# Aesthetic Clinician's Guide to Platelet Rich Plasma

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*Editor*

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# Preface

Hair loss has been an interest of mine for years since I started my residency over a decade ago at the Cleveland Clinic and had the opportunity to work with Dr. Wilma Bergfeld. While medical therapies were effective at treating hair loss, I saw many frustrated patients who were looking for additional results without surgery. As one of the early adopters of platelet rich plasma in 2016, I quickly realized the utility and benefit of the procedure and saw how satisfied my patients were with the outcomes.

The field of regenerative medicine is rapidly progressing, especially the role of platelet rich plasma in skin rejuvenation and hair restoration. The literature around this topic is progressing at a rapid rate so I wanted readers to have a place to reference the latest material of PRP in the dermatologic realm.

In our current climate, patients are educated about their aesthetic options, it is of utmost importance to be up to date on these topics. I chose common conditions that are being treated with PRP and picked experts, both dermatologists and plastic surgeons, who have extensive experience with PRP. This book will not disappoint, and I am honored to present this to you.

I hope you enjoy this first edition of *An Aesthetic Clinician's Guide to Platelet Rich Plasma* as much as I enjoyed creating it. A big thank you to our contributing authors for their educational articles!

Cleveland, OH, USA — Shilpi Khetarpal

# Acknowledgment

I have to start by thanking my parents, Drs. Sanjiv and Alpana Khetarpal, for being my role models and for supporting and guiding me to medicine and pushing me to my potential.

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# History of PRP

**Deborah Paul and Mara Weinstein Velez**

While the development of regenerative medicine has been present for decades, the popularization of tissue regeneration has emerged as a hot topic during the latter half of the twentieth century. Platelet-rich plasma (PRP), defined as an autologous platelet-rich concentrate of blood plasma, was conceptualized in the 1970s in the field of hematology to treat thrombocytopenia (Alves and Grimalt 2018). Since then, it has been widely used in the fields of dermatology, orthopedics, maxillofacial surgery, cardiac surgery, pediatric surgery, urogynecology, plastic surgery, and ophthalmology (Acebes-Huerta et al. 2020; Wu et al. 2016). Extracutaneous uses include the treatment of endometrial repair, in vitro fertilization, osteoarthritis, tendon repair, jaw osteonecrosis, deep sternal wound infections, and many others (Bos-Mikich et al. 2018). Orthopedics and maxillofacial surgery were early fields using PRP and account for an overwhelming portion of existing research; however, multiple meta-analyses have shown disappointing or inconsistent results. Popular culture and the media are largely responsible for revitalizing interests in PRP in the twenty-first century through the treatment of sport-related injuries in the field of orthopedics. Today, PRP is a multimillion-dollar industry with a projected growth estimated between 380 million and 4.5 billion over the course of 5–10 years as of 2018 (Hausauer and Humphrey 2020a; Jones et al. 2018).

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## 1 PRP in Hematology

As early as 1600 BCE, Egyptian Papyri studied different methods for controlling hemorrhage with the use of pressure, tourniquets, and ligatures (Michael 2019; Saber 2010). Over the years, wound compressive material and anticoagulants were developed to obtain hemostasis. During World War I, transfusion emerged as a promising treatment for acute trauma-related blood loss (Michael 2019). By World War II, the process and distribution of blood products were further streamlined (Michael 2019). In 2012, the PROPPR (Pragmatic, Randomized Optimal Platelet, and Plasma Ratios) trial further confirmed the early use of the transfusion of whole blood products to establish hemostasis in critically ill patients (Cardenas et al. 2018). Closely examining the different components of whole blood, platelets emerged as a unique cell that plays a critical role to establishing local hemostasis and participating in the inflammatory response at sites of injury. From this, various formulations have developed for application to other fields for both hemostasis and wound healing.

## 2 PRP in Orthopedics

In the past, PRP was used in orthopedics intraoperatively during knee arthroplasty for both wound healing and establishing hemostasis to minimize postoperative blood loss. Today, the majority of the clinical application of PRP is in the treatment of muscle-related injuries, tendinopathies, and ligament injury (Foster et al. 2009; Lynch and Bashir 2016; Middleton et al. 2012). It is especially popular in sport-related injuries to decrease recovery time. There are numerous randomized control trials showing a clinically significant improvement in recovery time in patients treated with PRP compared to placebo for epicondylitis (Wu et al. 2016). Similar findings have been seen in the treatment of Achilles tendinopathy, where increased local revascularizations from platelet granule growth factors have been attributed with the clinical improvement. Unfortunately, when examined closely, these studies are often limited, in that they are underpowered due to their small sample size (Jones et al. 2018). Despite this, PRP continues to be used in orthopedics given its favorable safety profile (Jones et al. 2018).

## 3 PRP in Maxillofacial Surgery

Nearly a decade after the development and application of PRP in hematology, PRP was applied to maxillofacial surgery (Alves and Grimalt 2018). There has been a unique focus on the use of leukocyte rich and fibrin matrix in maxillofacial surgery for increased immune function, antimicrobial function, and the slow release

PRP-related growth factors. Leukocyte-rich PRP is also preferred for the low costs and simple preparation. The use of the fibrin matrix has been shown to extend the release of growth factors well after 1 week compared to traditional PRP (Emer 2019). It has been used to promote wound healing in extraction sockets, to reduce alveolar ridge resorption, and to promote postoperative bone growth (Chou et al. 2020). PRP has also been shown to improve overall bone density and maturation leading to increased bone development (Wu et al. 2016).

## 4 Dermatologic Applications

In dermatology, PRP's primary application is in wound healing of chronic ulcers (commonly diabetic, leprosy ulcers and associated neuropathy, pressure and venous ulcers), skin rejuvenation, fat grafting, alopecia, acne, and scar repair (Emer 2019; Ayatollahi et al. 2017; Hausauer and Humphrey 2020b; Hesseler and Shyam 2019; Huang and Huang 2020). The available literature on the use of PRP for these diseases are rapidly emerging, primarily through anecdotal cases and more robust case series over the past decade (Hausauer and Humphrey 2020b). One of the earliest and most widespread use of PRP in dermatology outside of wound healing is in the treatment of alopecia. Although few randomized trials exist, the strongest evidence for its use is in the treatment of androgenetic alopecia (Chen et al. 2018; Dervishi et al. 2020; Giordano et al. 2017; Kramer and Keaney 2018; Mao et al. 2019; Picard et al. 2017). Although encouraging, confirmation studies are still needed through larger randomized control trials and high-powered studies. Standardization of PRP formulations used across studies continues to be a limiting factor to comparing studies and replicating results in clinical practice. Existing protocols often differ in the amount centrifugation cycles and plasma proteins, altering the final concentrate of platelets (Motosko et al. 2018).

## 5 PRP Subtypes and Definitions

Previously accepted terminology for PRP included platelet concentrate, platelet gel, or fibrin glue. These terms were discontinued since they erroneously implied that the collected product was solid, a gel formulation or always inclusive of fibrin, respectively (Foster et al. 2009). Today, PRP is the universally accepted term for this "platelet rich product in plasma" to be more inclusive of the different formulations used in clinical practice. Various subclassifications exist through different formulations based on additional products found in the final collection.

Clinical formulations of PRP used across disciplines vary in their concentration of platelets and other cell types, such as leukocytes, erythrocytes, and fibrin. While the consensus on optimal protocols and classifications remain debatable, four classifications of PRP are commonly used when defined in the literature:

leukocyte-poor platelet plasma (P-PRP) also known as pure PRP, leukocyte-rich platelet plasma (L-PRP), leukocyte-poor platelet-rich plasma with fibrin matrix (P-PRF), or leukocyte-rich platelet-rich plasma with fibrin (L-PRF) (Bos-Mikich et al. 2018; Dohan Ehrenfest et al. 2009). Other proposed classifications incorporate activation status with platelet and leukocyte concentration or the DEPA (dose, efficiency, purity, and activation) classification (Hausauer and Humphrey 2020a). In dermatology, the leukocyte-poor formulation, referred to as pure PRP is commonly used with the belief that less leukocytes minimize the risks of unwanted tissue reactivity (Wu et al. 2016). In contrast, it is not uncommon for the leukocyte-rich formulation to be preferred among orthopedic physicians for a possible anti-microbial effect (Middleton et al. 2012). Fibrin is preferred in autologous fat grafting for a delayed release of growth factors. PRP relies on the timely release of these growth factors through the degranulation of platelet alpha granules (Hausauer and Humphrey 2020a). This is controlled with various thrombolytics and activators.

## 6 Platelet Biology and Growth Factors

Platelets are unnucleated cells derived from bone marrow megakaryocytes (Merchan et al. 2019). When tissue injury occurs, platelets are the first to arrive as mediators of tissue repair due to their role in hemostasis, cytokine activation, and growth factor release (Wu et al. 2016). In PRP, a patient's whole blood is concentrated to produce a platelet concentration 2–5 times that of normal whole blood, often in numbers approaching 1,000,000 μl (normal range: 150,000/μl–350,000/μl) (Foster et al. 2009). Numbers higher than 2–5 times above baseline have not been shown to have increased efficacy (Foster et al. 2009). Through the centrifugation process, a platelet-rich plasma concentrate is generated with various growth factors, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factors (IGF1, IGF2), GM-CSF: Granulocyte macrophage colony stimulating factor and keratinocyte growth factor (KGF) (Bos-Mikich et al. 2018; Chicharro-Alcantara et al. 2018).

These growth factors, along with cytokines (interleukins), interact to promote tissue angiogenesis, collagen synthesis, cellular differentiation, hemostasis and establish vascular support through the various stages of wound healing (inflammation, proliferation, epithelization, and remodeling) (Foster et al. 2009; Chicharro-Alcantara et al. 2018). There are also bioactive molecules released by the alpha granules during degranulation, such as serotonin, histamine, dopamine, calcium, and adenosine (Foster et al. 2009). Histamine and dopamine, in particular, play an integral role in increasing vascular permeability to allow the movement of inflammatory cells and macrophages to the site of tissue damage (Foster et al. 2009). Serotonin also contributes to this permeability in addition to local vasoconstriction. Calcium is a cofactor in platelet aggregation (Foster et al. 2009).

