Platelet-rich fibrin: Its role in periodontal regeneration

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Abstract Platelets can play a crucial role in periodontal regeneration as they are reservoirs of growth factors and cytokines which are the key factors for regeneration of the bone and maturation of the soft tissue. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are autologous platelet concentrates prepared from patient’s own blood. Recent researches are being focused on the development of therapeutic alternatives which are easy to prepare, non-toxic or biocompatible to living tissues and economically cheap that might result in the local release of growth factors accelerating hard and soft tissue healing. PRF is a natural fibrin-based biomaterial prepared from an anticoagulant-free blood harvest without any artificial biochemical modification that allows obtaining fibrin membranes enriched with platelets and growth factors. Evidence from the literature suggests the potential role of PRF in periodontal regeneration and tissue engineering. The slow polymerization during centrifugation and fibrin-based structure makes PRF a better healing biomaterial than PRP and other fibrin adhesives. The main aim of this review article is to briefly describe the novel platelet concentrate PRF and its potential role in periodontal regeneration.

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KEYWORDS
Platelet-rich fibrin; Platelet-rich plasma; Regeneration; Tissue engineering; Growth factors

Contents
1. Introduction ................................................................. 118
2. Role of platelets in periodontal wound healing ..................... 118
3. What is PRF? .............................................................. 119

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1. Introduction

Periodontal disease is defined as a complex, multifactorial disease characterized by the loss of connective tissue attachment with destruction of periodontal tissues. The aim of periodontal therapy is to eliminate inflammatory process, prevent the progression of periodontal disease and also to regenerate the lost periodontal tissues. Periodontal regeneration is a complex multifactorial process involving biologic events like cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence. Periodontal regenerative procedures include soft tissue grafts, bone grafts, root biomodifications, guided tissue regeneration, and combinations of these procedures.

The current perspective is that regenerative periodontal therapies to date can only restore a fraction of the original tissue volume and have a limited potential in attaining complete periodontal restoration. Various biomaterials have been used for periodontal tissue regeneration in addition to autogenous and allogenic bone grafts but not a single graft material is considered as gold standard for the treatment of intrabony defects.

Periodontal wound healing requires a sequence of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. The disruption of vasculature during wound healing leads to fibrin formation, platelet aggregation, and release of several growth factors into tissues from platelets through molecular signals which are primarily mediated by cytokines and growth factors. There is evidence that the presence of growth factors and cytokines in platelets play key roles in inflammation and wound healing. Platelets also secrete fibrin, fibronectin, and vitronectin, which act as a matrix for the connective tissue and as adhesion molecules for more efficient cell migration. This has led to the idea of using platelets as therapeutic tools to improve tissue repair particularly in periodontal wound healing.

Platelet-rich fibrin (PRF) described by Choukroun et al. is a second-generation platelet concentrate which contains platelets and growth factors in the form of fibrin membranes prepared from the patient’s own blood free of any anticoagulant or other artificial biochemical modifications. The PRF clot forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest and shows a complex architecture as a healing matrix with unique mechanical properties which makes it distinct from other platelet concentrates. PRF enhances wound healing and regeneration and several studies show rapid and accelerated wound healing with the use of PRF than without it. PRF is superior to other platelet concentrates like PRP due to its ease and inexpensive method of preparation and also it does not need any addition of exogenous compounds like bovine thrombin and calcium chloride. It is advantageous than autogenous graft also because an autograft requires a second surgical site and procedure. Thus PRF has emerged as one of the promising regenerative materials in the field of periodontics. This review article explains the novel platelet concentrate PRF, its preparation, clinical applications and benefits and drawbacks over other biomaterials.

2. Role of platelets in periodontal wound healing

Platelets play a key role in wound healing and hence wound healing after periodontal treatment can be accelerated by the use of platelet concentrates. The wound healing process initiated by the formation of blood clot and after tissue injury in periodontal surgery causes adherence and aggregation of platelets favoring the formation of thrombin and fibrin. In addition, there is release of certain substances from platelets that promote tissue repair, angiogenesis, inflammation and immune response. Platelets also contain biologically active proteins and the binding of these secreted proteins within a

Figure 1 Test tube showing platelet-rich fibrin after centrifugation of blood.
developing fibrin mesh or to the extracellular matrix can create chemotactic gradients favoring the recruitment of the stem cells, stimulating cell migration, differentiation, and promoting repair. Thus the use of autologous platelet concentrates is a promising application in the field of periodontal regeneration and can be used in clinical situations requiring rapid healing.

