Review Article

Standardisation of Platelet-Rich Plasma in Clinical Practice

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Abstract

Platelet-Rich Plasma (PRP) has been widely used in many medical fields, including aesthetic medicine. It has gained popularity owing to the knowledge that PRP can promote wound healing and facilitate scar improvement or facial rejuvenation. Unfortunately, the evidence supporting PRP remains elusive due to inconsistencies in the literature and lack of standardisation in the PRP preparation protocols, administration, and documentation. Since its introduction, many authors have attempted to classify PRP, however until today; no consensus has been reached due to the confusion in the nomenclatures. PRP administration has also become a blind process whereby varying volumes or constituents of PRP are used, causing unpredictable and inconsistent outcomes. We aim to highlight this issue and call for standardisation in PRP administration protocol.

Keywords: Platelet-Rich Plasma, PRP, Classification, Aesthetic

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Recently, there has been a surge of platelet-rich plasma (PRP) usage for various indications in many medical fields, including dermatology, cardiology, plastic surgery, anesthesia, orthopedics, spine and sports medicines (Frautschi et al., 2017; Collins et al. 2021; Kelm and Ibrahim, 2022). PRP has also been getting much attention in the aesthetic world, as shown in Figure 1, mainly due to widespread commercial interest and social media (Kelm and Ibrahim, 2022). It is commonly used as an adjunct to augment the effect of blepharoplasty, micro-needling, facelift, fractional carbon dioxide laser, hair transplantation and fat grafting (Frautschi et al., 2017; Alves and Grimalt, 2017; Kelm and Ibrahim, 2022). PRP can also be used as a standalone product in its topical or injectable form to promote skin rejuvenation, scar improvement, de-pigmentation, wound healing and hair growth in alopecia (Frautschi et al., 2017; Alves and Grimalt, 2017; Kelm and Ibrahim, 2022). PRP utilization stems from the understanding that platelet-derived growth factors and cytokines promote wound healing and play critical roles in all three phases of the repair cascade: inflammation, proliferation, and remodeling (Everts et al., 2020; Kelm and Ibrahim, 2022).

However, there is a paucity in the evidence as most reported beneficial outcomes are anecdotal. Although the theoretical potentials of PRP were supported by many in vitro studies, the in vivo evidence remains inconclusive (Harrison and Alsousou, 2020). Researchers still cannot strongly validate the PRP advantageous effects in the human population because most evaluations of the different PRP preparations were derived from small case series, cohort studies without adequate control groups, or poorly designed clinical trials (Platelet-rich Plasma for Facial Rejuvenation (PPFPR), 2017). They often have unclear documentation on the PRP bio-formulations used, inconsistent dosing, and a lack of objective outcome measurements. Many systematic reviews also failed to find standardization in the PRP protocols available in the literature (Frautschi et al., 2017; PPFPR, 2017; Evert et al.,2020; Evans et al., 2022; Gentile and Garcovich, 2022; Kelm and Ibrahim, 2022).

These vast inconsistencies in the clinical practice are further compounded by the absence of clear regulations and legislation on PRP, the abundance of commercially available PRP preparation systems producing varying PRP compositions, and the lack of clear protocols for administering PRP (Fadadu et al, 2020; Evans et al., 2022; Gentile and Garcovich, 2022; Kelm and Ibrahim, 2022). Hence, the practicality of PRP is now questionable due to the heterogeneity of the evidence present.

The Basics of PRP Therapy

Platelet-rich plasma (PRP) is an autologous blood product created via centrifugation of whole blood, with the concentration of platelets usually five-fold greater than normal physiological levels. PRP was first used in the 1970s by hematologists as a transfusion product in thrombocytopenic patients, before making its way into other specialties (Collins et al, 2021; Kelm and Ibrahim, 2022).

The platelet's roles in wound healing cascade have been well studied. Platelets contain a rich source of growth factors and cytokines, including platelet-derived growth (PDGF), transforming growth factor (TGF) and vascular endothelial growth factor (VEGF). Following platelet activation, these factors are released, regulating cell proliferation, chemotaxis and angiogenesis. The provisional fibrin matrix formed also supports cellular migration, proliferation and differentiation (Evert et al.,2020). The justification for PRP treatment is that the injection of concentrated platelets containing plasma at the injury sites will accelerate wound healing and tissue