## 7 PRP Collection

Collection of PRP is completed the day of the procedure. A sample of 10–60 cc of whole blood is collected and combined with an anticoagulant to prevent premature coagulation (Hesseler and Shyam 2019). A centrifuge is used to separate whole blood into red blood cells (bottom layer), platelet-poor layer (top layer), a buffy coat (if using a double spin system), and lastly, a platelet-rich layer (Hesseler and Shyam 2020). This summarizes the most often initial step, additional cycles may be performed with varying protocols to yield the varying PRP subtypes discussed previously. The collected concentrate is then activated for use. Bovine thrombin and calcium chloride are common activators (Hausauer and Humphrey 2020a). Bovine thrombin however comes with a small risk of patients developing antibodies to bovine thrombin leading to a systemic immune coagulopathy (Foster et al. 2009). In cutaneous tissue, type I collagen (found in scar tissue, dermis, tendons, ligaments, and bone) is a natural activator that is as effective as bovine thrombin in activating the degranulation of alpha granules (Foster et al. 2009).

## 8 Conclusion

PRP was introduced in the 1970s in the field of hematology. Since then, it has been used across specialties with emerging interest in dermatology. It is an exciting and well-tolerated safe adjunctive treatment when traditional treatments fail. In dermatology, it is most promising in the treatment of chronic wounds with emerging data supporting its use in alopecia (androgenetic alopecia in particular) and skin rejuvenation. Future direction for PRP in dermatology is the application of its use in the treatment recalcitrant diseases, such as melasma, striae, and peri-ocular dark circles (Hausauer and Humphrey 2020b; Merchan et al. 2019). Interest is also developing in its use as an adjunctive topical to current treatment options. In acne scars, it is already being used with microneedling (Hashim et al. 2017; Peng 2019). Emerging research is promising and focuses on expanding the clinical application of PRP and standardizing treatment protocols.

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# Basic Science of PRP

**Abigail E. Meyers, Alison Jin, Grzegorz J. Kwiecien, and James E. Zins**

## 1 Introduction

It has been estimated that plastic surgeons and dermatologists performed more than 735,000 surgical hair restoration procedures for over 1.4 million non-surgical patients in 2019 alone (Keene et al. 2020). Platelet-rich plasma (PRP) has been shown to increase both hair count and density for androgenic alopecia (AGA) and alopecia areata (AA) in a number of recent studies (Semsarzadeh and Khetarpal 2020; Alves and Grimalt 2016; Evans et al. 2020; Bruce et al. 2020; Shapiro et al. 2020; Khaled Yaseer et al. 2020; Gentile et al. 2015, 2020; Gentile and Garcovich 2020; Cervelli et al. 2014; Dicle et al. 2020; Dubin et al. 2020; Torabi et al. 2020; Uebel et al. 2006).

In combination with PRP treatments, it has been shown that there is an average of 15.1% increased follicular unit density (FUD) (Uebel et al. 2006). Males and females have expressed positive results by patient-reported outcomes.

However, inconsistencies in preparation and administration protocols among published studies make the data difficult to objectively analyze. Many evaluations aimed at gauging efficacy are neither standardized nor validated, making comparisons among studies difficult to analyze. This includes non-standardized before and after photographs, non-validated patient satisfaction surveys, and other non-objective means of evaluation (Bruce et al. 2020; Dubin et al. 2020; Qu et al. 2019; Khatu et al. 2014). However, some studies using objective outcomes have demonstrated promising results after PRP, including hair-pull tests, hair diameter, mean hair count and density, and anagen-to-telogen growth cycle ratio (Alves and Grimalt 2016; Gentile et al. 2015; Cervelli et al. 2014; Khatu et al. 2014; Schiavone et al.

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2014; Singhal et al. 2015; Gkini et al. 2014; Takikawa et al. 2011; Puig et al. 2016; Ayatollahi et al. 2017; Garg 2016).

PRP is promoted for its autologous nature as a therapeutic treatment with few complications or side effects. It has been deemed safe as a monotherapy or adjuvant to light therapy, finasteride, and minoxidil (the only FDA-approved drugs), for treatment of androgenic alopecia in men and women (Bruce et al. 2020; Puig et al. 2016; Ayatollahi et al. 2017; Mapar et al. 2016; Rogers and Avram 2008; Avram and Finney 2019). PRP has also been demonstrated to enhance graft take following hair transplantation, citing optimal graft maintenance and thickening of existing hair follicles (Garg 2016; Rogers and Avram 2008; Avram and Finney 2019; Avram et al. 2017). Potential drawbacks are few and mainly stem from gaps in the literature. Hair regrowth may not be permanent, but data on long-term outcomes are insufficient to delineate exactly how long results are expected to last (Gkini et al. 2014; Shin et al. 2012).

Techniques generally involve harvesting, centrifugation, a potential platelet activation step, and injection into the affected area of the scalp. Numerous classification systems regarding the contents of PRP have been proposed, without an established consensus that facilitates comparison among the literature (Frautschi et al. 2017; Motosko et al. 2018; Stevens and Khetarpal 2019; Magalon et al. 2016; Dohan Ehrenfest et al. 2009; DeLong et al. 2012). PRP remains a term that encompasses the four main categories of pure PRP (P-PRP), leukocyte and PRP (L-PRP), pure platelet-rich fibrin (P-PRF), and leukocyte and platelet-rich fibrin (L-PRF) (Frautschi et al. 2017; Stevens and Khetarpal 2019; Dohan Ehrenfest et al. 2009). Preparations vary by contents, concentration and centrifugation method. For example, providers can use either a commercial kit or a manual method with a laboratory centrifuge (Stevens and Khetarpal 2019). Normal platelet concentration in the blood ranges from 150,000 to 450,000/μL (Frautschi et al. 2017). A baseline of at least one million platelets/μL has been deemed necessary, while 1.5 million platelets/μL have been demonstrated as optimal. Increasing concentrations beyond 1.5 million platelets/μL have been shown to diminish potential hair gains (Marx 2001; Giusti et al. 2009).

## 2 Mechanism of Action

The secretory alpha-granules within platelets are known to release a multitude of growth factors that propagate signals among stem cells in the follicular bulge, upregulate downstream signals associated with cellular proliferation, increase development of new follicles, and ultimately activate the anagen phase of hair growth (Semsarzadeh and Khetarpal 2020; Cervelli et al. 2014; Garg 2016; Frautschi et al. 2017; Alves and Grimalt 2018; Li et al. 2012a; Blair and Flaumenhaft 2009). Activated platelets release platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), connective tissue growth

factor (CTGF), and insulin-like growth factor-1 (IGF-1) (Alves and Grimalt 2016; Uebel et al. 2006; Frautschi et al. 2017; Stevens and Khetarpal 2019; Dohan Ehrenfest et al. 2009; Dhurat and Sukesh 2014; Fabi and Sundaram 2014; Kim et al. 2011; Li et al. 2012b). The growth factors activate downstream pathways that promote cell differentiation, chemotaxis, fibroblast proliferation, stimulate angiogenesis, regulate collagen synthesis, and inhibition of apoptosis. These functions are imperative to promote hair growth (Uebel et al. 2006; Kim et al. 2011; Gupta and Carviel 2016). Ninety-five percent of pre-synthesized growth factor secretion occurs within 1 h of injection and release has been shown to continue for 7 days (Marx 2001; Senzel et al. 2009).

The mechanisms by which PRP affects the hair cycle have been described by several authors, including in vivo and in vitro study on dermal papilla (DP) cells (Li et al. 2012a; Gupta and Carviel 2016). PRP provokes increased DP cell expression of FGF-7 (fibroblast growth factor 7) and β-catenin (Wnt), potent hair growth stimulators, and upregulation of BCL-2 (B-cell lymphoma-2), which inhibits apoptosis (Li et al. 2012a; Gupta and Carviel 2016). Activated PRP and subsequent growth factor release also initiates the ERK (extracellular signal-related kinase, also known as MAP kinase) and PKB (protein kinase B, also known as Akt) pathways, which promote transcription of genes involved in proliferation and cell survival (Cervelli et al. 2014; Alves and Grimalt 2018; Li et al. 2012a; Gupta and Carviel 2016). Angiogenesis, via cellular pathways downstream of VEGF and FGF, allows for increased follicular vascularization and is essential for active hair growth during the anagen phase (Garg 2016; Dohan Ehrenfest et al. 2009; Dhurat and Sukesh 2014; Mecklenburg et al. 2000). PRP ultimately increases blood flow, oxygenation, and activation of anti-apoptotic pathways, which allows for a prolonged anagen growth phase, as well as a faster transition from the telogen to anagen phase (Alves and Grimalt 2016; Gentile et al. 2015; Garg 2016; Stevens and Khetarpal 2019; Li et al. 2012a).

## 3 Classification Systems

PRP was first defined as a suspension of platelets in plasma with a higher than baseline blood concentration of platelets in oral surgery applications by Marx et al. in 2001 (Marx 2001). The four main categories of PRP (P-PRP, L-PRP, P-PRF, and L-PRF) were later introduced by Ehrenfest et al. in 2009. In an effort to make results comparable and replicable among studies, a number of classification systems have since been proposed. Magalon et al. introduced *DEPA* classification, which is based on *D*ose of injected platelets, *E*fficiency of production, *P*urity of PRP obtained, and the *A*ctivation process (Magalon et al. 2016). The orthopedic *PAW* classification system by DeLong et al. accounts for the absolute number of *P*latelets, manner of platelet *A*ctivation, and the presence or absence of *W*hite cells in the preparation (DeLong et al. 2012). These systems rely on complete platelet counts in both the whole blood and PRP end product.

