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# Platelet Concentrates - From Platelet Rich Plasma (Prp) And Platelet Rich Fibrin (Prf)

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**Abstract**
The efficacy of platelet concentrates in wound healing and the concentration of growth factors found within these platelets are the basis for the use Platelet rich plasma (PRP) and Platelet rich fibrin (PRF) F in tissue regeneration. The platelets collected in PRP are activated by the action of thrombin and calcium chloride while in PRF, no anticoagulant is used. The 3-dimensional fibrin network formed depends on the speed of the polymerization process which in turn governs the cellular migration and healing process.

**Key Words**
Platelet Rich Plasma, Platelet Rich Fibrin, Fibrinogen

## Introduction

Biological interventions in regenerative medicine fall into four main categories including gene therapy, tissue engineering, cell-based therapies, and platelet rich plasma (PRP) therapies, with different success in clinical translation. The process of wound healing is a complex and under explored area that deals with many cell types and growth factors. After bleeding, platelets begin to form a stable blood clot, releasing a variety of growth factors that induce and support healing and tissue formation. Administration of these growth factors may be combined with tissue regeneration techniques in the repair of hard and soft tissues. Fibrin glues and adhesives are well documented as natural biologic haemostats but pose a huge drawback of viral contamination due to which platelet concentrates are used, in lieu of fibrin glues.

One of the recent achievements in dental clinical research is the use of platelet concentrates to produce autologous fibrin adhesives such as Platelet rich plasma (PRP) and Platelet rich fibrin (PRF). These platelet concentrates obtained from the peripheral blood contains several growth factors and other cytokines that promote tissue regeneration. Natural human blood clot contains 95% red blood cells (RBCs), 5% platelets and less than 1% white blood cells (WBCs). It has now been established beyond doubt that platelet concentrates not only plays a role in controlling bleeding conditions like thrombocytopenia, severe oral haemorrhage associated with acute leukaemia, but also contains growth factors that stimulates healing of hard and soft tissues.[1] These fibrin adhesives can be derived autologously from the patient or can be obtained commercially, but with a small risk of disease transmission for the later one.

This article aims to discuss the platelet concentrates and their evolution from PRP to PRF along with the growth factors.

## Platelet Rich Plasma

PRP was first developed in the 1970s and first used in Italy in 1987 in an open heart surgery procedure by M. Ferrari.[2] PRP therapy began gaining popularity in the mid 1990s. It has since then been applied to many different medical fields such as cosmetic surgery, dentistry, sports medicine and pain management. Whitman et al first introduced it in dentistry in 1997.[3] It is such a talked about technology that there were numerous names to PRP like platelet concentrates (PC), autologous growth factors (AGF), plasma rich in growth factors (PRGF), platelet gel (PG), platelet rich fibrin matrix (PRFM) etc. PRP, an autologous plasma fraction of peripheral blood, is the simplest regenerative medicine intervention that is rapidly extending to multiple medical fields mainly due to the ease of use and bio safety that facilitates translation in humans. PRP is a component of blood in which the platelets are concentrated in a limited volume of plasma. Platelets are cytoplasmic fragments of the megakaryocyte in the bone marrow. A variety of molecules are stored in platelets granules either synthesized by their parent cell the megakaryocyte or captured in the circulation. In PRP technologies platelets are used because of their capability to function as vehicles for growth factors and cytokine delivery. Once platelets have adhered to injured vessels (by collagen), they release granules containing serotonin, thromboxane and adenosine to start the clotting process, which in turn leads to the formation of fibrin. PRP works via degranulation of the granules in platelets, which contains the synthesized and pre-packed growth factors. It is used to release growth factors like platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), insulin like growth factor 1 (IGF-1), platelet activating factor-4 (PAF-4), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) to the surgical site, that helps in wound healing and stimulate bone regeneration.[4] The major effects of PRP are derived from PDGF, which has been identified as an important protein for hard and soft tissue healing. A subfamily of TGF, is bone morphogenic protein (BMP). BMP has been shown to induce the formation of new bone in research studies in animals and humans. By adding PRP, and thus BMP, to the implant site with bone substitute particles, the

bone can be grown more predictably and faster than ever before. In particular, growth factors regulate cellular events in wound healing, such as proliferation, differentiation, chemotaxis and morphogenesis of tissues and organs which helps in tissue repair and regeneration. They are deposited in the extracellular matrix and are then released during matrix degradation. Their interaction with surface receptors on the target cells activates an intracellular signalling pathway that induces transcription of the messenger RNA and proteins needed for the regenerative process. These in turn causes the expression of a normal gene sequence of the cell such as cellular proliferation, matrix formation, osteoid production etc. Thus PRP acts through the stimulation of normal healing. A PRP blood clot contains 4% RBSs, 95% platelets and 1%WBCs.[5]