3. What is PRF?

PRF (platelet rich fibrin) was first developed in France for use in the field of 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 P7 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.

4. Historical background

Platelets are used as powerful tools for periodontal regeneration for the past two decades due to the key role of platelets in wound healing process. Although the use of fibrin adhesives is well documented from the past 30 years their use is still controversial due to the complexity in preparation and risk of cross-infection. After that concentrated platelet-rich plasma (cPRP) was developed with a less complex production protocol. It is prepared from the patient’s own blood and is activated by the addition of thrombin and calcium. The structure consists of a three dimensional biocompatible fibrin scaffold with a limited volume of plasma enriched in platelets. When PRP is activated the growth factors and proteins are released to the local environment accelerating postoperative wound healing and tissue repair. But the disadvantage of using PRP is that its properties can vary depending on the concentration of platelets, amount of leukocytes, the type of activator used and time of placement of fibrin scaffold after clotting. But there are certain risks associated with the use of PRP. The presence of bovine thrombin in PRP can result in the development of antibodies to the clotting factors V, XI and thrombin which can adversely affect the coagulation process. In addition, bovine thrombin preparations contain clotting factor V which can result in immune system activation when challenged with a foreign protein. Other drawbacks about the use of PRP include legal restrictions on handling the blood and also controversies in the literature regarding the benefits and clinical outcome of use of PRP. All these have led to the generation of a new family of platelet concentrate called platelet-rich fibrin which overcomes many of the limitations of PRP. PRF is a potent autologous regenerative material with many clinical applications in the field of periodontics as it accelerates both soft tissue and hard tissue healing.

5. Potential benefits of using PRF in periodontal regeneration

Platelet-rich fibrin is a second generation platelet concentrate which can enhance both soft and hard tissue healing. Its advantages over platelet-rich plasma include ease of preparation, ease of application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required). This considerably reduces the biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin. PRF also contains physiologically available thrombin that results in slow polymerization of fibrinogen into fibrin which results in a physiologic architecture that is favorable to wound healing. The cytokines which are present in platelet concentrates play an important role in wound healing. The structural

Figure 2 Platelet-rich fibrin after centrifugation.

Figure 3 Platelet-rich fibrin after collection.
configuration of PRF with respect to cytokine incorporation in fibrin meshes is different from that present in PRP. The natural polymerization in PRF results in increased incorporation of the circulating cytokines in the fibrin meshes (intrinsinc cytokines). These intrinsic cytokines will be having an increased lifespan and they will be released and used only at the time of initial cicatricial matrix remodeling which creates a long term effect. In PRP and other fibrin adhesives the presence of artificial additives like bovine thrombin and calcium chloride results in sudden fibrin polymerization causing loss of synergy between cytokines and fibrin with faster physiologic elimination of these cytokines.

The three dimensional organization of a fibrin network in PRF and PRP affects the biologic and mechanical properties of these platelet concentrates. During gelling of these fibrin structures, the fibrin fibrillae can be assembled in 2 ways, bilateral junctions or equilateral junctions. In PRP there are bilateral junctions with strong thrombin concentrations that allow thickening of fibrin polymer with a rigid network resulting in poor cytokine entrapment and cellular migration. But in PRF the equilateral junctions are present with weak thrombin concentrations forming a fine and flexible fibrin network which is more elastic in nature favoring cytokine entrapment and cellular migration.\(^6\) All these comparative parameters make PRF a better healing biomaterial than PRP and other fibrin adhesives.

Another added advantage of PRF is the presence of natural fibrin network in PRF which protects the growth factors from proteolysis.\(^12,22\) PRF also favors the development of microvascularization leading to a more efficient cell migration.