To account for additional variables that determine the efficacy of PRP, Frautschi et al. expanded upon *PAW* to a more comprehensive *FIT PAAW* classification system. It considers the *F*orce of centrifugation, *I*teration or sequence of centrifugation, *T*ime of centrifugation, *P*latelet concentration (baseline of patient's whole blood and final PRP product), *A*nticoagulant use, utilization of an *A*ctivator including type and amount, and the composition of *W*hite blood cells (Frautschi et al. 2017). Accounting for anticoagulant use is critical, as it could impact platelet yield and function via alterations in pH. Anticoagulant use is unique to this classification system (Araki et al. 2012; Fukaya and Ito 2014; Wahlström et al. 2007). Variations in centrifugation and activation methods can yield divergent results regarding platelet concentration and viability, with some protocols claiming optimized outcomes based upon these variables (Frautschi et al. 2017; Dugrillon et al. 2002; Amable et al. 2013; Kahn et al. 1976; Slichter and Harker 1976; Landesberg et al. 2000; Jo et al. 2013; Bausset et al. 2012; Tamimi et al. 2007; Mazzocca et al. 2012; Anitua et al. 2008; Kececi et al. 2014; Perez et al. 2014; Su et al. 2008; Fernández-Barbero et al. 2006). All these factors are critical for determining the eventual concentration of platelet-derived bioactive molecules and will influence the clinical efficacy of the ultimate PRP formulation (Whitman et al. 1997).

## 4 Applications

Androgenic alopecia (AGA) affects 45% of men and 35% of women over the age of 60 (Stevens and Khetarpal 2019). Current studies support the use of PRP in treatment of male and female pattern hair loss (Tabolli et al. 2013; Bruce et al. 2020; Gentile et al. 2015, 2020; Gentile and Garcovich 2020; Cervelli et al. 2014; Qu et al. 2019; Schiavone et al. 2014; Gkini et al. 2014; Puig et al. 2016; Rogers and Avram 2008; Avram and Finney 2019). Applications in alopecia areata, general hair loss, and as an adjuvant for pharmaceuticals or hair transplant have shown positive results (Uebel et al. 2006; Garg 2016; Avram et al. 2017; Kim et al. 2011; Khademi et al. 2019; Elghblawi 2018; Saxena et al. 2016; Alves and Grimalt 2020). Benefits emanate from the inherent autologous nature of PRP, making it a safe and well-tolerated treatment option. It is minimally invasive, particularly compared to follicular unit extraction (FUE) and follicular unit transfer (FUT) hair transplant procedures. There are few side effects, and no serious complications have been reported. Some patients experience mild inflammation, erythema, and skin tightness, though this is expected and dissipates quickly (Tian et al. 2019). Once the initial course is completed, annual injections are recommended to maintain the result (Motosko et al. 2018).

## 5 Preparation and Technique

The authors' technique for preparation and application of PRP involves a two-step centrifugation process in order to separate plasma from red blood cells and leukocytes and to further separate PRP from platelet-poor plasma (PPP) ("Platinum

Series Centrifuge," EmCyte Corporation, Fort Myers, FL). To avoid platelet activation during centrifugation, low-gravity is used. Delivery of PRP within 10 min is advised because 95% of the pre-synthesized growth factors are released within the first hour (Marx 2001). The authors do not use an external activating agent, as this step is arguably unnecessary and platelets may become activated endogenously upon interaction with dermal collagen, thrombin, thromboxane A2 ($TXA_2$), adenosine diphosphate (ADP), or platelet-activating factor (PAF) after injection (Frautschi et al. 2017; Cavallo et al. 2016). Use of anticoagulants is also optional. Superiority of one anticoagulant over another has not been demonstrated, though most recent studies report use of citrate as sodium citrate or acid citrate dextrose (Frautschi et al. 2017). Ten cc of sodium citrate is drawn up in a 60 cc syringe. Fifty cc of whole blood is then drawn from the patient, filling the syringe to 60 cc. Sessions are repeated monthly for the first 3 months, then continued quarterly for 1 year. A single treatment is administered once per year thereafter to prevent recurrent hair loss (Gentile et al. 2015; Motosko et al. 2018; Ferrando et al. 2017).

## 6 Validated Outcomes

Hairdex is a disease-specific validated scale used to assess quality of life (QoL) for patients with AGA or AA (Fischer et al. 2001). It consists of 48 questions, which represent five domains: symptoms, functioning, emotions, stigmatization, and self-confidence. Each domain and total average score is represented on a scale from 0 to 100 with lower scores indicating higher QoL.

A total of 533 patients in 2018 and 372 patients in 2019 have been treated with PRP by the Dermatology and Plastic Surgery Institute at Cleveland Clinic. In 2019, the authors started to prospectively evaluate outcomes of PRP injections with Hairdex questionnaire. Forty-one patients completed pre-PRP treatment questionnaire, 50 completed post-PRP treatment questionnaire, and 17 completed both. Mean age was 48 years and 55% were males. Thirteen percent of patients had one injection, 10% had two injections, 15% had three injections, and 62% had more than 3 injections. PRP was utilized in combination with hair transplantation as well as other non-surgical methods including pharmaceuticals (e.g., finasteride, minoxidil, spironolactone) and red-light therapies. PRP in combination with hair transplantation is also practiced (Table 1). In combination with PRP treatments, it has been shown that there is an average of 15.1% increased follicular unit density (FUD) (Marx 2001). Males and females have expressed positive results by patient-reported outcomes.

Hairdex is a disease-specific scale used to assess quality of life (QoL) for patients with AGA or AA (Fischer et al. 2001). The authors' preliminary data showed that hair loss most severely affect emotional life and self-confidence as indicated by the highest scores in these domains in both women and men (Table 1). Hair loss tends to affect emotional life of women more severely than men (score of 39.8 vs. 29.8, respectively; $p = 0.083$) (Table 1). Differences in QoL can be also observed between various age groups. Younger patients (less than 50 years sold) tend to have more

**Table 1** Demographics and quality of life of patients before initiating treatment with PRP. Considerable differences can be observed between male and female patients. Female patients were older and were more likely to have a prior history of smoking. They also had a trend toward worse emotional well-being related to hair loss

| | Male ($N = 42$) | Female ($N = 34$) | $p$-value |
|---|---|---|---|
| *Demographics* | | | |
| Age at first injection (years) | 42 ± 17 | 56 ± 15 | <0.001 |
| Smoking status | | | 0.046 |
| Current | 3% | 7% | |
| Former | 8% | 29% | |
| Never | 89% | 64% | |
| Hamilton class | | | n/a |
| I | 7% | n/a | |
| II | 31% | n/a | |
| III | 21% | n/a | |
| IV | 14% | n/a | |
| V | 21% | n/a | |
| VI | 3% | n/a | |
| VII | 3% | n/a | |
| Norwood class | | | n/a |
| I | n/a | 4% | |
| II | n/a | 65% | |
| III | n/a | 31% | |
| Prior treatment | | | |
| Minoxidil | 69% | 54% | 0.201 |
| Finasteride | 21% | 6% | 0.086 |
| Spironolactone | | | |
| Infrared lights | | | |
| Dietary supplements | 18% | 53% | 0.869 |
| Hair transplant | 3% | 0% | 0.371 |
| Prior treatment concurrent with PRP | 73% | 82% | 0.421 |
| *Preoperative quality of Life ([a]Hairdex)* | | | |
| Symptoms | 6.6 ± 2.0 | 6.5 ± 2.1 | 0.940 |
| Functioning | 23.6 ± 7.5 | 21.7 ± 8.9 | 0.423 |
| Emotions | 9.5 ± 1.6 | 9.1 ± 1.9 | 0.719 |
| Stigmatization | 7.9 ± 1.4 | 7.7 ± 1.8 | 0.782 |
| Self-confidence | 45.6 ± 16.5 | 58.3 ± 10.7 | 0.162 |
| Total | 37.0 ± 8.1 | 2.8 ± 0.5 | 0.365 |

All continuous variables presented as mean ± standard deviation. All categorical variables presented as percentage

[a]Each domain of the Hairdex questionnaire was scored on a scale 0–100 with higher score indicating worse quality of life (Here reference to Fischer et al. (2001) paper)

**Table 2** Quality of life of younger (less than 50 years old) and older patients (equal or above 50 years old) before initiating treatment with PRP. Younger patients were more likely to have more severe symptoms and worse self-confidence as compared to older patients

| | <50 years old ($N = 37$) | >= 50 years old ($N = 39$) | $p$-value |
|---|---|---|---|
| *Preoperative quality of life ([a]Hairdex)* | | | |
| Symptoms | 14.7 ± 15.1 | 8.3 ± 9.7 | 0.062 |
| Functioning | 22.3 ± 20.4 | 19.2 ± 18.9 | 0.557 |
| Emotions | 35.3 ± 22.2 | 33.9 ± 21.3 | 0.816 |
| Stigmatization | 19.6 ± 17.2 | 13.5 ± 14.1 | 0.155 |
| Self-confidence | 26.5 ± 18.8 | 19.7 ± 12.0 | 0.113 |
| Total | 26.5 ± 17.8 | 22.3 ± 15.1 | 0.341 |

All variables presented as mean ± standard deviation

[a]Each domain of the Hairdex questionnaire was scored on a scale 0–100 with higher score indicating worse quality of life (Here reference to Fischer et al. (2001) paper)

severe symptoms (score of 14.7 vs. 8.3, respectively; $p = 0.062$) and worse self-confidence (score of 26.5 vs. 19.7, respectively; $p = 0.113$) when compared to older patients (equal or more than 50 years old) (Table 2).

Preliminary data from 17 patients who completed questionnaires both before and after PRP treatments demonstrated a significant decrease in Hairdex score indicating improvement in overall quality of life following PRP injection (score of 25.0 vs. 19.3, respectively; $p = 0.02$). All Hairdex domains improved with treatment (Fig. 1). The domains that showed most pronounced improvement were emotions (score of 37.0 vs. 27.9, respectively; $p < 0.01$) and self-confidence (score of 21.2 vs. 17.0, respectively; $p = 0.05$) (Fig. 1). These results suggest that PRP is an effective treatment for both AGA and AA hair loss (Figs. 2, 3, 4). The recommended protocol below is based on the current literature and the authors' experience with positive results.

