

PLATELET-RICH FIBRIN: A NEW PARADIGM IN WOUND HEALING

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Submitted on: January 2017

Accepted on: January 2017

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Abstract

To assess the potential use and benefits of Platelet-Rich Fibrin (PRF) over Platelet-Rich Plasma (PRP), for wound healing post oral and maxillofacial surgeries. This article describes the evolution of this second generation platelet concentrate and its multiple uses in various surgical procedures. Around 5 ml of whole venous blood is collected from the patients in each of the two sterile vacutainer tubes of 6 ml capacity without anticoagulant. The vacutainer tubes are then placed in a centrifugal machine at 3000 revolutions per minute (rpm) for 10 minutes, and the middle fraction containing the fibrin clot is then collected 2 mm below lower dividing line, to obtain the PRF. Cavities filled with PRF post oral and maxillofacial surgical procedures, at the institute, showed faster healing in half the time as compared to physiologic healing. PRF, which belongs to a new second generation of platelet concentrates, with simplified processing, and not requiring biochemical blood handling, has several advantages over traditionally prepared PRP, which has been widely used for accelerating soft tissue and hard tissue healing. However, the preparation being strictly autologous, the amount of PRF obtained is limited.

Keywords: Platelet concentrate, platelet-rich fibrin, platelet-rich plasma, Vacutainer.

Introduction

One of the great challenges of clinical research has been the development of bioactive surgical additives, which help to regulate inflammation and increase the speed of healing process.^[1] The healing of hard and soft tissues both, is mediated by a wide range of intra and extra articular events, which in turn are regulated by various signaling proteins. Understanding of this entire process is still not complete;^{[2],[3]} however, it is known that

platelets play a crucial role not only in hemostasis, but also in the wound healing process.

Although use of fibrin adhesives in different fields has been documented since past 30 years,^[4] its use has remained controversial owing to the complexities involved-such as the lengthy and tedious production protocols, and risk of cross infections' amongst others. The development of technologies to obtain platelet concentrates lead to the formation of a new kind of fibrin

adhesive-concentrated Platelet-rich plasma (cPRP); however, because of legal restrictions on blood handling procedures, another family of platelet concentrate appeared in France-Platelet-rich fibrin (PRF).^{[1],[5]} This new biomaterial has proved to be a very good friend to Oral and Maxillofacial Surgeons, and has found numerous applications in other branches of dentistry as well, such as, Periodontics and Oral Implantology.^[5]

Fibrin - Natural Guide For Angiogenesis

Fibrin, which is the activated form of a plasma molecule called fibrinogen^[6] is a soluble fibrillary molecule and is massively present not only in the plasma, but also in the platelet *alpha*-granules. It plays a potential role in platelet aggregation during homeostasis and the fibrin matrix also has the property of angiogenesis.^{[7],[8]}

Also, fibrinogen is the final substrate of all coagulation reactions. Being a soluble protein, it is transformed into an insoluble fibrin by thrombin, while the polymerized fibrin gel constitutes the first cicatricial matrix of the injured site.^{[9],[10],[11]}

Platelet Concentrate Evolution

Platelets isolated from the peripheral blood are an autologous source of growth factors. In medical practice, platelet concentrate is derived from blood, and is used for the prevention and treatment of hemorrhages due to conditions like severe thrombocytopenia of central origin, such as due to medullary aplasia, acute leukemia, etc.^[5] The development of platelet concentrate as a bioactive surgical additive, stems from the use of fibrin adhesives.^[12] Since 1990, medical science has recognized several components in blood, which are a part of the natural healing process, and when added to wounded tissues or surgical sites, have the potential to accelerate wound healing. Fibrin glue was first described in 1970 and is formed by polymerizing fibrinogen with thrombin and calcium. It was originally prepared using donor plasma; however, because of the low

concentration of fibrinogen in plasma, the stability and quality of fibrin glue was low. These adhesives can be obtained autologously from the patient or can be obtained commercially, the latter carrying a small risk of disease transmission.

PRP is an autologous modification of fibrin glue, derived by methods that concentrate autologous platelets, and has been described and used in various applications with apparent clinical success. It is an easily available source of growth factors to support bone and soft tissue healing. PRP is a simple strategy to concentrate platelets or enrich natural blood clot. A natural blood clot contains 94% red blood corpuscles (RBCs), 5% platelets and 1% white blood corpuscles (WBCs), while PRP contains 95% of platelets.^[5] PRP obtained from autologous blood is used to deliver growth factors in higher concentration to the site of bone defect or a region requiring augmentation. The drawbacks of PRP include biochemical blood handling with addition of anticoagulants.

The PRF is a second generation platelet concentrate which is an improvement over traditionally prepared PRP.

