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RESEARCH ARTICLE

PLATELET-RICH FIBRIN- SECOND GENERATION PLATELET CONCENTRATE; AND ITS CLINICAL APPLICATIONS – A LITERATURE REVIEW

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ABSTRACT

Wound healing process involves the activity of leukocytes and platelets. The growth factors present in platelets are important to guide the regenerating cells to the area of healing. After the introduction of regenerative potentiality of platelets in 1974, various platelet concentrates platelet rich plasma (PRP) and platelet rich fibrin (PRF) were used as regenerative medicine preparations. Platelet rich fibrin (PRF) is an autogenous biomaterial consisting of growth factors and cytokines entrapped in a fibrin matrix. It combines the fibrant sealant properties along with growth factors thereby providing an ideal environment for wound healing and regeneration of tissues. PRF is produced with a simple method, low cost and easily available, which has been applied in different fields of dentistry, particularly oral and maxillofacial, orthopaedic and plastic surgery. The following review attempts regarding the technique of using PRF, focusing on its preparation, advantages, and disadvantages of using it in clinical applications.

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INTRODUCTION

Development of the bioactive surgical additives is one of the great challenges of clinical research which has been used to regulate inflammation and increase the speed of healing process (Balaram Naik *et al.,* 2013; Dohan *et al.,* 2006). A wide range of intra and extra articular events and various signaling proteins mediate and regulate the healing process of both hard and soft tissues. But understanding this entire process is still incomplete; however, it is known that platelets play a crucial role not only in hemostasis, but also in the wound healing process (Gassling *et al.,* 2009). In 1974, regenerative potentiality of platelets was introduced by Ross *et al.*, 1974 and described a growth factor from platelets. After activation of the platelets which are trapped within fibrin matrix, growth factors are released and stimulate the mitogenic response in the bone periosteum during normal wound healing for repair of the bone (Balaram Naik *et al.,* 2013; Vivek Gupta *et al.,* 2011; Gassling *et al.,* 2010) Healing of wound is initiated by clot formation and inflammation, followed by a proliferative stage which comprises of epithelialization, angiogenesis, granulation tissue formation and collagen deposition and finally collagen maturation and contraction. Growth factors are mitogenic (proliferative), chemotactic (stimulate directed migration of cells) and angiogenic (stimulate new blood vessel formation). Therefore, they appear to be critical to the wound-healing process (Megha Agrawal and Vineet Agrawal, 2014; Kanakamedala *et al.,* 2009). Platelet-Rich Fibrin (PRF) consists of a strictly autologous fibrin matrix rich in platelets, leukocyte cytokines, and various growth factors. This concept was described in France by Choukroun in 2001 (Eduardo Borie *et al.,* 2015), and it is a second-generation platelet concentrate used for its ability to enhance tissue repair and regeneration. It was introduced as a replacement for the Platelet-Rich Plasma (PRP), also known as the first-generation platelet derivatives, as it is simple, safe compared to PRP.

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PLATELET AND ITS CONCENTRATES – HISTORY AND EVOLUTION

Platelets, also referred to as thrombocytes, are a component of mammalian whole blood. Platelets are derived from bone marrow megakaryocytes, are discoidal anucleate cytoplasmic fragments, and are on average 2-3 μm in diameter. They are not actually true cells, but are circulating cell fragments. A normal platelet count for an adult human is 1.5-4 lakhs per μL in peripheral blood, and the average lifespan of a platelet is approximately 8-10 days. They contain more than 30 bioactive proteins, many of which have a fundamental role in hemostasis or tissue healing and are a natural source of growth factors (El-Sharkawy *et al.,* 2007; Albanese *et al.,* 2013). Seven

fundamental protein growth factors that are actively secreted by platelet initiate all wound healing process .During tissue repair, platelets that have been activated will release two different types of granules, the dense and α-granules (Eduardo Borie *et al.,* 2015). The dense granules have serotonin, adenosine triphosphotase, adenosine diphosphotase, and calcium. Platelets also contain α-granules, a few mitochondria, and two prominent membrane structures. The α-granules are spherical or oval structures with diameters ranging from 200-500 nm, each enclosed within a membrane. These α-granules activate signaling proteins, which ultimately result in gene expression for cellular proliferation, matrix formation, collagen synthesis, and osteoid production (Albanese *et al.,* 2013; Anitua *et al.,* 2006; Sanchez *et al.,* 2013). Growth factors stored in the α-granules of platelets include platelet derived growth factor, insulin-like growth factor, vascular endothelial growth factor, and transforming growth factor-β and exhibit chemotactic and mitogenic properties which assist in promoting cell proliferation and modulating cell functions that promote tissue healing and regeneration. Platelet concentrates have been in use for the past 30 years, were first utilized in transfusion medicine for the prevention of hemorrhage induced by severe thrombocytopenia and its use stems from the ability of the fibrin glue to enhance healing. Fibrin, the activated form of the fibrinogen, plays a determining role in the platelet aggregation during hemostasis.