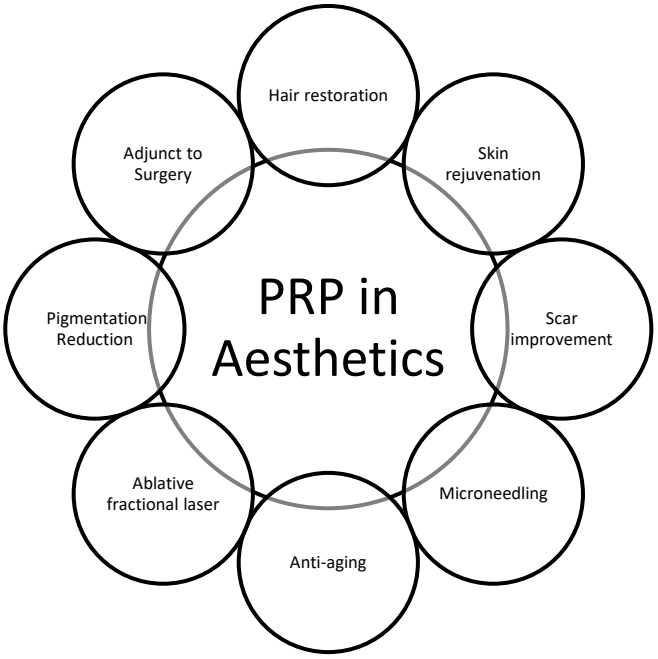

Figure 1: PRP utilization in Aesthetic Medicine

regeneration by releasing a supraphysiological amount of growth factors and cytokines mentioned above (Evert et al.,2020; Kelm and Ibrahim, 2022).

The ability of PRP to facilitate the synthesis of collagen and elastin, and stimulate follicular development allows PRP to be an attractive option for aesthetic treatment (Evans et al., 2022; Gentile and Garcovich, 2022; Kelm and Ibrahim, 2022). In hair growth, the growth factors released from the platelet can increase the transition from telogen to anagen, induce dermal cells proliferation, and promote hair follicle maturation. In skin rejuvenation, the available evidence suggests that PRP can soften the wrinkles, reduce the scar and pigmentation appearance, as well as accelerate wound healing (Kelm and Ibrahim, 2022).

Although there is no single standardized PRP protocol, most preparations follow these fundamental steps: (1) blood collection from the patient through venepuncture; (2) centrifugation to separate red blood cells and platelet-poor plasma from the 'buffy coat', a layer rich in white blood cells (WBCs) and platelets; (3) plasma aspiration; (4) potential second centrifugation to separate the White Blood Cells (WBCs) depending on the intent whether to keep or remove the WBCs; (5) selected supernatant removal; (6) mixing/resuspension of platelets; (7) activation with calcium chloride, thrombin or another agent; and (8) application (Frautschi et al., 2017; Fadadu et al, 2020).

The Rationale for Standardization in Clinical Practice

The PRP's platelet, WBCs, and growth factors concentration can differ significantly depending on the centrifugal spinning and preparation protocols (Kelm and Ibrahim, 2022). Two recent systematic reviews on PRP in alopecia found heterogenous PRP preparation protocols in their analysis, with substantial variability depending on the commercial PRP kits used, concentration and volume administered, and injection details (Evans et al., 2022; Gentile and Garcovich, 2022). The exact constituents of the PRP, or so-called the "PRP dose" administered to the patients, can also vary based on the individual clinician practice (Evans et al., 2022; Gentile and Garcovich, 2022). Some preparations may also be contaminated with red blood Cells (RBCs) (Evert et al.,2020).

As for the individual cellular composition of PRP, a dose-dependent relationship has been previously reported in platelet, suggesting a linear relationship between the platelet concentration and mesenchymal stem cells growth, fibroblasts proliferation, and collagen formation (Frautschi et al., 2017). The role of WBCs in PRP has also been controversial and subjected to ongoing debates. Some studies showed a positive effect of the WBC's cytokines from the antimicrobial properties and increased VEGF level, while others reported adverse effects from the reactive oxygen species they release, causing inflammation and tissue damage (Frautschi et al.,2017). When applied to local tissue, PRP containing RBCs can cause eryptosis or suicidal erythrocyte death. This response triggers the release of macrophage migration inhibitory factors, which inhibit stem cells' migration and fibroblast proliferation (Evert et al.,2020).

