An Update on the Protocols and Biologic Actions of Platelet Rich Fibrin in Dentistry

ABSTRACT

Platelet rich fibrin (PRF) is a surgical biologic additive that is prepared by manipulation of autologous blood. It has now evolved to become one of the most widely used platelet concentrate in dentistry. It has almost replaced Platelet rich plasma (PRP) for usage owing to its advantages such as being 100% autogenous, easy technique, time and cost effectiveness, superior & prolonged growth factor release. It finds varied applications in dentistry including management of gingival recession, for guided bone regeneration in periodontal, peri-implant and endodontic bone defects. Since its inception in 2001 by Choukroun & co-workers, there has been in-depth research regarding its clinical applications, biologic actions, various technique modifications and optimizations. Several modifications of the conventional protocol like the advanced PRF, injectable PRF, PRF lysate and Titanium-prepared PRF. Hence, the aim of this article to review the biological properties of platelet rich fibrin and the advancement in the PRF technology since its inception.

INTRODUCTION

Wound healing is a central concept in regenerative surgical sciences. Wound healing occurs as a result of complex interplay between various cells and signaling molecules. The participating cells include epithelial cells, osteoblasts, fibroblasts and the signals are those released from the platelets from the blood clot including various cytokines and growth factors.[1] Clinicians continuously strive to provide rapid and enhanced healing to the patients. The quest to enhance the natural healing response, leaves us in a constant search for alternatives. In the field of dentistry and oral & maxillofacial surgery, platelet concentrates have been applied since more than half a century. With the increase in our understanding of the biological properties of these concentrates, the initial protocols have undergone immense change. The currently used platelet concentrates are not only much more efficient than their predecessors in the terms of biological efficacy, but are also much less complicated, simple and effective.[2]

In the past decade, second generation platelet concentrates have gained immense popularity as bioactive surgical additives in the field of regenerative dentistry. Among the various platelet concentrates, currently, the most widely used concentrate in dentistry is platelet rich fibrin (PRF). The concept of platelet rich fibrin ushered in an era of completely autologous form of platelet concentrates with no addition of bovine thrombin or anticoagulants. It is prepared by manipulation of patients own blood without any other additives.

Keywords

Blood Platelets
Fibrin
Growth Factors
Leukocytes
Platelet-Rich Fibrin (PRF)
Platelet Concentrates

Authors

Dr Rucha Shah *
(MDS, FICOI)

Dr Triveni M G *
(MG: MDS)

Dr Raison Thomas *
(MDS, FICOI)

Dr Dhoom Singh MEHTA *
(MDS, FICD, FISOI)

Address for Correspondence

Dr Rucha Shah *
Email: srucha2k@yahoo.com

* *Bapuji Dental College & Hospital*

Received: 10.04.17
Accepted: 24.04.17
doi: 10.1922/EJPRD_01690Shah09

The advantages of PRF include that it is completely autologous, easy to prepare, inexpensive & much more efficient than its predecessors in terms of trapping platelets and sustained release of various growth factors.[3-6]

Since it was first described by Choukroun & co-workers, several modifications of the conventional protocols have been described.[3] Two most popular variants include advanced platelet rich fibrin (A-PRF) and injectable platelet rich fibrin (i-PRF).[7,8] There is limited literature pertaining to these modifications as well as the technical and biological aspects of PRF formation and action. The aim of this article to review the biological mechanisms for enhanced wound healing properties of PRF additives and the advancement in the PRF technology since its inception.

DEVELOPMENT

The development of platelet concentrates finds its origin in the concept of fibrin adhesives. Table 1 outlines the various techniques in chronological order as Platelet concentrates evolved.[7-12]

In 2001, Choukroun & co-workers introduced the protocol of PRF. This protocol was developed to overcome the French laws related to re-implantation of blood derived products. PRF is a completely autologous platelet concentrate produced without any external additive. The protocol includes centrifugation of freshly drawn blood without any anticoagulant in glass/glass based collection tubes which results in formation three layers i.e. red blood corpuscles at the bottom, platelet poor plasma at the top and PRF in between.[4] The same protocol has been modified to obtain A-PRF and i-PRF by modifying the centrifugation speed, time and the tube in terms of its design and material which the PRF is produced. [7,8]

FORMATION OF PLATELET RICH FIBRIN

When freshly drawn blood is collected in glass/glass coated plastic tubes without anticoagulant, the coagulation cascade starts immediately. The basic principle is to allow the blood to clot as it would physiologically. Under normal circumstances, the blood would coagulate to form a blood clot. However, in the PRF protocol, the blood is subjected to centrifugation at around 2700-3000 rpm for 12 minutes or approximately a force of 400g immediately after collection.[4,5] In the centrifuge, two processes are occurring simultaneously i.e. blood coagulation and separation of blood elements under the centrifugation force. In the process of centrifugation there are several forces acting on the blood. The centrifugal force acts towards the bottom of the tube whereas the frictional and buoyant force counteracts it. By exposing the blood to high revolutions for specific time in the centrifuge, a high amount of centrifugal force is generated and this exceeds that of the buoyant and frictional forces. This results in a net centrifugal force which acts away from the center of rotation i.e. towards the bottom of the tube.