Function and role of fibrin matrix

- ***Role of Fibrin in Angiogenesis***: Entrapment of cytokines in the 3-dimensional architecture of fibrin matrix results in their sustained release which is monumental in initiation of angiogenesis. The cytokines responsible for this action include the Fibrin growth factor (FGF), vascular endothelial growth factor (VEGF), angiopoietin, and Platelet derived growth factor (PDGF) within the fibrin gel. It is the rigidity of the fibrin matrix that is instrumental in the process of angiogenesis in response to FGF and VEGF stimulation.
- ***Fibrin Assisted Immune Response.*** Increased expression of CD11c/CD18 receptor on endothelial cells by fibrin aids in enhanced adhesion to endothelial cells and fibrinogen, and transmigration of neutrophils. Fibrin and fibronectin also modulate the wound colonization by the macrophages.
- ***Effect of Fibrin on Mesenchymal Stem Cells***. Fibrin matrix acts as a scaffold for the undifferentiated mesenchymal cells that facilitate the differentiation of these cells thus aiding in tissue regeneration.
- ***Effect of Fibrin on Osseous Tissue***. Direct interaction between fibrin and the osseous tissue lacks significant documentation. However, bone morphogenic proteins enmeshed in fibrin matrix have the ability to be released consistently highlighting the angiogenic, hemostatic, and osteoconductive properties. Consistent release of VEGF, FGF and PDGF helps in angiogenesis. Hemostasis is achieved through the ability of fibrin clot to trap circulating stem cells, allowing vascular and tissue restoration.

Classification of platelet concentrates (Dohan Ehrenfest *et al.*, 2009; Ehrenfest *et al.*, 2014)

This classification system was proposed by Ehrenfest *et al* and was given based on the fibrin architecture and cell content, preservation and activation on these products in dentistry and in various fields of medicine.

- **Pure Platelet-Rich Plasma (P-PRP)** or leucocyte-poor PRP products are preparations without leucocytes and with a low-density fibrin network after activation. (PRGF-Endoret technique)
- **Leucocyte-and PRP (L-PRP)** products are preparations with leucocytes and with a low-density fibrin network after activation. It is in this family that the largest number of commercial or experimental systems exist (such as Biomet GPS system).
- **Pure platelet-rich fibrin (P-PRF)** or leucocyte-poor platelet-rich fibrin preparations are without leucocytes and with a high-density fibrin network. These products only exist in a strongly activated gel form, and cannot be injected or used like traditional fibrin glues (Fibrinet)
- **Leucocyte-and platelet-rich fibrin (L-PRF)** or second-generation PRP products are preparations with leucocytes and with a high-density fibrin network (Intra-Spin L-PRF)

PLATELET RICH PLASMA (PRP)

The Platelet-Rich Plasma (PRP), an autologous product concentrating a large number of platelets in a small volume, was found to be an easily accessible source of growth factors to enhance the bone and soft-tissue healing. platelet rich plasma (PRP) was used as a method of introducing concentrated growth factors platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor 1 (IGF-1) to the surgical site, thereby enriching the natural blood clot in order to hasten wound healing and stimulate bone regeneration (Michael Toffler *et al.,* 2009; Soffer *et al.,* 2003). It differs from fibrin glue and other non-platelet tissue adhesives because of the presence of platelets, which has a unique ability to promote wound healing and enhance osteogenesis. PRP accelerates endothelial, epithelial, and other tissue regeneration, stimulates angiogenesis, supplements collagen synthesis, promotes soft-tissue healing, and enhances the hemostatic response to injury. And since it is an autologous blood product, it has no risk of transmitting infectious disease. A natural human blood clot consists of 95% red blood cells (RBCs), 5% platelets, less than 1% white blood cells (WBCs), and numerous amounts of fibrin strands. A PRP blood clot, on the other hand, contains 4% RBCs, 95% platelets, and 1% WBCs (Sunitha and Munirathnam, 2008). The PRP preparation protocol requires collection of blood with anticoagulant, centrifugation in two steps, and induced polymerization of the platelet concentrate using calcium chloride and bovine thrombin (Marx *et al.,* 1998; Weibrich *et al.,* 2003). PRP has been used in conjunction with different grafting materials in bone augmentation procedures since the day of its introduction.