### 6. Protocol for preparation of PRF

The classical technique for PRF preparation was invented by Dr. 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 or bovine thrombin during gelling.\(^6\)

A standard protocol for PRF preparation should be followed to obtain proper quantity and quality of the fibrin matrix, leukocytes, platelets, and growth factors. The equipment required for PRF preparation includes a PC-02 table centrifuge and a blood collection kit consisting of a 24 gauge butterfly needle and 9 ml blood collection tubes. A sample of blood is collected from patient without anticoagulant in 10 ml tubes which are immediately centrifuged at a rate of 3000 rpm for 10 min. During the centrifugation process, when the blood gets in contact with the test tube wall the platelet gets activated leading to the initiation of coagulation cascade. After centrifugation, the resultant product consists of three layers. The topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and RBCs at the bottom of the test tube (see Fig. 1). The fibrin clot obtained after centrifugation is removed from the tube and the attached red blood cells scraped off from it and discarded (see Fig. 2). PRF can also be prepared in the form of a membrane by squeezing out the fluids present in the fibrin clot.

The duration of time between blood collection and centrifugation process is an important parameter affecting the success and clinical outcome of this procedure. The slow handling of blood to centrifugation process will result in diffuse polymerization of fibrin leading to the formation of a small blood clot with irregular consistency. Hence a reproducible protocol for PRF production should be followed to obtain a clinically usable fibrin clot with massive enmeshment of platelets (see Fig. 3).

### 7. Clinical applications

PRF is a powerful healing biomaterial with inherent regenerative capacity and can be used in various procedures such as for the treatment of periodontal intrabony defects,\(^{10,11}\) treatment of furcation,\(^{22}\) sinus lift procedures\(^{24}\) and as a scaffold for human periosteal cells in vitro, which finds application in the field of tissue engineering.\(^{25}\)

### 8. Evidence for the role of PRF in periodontal regeneration

PRF is enriched with platelets, growth factors and cytokines increasing the healing potential of both hard and soft tissue.\(^4,5\) There are only a few references in the literature about the biologic properties of PRF when compared to other platelet concentrates. The literature mostly contains animal and human studies of the experimental use of PRF and only limited in vitro studies have been carried out on the effects of PRF on cell proliferation. Inspite of the lack of scientifically proven clinical benefits, PRF is considered as a healing biomaterial and is commonly used in implant and plastic periodontal surgery procedures to enhance bone regeneration and soft-tissue wound healing.\(^{26,27}\)

According to Choukroun et al. PRF was initially used in implant surgery to enhance the healing properties of the bone.\(^7\) PRF can promote the healing of osseous defects by the following mechanisms. According to Chang et al. PRF promotes the expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and stimulates the production of osteoprotegerin (OPG) which in tum causes proliferation of osteoblasts.\(^28\) Another study by Huang et al. reported that PRF stimulates the osteogenic differentiation of the human dental pulp cells by upregulating osteoprotegerin and alkaline phosphatase expression.\(^29\) PRF also releases growth factors such as platelet-derived growth factor and transforming growth factor which promote periodontal regeneration.\(^30\) Chang et al. in a study reported that PRF stimulates cell proliferation in a specific manner.\(^30\) PRF induces cell proliferation of osteoblasts, periodontal ligament cells and growth factors during a 3-day culture period and suppressed oral epithelial cell growth. These cell type-specific actions may be beneficial for periodontal regeneration.