Table 1. Chronological evolution of platelet concentrates.

S no	Name	Proposed by	Technique	Drawbacks
1	Platelet concentrates	1970's	Donor plasma which was then mixed with thrombin and calcium which led to polymerization of fibrinogen	Poor stability or risk of disease transmission in case of commercially available products
2	Autologous fibrin glue	Tayapongsak 1994	Pre-operative (one to three week before procedure) collection of blood followed by around 30 minutes (ammonium sulphate precipitation technique) to 48 hours (cryopecipitate technique) of handling.	Technique was long and complex The amount of concentrate obtained was quite less as compared to the amount of blood collected (2ml from 75ml blood in ammonium sulfate concentrate technique and 10-15ml concentrate from 250ml of blood).
3	Platelet rich plasma	Whitman 1997	Double centrifugation of autologous blood with anticoagulant. It consisted of a soft spin followed by which the blood would separate into red corpuscular base, buffy coat and the platelet poor plasma. The last two components were aspirated and re centrifuged at a hard spin after which PRP was collected in the bottom of the tube.	Bovine thrombin which could give rise to life threatening coagulopathies in rare cases
4	Plasma rich in growth factors	Anitua & co-workers 1999	Autologous blood with anticoagulant was centrifuged at 460G for 8 mins and this resulted in collection of plasma rich in growth factors (PRGF) at the bottom of the tube. This PRGF was then taken from the bottom of the tube and cacl2 was added (0.05ml/ml of PRGF). This led to coagulation in around 10 minutes and a gelatinous PRGF was obtained.	Led to incomplete activation of platelets and low levels of growth factors release.

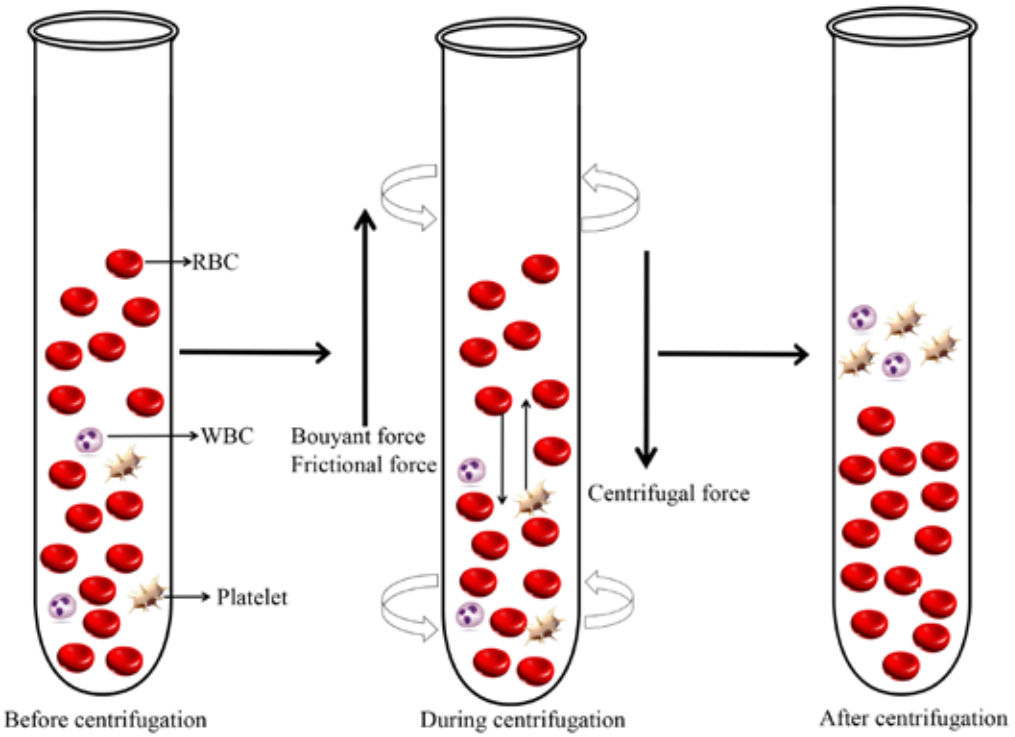

Figure 1: Formation of PRF. Before centrifugation, all cells are mixed. During centrifugation centrifugal force is directed towards the bottom of the tube and buoyant and frictional forces oppose it. There is a net centrifugal force and this is dependent on the mass of the particles. Hence, the RBC's which have higher mass are pulled towards the bottom of the tube and WBC's and platelets along with plasma which have comparatively lower mass reach the top of the tube. This eventually excludes the RBC's from coagulation and the clot hence formed is PRF.