As we understand more about the role of platelet concentration, WBC's influence, and the deleterious effect of RBC, several attempts to characterize the PRP processed manually or from the commercially available systems have been made. Fadadu et al. reported no standardization in all thirty-three PRP preparation systems they studied. The final cellular concentration also varied significantly across the systems. In addition, only eleven systems met the definition of PRP as defined by Marx et al. as having a minimum platelet concentration of 1 000 000 platelets/μL, and only ten systems met the criteria that PRP should have a concentration of at least five times than the baseline. Surprisingly, 3 of the 33 systems reviewed even produced PRP with platelet counts less than the whole blood baseline level (Fadadu et. al., 2019)

Many studies do not document the concentration of platelets within the patient's baseline whole blood and the final PRP preparations, and it is also being reflected in our daily clinical practice (Frautschi et al., 2017). PRP administration has become a blind process where unknown volumes or concentrations of active bio formulations are used. Inadvertently, even if there are any tangible good outcomes, they are often inconsistent and subjected to presumption (Frautschi et al., 2017).

However, in practice, we are aware that the precise determination of the PRP compositions is not straightforward. The terminology is somewhat confusing, and many reports in aesthetic use the broad term of PRP for simplicity. Several authors have attempted to classify PRP formulation (Table 1).

Table 1: PRP Classification

Author (year)	Classification
Ehrenfest et al. (2009)	Leukocyte-Fibrin density
DeLong et al. (2012)	PAW
Mautner et al. (2015)	PLRA
Magalon et al. (2016)	DEPA
Lana et al. (2017)	MARSPILL

Dohan Ehrenhest et al. in 2009 classified the PRP according to 2 qualitative parameters: presence or absence of cell content (such as leucocytes) and the fibrin architecture, namely

pure platelet-rich plasma (P-PRP), leukocyte and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leukocyte and platelet-rich fibrin (L-PRF), as shown in Table 2. However, as it did not consider the role of other cellular subpopulations such as RBCs and neutrophils (Collins et al, 2021), DeLong et al. introduced a more quantitative classification, the PAW classification (Platelets, Activation, White blood cells) in 2012, as depicted in Figure 2. Unfortunately, the PAW classification still did not address the role of RBC; hence Mautner et al. in 2015 proposed the PLRA classification (Platelet count, Leukocyte content, RBC content, activation) as illustrated in Table 3, which was the first system to specify the volume of PRP administered and the absolute platelet concentration. In 2016, Magalon et al. proposed another classification system based on the quality of the preparation, the DEPA classification (Dose, Efficiency, Purity, Activation), as illustrated in Table 4. It analyses some additional aspects of the preparation process (efficiency and purity). However, it does not address the critical quantitative measurement of the different cell types as the PLRA (Collins et al.,2021).

The latest classification, introduced by Lana et al., was the MARSPILL classification (Method, Activation, RBCs, Spin, Platelet concentration, Image guidance, Leukocyte concentration, Light activation). As demonstrated in Table 5, it incorporates several aspects of the manufacturing process and the subgroups of cellular components. They also introduced the novel concept of light activation and image guidance in administering the PRP (Lana et al.,2017). Nonetheless, the final element of this classification is probably not applicable in some aesthetic procedures, such as the application of PRP following laser or micro-needling treatments.

Despite these classifications, none of these systems is universally accepted or used extensively as they are still unable to address the variability in the production protocols and the ongoing confusion with the nomenclatures among clinicians (Rossi et al.,2019).

The need for standardization in PRP formulations can probably be met by freeze-drying PRP (FD-PRP). Freeze-drying, or lyophilization, is a technology of freezing followed by water sublimation and subsequent removal of water vapor (Andia et al., 2020). Freeze-dried PRP (FD-PRP) can be prepared on an autologous basis or derived from a single or pooled healthy donors to produce allogenic FD-PRP, often followed by sterilization by gamma radiation (Andia et al., 2020).

The quality of FD-PRP is determined by the platelet integrity and the activity of plasma coagulation factors or cytokines after rehydration. Andia et al. showed that freeze-drying preserves the platelet function, cytokine concentration, and function (Andia et al., 2020). This technology can accurately determine the platelet concentration and cytokine growth factor levels, facilitating standardized treatment protocols. FD-PRP can also be formulated to achieve specific parameters or concentrations and fabricated by combining with other biomaterials or elements to control the cytokine release and match the specific local tissue requirement. Besides, FD-PRP is stable at room temperature and can be stored for several months (Andia et al., 2020).

Despite the advantages, the high cost of FD-PRP production and limited processing and storage facilities may be the limiting factors (Andia et al., 2020). Nevertheless, this is an alternative worth exploring in the quest for consistency and standardization in PRP therapy.