**Technique of PRP Preparation**

PRP preparation involves the collection of the patient's whole blood (that is anticogaulated withcitarte dextrose) before undergoing two stages of centrifugation designed to separate the PRP aliquot from platelet poor plasma and red blood cells.[6] In humans, the typical baseline blood platelet count is approximately 200,000 per uL; therapeutic PRP concentrates the platelets by roughly five-fold.[7] There is, however, broad variability in the production of PRP by various concentrating equipment and techniques.[8]

Venous blood is taken with anticoagulant to avoid platelet activation and degranulation. Most frequently used anticoagulant is sodium citrate which chelates calcium ions, in doing so preventing proR08; thrombin conversion into thrombin. Other anticoagulants (i.e. heparin, EDTA) are avoided because they may compromise platelet stability and activation. Sodium citrate makes the plasma acidic and some protocols recommend buffering the PRP back to a physiologic range prior to injection. Importantly, PRP activation is needed to induce the secretion of granule contents i.e. platelet secretome. This occurs spontaneously in blood but is inhibited if the blood is withdrawn in tubes containing anticoagulants. Reversion of anticoagulants inhibition of coagulation and platelet activation can be achieved by several procedures. One possibility is the addition of calcium or thrombin/Ca2+ to cleave fibrinogen with subsequent polymerization of fibrin monomers. Alternatively, physiological activation can be achieved by injecting the inactivated PRP, which once in contact with collagen and other tissue factors will get activated.

The first centrifugation of the two stage centrifugation process (''hard spin'') separates the blood in 3 layers. The bottom layer is of RBCs which makes up 55% of total volume. The top layer is the acellular plasma layer which is made up fibrinogen and is low in platelets. It is called platelet-poor plasma (PPP) and constitutes 40% of total volume. Below this is an intermediate layer rich in platelets (buffy coat). It constitutes only 5% of total volume. This will contribute to PRP. At this stage, the top and the intermediate layer (composed of PPP, PRP) along with some RBCs are aspirated using a sterile syringe. This aspirant is then subjected to second spin (soft spin) which is longer and faster. Again 3 layers are formed. At the bottom of the tube, some residual RBCs are trapped. Above it is the buffy layer of PRP and at the top, the acellular plasma (PPP). The top layer of PPP is aspirated and discarded leaving behind PRP **(Figure 1)**. PRP is then mixed with bovine thrombin and calcium chloride at the time of use which helps in gelation of PRP. Calcium chloride nullifies the action of anticoagulant and thrombin converts firbrinogen to fibrin. [9]

**Safety concerns in relation to PRP which resulted into development of PRF**

PRP is inherently safe because of its autogenous preparation, utilizing the patient's own blood in a significantly small quantity. For this reason, it is safe and there have been no published references relating to the risk of infections, disease transmission (such as HIV, hepatitis, or Creutzfeldt-Jacob disease), immunogenic reactions or any other adverse effects which exist with allografts or xenografts. But the use of bovine thrombin for gel formation after isolation of PRP may be associated with the development of antibodies to the factor V, XI and thrombin, resulting in the risk of life threatening coagulopathies. Marx et al[7] in their article stated that the second set of bleeding episodes in the patients who developed coagulopathies were not due to antibodies against bovine thrombin but instead due to antibodies that developed to bovine factor Va that was a contaminant in certain bovine thrombin commercial preparations.

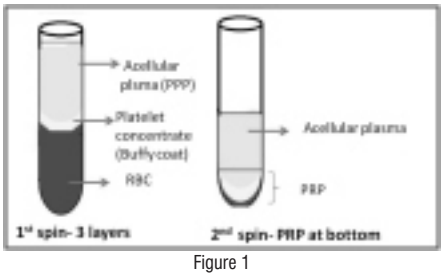

Figure 1

However recombinant human thrombin, autologous thrombin or perhaps extra-purified thrombin can be used as alternate modes for preparation of PRP. PRP has been used in conjunction with different grafting materials in bone regeneration procedures, but the results are controversial and need more studies to prove its efficacy for the same. PRP can be prepared either from autologous or allogenic source. Majority of studies documented have used autologous platelets preparations as they are more acceptable to the patient and carry lower risk of transmission of viral infections.