## 7 Getting Started with PRP

Necessary supplies to get started include a blood draw kit, test tube, centrifuge, syringe, rigid 90-mm needle, vacutainer transfer device, and optional chiller. Alternatively, you may elect to use commercially available ready-to-use disposable PRP kits, which range from $175 to $1150 per kit (Kececi et al. 2014; Akhundov et al. 2012). While there is ongoing debate regarding the optimal preparation of PRP for treatment of hair loss, the following are common steps shared by manual protocols in studies reporting promising outcomes:

Procedure (Fig. 5):

1. Take "before" photos highlighting affected areas of hair loss.
2. Collect blood with an anticoagulant (e.g. citrate, ethylenediaminetetraacetic acid (EDTA), trisodium phosphate, heparin, or anticoagulant dextrose solution

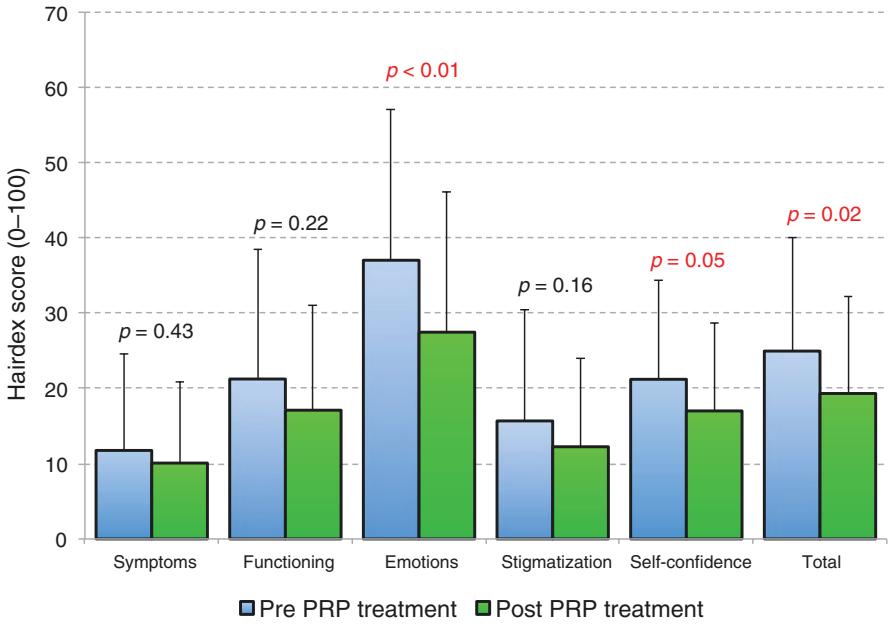


**Fig. 1** Comparison of quality of life of 17 patients who completed both pre- and post-treatment Hairdex questionnaire. A total Hairdex score significantly decreased indicating improvement in overall quality of life. All domains improved with treatment. The domains that showed most pronounced improvement were emotions and self-confidence

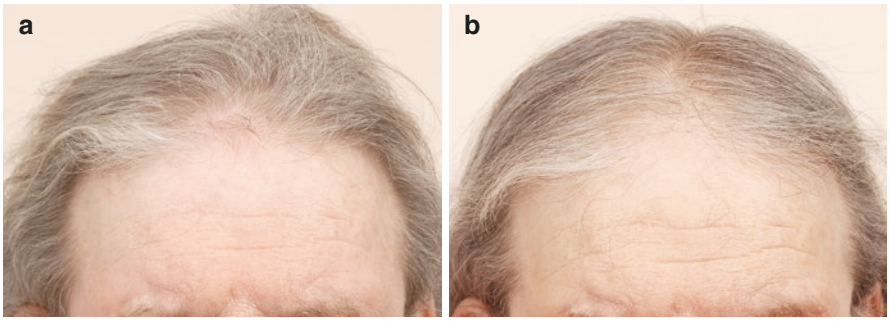


**Fig. 2** A 70-year-old female with androgenic alopecia before and 1 month after their first PRP treatment

A (ACD-A), which is commonly used in commercially available kits) (Cervelli et al. 2014; Frautschi et al. 2017; Araki et al. 2012; Fukaya and Ito 2014).

3. Centrifuge whole blood for 2 min (PurePRP SP Spin 1 on EmCyte centrifuge) to separate into two major layers: plasma and red blood cells (RBCs) top to bottom.
4. Using 60 cc syringe, aspirate the platelet plasma suspension (PPS) until RBC fills the aspirating pipe.

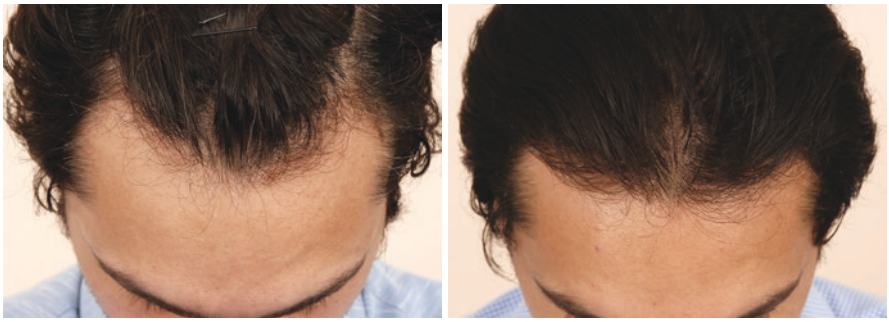

**Fig. 3** A 32-year-old male with androgenic alopecia before and 12 months after four PRP treatments

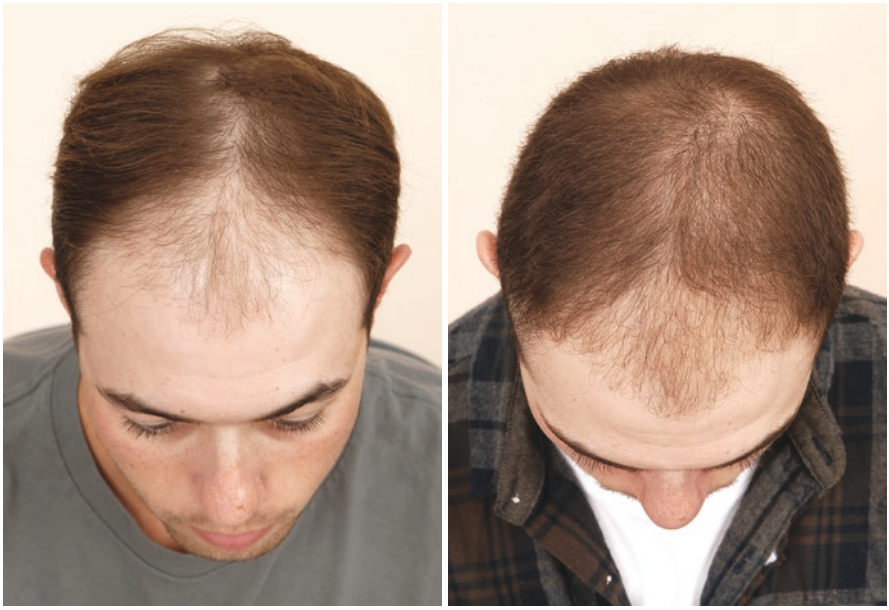

**Fig. 4** A 24-year-old male with androgenic alopecia before and 7 months after four PRP treatments

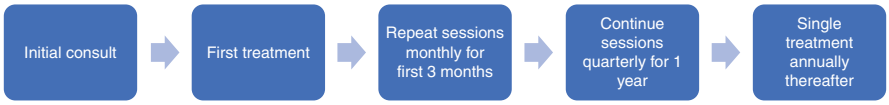


**Fig. 5** PRP protocol timeline

5. 5. Transfer PPS into the concentrating accessory for 6 min second spin (PurePRP SP Spin 2 on EmCyte centrifuge).
6. Platelet concentrate buffy coat separates out at the bottom of the concentrating accessory. Aspirate platelet-poor plasma (PPP) from the concentrating accessory and leave 7 cc of plasma.
7. Swirl to resuspend the platelet buffy coat into the plasma.
8. Tilt to immerse the Aspirating Pipe into the PurePRP for 2.5–5 cc three times.
9. Extract the pure PRP into the 3 cc syringe.
10. *Optional* exogenous activation: addition of platelet activation agent (e.g., calcium gluconate, calcium chloride, thrombin) at a ratio of 0.1 cc activator per 0.9 cc of plasma (Gentile et al. 2015; Cervelli et al. 2014; Khatu et al. 2014; Singhal et al. 2015; Gkini et al. 2014; Stevens and Khetarpal 2019; Magalon et al. 2016; Cervelli et al. 2009). We do not endorse an activation step. Activation prior to injection has not been clearly shown to improve growth factor release or efficacy, as platelets will ultimately become activated endogenously (Frautschi et al. 2017; Cavallo et al. 2016).
11. Injection of PRP using a hypodermic needle in a subcutaneous plane using the nappage technique of approximately 1 cm intervals between injection sites in the areas of hair thinning. A total of 7.5–10 cc is typically injected into the scalp in our experience.
12. Post-procedure photographs taken after each treatment interval.
13. Repeat sessions monthly for the first 3 months, then quarterly for 1 year.
14. Annual treatments are continued to maintain the result.