The force of centrifugation exerted is directly proportional to the mass of the individual particle. Hence, under the centrifugation force, RBC's, which have relatively higher mass settle towards the bottom of the tube. Whereas WBC's, platelets and plasma along with its clotting factors which have comparatively lower mass are pushed towards the top of the tube *(Figure 1)*.[13] While this separation is occurs, another process that happens simultaneously is blood coagulation owing to the absence of an anticoagulant.

By the time the final steps of coagulation cascade i.e. conversion of prothrombin to thrombin and fibrinogen to fibrin occurs, the factors required for coagulation are all present in the plasma, which is now located at the top of the tube near platelets under the force of centrifugation. This occurs in the early part of the centrifugation cycle (before approximately 2-3 mins).[8] If the centrifugation is stopped at this point the different components may get mixed again as there would be no clot owing to the fact that the coagulation cycle would not be completed. Once this separation is achieved, the rest 6-8 minutes of the centrifugation cycle is to maintain the separation and let clotting proceed. Hence, RBC's which do not contribute significantly to healing of a wound are effectively excluded from the blood clot under the centrifugation force, and the clot now consists mainly of platelets (1.5 to 3 lakh/ml in a blood clot to around 10 lakh/ml in PRF) and fibrin.[14,15] The normal time taken for the coagulation to complete is around 8 mins and hence all the protocols to produce the PRF concentrates have a duration similar to these.

BIOLOGIC EFFECTS OF PRF

PRF contains a strong natural fibrin matrix which has been formed physiologically without the addition of any external factors. Macroscopically, PRF is mainly composed of yellow fibrin body and terminal RBC stub with an Intervening platelet rich whitish buffy coat. Microscopically, it constitutes a three dimensional structure made up of fine, flexible, mature & dense polymerized fibrin strands. In the red part, predominantly normal shaped RBC's are found entrapped in relatively immature fibrin network.[16] At the junction of red stub and fibrin body, lymphocytes with irregular surface along with numerous platelets aggregates are observed. In the buffy coat part large & dense platelet clusters are seen which indicate that they are in state of aggregation and clotting. In the rest of the fibrin body, thick mature fibrin strand running parallel to each other are observed. Platelets are concentrated in the first millimeter after the red part and constantly decreases as the distance from the red end increases. They completely cease beyond half of the yellow fibrin clot. The protocol of PRF equipoises the loss of platelets and growth factors by enhancing its content by upto 97% of platelets and >50% of leukocytes from a given blood sample.[17,18]

The main cell responsible for the biologic activity of PRF is the platelet. They contain granules including alpha granules, dense granules and glycogen granules. The alpha granules are the main granules that contribute to wound healing by the virtue of the various growth factors they contain. These include platelet derived growth factors (PDGF), transforming growth factor-b (TGF-b), vascular endothelial growth factor (VEGF), insulin like growth factor-1 (IGF-1), epidermal growth factor (EGF) etc.[18,19] They then reach the target cells, bind to transmembrane receptors and activate various intracytoplasmic proteins to cause action related gene expression which has effects like cell mitosis or collagen production.[20,21] All these growth factors together with the unique slow remodeling 3-d structure are responsible for the accelerated healing events that PRF mediates *(Figure 2)*.

The sudden polymerization and bilateral junctions in PRP causes entrapment of activated platelets in the fibrin network and the cytokines and growth factors thus produced are contained extrinsically in colloidal suspension between the fibrin network. Hence, majority of the growth factors are released over the first hour. In contrast to that, the physiologic polymerization in case of PRF causes enhanced entrapment of circulating/intrinsic cytokines within the fibrin network. These molecules are trapped in the fibrin meshwork and are released only when the cicatricial matrix remodeling occurs. Also, due to this intrinsic entrapment, even when we compress the gel to form a membrane, the cytokines are not lost in the exudate but are retained within the membrane This allows the cytokines to be stored and released slowly thus ensuring that the growth factor supply is maintained at constant rates for a long period of time (upto 28 days). The biologic actions of PRF matrix have been summarized in Table 2.[22-26]

PRF PREPARATION PROTOCOL

The classic PRF protocol was suggested by Choukroun & co-workers *(Table 3)*. The basic protocol of producing PRF requires around 10 ml of blood to be collected from the patient without anticoagulant in a glass/ glass coated plastic tubes. After collection, the blood is quickly subjected to centrifugation at 2700 rpm for 12 minutes. After the completion of cycle, the blood in the tube gets separated into three distinct layers; platelet poor plasma at the top, PRF in the middle and a red blood corpuscular base in the bottom.[24,25] The PRF is then carefully retrieved from the test tube and the RBC base is carefully removed to retain a small part of it in the clot. The clot thus obtained can be used as it is, compressed to form plugs for implantation into the socket.