Platelet - Rich Fibrin -A Natural Fibrin Matrix

PRF is an immune and platelet concentrate collecting on a single fibrin membrane, containing all the constituents of a blood sample which are favorable to healing and immunity.^[6] This new biomaterial looks like an autologous cicatricial matrix, which is neither like fibrin glue nor like a classical platelet concentrate. It is simply centrifuged blood without any addition.^[1] PRF consists of a fibrin matrix polymerized in a tetra molecular structure, with incorporation of platelets, leucocytes, cytokines, and circulating stem cells.^{[7],[13]} Clinical studies reveal that this biomaterial would be a favorable matrix for the development of a coherent healing, without any inflammatory excess. PRF in the form of a platelet gel can be used in conjunction with bone grafts,

which has several advantages, such as promoting wound healing, bone growth and maturation, wound sealing and hemostasis, and imparting better handling properties to graft materials.^[5] It can also be used as a membrane. Many clinical trials suggest the combination of bone grafts and PRF to enhance bone density.^[5]

Preparation of Platelet - Rich Fibrin

The PRF preparation protocol is very simple and armamentarium required is same as that of PRP. Around 5 ml of whole venous blood is collected in each of the two sterile vacutainer tubes of 6 ml capacity without anticoagulant. The vacutainer tubes are then placed in a centrifugal machine [Figure 1] at 3000 revolutions per minute (rpm) for 10 minutes, after which it settles into the

following layers: red lower fraction containing red blood cells, upper straw colored cellular plasma and the middle fraction containing the fibrin clot [Figure 2]. The upper straw colored layer is then removed and middle fraction is collected, 2 mm below lower dividing line, which is the PRF [Figure 3]. The mechanism which is followed here is that, fibrinogen which is initially concentrated in the high part of the tube, combines with the circulating thrombin due to centrifugation, to form fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and a cellular plasma at top. Platelets are trapped massively in the fibrin meshes [Figure 4]



Figure 1: Centrifuge machine used for preparation of Platelet-rich fibrin from blood samples in the vacutainer tubes.



Figure 2: Blood in the vacutainer tubes after centrifugation at 3000 rpm for 10 minutes divided into three fractions; lower fraction of red blood cells, middle fraction containing fibrin clot and upper a cellular plasma fraction



Figure 3: Isolated Platelet-rich fibrin by separating the middle fraction from the centrifuged blood, 2 mm below the lower dividing line



Figure 4: Platelet-rich fibrin ready for use

The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. In fact, without anticoagulant, the blood sample starts to coagulate almost immediately upon contact with the tube glass, and it does take a minimum of few minutes of centrifugation to concentrate fibrinogen in the middle and upper part of the tube. Quick handling is the only way to obtain a clinically usable PRF clot.

PRF protocol makes it possible to collect a fibrin clot charged with serum and platelets. By driving out the fluids trapped in the fibrin matrix, practitioners can obtain very resistant autologous fibrin membranes.

Why platelet-rich fibrin over platelet-rich plasma?

PRF does not require biochemical handling of blood ^{[5], [14], and [15]} [Table 1].

PRF preparation is a simplified and cost effective process over PRP. ^{[5],[14],[15]} [Table 1].

PRF eliminates redundant process of adding bovine thrombin to promote conversion of fibrinogen to fibrin which is necessary in PRP. ^{[1],[5],[14],[15],[16]} The use of anticoagulants also is avoided.

Conversion of fibrinogen to fibrin takes place slowly with small quantities of physiologically available thrombin present in the blood sample itself. Thus a physiologic architecture, which is very favorable to the healing process, is obtained due to slow polymerization. The fibrin network generated here is very similar to a natural one, and leads to a more efficient cell migration and proliferation, and thus cicatrisation. ^{[5],[17]} Slow polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and organic chains in the fibrin meshes.

This result would imply that PRF, unlike the other platelet concentrates would be able to release cytokines during the fibrin matrix

remodeling. Such a mechanism might explain the clinically observed healing properties of PRF. And also, PRF has a supportive effect on the immune system.^{[1],[2],[5],[17],[18]} Studies showed PRP has limited potential to stimulate bone

regeneration as it releases growth factors quickly, just before the cell outgrowth from the surrounding tissue.^{[17],[18],[19]} It is also been demonstrated that bovine thrombin which is used for PRP preparation may have toxic effects on the body cells.