Diss et al. in a 1 year prospective study on osteotome sinus floor elevation using Choukroun’s platelet-rich fibrin grafting material clearly demonstrated that fibrin matrix of PRF directly promotes angiogenesis.\(^31\) PRF when used as a membrane for guided tissue regeneration as a grafting material creates an improved spacemaking effect which facilitates cell events that are favorable for periodontal regeneration leading to mineralized tissue formation. PRF is having an inherent osteoconductive and/or osteoinductive property which is beneficial for regeneration of the bone. Sanchez et al. in an experimental study compared the influence of PRP and PRF on proliferation and differentiation of osteoblasts and he reported that the affinity of osteoblasts to the PRF membrane appeared to be superior than the affinity of osteoblasts to PRP.\(^32\) Sharma et al. conducted a randomized controlled clinical trial for the treatment of 3-wall intrabony defects in chronic periodontitis patients with plateletrich fibrin and reported a statistically significant improvement in pocket depth reduction and bone fill in test group than in controls.\(^33\) A similar study was conducted for the treatment of mandibular degree II furcation defects.
with plateletrich fibrin and showed a significant improvement in pocket depth reduction, gain in clinical attachment level and bone fill in test group when compared to controls.28 Thorat et al. investigated the clinical and radiological effectiveness of autologous PRF in the treatment of intrabony defects of chronic periodontitis patients and reported a greater reduction in pocket depth, more gain in clinical attachment level and greater intrabony defect fill at sites treated with PRF than those treated with open flap debridement alone.31 Another randomized controlled clinical trial was done in three treatment groups comprising of OFD (open flap debridement) + PRF, OFD + PRF + HA (porous hydroxyapatite graft) and OFD alone as control. This study showed a significant bone fill in plateletrich fibrin treated group than in controls and a significant bone fill and gain in clinical attachment level in plateletrich fibrin combined with porous hydroxyapatite graft than in control group.32 A comparative evaluation between plateletrich fibrin and plateletrich-plasma for the treatment of three-wall intrabony defects was done and showed a greater bone fill in PRF treated group than in PRP treated group.33 The effect of plateletrich fibrin on human periodontal ligament fibroblasts and application in periodontal infrabony defects was studied by Chang et al. and reported that PRF was found to increase extracellular signal-regulated kinase phosphorylation and osteoprotegerin in periodontal ligament fibroblasts and upregulation of alkaline phosphatase activity. Also, infrabony defects exhibited pocket reduction and clinical attachment gain after six months with bone fill in defects.34–38

9. Evidence for the role of PRF in tissue engineering

The α-granules present in platelets contain growth factors like platelet derived factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF).39 Platelet derived growth factor (PDGF) has an important role in periodontal regeneration and wound healing40 and receptor for PDGF is present on gingiva, periodontal ligament and cementum and it activates fibroblasts and osteoblasts promoting protein synthesis.37 PDGF also functions as a chemoattractant for fibroblasts and osteoblasts in gingiva and periodontal ligament resulting in their activation.38 PRF promotes angiogenesis because as it has low thrombin level optimal for the migration of endothelial cells and fibroblasts. PRF entraps circulating stem cells due to its unique fibrin structure. This property of PRF finds application in healing of large osseous defects where there is migration of stem cells differentiating into osteoblast phenotype.26 PRF also helps in facilitating adhesion and spreading of cells, regulates gene expression of growth factors, growth factor receptors, proteins, and determines the outcome of a cell's response to growth factors due to the presence of collagen, fibronectin, elastin, other non-collagenous proteins, and proteoglycan in the extracellular matrix of PRF.39 The use of PRF as a tissue engineering scaffold was investigated by many researchers for the past few years. In a study by Gasstl 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.30 PRF has immune functions like chemotaxis as leukocytes present in PRF degranulates during activation and releases cytokines like IL-1, IL-4, IL-6 and TNF-α. PRF also contains anti-inflammatory cytokine such as IL-4 which requires further research.30 Thus PRF is a potential tool in tissue engineering but clinical aspects of PRF in this field requires further investigation.

10. Drawbacks of PRF

The main shortcoming of PRF is its preparation and storage. The clinical benefit of PRF depends on time interval between speed of handling between blood collection and centrifugation as PRF is prepared without any addition anticoagulants. Another main disadvantage of PRF is its storage after preparation.39 Also PRF membranes should be used immediately after preparation as it will shrink resulting in dehydration altering the structural integrity of PRF. Dehydration also results in the decreased growth factor content in PRF41 and leukocyte viability will be adversely affected altering its biologic properties. PRF when stored in refrigerator can result in risk of bacterial contamination of the membranes. These limitations with the use of PRF can be circumvented by sticking onto a standard protocol for preparation and preservation.

11. Future directions

In the future more studies should be carried out to correlate the clinical outcome of PRF with its biologic mechanisms which opens novel applications of this autologous platelet concentrate. There are only limited studies in the literature on the effect of PRF on cell proliferation and other biologic effects. Therefore, more studies should be conducted which open newer strategies for the use of this platelet concentrate.

12. Conclusion

PRF by Choukroun’s technique is a simple and inexpensive technique for the successful regeneration of periodontal tissues. The main advantage is that PRF preparation utilizes the patient’s own blood reducing or eliminating disease transmission through blood. In the future more studies and clinical trials are needed to investigate potential applications of PRF in the field of periodontal regeneration and tissue engineering and to extend its clinical applications.

Conflict of interest

The author declared that there is no conflict of interest.

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