They can also be compressed manually or using a PRF box to form membranes. The clots can also be cut into small pieces and mixed with bone grafts.

Since PRF is 100% autologous and contains no external additives, the pattern of PRF formation is completely physiological; the polymerization occurs slowly, naturally and progressively in presence of physiologic thrombin.[5] This is in contrast with other platelet concentrates, particularly the first generation concentrates where the polymerization of fibrin occurs in presence of exogenous and un-physiologic thrombin in quite a rapid manner. Due to the physiologic mode of polymerization, PRF has some unique properties.[5,6,25,26]

MODIFICATIONS OF PRF TECHNIQUE

Two major modifications of the PRF technique have been suggested in the protocol over the last few years. The variations are in protocol related to the revolutions or time or the design and the material of the tube. Table 3 presents the various protocols for PRF. Both the protocols for leukocyte & PRF (L-PRF) & advanced PRF (A-PRF) lead to formation of platelets clots.

ADVANCED PRF

Leukocyte and PRF (L-PRF) is produced at a speed of 2700 rpm for 12 minutes in sterile glass based plastic tubes.[25] For formation of A-PRF, slower speed (1500 rpm) and more time (14 mins) is used in sterile plain glass-based vacuum tubes (A-PRF10 tubes). The authors propose that such a protocol leads to enhanced B- & T-lymphocyte entrapment, more even distribution of platelets, neutrophils. Also, the authors say that the number of viable cells including platelets are much higher in A-PRF. There is better deployment of resident monocyte, macrophages and lymphocytes.[7] Clinically, this would be beneficial as it would translate into increased amount of growth factor and cytokine release. However, some studies have provided contradictory results. Pinto & co-workers demonstrated that A-PRF protocol produced lighter, shorter, narrower clot with light polymerization and more squashed bodies. When the amount of growth factors (TGF, PDGF-bAB, VEGF) released from A-PRF were compared to that of L-PRF it was found that the levels were less than half of those from L-PRF.[25-27] However, in another study it was observed that A-PRF released significantly higher total quantities of growth factors when compared to traditional PRF.[28] There is limited literature on the comparison between the two protocols and more studies are required to ascertain the benefits and limitations of L-PRF vs A-PRF.

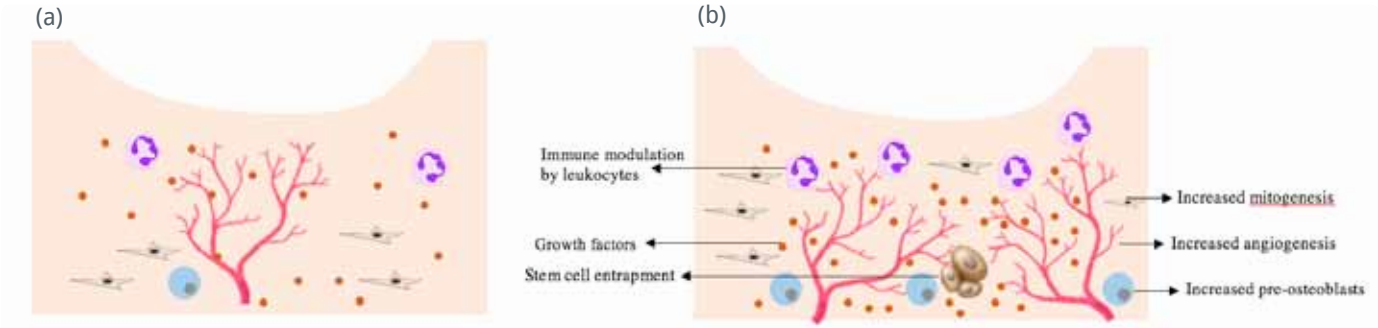

Figure 2: Healing in the presence of PRF (b) demonstrates increased amount of growth factors, stem cell entrapment, mitogenesis, angiogenesis and pre-osteoblasts as compared to that without PRF (a) leading to enhanced the wound healing.

ejprd.org - Published by Dennis Barber Journals.

