REVIEW ARTICLE

Platelet-rich Plasma: A Recent Review

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Abstract

Platelet-rich plasma (PRP), prepared from autologous blood, is commonly used in various pathologies like hip and knee osteoarthritis, rotator cuff pathology, epicondylitis, tendinitis, fracture healing and back pain, for tissue regeneration, wound healing, scar revision, skin rejuvenating effects, and also in treatment of alopecia. It is one of the important products, which is nowadays being used by pain physicians aggressively. But, the preparation protocols vary and there are many factors that influence the composition of PRP. The aim of this review is to find the details of PRP and the standard preparation protocol, if any.

Keywords: Centrifugation, Growth factor, Platelet, Platelet-rich plasma.

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Platelet

The cytoplasmic fragment of megakaryocytes is known as platelet. It is formed in the bone marrow. Normal platelet count ranges from 150,000 to 350,000 platelets/μL of blood. It is approximately 2 μm in diameter and contains more than 800 proteins and molecules. These are chemokines, membrane proteins, metabolites, messenger molecules, growth factors, and various soluble proteins, which again includes more than 30 bioactive proteins. On activation of platelets, degranulation and release of its contents occur.[1,2]

Activation

Platelet activation can be exogenous or endogenous. Exogenous activators can be both mechanical (freeze thawing cycles) and chemical (thrombin and calcium chloride). Chemical activators are used to activate platelets and stimulate degranulation to release its growth factors (GFs). Study showed that the use of bovine thrombin resulted in life-threatening coagulopathies.[3] Endogenous activation is done by using nonactivated platelet-rich plasma (PRP) and allowing local tissue factors to initiate the process. Some authors used activated platelets whereas some used platelets without prior activation and arguing for the better results.[4] As soon as PRP is administered, the platelets are automatically released and ready to exert their function, so such aggregators may not be necessary. As collagen is present in the soft tissue and is a natural activator, exogenous activation is not required when PRP is used in the soft tissue.[5] There is no any recommendation regarding the platelet activation method and use of an agonist to prepare PRP.[6]

Within 10 minutes of platelet activation, 70% of their stored growth factors is secreted. Within the first hour, it reaches near to 100%. Until their depletion and death, for about 8 days, platelets can synthesize more amounts of growth factors. Therefore, it is suggested to clinicians not to activate or clot PRP in advance.[7]

Biochemical Milieu

Biochemical milieu after platelet activation and the resulted actions are related to multiple physiologic signaling mechanisms. Activation of platelets resulted in degranulation and release of platelet-derived growth factor (PDGF), transforming growth factor β1(TGF β1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and insulin-like growth factor 1 (IGF1). These growth factors help in chemotaxis, cell differentiation and proliferation (fibroblast, myoblast, mesenchymal, and epithelial cells production and proliferation), synthesis of proteoglycans and extracellular matrix, synthesis of collagen, osteoid production, hair growth promotion, and angiogenesis. These growth factors also stimulate synovial fibroblast and increase hyaluronic acid synthesis. Inhibition of nNFKB gene activation by the interleukin 1 receptor antagonist involves in inflammatory and apoptosis pathways. The cellular response is prevented when mediators such as soluble receptors of TNF-R1 bind to its component TNF-α. Balance between synthesis and degradation of proteoglycans and increase in chondrocyte proliferation are taken care by insulin-like growth factors, PDGF and TGF-β1.

An endogenous internal signal protein pathway is activated when growth factors are attached to the transmembrane receptors (expressed by mesenchymal cells, fibroblasts, osteoblasts, epidermal, and endothelial cells), resulting in expression of a gene that involves in the above-mentioned functions.[8–12]

PRP

Platelet-rich plasma is defined as a portion of the plasma fraction of autologous blood with a platelet concentration above the baseline (before centrifugation).[13] Platelet-rich plasma contains cell adhesion molecules like fibrin, fibronectin, and vitronectin, needed for cell migration seen during osteoconduction, osseointegration, and epithelialization of wound. The level of these molecules in PRP is same as in normal blood clot (200–400 μg/mL). It also contains full complement of clotting factors at their normal, physiologic levels.[14] Initiation of healing and hemostasis are the two main functions