## 8 Discussion

Platelet-rich plasma is an increasingly popular procedure, with almost 1000 procedures performed at Cleveland Clinic in 2018 and 2019. It has been proven as a safe treatment to slow hair loss, increase the density of hair follicles with minimal side effects (Avram and Finney 2019). From the authors' experience, multimodal therapy using PRP in combination with pharmaceuticals (i.e., finasteride, minoxidil, or spironolactone). Variables that need to be considered regarding preparation and protocol include potential commercial kit use, or manual preparation with one versus two step centrifugation, variable centrifugation forces, number and length of cycles, use of activators (i.e., thrombin or calcium chloride) versus endogenous activation upon exposure to dermal collagen and thrombin, and ultimate composition of injected PRP. Whether it is beneficial to include leukocytes and red blood cells in the ultimate PRP injection composition remains controversial. It is also unclear if neutrophils and red blood cells improve collagen synthesis or promote undesirable levels of reactive oxygen species and inflammation (Stevens and Khetarpal 2019; Magalon et al. 2016; Dohan Ehrenfest et al. 2012; Zhou et al. 2015; Giusti et al. 2018). While clinically significant benefits for hair growth have been demonstrated by a number of studies using a variety of treatment protocol variations and time

intervals between injections, no one protocol has been shown to be clearly superior to another. The use of standardized treatment protocols and validated patient-reported outcomes would help clarify this issue.

Ideally, in addition to standardized protocols, patient-reported outcomes should be coupled with objective measures of the results of treatment including hair diameter measurements, hair-pull traction tests, and mean hair count and density evaluation (Semsarzadeh and Khetarpal 2020; Frautschi et al. 2017; Motosko et al. 2018; Badran and Sand 2018). The study quality in the current literature is varied. Many reports lack very basic information such as initial patient platelet count, and ultimate PRP count at time of treatment. Without such basic information, little can be gleaned from such studies (Frautschi et al. 2017).

In addition, it is difficult to distinguish which individual therapy is most efficacious for each patient. There is a paucity of studies with follow-up greater than 1 year, so long-term efficacy of treatment is unclear (Gkini et al. 2014; Shin et al. 2012). Finally, although promising, much remains unknown regarding the value of PRP for treatment of hair loss, in particular, and aesthetic applications, in general. Future studies should emphasize objective and standardized treatment measures combined with validated patient-reported outcomes (Motosko et al. 2018). This would allow rigorous analysis of utility of PRP in the treatment of this very common health problem.

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# Preparation Systems


**Steven Krueger and Omer Ibrahim**

## 1 Introduction

Initially developed for use in the field of orthopedic surgery, the first FDA-cleared platelet-rich plasma (PRP) collection device came to market in 1999. The first-generation devices that followed were cumbersome, required large-volume blood draws, and produced significant pain at the application site. As new applications for the use of PRP were discovered, the preparation systems evolved to become more user-friendly and yield a less inflammatory product.

As a relative newcomer to the field of aesthetic medicine, PRP has several applications including hair restoration, skin rejuvenation, and scar revision, among others. One of the most widely debated topics within the field of PRP revolves around the optimal PRP preparation strategy. Because of the significant variation between the existing preparation systems, clinical trials often report different degrees of response. It is difficult to directly assess and compare research studies given the heterogeneity among the preparation systems and the lack of standardization of this therapy. This chapter highlights multiple factors to consider when evaluating a PRP preparation system.

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## 2 Overview

The basic procedure for preparing PRP involves drawing blood from the patient, isolating platelets via centrifugation, an optional step of activating the platelets, and administering PRP into the target tissue (Kramer and Keaney 2018). Each of these steps in the preparatory protocol introduces an additional source of variation that affects the composition and efficacy of the final product. The initial volume of whole blood collected, method of anticoagulation, centrifugation parameters, method of platelet activation, and administration technique can each impact treatment outcomes for a given PRP preparation system.

Multiple systematic reviews have concluded that most studies inconsistently report on the variables discussed in this chapter (Kramer and Keaney 2018; Frautschi et al. 2017). For example, in a review of the literature regarding the use of PRP in aesthetic surgery, the authors found that baseline platelet concentration of patients' whole blood was not documented in any of the examined studies, and final platelet concentration of the PRP was only documented in 18% of studies (Frautschi et al. 2017). In a systematic review of clinical trials using PRP for hair restoration, the authors found a wide variation in preparation protocols and final product composition, as well as a lack of consistent reporting of these factors (Kramer and Keaney 2018). Only 21% of studies provided comprehensive reporting of all PRP preparation factors chosen by the authors, including whole blood volume, the specific anticoagulant used, the processing machine used, number of spins, spin rate, spine time, and platelet activation method (Kramer and Keaney 2018). A systematic review of the clinical orthopedic literature demonstrated a similar degree of variation and inconsistency of the reporting of PRP preparation protocols and composition (Chahla et al. 2017).

These reviews advocate that future studies include a precise documentation of a number of factors to allow proper characterization of a dose–response relationship and to establish clinical guidelines for the proper preparation of PRP. It is essential for aesthetic clinicians to maintain a keen understanding of the components of a PRP preparation system in order to critically evaluate the scientific literature on this topic and to offer the most effective treatment regimen to patients.

## 3 FDA Regulation

Given the rising popularity of PRP, aesthetic clinicians should understand the role of the US Food and Drug Administration (FDA) in regulating its use. According to the FDA, products being sold for the purpose of collecting PRP for point-of-care autologous applications are considered Class II medical devices. As an autologous human blood product, PRP is regulated by the FDA's Center for Biologics Evaluation and Research (CBER) division (Beitzel et al. 2015). The CBER is responsible for regulating human cells, tissues, and cellular and tissue-based products, and the process

for regulating these products is described in 21 CFR 1271 of the FDA's Code of Regulations (Electronic Code of Federal Regulations (eCFR) 2020). Prior to selling a PRP preparation system in the United States, companies must obtain a 510(k) clearance from the CBER. The 510(k) application allows medical devices that are "substantially equivalent" to a currently marketed device to come to the market. New PRP collection devices need only demonstrate that platelets can be isolated at a greater concentration compared to whole blood (Kramer and Keaney 2018).

Under these regulations, PRP falls into the category of "minimally manipulated tissue" and is therefore exempt from the FDA's traditional regulatory pathway, which includes animal studies and clinical trials. There is some concern about whether activated PRP, which could theoretically be considered "more than minimally manipulated", qualifies for the same exemption, but the FDA has made no attempt to regulate this practice to date. This lack of regulation has led to an explosion of PRP preparation systems on the market. Indeed, at the time of this writing, over 20 devices have received FDA clearance.

The numerous PRP systems currently on the market have FDA clearance for producing PRP intended to enhance bone grafts in orthopedic surgery. The use of PRP outside of this indication, such as for hair restoration or skin rejuvenation, is therefore considered "off-label." Clinicians may use PRP off-label for aesthetic indications, but per FDA guidelines, they "have the responsibility to be well informed about the product, to base its use on firm scientific rationale and on sound medical evidence, and to maintain records of the product's use and effects" (U.S. Food and Drug Administration 2020).

## 4 Classification Systems

A number of classification systems have been proposed in an attempt to systematize the reporting of the clinical efficacy of PRP, but they can also be used to compare the devices currently being marketed (Dohan Ehrenfest et al. 2009; Harrison 2018; Magalon et al. 2016). One such example is referred to as the DEPA classification (Fig. 1), which accounts for the *dose* of injected platelets (measured by concentration or the total number of platelets relative to whole blood), the *efficiency* of production (measured by the platelet yield, or the percentage of total platelets recovered), the *purity* of PRP (measured by the amount of leukocytes and erythrocytes found in the final product), and the *activation* of PRP (i.e., with an exogenous chemical activator such as calcium) (Magalon et al. 2016).

Another classification system uses the acronym FIT PAAW (Frautschi et al. 2017). The FIT PAAW classification (Fig. 2) is composed of seven key components: (1) the Force of centrifugation, (2) the Iteration or sequence of centrifugation, (3) the Time of centrifugation, (4) Platelet concentration (baseline of patient's whole blood and final PRP product), (5) Anticoagulant use, (6) the utilization of an Activator including the type and amount, and (7) the composition of White blood cells.

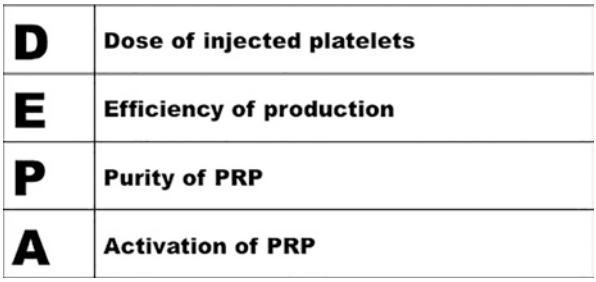


**Fig. 1** DEPA classification system. (Modified from Magalon et al. 2016)

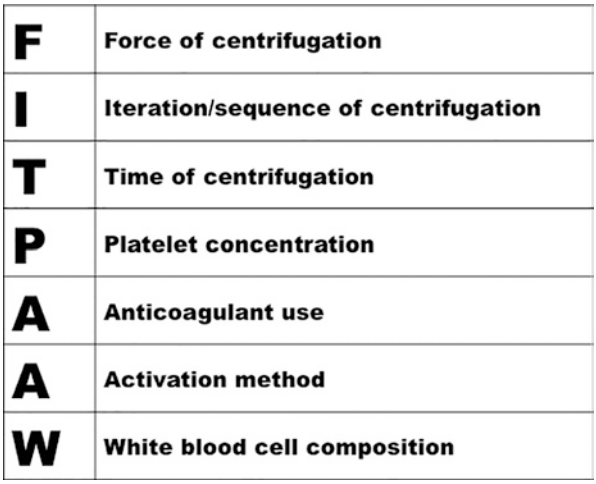


**Fig. 2** FIT PAAW classification system. (Modified from Frautschi et al. 2017)

These easy-to-remember classification systems will help the aesthetic clinician to choose a PRP preparation system that will provide a reliable, replicable, and effective product.

## 5 Device Selection Criteria

Because of the significant heterogeneity and lack of standardization between the numerous devices available for PRP preparation, there are several factors to consider when selecting which device to use in an aesthetic medical practice.