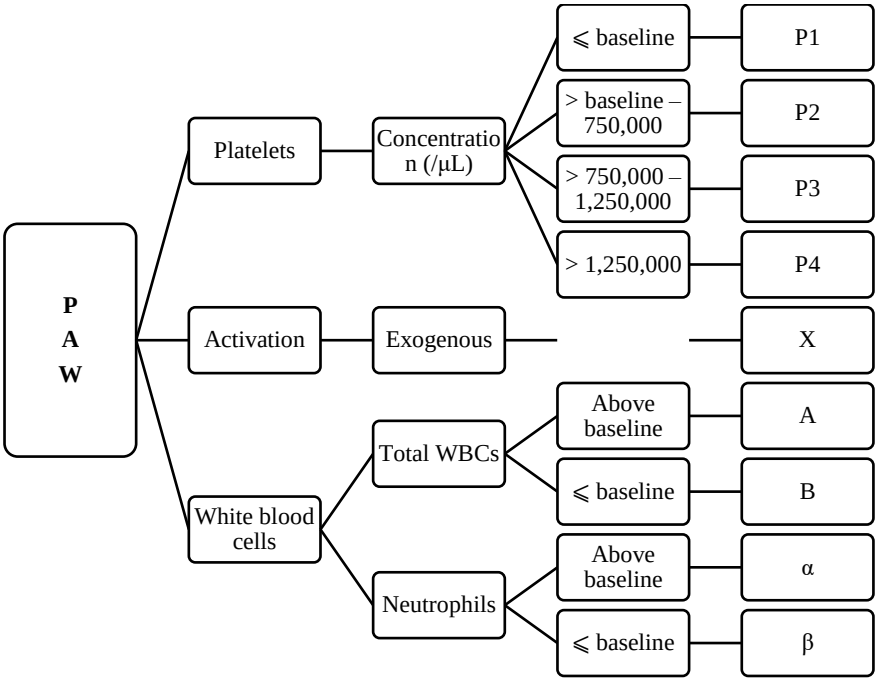

Figure 2: PLRA Classification (2015)

Table 2: Ehrenfest classification (2009)

	Preparation	Leukocyte	Fibrin Density
P-PRP	Pure platelet-rich plasma	Poor	Low
L-PRP	Leukocyte and platelet-rich plasma	Rich	Low
P-PRF	Pure platelet-rich fibrin	Poor	High
L-PRF	Leukocyte and platelet-rich fibrin	Rich	High

Table 3: PLRA Classification (2015)

	Criteria	Final Score
Platelet count	____P Volume Injected	_____M Cells/µL
Leucocyte content	> 1% < 1%	+ –
Red blood cell content	> 1% < 1%	+ –
Activation	Yes No	+ –

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Table 4: DEPA Classification (2016)

Criteria	Subgroup	Description
Dose of injected platelets	A: Very high B: High C: Medium D: Low	> 5 Billion injected platelets 3–5 Billion injected platelets 1–3 Billion injected platelets < 1 Billion injected platelets
Efficiency of production	A: High B: Medium C: Low D: Poor	Recovery rate in platelets > 90% Recovery rate in platelets 70–90% Recovery rate in platelets 30–70% Recovery rate in platelets < 30%
Purity of PRP	A: Very pure B: Pure C: Heterogenous D: Whole blood	Platelets in PRP > 90% Platelets in PRP 70–90% Platelets in PRP 30–70% Platelets in PRP < 30%
Activation process	-	Autologous thrombin Calcium chloride

Table 5: MARSPILL Classification (2017)

Acronym	Description
Method	Handmade (H) Machine (M)
Activation	Activated (A+) Not activated (A-)
Red blood cells	Rich (RBC-R) Poor (RBC-P)
Spin	One spin (Sp1) Two spins (Sp2)
Platelet number	**Folds from baseline:** PL 2–3 PL 4–6 PL 6–8 PL 8–10
Image guided	Guided (G+) Not guided (G-)
Leukocyte concentration	Rich (Lc-R) Poor (Lc-P)
Light activation	Activated (A+) Not Activated (A-)

Conclusion

We strongly believe that standardization of PRP therapy is the most crucial step in determining the efficacy of PRP in aesthetic surgery and other specialities. Future studies must address all the variables in PRP preparation to facilitate consistency in outcome reporting. With this, large-scale RCTs can be undertaken to compare the therapeutic effects between different PRP preparation protocols, concentrations, amounts, and techniques used.

Conflict of interest

None to declare

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