Table 2. Biologic actions of PRF

Effect	Mediated by	Action
Angiogenesis	Vascular endothelial growth factor (VEGF), angiopoetin, platelet derived growth factor (PDGF), basic-fibroblast growth factor (FGF-b).	• Cells in the wound vicinity to migrate, divide and change phenotype • stimulates expression of $\alpha 5\beta 3$ intergrin on the endothelial cells which promotes the binding of endothelial cells to fibrin, fibronectin & vitronectin.
Mitogenesis	TGF-β Fine & flexible trimolecular/ equilateral junctions	• Mitogen for cells including fibroblasts, marrow stem cells, endothelial cells, pre-osteoblasts, mesenchymal cells • Inhibitory effect on osteoclasts • Enhanced cytokine entrapment, promotes rapid cellular migration
Immunomodulatory effects	Fibrin and its degradation products Fibronectin Leukocytes IL-4	• Stimulate migration, phagocytosis and enzymatic degradation by neutrophils • Increases the expression of CD11C/CD18 receptor on neutrophils which mediates adhesion to endothelium and fibrinogen • Releases certain chemotactic factors which regulate wound colonization by macrophages • Increased degranulation to release several molecules including IL-1, IL-4, IL-6 and TNF-α • Coherent healing without inflammatory excess
Wound recolonization	Fibrinogen, fibronectin, vitronectin and tenascin Fibrin	• Undergoes degradation and allows epithelial cell migration on wound margins • Binds to several molecules including fibronectin, PDGF & TGF-b through the $\alpha V\beta 3$ integrin • Promotes the migration of fibroblasts
Osteogenic effect		• May upregulate the expression of alkaline phosphatase and osteoprotegerin • Enhance the expression of phosphorylated extracellular signal-regulated protein kinase, osteoprotegerin and alkaline phosphatase activity
Entrapment of stem cells		Even though the intrinsic content of stem cells in quite low, it has been hypothesized that the fibrin clot may act like a trap for circulating stem cells which may converge to a secretory phenotype allowing vascular and tissue restoration.

Table 3. PRF preparation protocols

PRF	Described by (year)	RPM	Time (minutes)	Tube
Leukocyte and Platelet rich fibrin (L-PRF)[3]	Choukroun 2004	2700	12	Glass coated tube
Advanced platelet rich fibrin (A-PRF)[6]	Ghanaati 2014	1300	14	Patented
Advanced platelet rich fibrin + (A-PRF+)[6]	Fujioka-Kobayashi, Miron 2016	1300	8	Same as A-PRF
Injectable platelet rich fibrin (I-PRF)[7]	Mourão 2015	700	3	Non coated

ADVANCED PRF +

Another modification of A-PRF has been suggested by Fujioka-Kobayashi & co-workers in 2016 where they have reduced the centrifugation time to 1300 rpm for 8 minutes. They have called this modification as A-PRF+. The authors argue that less time would result in a decrease in the amount of forces that the cells of the blood would be exposed to & hence, would increase the number of cells contained in the PRF matrix. When they assessed the PRF produced by this protocol to L-PRF and A-PRF in terms of growth factor release, biocompatibility & cellular activity, they observed that A-PRF+ demonstrated highest release of PDGF, TGF- β1, EGF and IGF. Also, in culture, gingival fibroblasts exposed to A-PRF+ revealed significantly higher levels of PDGF, TGF-b and collagen-1 at three and seven days measured in terms of m-RNA expression.[29]

These results suggest that reduction in the speed leads to PRF with better growth factor release.

The authors justify these finding based on the fact that by high centrifugation speed tends to push the cells including platelets and leukocytes away from the PRF clot. By lowering the centrifugation speed (and in-turn the g-force that was being applied), not only a more uniform platelet distribution can be achieved but also the number of the neutrophilic granulocytes trapped in the PRF was enhanced. This increased cellularity of the PRF would translate into increased macrophage differentiation which leads to increased osteoblastic differentiation. The increase in the growth factor release was attributed to possible increase in the number of leukocytes entrapped due to low centrifugation forces. These results demonstrate that use of low speed to produce PRF would optimize growth factor production and cellular response to PRF.[29]

INJECTABLE PRF

One of the latest developments in the PRF technology is the production of injectable PRF (i-PRF).[8] As compared to PRP, one drawback that limits the applications of PRF is that PRF is obtained as a gel form which is not conducive to be injected. PRP as an injection has several applications such as knee arthroplasty, facelift surgeries, decrease the incidence of sternal infections after cardiac surgeries, sports injuries, tendon/ligament injuries, osteoarthritis, meniscal healing, alopecia, musculoskeletal regenerative procedures, acne etc.[30,31] All these applications are based on the actions of the autologous growth factor released by platelets contained in the PRP to boost the localized healing.[27] In previous studies it has been observed that the growth factors in PRF are released in a sustained fashion over longer periods ranging from 7-21 days and have a stronger and more durable effect on cell proliferation and differentiation.[32] From this information, it can be deduced that as a bioactive material, PRF certainly has several advantages over PRP. Also, PRF is devoid of the drawbacks related to bovine thrombin including development of antibodies to the factors V, XI & thrombin and chances of life threatening coagulopathies.[30] Hence, an injectable variety of PRF theoretically would be a superior alternative to PRP for the abovementioned applications.