**Contraindications**

Certain absolute contraindication associated with use of PRP are Platelet dysfunction syndrome, Critical thrombocytopenia, Hemodynamic instability, Septicemia, Local infection at the site of the procedure, Patient unwilling to accept risks. Relatively fewer conditions can Contradict its use like- Consistent use of NSAIDs within 48 hours of procedure, Corticosteroid injection at treatment site within 1 month, Systemic use of coriticosteroids within 2 weeks, Tobacco use, Recent fever or illness, Cancer, especially hematopoetic or of bone, HGB < 10 g/dl, Platelet count < 105/ul.

**Platelet Rich Fibrin**

PRF was first developed in France by Choukroun et al.[10] for use in oral and maxillofacial surgery. Choukroun's platelet-rich fibrin (PRF) is a leukocyte and platelet rich fibrin biomaterial with a specific composition and three-dimensional architecture. PRF is classified as a second generation platelet concentrate as it is prepared as a natural concentrate without the addition of any anticoagulants. PRF is often called Choukroun's PRF as there are other platelet concentrates with similar names such as Vivostat PRF (considered a pure platelet-rich plasma) or Fibrinet PRF (without leukocytes). PRF has a dense

fibrin network with leukocytes, cytokines, structural glycoproteins and also growth factors such as transforming growth factor β1, platelet-derived growth factor, vascular endothelial growth factor and glycoproteins such as thrombospondin-1 during l78; 7 day. Leukocytes that are concentrated in PRF scaffold play an important role in growth factor release, immune regulation, anti-infectious activities, and matrix remodeling during wound healing. The slow polymerization mode of PRF and cicatricial capacity creates a physiologic architecture favorable for wound healing.It is a fibrin matrix in which platelet cytokines, cells and growth factors may be released after a certain time and that can serve as a resorbable membrane. It has been shown to have several advantages over traditional PRP. Its main advantages include no biochemical handling of blood, simplified and cost-effective process, no use of bovine thrombin and non requirement of anticoagulants, favourable healing due to slow polymerization, more efficient cell migration and proliferation. PRF also has supportive effect on immune system and helps in haemostasis.

**Technique of PRF Preparation**

Venus blood is collected and placed in a centrifuge at 3000 rpm for 10 minutes after which the following 3 layers are formed- top layer of straw coloured acellular plasma, middle layer of PRF and a bottom layer containing RBCs. The top layer is then removed and the middle portion is collected 2 mm below the lower dividing line which is PRF **(Figure 2)**. Since, there is no anticoagulant present, the coagulation process starts almost immediately when the blood comes in contact with the tube. So the success of this process lies in the speed with which the blood is collected and transferred to the centrifugal machine. Initially fibrinogen is in the upper part of the tube but when it comes in contact with thrombin, fibrin is formed in the middle layer in between RBCs at the bottom and the acellular plasma at the top. PRF can be obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.

Although PRF belongs to a new generation of platelet concentrates, it is in the first place, a fibrin technology. This is because of the gelling mode. PRP uses bovine thrombin and calcium chloride for coagulation. So there is sudden fibrin polymerization which leads to tetra-