Comparison of platelet concentrates

Sample	Red blood cells (%)	Platelets (%)	White blood Cellls (%)
Blood clot	95	5	Less than 1
Platelet Rich Plasma (PRP)	4	95	1
Platelet Rich Fibrin (PRF)	2	97	1

Advantages of PRP

- Accelerates postoperative wound healing and tissue repair, rapid revascularization and re-epithelialization.

- Because it is autologous, it eliminates concerns about immunogenic reactions and infectious diseases transmission.

PLATELET RICH FIBRIN (PRF)

PRF is obtained centrifugally by autologous peripheral blood, without adding any biological agents. Its chief advantages include ease of preparation and lack of biochemical handling of blood, which makes this preparation strictly autologous.

Preparation of PRF (Vivek Gupta *et al.,* 2011; Sunitha and Munirathnam, 2008; Anilkumar *et al.,* 2009)

The classical technique for PRF preparation was invented by Dr. Joseph Choukroun in 2000. It is the current PRF technique authorized by the French Health Ministry in which PRF is prepared without using an anticoagulant during blood harvesting. For preparation of PRF, blood sample is collected from the patient without anticoagulant using a butterfly needle and 10 ml blood collection tubes. After collection of blood, it is immediately centrifuged on a table-top centrifuge at a rate of 3000 rpm for 10 minutes. After centrifugation, 3 layers are obtained in the test tube (FIG-1 &2). The topmost layer consisting of a cellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube. The middle layer of PRF clot is then removed with sterile tweezers and separated from the underlying RBC layer using scissors and then transferred on a sterile dish and stored in a refrigerator. It is supposed that the junction of PRF to the RBC layer is rich in growth factors and therefore this region is preserved.

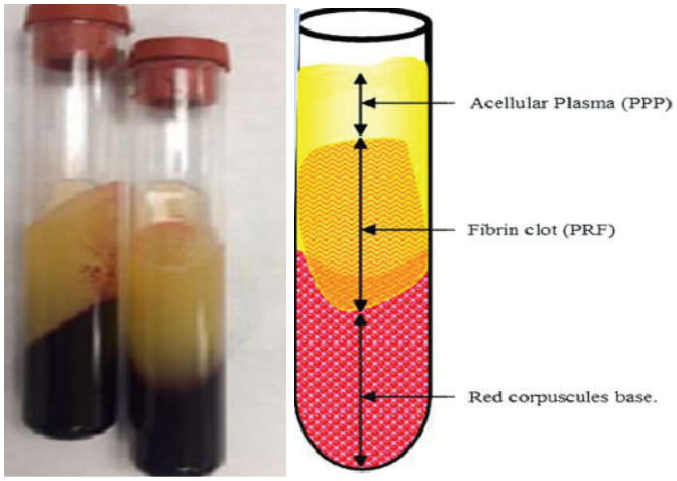

Figure 1. Blood in the vacutainer tubes after centrifugation at 3,000 rpm for 10 min divided into three fractions; lower fraction of red blood cells, middle fraction containing fibrin clot, and upper acellular plasma fraction

Because of the absence of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore, for successful preparation of PRF, speedy blood collection and immediate centrifugation before the clotting cascade is initiated, is absolutely essential. PRF results from a natural and progressive polymerization which occurs during centrifugation. The slow handling of blood to centrifugation process will result in diffuse polymerization of brin leading to the formation of a small blood clot with irregular consistency.

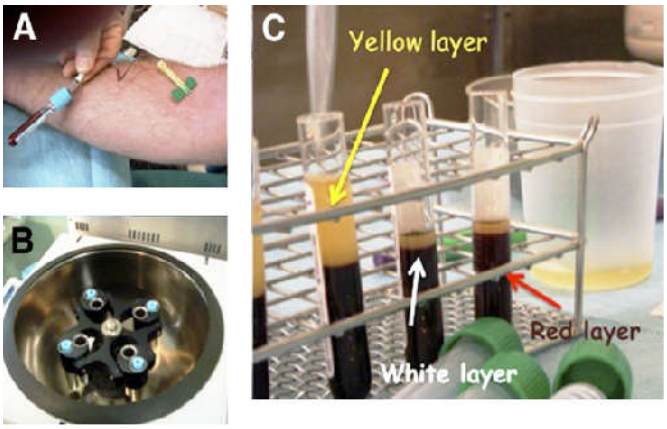

Figure 2. Collection of blood, centrifuge machine and blood in vacutainer tube after centrifugation