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of platelets. They take part in physical blood clot formation and thus not present in serum. Therefore, anticoagulated blood is require to produce PRP. Ethylenediaminetetraacetic acid (EDTA) was found to damage platelet membrane so not recommended. Metabolic needs and undamaged separation of viable platelets can be achieved by addition of anticoagulant citrate dextrose-A (ACD-A). The citrate is bound with calcium for anticoagulation. Buffering is done by dextrose. Other ingredients also support platelet metabolism. In citrate phosphate dextrose (CPD), there is less supportive ingredients for platelet metabolism. It can also be used to form PRP but is said to be 10% less effective in maintaining the viability of platelets.[15]

Use of PRP

In 1970s, PRP transfusion was done to treat thrombocytopenia.[16] Later on, after 10 years, it was used in maxillofacial surgery as platelet-rich fibrin (PRF).[17] Since then, use of PRP is widely studied in maxillofacial, cardiovascular, pediatric, ophthalmic, and plastic surgery, musculoskeletal and sports medicine, hip and knee osteoarthritis, rotator cuff pathology, epicondylitis, tendinitis, fracture healing, back pain, etc.[18] More recently, dermatologists are using PRP as a tissue regenerator in wound healing and revision of scar as well as in alopecia. A pro-inflammatory biochemical environment and high protease activity decreasing effective GF concentration in chronic ulcers impair its healing. Injection of PRP in dermis and subdermis induces augmentation of soft tissues and fibroblasts, deposition of newer collagen, and formation of newer blood vessels and adipose tissue.[19] Its use also showed improvement of burn scars, postsurgical scars, and acne scars with increase in collagen and elastic fibers ultimately improving the quality of skin.[4,20,21] Also, use of PRP in androgenic alopecia or alopecia areata for promoting hair growth and lengthening the anagen phase has been studied but lack of randomized controlled trials (RCTs) was observed by recent meta-analysis.[22,23] It can also be used in various forms, as a biological membrane, as a spray on the soft tissue surface, or as a mixture with the bone graft. It can be layered in or applied on the top of a graft.

Gato-Calvo et al.[24] not only suggested to explore allogenic PRP and its safe use (by eliminating its allergenic potentiality) but have also raised concern for regulatory issues to make it easily available in larger number of patients with hematological disorders. They concluded that role of PRP in osteoarthritis is still an open debate if clinical efficacy is not further standardized.

Once formed, in the anticoagulated state, it remains stable and sterile for 8 hours. Therefore, this property makes its use effective even in prolong surgeries. It is easy to prepare and administer, and well tolerated with almost negligible side effects or complications. It is said to be less aggressive than intraarticular corticosteroid injection or surgery.[25,26]

Preparation Techniques

There are two techniques to prepare PRP:[4]

- Open technique: Exposure of the product to the working environment with contact to different materials like pipettes or product-collection tubes. Product contamination during microbiological handling must be guaranteed.
- Closed technique: Commercial devices with CE marking involving centrifugation is used and thus environmental exposure to product is not there (recommended).

Working Definition

The concentration of platelets in PRP varies in many studies that ranges from 2.5 to 8 times above the baseline value. It ranges from 2.67 to 7.4 times in the available literatures.[6]

It is difficult to quantify a fold increase in the plasma platelet concentration above the baseline because platelet concentration in PRP may increase more fold in individual with low platelet count when compared to individual with high platelet count.[5] However, the ideal concentration of platelets in PRP is not yet defined properly. The PRP platelet concentration of 1,000,000 platelets/μL is scientifically proven to enhance bone and soft tissue healing; same concentration of platelets in a volume of 5 mL plasma is the working definition of PRP today.[15] Enhancement of wound healing cannot be relied upon lower concentrations. Similarly, further enhancement of wound healing has not been shown by more concentrations yet. Also, it is found that age and gender have no significant alteration in platelet concentration in PRP, but it does affect with hematocrit and total platelet count.[27,28]