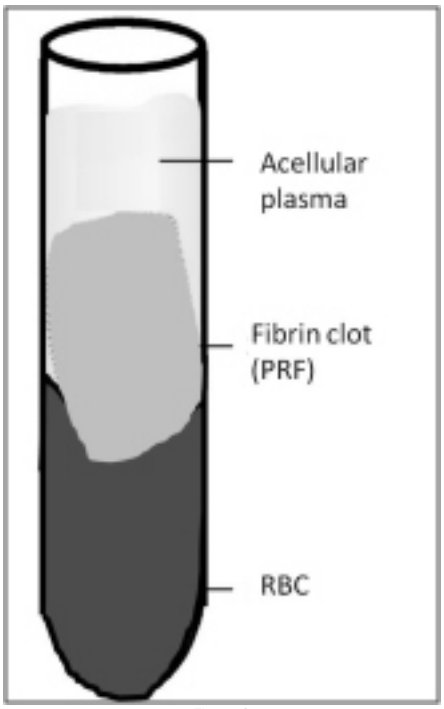

Figure 2

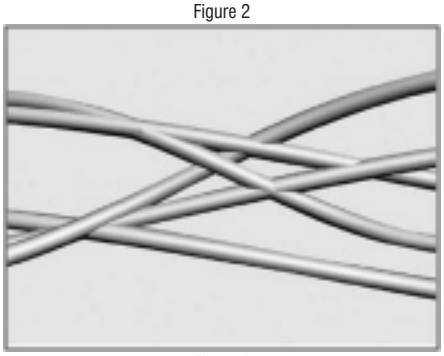
Figure 3

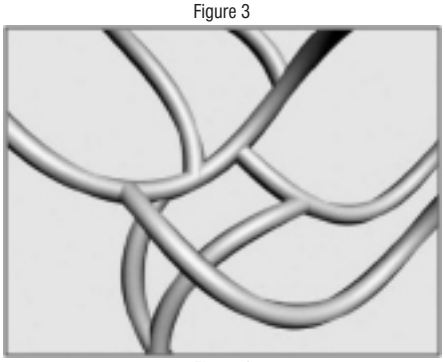
Figure 4

molecular or bilateral junction type of organization of fibrin network which is a rigid fibrin network not very favourable to cytokine enmeshment and cellular migration **(Figure 3)**. On the other hand, PRF has a characteristic of polymerizing naturally and slowly during centrifugation. So, a tri-molecular or equilateral junction type of organization of fibrin network is formed which is flexible and elastic and supports cytokine enmeshment and cellular migration **(Figure 4)**.

**Clinical Applications**

PRP has become a valuable adjunct to promote healing in many procedures in dental and oral surgery. These include - healing the alveolar socket after tooth extraction,surgical repair of the alveolar cleft, treatment of infrabony periodontal defects and periodontal plastic surgery, as well as procedures relating to the placement of osseo-integrated implants. In such procedures, the adhesive nature of PRP facilitates the easier handling of graft material, with more predictable flap adaptation and hemostasis, and a more predictable seal than is the case with primary closure alone. Recently, the use of PRP has also been proposed in the management of bisphosphonate-related osteonecrosis of the jaw (BRONJ) or avascular necrosis, which is caused by other factors (e.g. radio-osteonecrosis), with the aim of increasing wound healing and bone maturation.

Aging patients are usually the elective patients for PRP procedures. Such patients are generally considered as special needs patient, requiring stimulation for healing; age is considered an important determinant of tissue repair, which is the main cause of delayed healing. Moreover, elderly patients are mostly subject to systemic diseases which influence their response to postsurgical healing in terms of coagulation and tissue repair. The improvement in quality of life of aging patients in recent decades has increased the demand for longevity of dental health.

**Advantages of PRP and PRF**

They are smeared thickly on the wound after the procedure by the dentist and actually seal the wound away from infectious agents, lowering the risk of problems. The saturation of the wound with PRP & PRF helps increase tissue synthesis due to its growth factors, and this in turn results in faster tissue regeneration. Speedier healing decreases the risk of later infections, complications, and discomfort. Since PRP harvesting is done in the doctor's office, the patient need not incur the expense of the harvesting procedure in hospital or at the blood bank. Disease transmission is non-issue since the blood is harvested from the patient's own blood supply. The amount of blood needed is small and can be collected during a routine outpatient procedure. PRP is easy to handle and actually improves the ease of application of bone substitute materials and bone grafting products by making them more

gel-like.

**Limitation in platelet concentration research**

Even though biotechnology has lead us to extensively use platelet concentrations inwound healing and tissue repair, there is still lack of evidence to support. Blinded, multicentric, randomized controlled studies with large sample sizes to establish their therapeutic efficacy is the need of hour. There are no uniform regulatory norms established for collection, techniques for preparation and application of platelet concentrates across the world. In addition, the threshold dose required for therapeutic benefit is also undefined for different clinical indications. Hence, guidelines for the same need to be laid down by regulatory authorities to ensure its rational use. Although no adverse effects have been reported till date, patient safety still remains a major concern. All this emphasizes the role of transfusion medicine specialty in assuring quality, consistency, and safety of the product. It is however necessary to have more understanding of all the components.

**Conclusion**

Platelet concentrates are going to be a boom in tissue regeneration in a few decades. PRP research may eventually lead to superior therapies. Also the reduction in treatment time and cost effectiveness analysis comparing the potential economic benefit of PRP to alternative therapies in tissue repair and regenerations, should impact clinical and financial decisions.Platelet-rich plasma (PRP) and PRF are new approaches to tissue regeneration and they are becoming a valuable adjunct to promote healing. Studies conducted on humans have yielded promising results regarding the application of PRP and PRF to many dental and oral surgical procedures (i.e. tooth extractions, periodontal surgery, and implant surgery).

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