PRF Membrane (Michael Toffler *et al.,* 2009; Tolga Fikret Tözüm *et al.,* 2003)

PRF membrane can be obtained by squeezing out the liquids present in the fibrin clot. Liquid removal from the PRF fraction can be done through mechanical pressure between gauze layers resulting in a fairly solid, gel-like material that can be used in various clinical applications as a filling material or as a suturing membrane. PRF membrane can also be prepared by compressing PRF clot in special tools like "PRF Box" (Fig- 3) resulting in standardized membranes of constant thickness and size along with PRF exudate. PRF exudate contains good amount of growth factors, matrix glycoproteins (fibronectin, vitronectin etc.) and proteins specialized in increasing cell attachment to biomaterials and titanium.

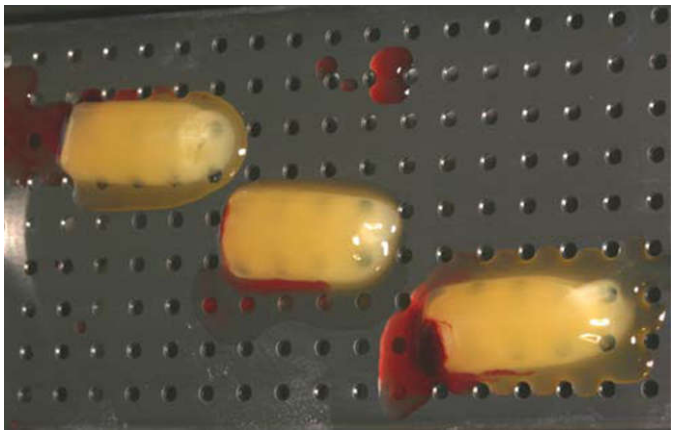

Figure 3. PRF is placed on the grid in the PRF Box

Wound healing consists of three phases (Megha Agrawal and Vineet Agrawal, 2014)

1. Inflammatory phase (1-4 days) (substrate-preparation phase)
2. The proliferation phase (2-22 days) (collagen-building phase)
   - Epithelialization
   - Angiogenesis
   - Granulation tissue formation
   - Collagen deposition
3. Maturation (remodeling phase) (6-12 months)
   - Collagen maturation and contraction

Table 1. Growth factors and cytokines present in PRF and their role in inflammation and wound healing

GROWTH FACTORS & CYTOKINES	
1. Vascular Endothelial Growth Factor (VEGF)	Initiates angiogenesis
2. Fibroblast Growth factor (FGF)	Osteoblast proliferation Enhances wound healing
3. Insulin like Growth Factor -1 (IGF-1)	Osteoblast proliferation Enhances wound healing
4. TGF-β	Proliferation of osteoblasts Collagen type-1 production Woven bone formation Stimulates angiogenesis
5. IL-1	Stimulates T-helper cells, inflammatory Mediator
6. IL-6	B cell differentiation, T cell activator, antibody secretion stimulation, inflammatory and remodeling mediator
7. IL-4	Proliferation and differentiation of activated B cells, moderates inflammation, increases fibroblast synthesis of fibrillary collagen
8. TNF-α	Monocyte activator, stimulate fibroblast remodeling, increase phagocytosis and neutrophil toxicity, modulates IL-1 and IL- 6 expression

These growth factors are released from platelets when activated by a stimulus or aggregated by activators.

Role of PRF in Wound Healing

- Prolonged release of growth factors at the wound site
- Proliferation of fibroblasts and osteoblasts
- Promotes angiogenesis
- Induces collagen synthesis
- Guides in wound coverage
- Mechanical adhesion by fibrin
- Trapping of circulating stem cells
- Regulation of immunity

Advantages of using PRF

- Its preparation is a simplified and efficient technique, with centrifugation in a single step, free and openly accessible for all clinicians.
- It is obtained by autologous blood sample.
- Minimized blood manipulation.
- It does not require the addition of external thrombin because polymerization is a completely natural process, without any risk of suffering from an immunological reaction.
- It has a natural fibrin framework with growth factors within that may keep their activity for a relatively longer period and stimulate tissue regeneration effectively.
- It can be used solely or in combination with bone grafts, depending on the purpose.
- Increases the healing rate of the grafted bone.
- It is an economical and quick option compared with recombinant growth factors when used in conjunction with bone grafts.
- Used as a membrane, it avoids a donor site surgical procedure and results in a reduction in patient discomfort during the early wound-healing period.