Recently, such an injectable or liquid variety of PRF has been developed. For producing i-PRF, blood is drawn without anticoagulant in plastic tubes without any coatings and centrifuged at around 700 for 3 minutes.[7, 33] Another set of authors have proposed a similar protocol where they centrifuge plain blood in non-coated test tubes at 2400-2700 rpm for around 2 minutes. The supernantant is collected and they have named it concentrated growth factors (CGF).[34] Both the methods essentially work on the same principle and hence can be considered variants of same concentrate. The time is considerably shorter than other two protocols (i.e. L-PRF & A-PRF). This can be attributed to the fact that for i-PRF/CGF only the separation of blood components is desired which happens in initial 2-4 minutes. Plastic tubes have a hydrophobic surface and do not efficiently activate the coagulation process.[35] Hence, all the blood components that are required to form a good platelet concentrate (plasma containing all clotting factors & platelets) reach the top of the tube under the centrifugation force in the first 2-4 minutes. The separated plasma and platelets form a light yellow colored layer which is situated at the top of the tube. This is then aspirated and amounts to a partially active injectable form. Currently, it has been used for mixing with bone grafts, which on completion of the coagulation process forms a gel-putty consistency with the graft particles incorporated in the graft.[8,34] The graft thus formed has a good workable consistency, leads to decreased leaching of the graft as it is tightly encapsulated in the fibrin matrix *(Figure 3)*. Mixing the bone graft with i-PRF also gives the benefit of growth factor release at the recipient site which would otherwise be missing in a normal bone graft. This has the potential to convert any osteoconductive graft to osteopromotive (due to the presence of platelets & growth factors) which would translate into faster and better efficiency of bone formation.

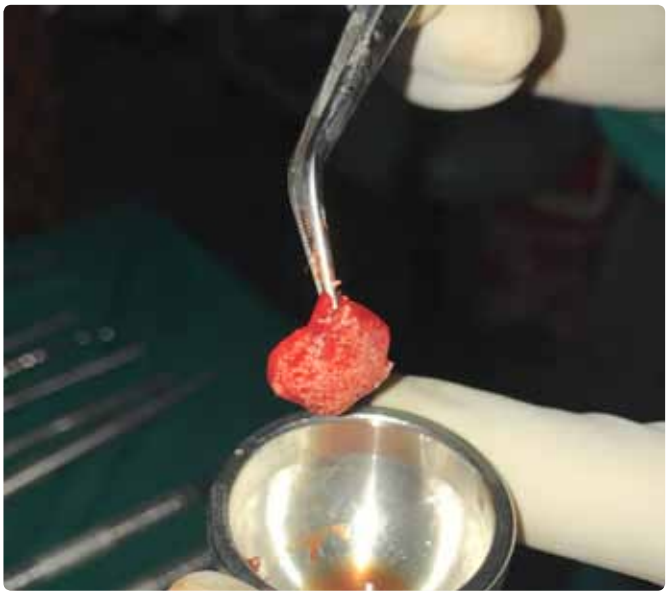

Figure 3: i-PRF mixed with bone graft forms a workable mass

In a study by Miron & co-workers on the comparison of PRP with i-PRF, it was found that though the initial growth factor release including that of PDGF-AA, PDGF-AB, EGF and IGF-1 was higher by PRP, the the total release of the factors at the end of ten days was significantly higher in i-PRF. PRP demonstrated higher levels of VEGF & TGF--b at ten days as compared to i-PRF. PRP & i-PRF demonstrated similar tissue compatibility, PRP was associated with higher cellular proliferation, whereas i-PRF demonstrated higher cellular migration. Also, in cell culture, i-PRF induced significantly m-RNA expression of TGF--b and collagen-1 at 7 days. This preliminary data hints that i-PRF may have comparative or slightly superior biologic properties as compared to that of PRP.[33]

Another kind of graft that has been obtained with i-PRF is the PRF block. For its preparation, i-PRF is mixed with a combination of bone graft and shredded PRF clot. This enhances the volume of the graft.

However, the literature pertaining to i-PRF & its clinic-biological effects is limited. More studies are required to further asses its properties and applications.

PRF LYSATE

A newer application of PRF based products is the PRF Lysate. In this, after PRF preparation, it is incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air and the exudate thus collected has been referred to as PRF lysate. It is said to be a good source of several growth factors including PDGF, TGF, VEGF & EGF.[36] In a study, it has used to reverse the damage caused by chronic UV radiation exposure to human dermal fibroblasts by significantly increasing the proliferation rates, migration rates, and collagen deposition equal to those of normal fibroblasts.[36,37] This is a relatively new application and not many studies are there on the same. Further studies may investigate potential applications of PRF lysate.