In addition to concentration, considering other parameters is equally important. Like, which type of PRP should be used in different pathologies is defined by the presence or absence of leukocytes and their activation.[4] Preparation methods, collected whole blood volume, relative force and time of centrifugation, and single or double centrifugation spins all affect the concentration and quality of PRP. Numerous protocols have been there in the literature describing the optimal conditions for centrifugation to get the best platelet yield. However, there is no general consensus for which method is superior, how many number of centrifugations required, or at what speed and how long should be the duration of spin.[6]

Preparation Methods

These include single and double centrifugation, blood selective filtration procedures, and manual or automatic systems operated in open or closed circuits. By centrifugation method, PRP is prepared by differential centrifugation either by the PRP method or by the buffy-coat method.[29]

In the PRP method, red blood cells (RBCs) are separated by initial centrifugation with soft spin, which is then followed by a second high-speed centrifugation (hard spin) to concentrate platelets. With this technique, upper two-third is platelet-poor plasma (PPP) and lower one-third is PRP. At the bottom, platelet pellets are formed.

In the buffy-coat method, whole blood with anticoagulant undergoes the first high-speed spin (hard spin) step at constant acceleration to separate RBCs from the remaining blood volume. Three layers formed. Platelets and WBC are present in the upper layer, the middle thin layer is rich in WBCs, and a buffy coat and a bottom layer consisting mostly of RBCs. The upper layer and the superficial buffy coat are transferred to the other tube to produce pure PRP (P-PRP). The whole buffy-coat layer and few RBCs are transferred to produce leukocyte-rich PRP (L-PRP). Then, the soft spin is performed to help formation of soft pellets (erythrocyte-platelet) at the bottom of the tube. Platelet-poor plasma at the upper portion of volume is removed. Pellets are homogenized at lower one-third (5 mL of plasma) to produce PRP. With only 10 mL of whole blood volume, the buffy coat formed as a very thin layer, which causes difficulty to separate it from the underlying RBC layer.

Immediately after centrifugation, concentrated platelets will slowly diffuse into the PPP reducing the total amount of platelets in prepared PRP. So, PRP must be separated from the PPP as early as possible.

Attempt to prepare PRP with a single spin may not produce a true PRP. Instead, it produces the mixture of PRP and PPP with very low platelet counts. As RBC will interfere during fine separation, single spin cannot adequately concentrate platelets regardless of their rate and time. Single spin centrifuges are designed especially for the diagnostic purpose and not for PRP production. Many such machines are not approved by the FDA as these produce insufficient platelet yield, damage platelets, and not using pyrogen-free test tubes. But, some studies showed single spin to be better whereas other showed double spin to be better. Same PRP separation systems with multiple samples from same individual also showed difference in the growth factors' content.[6] Various protocols for the platelet yield were extensively summarized in few of the studies.[4,6,29,30]

Nowadays, numerous commercial PRP kits are available. The cost of PRP kits, prepared from centrifugation based in the closed system, is expensive and varies from US$300.00 to US$1500.00. The task to assess which kit is better for PRP preparation is very difficult as the ability to collect and concentrate platelets, method, and centrifugation time varies with their systems. Recently, in search of low-cost and quality PRP, a "turn down-turn up PRP protocol with double spin and closed system" was developed. Author claimed that it cost only US$10.00 (excluding equipment and lab personal expenses) and is similar to current quality PRP preparation standards of clinical trials, suggested by the American Academy of Orthopedic Surgeons that also presented the minimum information for studies evaluating biologics in orthopedics (MIBO) statement.[30]

The Turn Down-Turn Up PRP Protocol - Double Spin-Closed System[30] is as follows:

- About 8.5 mL of venous blood is collected into a vacuum tube with 1.5 mL of acid citrate dextrose (ACD).
- Remaining vacuum in the tube is equalized.
- With the tube cap facing down, it is centrifuged at 200 "g" for 15 minutes.
- Remove the tube from machine, and maintain it in the downward position without any turn.
- Maintaining sterility, aspirate 3.5 mL of the hematic layer through cap.
- The tube is turn upright with the cap facing up.
- The tube with lid up is centrifuged at 1600 "g" for 10 minutes.
- About 3.5 mL of the upper portion of the material (PPP) is aspirated.
- About 1–2 mL of the lower portion of the tube (PRP) is aspirated.

