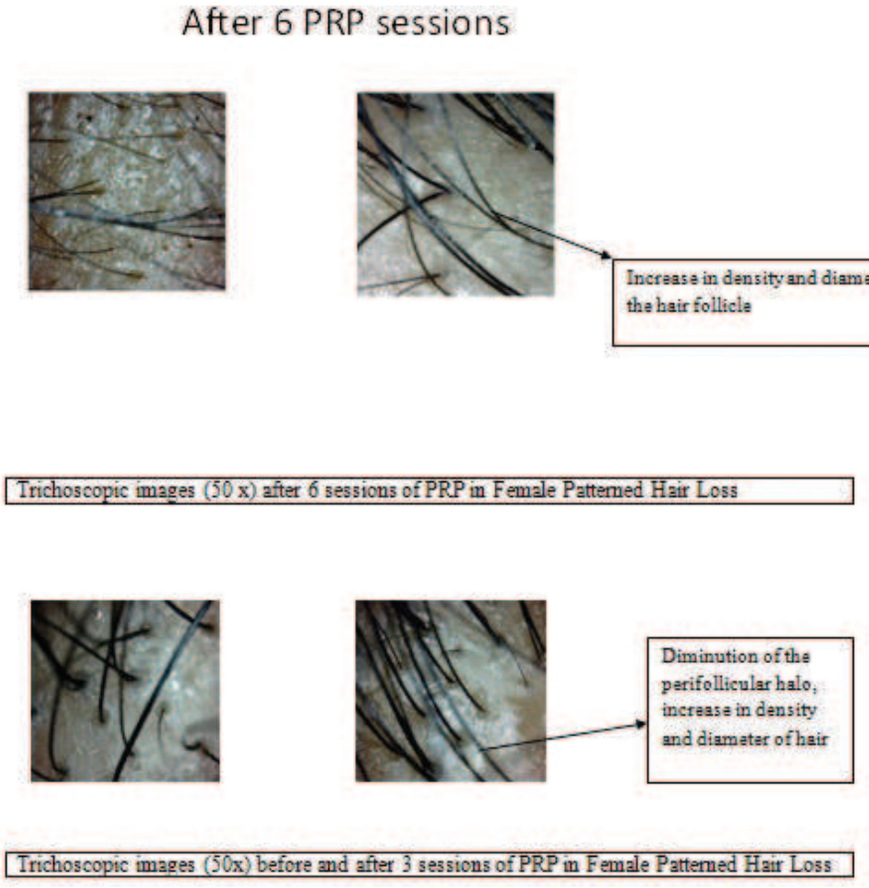


**Figure 2.** Trichoscopic images (50×) after six sessions of PRP in female patterned hair loss. Trichoscopic images (50×) before and after three sessions of PRP in female patterned hair loss.

6 months, whereas only 20% of non-PRP group had similar growth. PRP group had much denser and lengthier follicle growth. Also, in PRP group, number of multiple grafts was more, shafts were longer, better texture of hair was seen, posttransplant catagen fall was less and there was absence of redness after 3 months. PRP therapy during hair transplant was found to play a significant role in regrowth of hair and remarkably improved the density and quality of hair growth 8 months after transplantation (**Figure 3**).


**Figure 3.** Pre- and post-procedural photographs of a patient 6 months after undergoing follicular unit extraction (F.U.E.) hair transplant with PRP injections during the transplant.

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## 8. Alopecia areata

A double-blinded, placebo and active-controlled, half-head, parallel group study on 45 patients designed to evaluate the efficacy of PRP in alopecia areata concluded that PRP is a safe and alternative treatment for AA. PRP was found to significantly increase hair regrowth and to decrease hair dystrophy and burning or itching sensation without any side effects. Ki-67 levels, which are a cell proliferation marker, were significantly higher in PRP group [24].

Singh found in their study that out of 20 patients with alopecia areata treated with PRP, only one had a relapse. There were no side effects, and all patients well tolerated the procedure [25].

### 8.1. Scars and dermal augmentation

A study done to determine the efficacy of single injection autologous PRFM for deep nasolabial folds (NLFs) correction concluded that PRFM provides significant long-term deep NLFs diminution. No fibrosis, irregularity, hardness, restricted movement or lumpiness was seen [26].

PRP has a high concentration of platelets with neovascularization properties and, thus, has the potential to promote survival of fat graft. Fat graft volume and weight were found to be significantly higher in the PRP group than in the control group. Histologic evaluation showed greater vascularity, fewer cysts and vacuoles, and lesser fibrosis in the PRP group [27].

#### *8.1.1. Post-acne atrophic scars*

PRP injections combined with fractional carbon dioxide resurfacing provide good results in treatment of acne scars as revealed by a simultaneous split face trial [28].

#### *8.1.2. Our experience*

We find combination of PRP therapy with Er:YAG laser resurfacing to be effective therapeutic modality for the treatment of post acne atrophic scars. This combination also helps in decreasing the downtime and incidence of adverse effects like post-inflammatory hyperpigmentation which are conventionally associated with usage of Er:YAG lasers alone. So, higher fluences can be used for treatment with less chances of post-inflammatory hyperpigmentation (**Figure 4**).