TITANIUM-PRF

Another avenue which has been investigated is the usage of different materials for blood processing during PRF preparation. Recently, Tunali & co-workers used medical grade titanium tubes to produce PRF and called it T-PRF. On examination it was observed that T-PRF samples seemed to have a highly organized network with continuous integrity compared to the L-PRF samples. Also, T-PRF fibrin network was observed to cover larger area than L-PRF fibrin network; and the fibrin was also thicker in the T-PRF samples.[38] In a human study, T-PRF was obtained from centrifugation of 20 ml blood at 2800 rpm for 12 minutes in medical grade titanium tubes. When the same was applied for palatal mucosal wound healing, it was found that T-PRF membranes exhibited positive effects on palatal mucosal wound healing.[39] Further research is required to find the best bio-material for PRF processing which would enhance the biologic properties of PRF.

All the modifications of PRF aim at maximizing the growth factor mediated biologic effects and the cellular activity. All the modifications have been proposed quite recently. Further animal and clinical studies would throw more light on the efficacy of these modifications.

CLINICAL CONSIDERATIONS IN DENTISTRY

In dentistry, PRF is being widely accepted and used for varied applications. It has been used as a sole material with good success rate for sinus lift & socket preservation. As an adjunct it has been used for all grafting applications including guided tissue regeneration in intra-bony defects and Grade II furcation involvements, guided bone regeneration in cases of socket preservation/augmentation, for combined endodontic-periodontal lesions, hard and soft tissue augmentation around implants, to be mixed with bone grafts to increase their volume & bio-activity etc.[1,2,6] It has also been used as a scaffold to culture human periosteal cells for tissue engineering purposes.[40] PRF has several advantages i.e. it is completely autologous, safe, easy to prepare, requires minimal biochemical handling of blood, & cost effective. However, it has a few limitations such as short half-life, limited availability and non-feasibility for tissue banks.

Few systematic reviews on the application of PRF have been performed. Shah & co-workers in their systematic review on effectiveness of PRF for intrabony defects found that there is a significant increase in clinical attachement level (CAL), IBD and PPD as compared to open flap debridement alone.[41] Also, Ali & co-workers in their systematic review found that PRF was good to be used as a sole filling material for sinus augmentation when used with simultaneous implant placement. Also, it enhanced the maturation of the graft when placed with demineralized freeze dried bone grafts.[42] Moraschini & Barboza in their systematic review on the efficacy of PRF as a graft for gingival recession found that it had no beneficial effects in terms of root coverage, clinical attachment level & keratinized mucosa width.[43] These systematic appraisals of literature indicate that PRF is a beneficial bioactive adjunct to be used in regenerative dentistry.

CONCLUSION

PRF as a biologic surgical additive has been successfully used for varied applications in dentistry. Technological advancements in the field of PRF such as i-PRF have paved way for the versatility in the applications of the platelet concentrates. With the increase in our understanding about the biology of PRF, in future we can expect improved additives which will further enhance the wound healing experience.

REFERENCES

1. Polimeni G, Xiropaidis AV, Wikesjö UM. Biology and principles of periodontal wound healing/regeneration. *Periodontol* 2000 2006;**41**:30-47.
2. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: a radiologic and histologic study at 6 months. *J Periodontol* 2009;**80**:2056-64.

ejprd.org - Published by Dennis Barber Journals.

3. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: Le PRF. *Implantodontie* 2001;**42**:55–62.
4. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J *et al.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:e37-44.
5. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II:platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:e45-50.
6. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, *et al.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:e51-5.
7. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, *et al.* Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 2014;**40**:679-89.
8. Mourão C, Valiense H, Melo E, Mourão N, & Maia M. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. *Revista do Colégio Brasileiro de Cirurgiões* 2015;**42**:421-23.
9. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg* 1997;**55**:1294–99.
10. Soffer E, Ouhayoun JP, Anagnostou F. Fibrin sealants and platelet preparations in bone and periodontal healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;**95**:521–8.
11. Tayapongsak P, O›Brien DA, Monteiro CB, Arceo-Diaz LY. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. *J Oral Maxillofac Surg* 1994;**52**:161-5; discussion 166.
12. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants* 1999;**14**:529-35.
13. García-Alonso A, Senis Y. Platelet Proteomics: Principles, Analysis, and Applications. John Wiley & Sons , *New Jersey*. 2011 pp 67-73.
14. Marx RE. Platelet-rich plasma (PRP): What Is PRP and What Is Not PRP? *Implant Dent.* 2001;**10**:225–8.
15. Marks V, Cantor T, Mesko D, Pullmann R, Nosalova G. Differential Diagnosis by Laboratory Medicine: *A Quick Reference for Physicians. Springer-Verlag Berlin Heidelberg, Germany*, 2002 pp 427
16. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun›s platelet-rich fibrin clot and membrane. *J Periodontol.* 2010;**81**:546-55.
17. Dohan Ehrenfest DM, Bielecki T, Jimbo R, Barbé G, Del Corso M, Inchingolo F, Sammartino G. Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-rich plasma (P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF). *Curr Pharm Biotechnol.* 2012;**13**:1145-52.
18. Michelson AD. Platelets. 3rd ed. Amsterdam: Academic Press, 2013. Ch 17
19. Blair P, Flaumenhaft R. Platelet α–granules: Basic biology and clinical correlates. *Blood reviews.* 2009;**23**:177-89.
20. Golebiewska EM, Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. *Blood Reviews* 2015;**29**:153-62.
21. Roy S, Driggs J, Elgharably H, Biswas S, Findley M, Khanna S, *et al.* Platelet-rich fibrin matrix improves wound angiogenesis via inducing endothelial cell proliferation. *Wound Repair Regen* 2011;**19**:753-66.
22. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:e56-60.
23. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *et al.* Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**:299-303.
24. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunite´en paro-implantologie: le PRF. *Implantodontie* 2000;**42**:55-62.
25. Ehrenfest DM, Kang B, Corso MD, Nally M, Quirynen M, Wang HL*et al.* The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a Leukocyte- and Platelet-Rich Fibrin (L-PRF) clot and membrane. Part 1: evaluation of the vibration shocks of 4 models of table centrifuges for L-PRF. *Poseido* 2014;**2**:129-39.
26. Pinto NR, Pereda A, Jiménez P, Corso MD, Kang B, Wang HL, *et al.* The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a Leukocyte- and Platelet-Rich Fibrin (L-PRF) clot and membrane. Part 2: macroscopic, photonic microscopy and Scanning Electron Microscopy analysis of 4 kinds of L-PRF clots and membranes. *Poseido* 2014;**2**:141-54.
27. Ehrenfest D, Corso M, Kang B, Lanata N, Quiryen M, Wang H, *et al.* The impact of centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a leukocyte-and-platelet rich fibrin (L-PRF) clot and membrane. Part 3: comparison of the growth factors content and slow release between the original L-PRF and the modified A-PRF (Advanced Platelet-Rich Fibrin) membranes. *Poseido* 2014;**2**:155-66.
28. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, *et al.* Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clin Oral Investig* 2016 Jan 25 [Epub ahead of print] PubMed PMID: 26809431.
29. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. *J Periodontol* 2017 Jan;**88**(1):112-121.
30. Jain NK, Gulati M. Platelet-rich plasma: a healing virtuoso. *Blood Res.* 2016;**51**:3-5.
31. Maria-Angeliki G, Alexandros-Efstratios K, Dimitris R, Konstantinos K. Platelet-rich plasma as a potential treatment for noncicatricial alopecias. *Int J Trichology*. 2015;**7**:54-63.
32. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009;**108**:707-13.
33. Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, Ghanaati S, Choukroun J. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? *Clin Oral Investig*. 2017 Feb 2. doi:10.1007/s00784-017-2063-9. [Epub ahead of print]
34. Sohn DS, Huang B, Kim J, Park WE, Park CC. Utilization of autologous concentrated growth factors (CGF) enriched bone graft matrix (sticky bone) and CGF-enriched fibrin membrane in implant dentistry. *J Implant Advanced Clin Dent* 2015;**7**:11-8.

35. Bowen RAR, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. *Biochemia Medica* 2014;**24**:31-44.

36. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;**108**:707–713.

37. Wirohadidjojo YW, Budiyanto A, Soebono H. Platelet-Rich Fibrin Lysate Can Ameliorate Dysfunction of Chronically UVA-Irradiated Human Dermal Fibroblasts. *Yonsei Med J* 2016;**57**:1282-5.

38. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Yaprak E, Toker H *et al*. A novel platelet concentrate: titanium-prepared platelet-rich fibrin. *Biomed Res Int* 2014;209548.

39. Ustaoğlu G, Ercan E, Tunali M. The role of titanium-prepared platelet-rich fibrin in palatal mucosal wound healing and histoconduction. *Acta Odontol Scand*. 2016;**74**:558-64.

40. Gassling V, Douglas T, Warnke PH, Acxil Y, Wiltfang J, Becker ST. Platelet-rich fibrin membranes as scaffolds for periosteal tissue engineering. *Clin Oral Implants Res* 2010;**21**:543–9.

41. Shah M, Deshpande N, Bharwani A, Nadig P, Doshi V, Dave D. Effectiveness of autologous platelet-rich fibrin in the treatment of intra-bony defects: A systematic review and meta-analysis. *J Indian Soc Periodontol* 2014;**18**:698-704.

42. Ali S, Bakry SA, Abd-Elhakam H. Platelet-rich fibrin in maxillary sinus augmentation: A systematic review. *J Oral Implantol* 2015;**41**:746-53.

43. Moraschini V, Barboza Edos S. Use of platelet-rich fibrin membrane in the treatment of gingival recession: A systematic review and meta-analysis. *J Periodontol* 2016;**87**:281-90.