First and foremost, one must consider whether a device has been FDA-cleared and is being sold and used according to federal regulations. It is important to note who manufactures the device and where it is made. The type of device should also be considered, including whether it employs a single-spin, double-spin, or computer-aided centrifugation system and whether collection tubes are positioned horizontally or at a fixed angle (details discussed herein). As previously mentioned, DEPA and other classification systems are being used more frequently to differentiate between

the PRP preparation systems. Validated cellular characteristics of the PRP product, in the form of peer-reviewed publications and videos, should also be assessed. It is important to investigate each device's history and safety track record, ideally taking into account any scientific literature validating off-label uses, anecdotal accounts from trusted colleagues who have used the device, and the patient experience.

Additional considerations to be made when selecting a PRP preparation device include costs, the quality of training provided by the manufacturer, ease-of-use, ongoing technical support, and any marketing resources provided.

Based on a review of published studies in the dermatologic literature, one group of authors recommends a single-spin system that produces pure PRP with a mean platelet concentration that is three- to sixfold higher than that of whole blood and excludes most granulocytes (Conic et al. 2018).

## 6 Costs

The key technology, and therefore the main expense, involved in a PRP preparation system is the collecting tube (and the separator gel that is often included within it, discussed later). In one study comparing different commercial PRP preparation systems, the price of the disposable kit used for the preparation of PRP varied from $50 to $500, with a median of $120 (Kushida et al. 2014). The volume of the collecting tube may vary. Most patients require one tube per treatment session. A metric that may be more beneficial when comparing the economics of various devices is the price per 100 million platelets retrieved. For example, if Company A charges $150 per collecting tube while Company B charges $90, but Company A's system costs $4–5 per 100 million platelets retrieved while Company B's system costs $10, then Company A is likely a more economical choice.

Centrifuge devices tend to be standardized and relatively inexpensive. Some manufacturers that produce large-volume collecting tubes may require a customized centrifuge system. Companies may offer to provide the centrifuge at a cheaper price or free of charge with the purchase of a certain number of collection kits.

## 7 Types of FDA-Cleared Systems

PRP preparation devices can be divided into simple systems, characterized by small-volume blood draws and a single-spin (SS) centrifugation cycle, and complex systems, characterized by large-volume blood draws and a double-spin (DS) centrifugation cycle. DS protocols result in higher concentrations of platelets and leukocytes (Hausauer and Humphrey 2020). Most PRP systems used for dermatologic purposes are SS systems.

Single-spin (SS) systems yield relatively pure PRP that contains essentially no erythrocytes and fewer leukocytes, and is therefore considered less inflammatory and

more ideal for dermatologic applications. Clinical experience and research demonstrate that ideal cosmetic outcomes in skin and hair require minimal inflammation. Most SS systems contain a separator gel in either a large or small collection tube. These devices can generate PRP that contains, on average, a 1- to 1.5-fold higher concentration of platelets compared to whole blood. Larger tubes that involve a second step of removing platelet-poor plasma (PPP) from the final product can achieve concentrations that are up to 2.5- to threefold higher compared to whole blood. Because SS systems produce plasma that is devoid of erythrocytes, the final product takes on a gold or straw color (Fig. 3).

Double-spin (DS) systems can generate PRP that contains, on average, a three- to ninefold higher concentration of platelets compared to whole blood. However, the final product contains higher numbers of erythrocytes and granulocytes, which are generally felt to be disadvantageous for dermatologic applications. Most DS systems tend not to employ the use of a separator gel, and instead rely on the formation of a buffy coat, which stores the highest concentration of platelets, but also a significant number of erythrocytes and leukocytes. This yields a more cell-rich product that takes on a red color (Fig. 4). Some of these devices are equipped with a computer-aided centrifugation system that requires a highly trained operator. Complex centrifugation devices can serve as bone-marrow-concentrating systems and are generally more appropriate for orthopedic and other intraoperative indications. Typically, a large volume of blood is required for these systems. The kits needed for

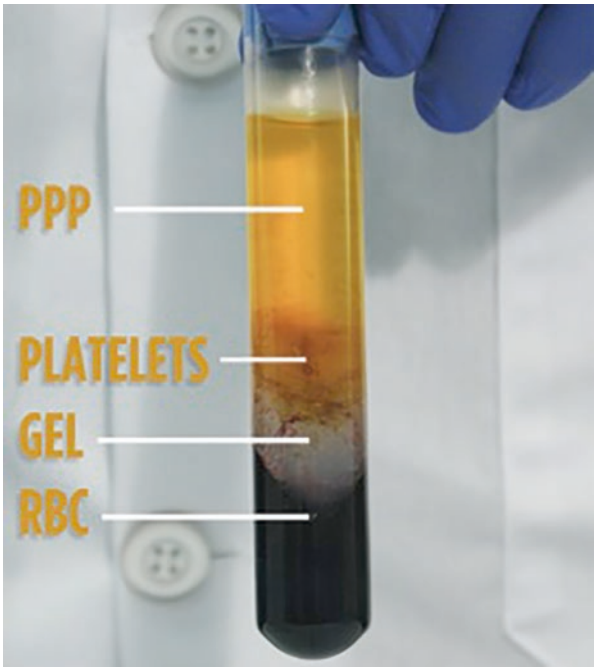


**Fig. 3** Example of single-spin platelet-rich plasma (PRP) collection tube after centrifugation: platelet-poor plasma (PPP) resides at the top of the tube, PRP resides in the middle of the tube above a thixotropic separator gel, and the red blood cells (RBCs) remain separate below the gel. (With permissions from and copyright retained by Eclipse)

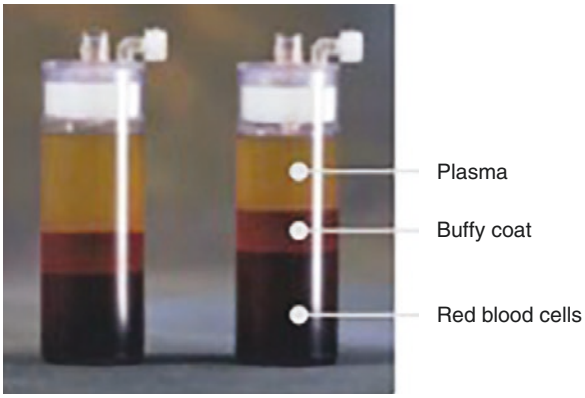


**Fig. 4** Example of a final PRP product that contains a high number of erythrocytes. (With permissions from and copyright retained by Eclipse)

DS systems tend to be more expensive than their SS system counterparts. In general, DS systems are felt to be too complicated for a busy outpatient dermatology practice.

## 8 Preparatory Procedure

### *8.1 Tubes*

A standard PRP collection tube features a rubber cap that provides a vacuum seal. Tubes are made of glass or plastic. While glass tubes are at risk of breaking, they are less porous than plastic tubes, and therefore may be less likely to lose their vacuum seal over time. A manufacturer's tube may be coated with a proprietary material to prevent platelets from adhering to the walls of the tube. Most tubes used for dermatologic applications contain a separator gel plug and an anticoagulant (each discussed herein). A buffer may be included in the tube, which creates a more alkaline environment that brings the pH back into a physiologic range (Arora and Agnihotri 2017). This may enhance growth factor activity and decrease pain associated with PRP administration.

### *8.2 Anticoagulants*

PRP collection tubes must contain an anticoagulant in order to prevent the initiation of the clotting cascade as whole blood is drawn into the tube. Examples of anticoagulants include citrate, sodium citrate, trisodium citrate, trisodium phosphate, anticoagulant citrate dextrose-A (ACD-A), citrate phosphate dextrose (CPD), heparin, and ethylenediaminetetra-acetic acid (EDTA) (Frautschi et al. 2017; Arora and Agnihotri 2017). Because EDTA appears to suppress platelet degranulation, it is not recommended for PRP preparation. The majority of PRP studies that report on

anticoagulation of collected blood use sodium citrate or ACD-A. However, specific documentation of anticoagulation is often lacking in these studies, which is problematic (Frautschi et al. 2017). Not only could the choice of anticoagulant impact the platelet yield but also platelet function through alterations in pH.

## *8.3 Separator Gels*

Most PRP collection tubes used for aesthetic indications contain a thixotropic separator gel (Fig. 3). This unique property allows the gel to become less viscous when subjected to an applied stress, and then regain its viscosity when that stress is removed. The separator gel becomes temporarily fluid when centrifuged at high speeds, and can therefore migrate according to its relative density. The ideal separator gel would have a relative density lower than that of an erythrocyte or granulocyte, but higher than that of a platelet, thus facilitating a separation between desired and undesired components of whole blood and allowing easy access to the PRP that lies above it. The separator gel is a key element of most PRP preparation systems, and is often considered part of a manufacturer's intellectual property.

## *8.4 Centrifugation*

The common step that unites all methods of PRP preparation is the use of differential centrifugation to separate the patient's whole blood into layers (Frautschi et al. 2017). Centrifugation utilizes the physical principle of Stokes Law, which states that the sedimentation rate of a particle suspended in a liquid is proportional to the particle's size and relative density (Arora and Agnihotri 2017). Relative density, also known as specific gravity, is defined as the ratio of the density of a given substance to the density of a standard material (such as water for a liquid). Once the centrifugation cycle begins, heavier components with a higher density settle lower in the collection tube, while lighter components with a lower density settle above them. The components inside a PRP collection tube include erythrocytes, leukocytes, platelets, plasma, and in most systems used for dermatologic purposes, a separator gel (discussed previously). After centrifugation, these components can typically be found in a predictable order (Fig. 3).

There are three main variables that affect the recovery of platelets from whole blood during centrifugation: rotor size, centrifuge speed, and duration of centrifugation. Since rotor size generally remains fixed for a given centrifuge device, the other two variables (centrifuge speed and duration) can be adjusted to alter the final composition of the PRP product (Arora and Agnihotri 2017). Studies have shown that the speed and number of revolutions influence platelet and growth factor concentration and activity.