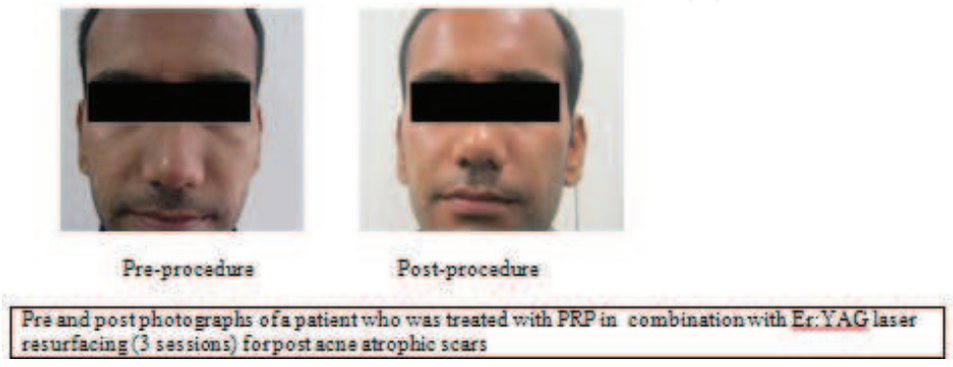


**Figure 4.** Pre- and post-photographs of a patient who was treated with PRP in combination with Er:YAG laser resurfacing (three sessions) for post-acne atrophic scars.

*8.1.3. Skin rejuvenation (vampire facelift)*

A study on skin rejuvenation with autologous platelet-rich plasma demonstrated good results with increase in the skin homogeneity and the patient satisfaction without serious side effects [29].

PRP enhances gene expression of matrix molecules, such as collagen and stimulates fibroblast proliferation ex vivo in the experimental models, thereby increasing total protein synthesis. PRP enhances elastin production by fibroblasts and stimulation of myofibroblast [29].

PRP and activated platelet poor plasma (aPPP) treatment increased the proliferation of human dermal fibroblasts, increased procollagen type I carboxy-terminal peptide production by human dermal fibroblasts, increased expression of type 1 collagen, alpha1 and type 1 collagen, alpha2, increased Matrix Metalloproteinase-1 (MMP-1) and MMP-3 proteins expression. MMPs digest various structural components of the ECM and are centrally involved in dermal remodeling. PRP can be topically applied or directly injected into the skin [7].

Skin remodeling can be enhanced by increasing the penetration and inducing mild inflammatory reactions through use of microneedles and lasers along with PRP [7].

PRP helps in remodeling of the skin.

As an adjuvant to lasers or microneedling, it is usually done once in every 4–6 months for 1 year and then yearly as maintenance therapy [30].

Fractional nonablative (Erbium glass) laser therapy combined with topical application of PRP resulted in objective improvement in elasticity of skin, a lower erythema index and an increased density of collagen. Histologically, an increase in length of dermo-epidermal junction, in amount of collagen and fibroblasts, was seen in the treated skin [31].

PRP can be combined with fractional ablative lasers (carbon dioxide) for treatment of deep wrinkles and severe photodamaged skin. The combination helps in reducing transient adverse effects like erythema and decreases the downtime, leading to rapid healing [32].

PRP injections once a month for 3 months, have shown good results for infraorbital rejuvenation, without any obvious adverse effects in a split face blinded trial [33].

*8.1.4. Our experience*

We found combination of PRP therapy with Er:YAG laser to be efficacious for facial and periorbital rejuvenation without much adverse effects. It is performed once every month for a total duration of 3 months. It promotes neocollagenogenesis and provides an environment for vascularization, leading to reduction in fine lines and improvement in texture and complexion as seen in **Figure 5**.

**8.2. Striae distensae**

For treatment of striae distensae, a combination of intradermal radiofrequency (RF) device with autologous PRP was synergistically effective, had fewer adverse effects and was well tolerated.

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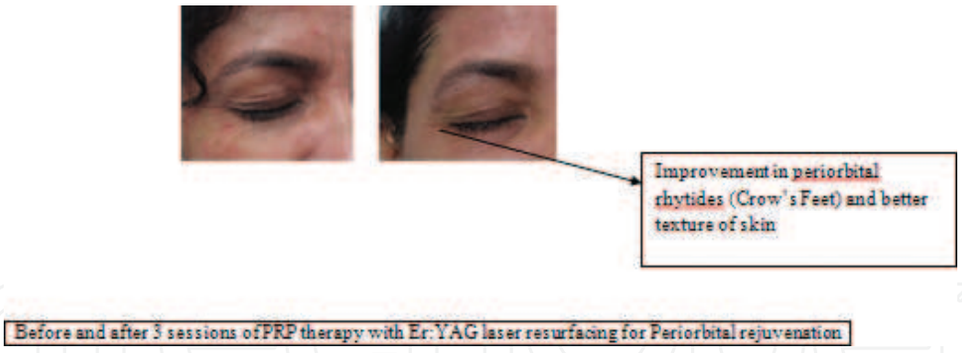


**Figure 5.** Before and after three sessions of PRP therapy with Er:YAG laser resurfacing for periorbital rejuvenation.

No significant side effects other than transient bruising were noted. Bipolar RF generated thermal energy, thereby denaturing the elastic fibers and collagen bundles, while PRP stimulated wound healing, leading to synergism and good cosmesis [34].

Combined enhanced penetration platelet-rich plasma and ultrasound following plasma fractional radiofrequency were also found to be useful in treatment of striae distensae in a study [35].

## 9. Conclusion

PRP therapy is one of the most upcoming forms of regenerative medicine with the potential to improve the homeostasis of the treated cells and tissues, provided that harvesting standards are maintained. Since it belongs to one's own body, safety is always ensured unlike various plant- and animal-derived stem cells or medications which may prove deleterious to human body. There is negligible risk of reacting to one's own cells and the procedure is minimally invasive with down time of or two days. It can give very promising results for facial rejuvenation, and nonsurgical face lifts minimizing and delaying the requirement of botulinum toxin and fillers. Results are, similarly, quite promising in burn and scar removal as monotherapy or in combination with lasers and micro-needling. PRP therapy also helps in hair strengthening and regrowth as monotherapy or in combination with hair transplant.

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