Classifications of PRP

Studies on classification of PRP have been done extensively.

Dohan Ehrenfest et al., in 2009, classify PRP on basis of its cell and fibrin.[31] It was largely cited, advocated, and validated by a multidisciplinary consensus conference published in 2012.[32] It is categorized as:

- Pure platelet-rich plasma (P-PRP) or leukocyte-poor PRP products (absence of leukocytes but with the low-density fibrin network after activation), e.g., cell separator PRP, Vivostat PRF, or Anitua's PRGF
- Leucocyte and PRP (L-PRP) products (presence of both leukocytes and low-density fibrin network after activation), e.g., Curasan, Regen, Plateltex, SmartPReP, PCCS, Magellan, or GPS PRP
- Pure platelet-rich fibrin (P-PRF) or leukocyte-poor platelet-rich fibrin (absence of leukocytes but with high-density fibrin network), e.g., Fibrinet
- Leucocyte and platelet-rich fibrin (L-PRF) or second-generation PRP products (presence of both leukocytes and high-density fibrin network), e.g., Choukroun's PRF

In 2012, Mishra et al.[33] classify PRP based on:

- Absolute platelet concentration (P)
- Activation method
- Presence or absence of WBCs and neutrophils relative to baseline

Classification on basis of Leucocytes concentration:[6]

- Leukocyte-rich PRP preparation: Concentration of WBC greater than 100% that of whole blood.
- Leukocyte-poor PRP preparation: Concentration of WBC lesser than 100% that of whole blood

The platelets, activation method and white blood cells (PAW) classification system for PRP was also produced depending on absolute concentration of platelet, activation method, and presence or absence of WBCs.[34] It is categorized as follows:

- Increased WBCs and no activation
- Increased WBCs and activated
- Minimal/no WBCs and no activation
- Minimal/no WBCs and activated

Platelet enrichment factor-PRP with platelet concentration: (1) more or equal to five times baseline, (2) less than five times the baseline.

Many of these previous classifications had not included the obtained final volume of PRP, amount of platelets, and the presence or absence of RBCs in it.

The Dose, Efficiency, Purity, Activation (DEPA) classification was proposed by Magalon et al.[35] in 2016 that focuses on platelets quantity in PRP kits, product purity, and preinjection platelet activation, the detail of which is as follows:

- Dose of injected platelets (platelet concentration in PRP is multiplied by the obtained PRP volume). It is divided as:
  - Very high dose (>5 billion)
  - High dose (3–5 billion)
  - Medium dose (1–3 billion)
  - Low dose (<1 billion)
- Production efficiency (percentage of recovered platelets in PRP from the blood). It is divided as:
  - High device efficiency (>90% recovered)
  - Medium device efficiency (70–90% recovered)
  - Low device efficiency (30–70% recovered)
  - Poor device efficiency (<30% recovered)
- PRP purity (correlates to the relative composition of platelets, WBCs, and RBCs in the obtained PRP). On comparing the platelet with RBCs and leukocytes, percentage of platelets in PRP is obtained. It is categorized into:
  - Very pure PRP (presence of >90% platelets)
  - Pure PRP (presence of 70–90% platelets)
  - Heterogeneous PRP (presence of 30–70% platelets)
  - Whole blood PRP (presence of <30% of platelets)
- Process of activation (activation if done using exogenous clotting factor like autologous thrombin or calcium chloride).

Above latest classification is thought to cover the previously missing points. However, the quantity is not easy to define by the clinicians. Registration for quantification is required to be done in each CE medical device used to prepare PRP.[6]

Conclusion

Various protocols have been used for PRP preparation. Many factors influence its composition. Considering its preparation methods, techniques, volume of whole blood requirement, centrifugation spins, platelet activation process, presence or absence of RBCs and leukocytes and their numbers, type of growth factors and their effective levels in PRP, PRP volume needed in different pathologies, and many more such factors, no any general consensus could be made so far. Many clinical trials are required to formulate the standard protocol, guidelines, or the recommendations for safe and effective use.

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