Centrifugal acceleration forces can affect the integrity and viability of platelets, which in turn impact the concentration of growth factors and cytokines delivered into the target tissue (Denfors et al. 1991). A high force, also known as a "hard spin," will cause settling down of all cellular components within the whole blood, and therefore tend to yield PPP in the supernatant. A relatively lower force, known as a "soft spin" or "light spin," will keep platelets suspended in the plasma while larger cells settle more rapidly (Arora and Agnihotri 2017).

Centrifugation systems may also differ in the position of the collection tube during the spin cycle. In fixed angle spin systems, the tube is placed at an approximately 45° angle, while a horizontal spin system positions the tube at an approximately 90° angle from the central vertical axis of the device. A fixed angle spin system forms an ellipse-shaped gel that provides enough separation to allow for a normal resuspension technique involving multiple inversions, producing a high platelet yield of approximately 80% (Fig. 5). A horizontal spin system forms a circle-shaped gel that provides a thicker separation to allow for more aggressive agitation of the tube, which generates a slightly higher platelet yield that can reach up to 90% (Fig. 6). Horizontal spin systems tend to be more costly and take up more space than fixed angle spin systems.

**Fig. 5** Example of a fixed angle spin system. (With permissions from and copyright retained by Eclipse)

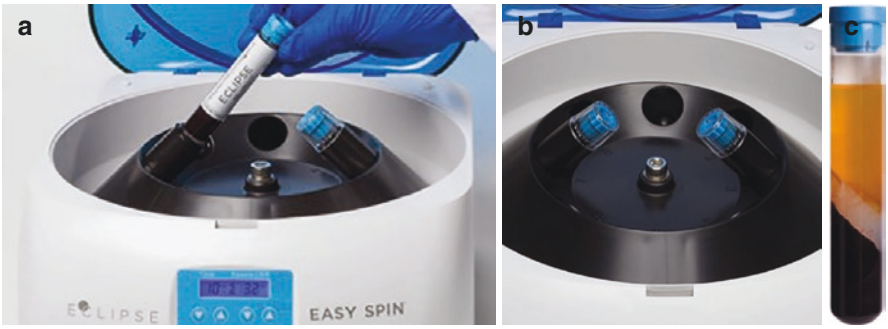


**Fig. 6** Example of a horizontal spin system. (With permissions from and copyright retained by Eclipse)

## *8.5 Platelet Activation*

The activation of PRP stimulates the release of growth factors from platelets. Anything that causes platelets to aggregate will stimulate their degranulation and subsequent release of growth factors. This can be achieved in several ways: physically, chemically, exogenously, and/or endogenously.

Physical methods of platelet activation include centrifugation and agitation. Shear forces caused by fluid flow can cause platelet activation (Arora and Agnihotri 2017). Double freeze-thaw cycles also activate PRP by causing membrane disruption due to temperature shock leading to the subsequent release of intracellular cytokines.

Endogenous platelet activation and degranulation can occur upon contact with a variety of materials including fibrillar collagen, basement membranes of cells, tissue thrombin, thromboxane A2, adenosine diphosphate, or platelet-activating factor (Frautschi et al. 2017; Arora and Agnihotri 2017). When inactivated PRP is injected into connective tissues, it comes into contact with collagen and tissue thromboplastin (tissue factor) and becomes activated. Interactions with thrombin result in an immediate release of cytokines from PRP, while interactions with collagen demonstrate a more sustained release pattern (Harrison et al. 2011). Procedures, such as microneedling or laser resurfacing, have been used concomitantly with PRP to produce controlled tissue damage that stimulates endogenous platelet degranulation (Kramer and Keaney 2018).

The addition of chemical activators to PRP is theorized to enhance the release of growth factors from platelets (Hausauer and Humphrey 2020). Examples of exogenous chemical activators include calcium chloride, calcium gluconate, and bovine-derived thrombin. Thrombin is the most potent platelet activator. One review found that calcium chloride was the most frequently used exogenous chemical activator (Frautschi et al. 2017).

It is important to understand that activated PRP begins to exert its therapeutic effects immediately. It is believed that approximately 70% of growth factors are secreted from platelets within the first 10 min after activation, and almost 100%

have been secreted within an hour (Arora and Agnihotri 2017). Bearing this in mind, activation of PRP prior to injection may be better suited for addressing local issues, such as wound healing. For applications such as hair restoration, it is hypothetically more desirable to have diffusion of the product into the surrounding tissues upon administration, and thus exogenous activation may not be beneficial. Furthermore, PRP begins to clot in the tube or syringe immediately upon activation, and thus the injector is under pressure to perform the procedure rapidly. The "sticky" nature of activated PRP may make it more difficult to inject and could theoretically increase the risk of vascular occlusion. Finally, as previously stated, it is unclear whether the use of activated PRP violates the FDA's "minimal manipulation" standard.

Because studies assessing the benefit of using activated versus non-activated PRP have led to inconsistent results, there is no clear consensus on the advantages and disadvantages of exogenous PRP activation. Nevertheless, a majority of studies do include an activation step at the time of application (Frautschi et al. 2017). Standardization of platelet activation protocols is needed to accurately compare the degree of growth factor release between preparation systems.

# 9 Final Product Characterization

## *9.1 Concentration of Platelets and Growth Factors*

PRP is defined by its higher concentration of platelets in a smaller volume of plasma compared to whole blood. On average, approximately 80% of the platelets collected in whole blood are retrievable after centrifugation. After the various components of whole blood move throughout the collection tube during centrifugation, the large majority of platelets are found in the lower part of the plasma fraction, and most platelets are resting on top of the separator gel. This allows differentiation between PPP and PRP. It is possible to increase the concentration of the final product by removing some amount of PPP prior to resuspension of the plasma, but then a smaller total volume of product is available for administration. In this scenario, the total number of platelets retrieved stays constant. While multiple studies and marketing of PRP preparation systems focus on the final concentration of platelets available for administration, a more appropriate dosing measurement would be the total number of platelets being administered in a treatment session. Larger collection tubes are the only way to increase the total number of platelets available for administration. Multiple companies have developed larger collection tubes.

When platelets aggregate and become activated, they release more than 800 different proteins into the surrounding media, several of which are known as growth factors (Arora and Agnihotri 2017). Growth factors are stored in alpha granules contained within platelets. Upon platelet degranulation, these small molecules are

delivered locally and act to modify the inflammatory response and stimulate cell proliferation and differentiation within the target tissue (Gupta and Carviel 2016). This is the supposed mechanism for the role of PRP in regenerative medicine. These growth factors include insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF), among others (Frautschi et al. 2017; Gupta and Carviel 2016; Piccin et al. 2017).

A study in Japan directly compared seven different commercial PRP preparation systems and found significant differences in the platelet and growth factor concentrations remaining in the final product (Kushida et al. 2014). More studies of this nature may help to objectively compare available devices.

It is still unclear whether higher concentrations of platelets and/or growth factors correlates to increased efficacy. Two studies found that intraindividual concentrations of platelets and growth factors were relatively consistent, while the interindividual concentrations varied to a higher degree (Rodrigues et al. 2019; Siah et al. 2020). These studies found no correlation between concentrations and clinical outcomes. Interestingly, PRP that is overly concentrated with platelets and/or growth factors has been shown to provide no additional benefit and may actually have an inhibitory effect on tissue function (Arora and Agnihotri 2017; Dhillon et al. 2012; Giusti et al. 2009; Rughetti et al. 2008; Weibrich et al. 2004). While the cocktail of growth factors found in PRP demonstrates a net positive effect, it is known that individual growth factors can be deleterious (e.g., initiate cell death responses).

Normal human platelet levels fall within a range from 150,000 to 450,000 cells per microliter. This threefold difference will almost certainly impact the final platelet concentrations of PRP produced from two different patients regardless of the preparation technique. Simply reporting that a system generates PRP that is concentrated threefold does not provide accurate dosage information. Despite this fact, the vast majority of studies do not document the baseline concentration of platelets within patients' whole blood or the final platelet concentration in the PRP product (Frautschi et al. 2017). Without these two values, PRP administration becomes a somewhat blind process utilizing unknown levels of bioactive materials. This gap renders a carefully calculated dose–response relationship, which is needed to establish clinical indications, treatment guidelines, and adverse events, unobtainable. Therefore, reporting of platelet concentrations in both whole blood and PRP should be included in all future clinical studies.

Aside from the concentration of platelets, it is also important to consider individual patient factors that may affect platelet function. For example, a variety of drugs (e.g., aspirin) and patient comorbidities (e.g., chronic renal insufficiency) can affect platelet function, and thus influence the efficacy of PRP irrespective of the dose of platelets being administered.

### *9.2 Concentration of Other Cell Lineages*

The final PRP product will contain other cell lineages in addition to platelets, including leukocytes and erythrocytes. These other cells likely affect the therapeutic outcome of PRP either positively or negatively depending on the aesthetic or medical indication (Hausauer and Humphrey 2020). For example, some surgical double-spin systems yield high concentrations of leukocytes and erythrocytes, which may not affect the intraoperative application of PRP in major surgeries where inflammation and active bleeding are already present, but can be detrimental when PRP is used for hair restoration due to the inflammation that is generated. PRP that contains a high percentage of inflammatory white blood cells produces a persistent burning pain upon application to the treatment site. While this may be insignificant for orthopedic surgery patients under general anesthesia, it is not ideal for awake patients in the outpatient dermatology clinic. Systems that produce a less inflammatory product are thus more widely used for aesthetic indications. While monocytes, which become tissue macrophages, are considered non-inflammatory and may aid in the regenerative process, it is not currently possible to separate these cells from granulocytes, which are considered to be more inflammatory and destructive.

Currently, there are no controlled studies comparing the effects of leukocyte-rich and leukocyte-poor PRP (Kushida et al. 2014). Some authors have recommended against the inclusion of leukocytes in PRP to avoid inflammation and damage within the exposed tissue, whereas others have suggested a positive effect from increased antimicrobial properties (Frautschi et al. 2017).