Limitations of using PRF (Zhao and Ding, 2001)

- The final amount available after centrifugation is low because it is autologous blood.
- The success of the PRF protocol depends directly on the handling, mainly, related to blood collection time and its transference for the centrifuge.
- Need of using a glass-coated tube to achieve clot polymerization.
- Possible refusal of treatment by the puncture required for blood collection.

Advantages of PRF over PRP (Megha Agrawal and Vineet Agrawal, 2014)

1. Simple and cost effective method of preparation of PRF
2. Eliminates the use of bovine thrombin and thereby reduces the chances of cross infection and coagulopathies
3. Slow natural polymerization of PRF on contact with glass particles of the test tube results in physiologic thrombin concentration, while in PRP, there is sudden fibrin polymerization depending on the amount of surgical additives (thrombin and calcium chloride) (Prakas and Thakur, 2011).
4. Fine and flexible 3-D structure of PRF more favourable to cytokine enmeshment and cellular migration (Prakas and Thakur, 2011).
5. 3-D network-connected tri-molecular or equilateral junctions in PRF allows the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration; while 3-D organization of PRP consists of a fibrin network condensed tetra molecular or bilateral junctions constituted with strong thrombin concentrations which allows the thickening of fibrin polymers leading to a rigid network, not very favourable to cytokine enmeshment and cellular migration.

- PRF has supportive effect on immune system
- The mitogenic effects of PRP are only limited to augmentation of the normal healing process and is theoretically not mutagenic, as the GFs released do not enter the cell or its nucleus, but only bind to the membrane receptors and induce signal transduction mechanisms.
- PRF helps in hemostasis (Naik *et al.,* 2013).

Current applications of PRF in dentistry (Megha Agrawal and Vineet Agrawal, 2014)

In recent times a lot of research has been done on PRF and numerous cases have been reported regarding the use of PRF clot and PRF membranes.

a.Oral and maxillofacial surgery

- Filling in avulsion sockets, bony defects etc.
- Bone augmentation in sinus lifts for posterior maxilla
- augmentation for implants
- Ridge preservation
- Protection and stabilization of graft materials during ridge augmentation procedures.
- Guided bone regeneration
- biomaterial impregnation, rinsing surgical sites, hydration of graft materials and for storage of autologous grafts.

b.Endodontics

- In treatment of open apex in regenerative pulpotomy
  - For regeneration of pulp-dentin complex
  - In combination with MTA to create root end barriers in apexification procedures to prevent extrusion of material
- To fill in bony defect after periapical surgery
- Used in treatment of combined endodontic and periodontic lesion

c.Periodontics

- For treatment of intrabony and furcation defects
- For treatment of gingival recession
- Guided tissue regeneration
- Periapical lesions

d.Tissue engineering (Megha Agrawal and Vineet Agrawal, 2014)

- Study by Gassling et al. reported that PRF appears to be superior to collagen as a scaffold for human periosteal cell proliferation and PRF membranes can be used for in vitro cultivation of periosteal cells for bone tissue engineering. Thus PRF is a potential tool in tissue engineering but clinical aspects of PRF in this eld requires further investigation.

General Applications of PRF

1. PRF promotes dentinogenesis by stimulating cell proliferation and differentiation of dental pulp cells.
2. Application in facial plastic surgery (Zhao and Ding, 2001; Arshdeep *et al.,* 2014);

   - Facial volumization,
   - Superficial rhytides,
   - Acne scars,
   - Androgenetic alopecia,
   - Alopecia areata,
   - Lichen sclerosus (Jeong *et al.,* 2011),
   - Rhinoplasty,
   - Facial esthetic lipostructure,
   - Rhytidectomy,
   - Depressed scar,
   - Dermal augmentation
   - Provide significant long-term diminution of deep nasolabial folds

3. Healing of severe non healing lower-extremity ulcers.
4. Repair of articular cartilage defects and to augment Achilles tendon.

PRF membrane functionalized by incorporation alkaline phosphatase induces the mineralization of PRF. Thus, PRF can also be a suitable material for bone replacement.

CONCLUSION

In vitro and *in vivo* studies have demonstrated safe and promising results, without contradictory findings, related to the use of PRF alone or in combination with other biomaterials. Only a perfect understanding of its components and their significance will enable us to comprehend the clinical results obtained and subsequently extend the fields of therapeutic application of this protocol. It has several advantages and possible indications to be used in various fields of medicine. Currently, platelet-rich fibrin seems to be an accepted minimally invasive technique with low cost.

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