PRP that contains a high concentration of erythrocytes can lead to bruising, while low concentrations of erythrocytes significantly reduce this risk, which is a major advantage when PRP is being used for cosmetic applications. Additionally, erythrocytes found in PRP are theorized to lead to the formation of reactive oxygen species, vascular injury, and cell death (Everts et al. 2019).

## 10 Warnings

The relative lack of standardization and regulation among PRP preparation systems has allowed a space in the market for untrustworthy practices. It is important for clinicians to be aware of such illicit behavior in order to avoid potentially unsafe patient care. Currently, there are PRP kits being sold online without FDA clearance. Counterfeit tubes that closely mimic the packaging of more reputable manufacturers have been found on popular e-commerce websites. Additionally, in vitro diagnostic (IVD) tubes have been wrongfully used and marketed as a PRP preparation system. These tubes, commonly referred to as “tiger tops,” are manufactured for routine lab work and are not FDA-cleared for autologous use at the point-of-care. The use of IVD tubes causes a large portion of platelets to be lost below the gel during centrifugation, resulting in PPP. The final platelet count or concentration becomes

unpredictable when using IVD tubes and changes based on the method of the operator. Most importantly, IVD tubes may contain pyrogens, or substances that induce fever. Labels for IVD tubes include explicit warnings against their use for the collection of materials to be injected into patients and encourage precautions to be taken to prevent possible backflow from the tube during blood draws given the presence of chemical additives within the tube. While medical devices indicated for autologous procedures must be manufactured in a pyrogen-free environment, IVD tubes are not held to the same standard. Despite undergoing a sterilization process that ensures the absence of viable living bacteria, IVD tubes may contain pyrogens, such as lipopolysaccharide and other endo- and exotoxins, that are released and deposited upon bacterial cell lysis.

## 11 Conclusion

The authors recommend that clinicians providing PRP to patients be adequately trained, provide detailed written consent forms to patients, and use a kit that has been FDA-cleared. The kit should have an excellent record of safety and effectiveness supported by published studies, and appropriate technical support should be provided by the manufacturer. It is important to be able to compare the available preparation systems, such as with the DEPA classification, when selecting a device for purchase. Clinicians should be confident in the final PRP product being administered to patients.

## 12 Future Directions

In search of the ideal PRP preparation system, future studies must carefully describe the preparatory protocols used in order to accurately interpret reported outcomes. Classification systems such as DEPA or FIT PAAW may facilitate this more standardized approach to the field. There is a great need for larger scale, randomized controlled trials that compare different preparatory procedures, final product compositions, and administration techniques to establish a standard of care for the safe and effective use of PRP in aesthetic medicine. Until a standard preparatory protocol is established, the reported efficacy and subsequent applicability of PRP will continue to vary.

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# Microneedling + PRP (for Rejuvenation, Acne Scarring)


**Aunna Pourang and Rohit Kakar**

## 1 Introduction

Microneedling (MN), also known as percutaneous collagen induction therapy, is a minimally invasive technique utilizing several fine-gauge needles to penetrate the skin for the treatment of medical and cosmetic dermatologic conditions. Fernandes developed the first rolling microneedle device, and Liebl patented the Dermaroller™ device shortly after (Fernandes 2005; DERMAROLLER). These pioneers of MN based their inventions on the subcision and needle microdermabrasion techniques developed by Orentreich and Orentreich and Camirand and Doucet, respectively (Orentreich and Orentreich 1995; Camirand and Doucet 1997). More recently, the procedure has been combined with topicals, such as platelet-rich plasma (PRP), to potentiate its effects.

Microneedling results in a controlled wound healing response of inflammation, proliferation, and remodeling that develops from micro wounds of the skin created via needle puncture (Fabbrocini et al. 2009). This process is thought to be initiated by superficial capillary disruption that leads to platelet activation and subsequent release of growth factors (GF) and cytokines, including platelet-derived GF, fibroblast GF, transforming growth GF α and ß, and vascular endothelial GF (Fabbrocini et al. 2009). Some authors have also proposed that the controlled injury induced by MN may lead to altered expression of tissue remodeling genes, as well as shifts in trans-epithelial bioelectric potentials that result in GF release and neocollagenesis (Liebl and Kloth 2012; Schmitt et al. 2018). Histologically, MN has been

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demonstrated to trigger epidermal thickening, increased elastin, and increased deposition of collagen in a normal lattice pattern (Fabbrocini et al. 2009; Velnar et al. 2009; Aust et al. 2008a).

Traditional MN utilizes devices such as manual rollers and automated pens to achieve the aforementioned micro wound effect. A variety of topical agents are commonly used with traditional MN to allow the needles to glide and avoid trauma, such as hyaluronic acid and water-based gels. Additional and more advanced MN devices build upon this by employing radiofrequency to further augment neocollagenesis and neoelastogenesis or by infusing active ingredients for transdermal drug delivery (Sahni and Kassir 2013; Chandrashekar et al. 2014; Vejjabhinanta et al. 2014; Cho et al. 2012; Lee et al. 2012). Fractional radiofrequency microneedling (FRM) devices use needles that are either uncoated, to deliver energy along the entire needle, or coated, where energy is only released below the epidermis (Duncan 2018).

Topicals, such as PRP, filler, and peels, can be applied during the MN procedure to further augment the procedure's effects (Leheta et al. 2014a; b; Sharad 2011). Growth factors contained within PRP include platelet-derived GF, transforming GF-β, vascular endothelial GF, epidermal GF and fibroblast GF, which are akin to the GFs released at the sites of platelet activation induced by MN wounds (Cole et al. 2010). Similarly, PRP's GFs induce a wound-healing response by stimulating fibroblasts, neocollagenesis, neoangiogenesis, hyaluronic acid production, and recruitment of mesenchymal stem cells which differentiate at the application site (Fabbrocini et al. 2009; Velnar et al. 2009; Aust et al. 2008a; Ramaut et al. 2018). Combining MN with PRP may enhance PRP absorption in the skin and promote synergy of the GFs released by both (Hom et al. 2007; Fabbrocini et al. 2011a). Consequently, the complementary effects of MN and PRP on acne scars, post-traumatic scars, striae distensae, melasma, and skin rejuvenation are explored herein.

## 2 Scars

Studies have demonstrated how needling procedures can break up the parallel collagen bundles of the superficial layer of the dermis in scars and induce neocollagenesis in an organized, normal lattice pattern (Fabbrocini et al. 2009; Aust et al. 2010). As a result, improvements in post-acne scars, post-traumatic scars (burns), post-varicella scars, and post-herpetic scars have been observed with MN treatments (Schwarz and Laaff 2011; Majid 2009; Costa and Costa 2014; Park et al. 2012; Harris et al. 2015). The literature has demonstrated that such results are further augmented for post-acne scars and post-traumatic scars by the addition of PRP (Chang et al. 2020; Agamia et al. 2020).

## *2.1 Acne Scars*

Although MN without PRP has been shown to improve acne scarring, monotherapy with intradermal PRP does not appear to be as effective; consequently, it is the combination that has demonstrated the most utility (Chang et al. 2020; Gawdat et al. 2014; Porwal et al. 2018). In a systematic review and meta-analysis, Chang et al. demonstrated the efficacy of MN with PRP in the treatment of acne scars across five studies (Chang et al. 2020); 226 patients with atrophic acne scars who underwent 3 to 6 MN sessions over 3 to 4 weeks had significantly better clinical improvement, higher satisfaction rates, and a shorter duration of post-procedural erythema than did patients who underwent MN alone (Baumann 2007).

The combination of MN and PRP in the treatment acne scarring also appears to affect quality of life. In a study evaluating the efficacy of MN with PRP in patients with ice pick, boxcar, and rolling acne scars, a greater improvement in the Dermatology Quality of Life (DLQI) score was noted in the group who underwent treatment with intradermal PRP with MN than those who had MN alone (Porwal et al. 2018). Similarly, in a study comparing FRM with PRP to FRM alone in the treatment of atrophic acne scars, the former group reported higher DLQI scores despite similar efficacies (Chowdhary et al. 2019).

PRP with MN has also demonstrated similar or superior efficacy when compared to other topicals used with MN (Pawar and Singh 2020; Chawla 2014). Pawar et al. compared post-MN application of PRP to the post-MN application of topical insulin among subjects with atrophic acne scars (Pawar and Singh 2020). Three months after four sessions at monthly intervals, both groups demonstrated a comparable and statistically significant improvement without any scarring or dyspigmentation. Icepick and boxcar scars responded better to topical insulin, however (Pawar and Singh 2020). Interestingly, insulin appears to lead to organized neocollagenesis via stimulation of VEGF through activation of PI3K/AKT pathways (AbdelKader et al. 2016; Azevedo et al. 2016). In a split-face study comparing intraprocedural application of PRP with MN to vitamin C with MN for acne scars, overall results were better in the MN with PRP group (Chawla 2014). Patients were also more satisfied with the results of the MN with PRP-treated side. In particular, MN combined with PRP improved boxcar and rolling scars but had limited efficacy in treating ice pick scars. Vitamin C, however, was noted to improve post-inflammatory hyperpigmentation secondary to acne (Chawla 2014).

When compared to chemical reconstruction of skin scars (CROSS), MN with PRP appears to be as effective with less downtime and side effects. In a study evaluating MN with intraprocedural topical PRP, intradermal-only PRP, and 100% topical trichloroacetic acid (TCA) via the CROSS method for the treatment of atrophic acne scars, all three groups showed a statistically significant improvement (Nofal et al. 2014). While some patients in the MN with PRP group experienced temporary erythema and edema, they were able to resume work the same day. Of note, intradermal PRP was associated with pain and bruising, and some of the patients in the TCA group experienced crusting and post-inflammatory hyperpigmentation (Nofal et al. 2014).