Advantages of Platelet-rich fibrin over Platelet-rich plasma	Disadvantages of Platelet-rich fibrin
No biochemical handling of blood Simplified and cost effective process and use of bovine thrombin and anticoagulants not required Favorable healing due to slow polymerization More efficient cell migration and proliferation PRF has supportive effect on immune system PRF helps in haemostasis	Amount available is low, because of autologous blood Quick handling of blood is needed, immediately after collection
PRF = Platelet-rich fibrin	

Table 1: The advantages of Platelet-rich fibrin over Platelet-rich plasma and disadvantages of Platelet-rich fibrin

Platelet-Rich Fibrin In Oral And Maxillofacial Surgery

PRF was first developed in France by Choukroun *et al.* for specific use in Oral and Maxillofacial surgery. PRF can be considered as a natural fibrin based biomaterial, favorable to the development of a micro vascularisation and is also able to guide epithelial cell migration to its surface. The interest in such a membrane, mainly to protect open wounds and accelerate healing, is evident.^{[20],[21]} Its utilization seems to be of high interest in cases of infected wounds as this matrix also contains leucocytes, and promotes their migration. Fibrin and its degeneration products help in natural support to immunity by modulating cluster of differentiation receptors, such as,

CD11/CD18 receptors etc. With this in mind, PRF is routinely used at our institute in various surgical procedures, e.g., post enucleation of large periapical lesions [Figure 5], surgical removal of impacted third molars [Figure 6] and impacted canines [Figure 7], preprosthetic surgeries, graft stabilization in various procedures, placements of implants in borderline cases, etc. with satisfactory results. Cavities filled with PRF showed complete healing in half the time as compared to time required for physiologic healing. Our clinical experience together with various *in vivo* and *in vitro* studies in literature, confirms the potential of PRF as a good healing biomaterial.^[7]



Figure 5: Platelet-rich fibrin application in periapical lesion in the region of upper anterior teeth



Figure 6 :Platelet-rich fibrin application in Mandibular Third Molar socket area



Figure 7: Platelet-rich fibrin application in cavity created post surgical removal of impacted right permanent maxillary canine

Discussion

PRF was first used by Choukroun *et al.* in France and belongs to a new generation of platelet concentrate. Simplified processing technique not requiring biochemical blood handling makes it superior to PRP. PRF can be used to promote wound healing, bone

growth, graft stabilization, wound sealing, and hemostasis. Because the fibrin matrix is better organized, it is able to more efficiently direct stem cell migration and the healing program.^{[20],[21]} *In vitro* release of growth factors from PRF and the results of *in vivo* studies has now put forward a

proposal to optimize the clinical application of PRF. [22] *In vitro* studies has shown better results of PRF over PRP. [14] The findings by Wiltfang *et al.* from a series of clinical trials showed encouraging results. Dohan *et al.* proved a slower release of growth factors from PRF than PRP and observed better healing properties with PRF. [1] In a study by Bensaïd *et al.* [23] it was observed that the cells are able to migrate from the fibrin scaffold; while Kawamura and Urist demonstrated that PRF may act as a supportive matrix for bone morphogenetic protein as well. [19] Even at our institute, the clinical results of using PRF in various surgical procedures were promising and satisfactory; however, an in-depth statistical evaluation still needs to be done.

Conclusion

Thus, with this article we can conclude that the new and recent generation of platelet concentrate-PRF, would be a good friend to Oral and Maxillofacial Surgeons in the near future. The clinical experience also confirms that PRF can be considered a healing biomaterial, as it features all the necessary parameters permitting optimal wound healing. It already has a list of intraoral applications, and numerous extra oral applications can also be imagined. PRF can be used for all types of superficial cutaneous and mucous healing. [8] This material is already being used widely in France, and considering its advantages, its popularity should increase here too. More clinical, histological and statistical studies are now required from different parts of the world to understand the benefits of this new platelet concentrate better.

However, it cannot be ignored that since it is obtained from an autologous blood sample, the quantity of PRF produced is low and only a limited volume can be used. This fact limits the systematic utilization of PRF, as in general surgery.

References

1) 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
- 2) Gabling VL, Acil Y, Springer IN, Hubert N, Wiltfang J. Platelet-rich plasma and Platelet-rich fibrin in human cell culture. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108:48-55
- 3) Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *ThrombHaemost* 2004; 91:4-15.
- 4) Gibble JW, Ness PM. Fibrin glue: The perfect operative sealant? *Transfusion* 1990; 30:741-7.
- 5) Sunitha R, Munirathnam N. Platelet-rich fibrin: Evolution of a second-generation platelet concentrate. *Indian J Dent Res* 2008; 19:42-6.
- 6) Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. *Ann N Y AcadSci* 2001; 936:11-30.
- 7) 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.
- 8) Dvorak HF, Harvey VS, Estrella P, Brown LF, McDonagh J, Dvorak AM. Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. *Lab Invest* 1987; 57:673-86.
- 9) Clark RA. Fibrin and wound healing. *Ann N Y Acad Sci* 2001; 936:355-67.
- 10) Collen A, Koolwijk P, Kroon M, Van Hinsbergh VW. Influence of fibrin structure on the formation and maintenance of capillary like tubules by human microvascular endothelial cells. *Angiogenesis* 1998; 2:153-65.

- 11) Van Hinsbergh VW, Collen A, Koolwijk P. Role of fibrin matrix in angiogenesis. *Ann N Y Acad Sci* 2001; 936:426-37.
- 12) Carlson ER. Bone grafting the jaws in the 21st century: The use of platelet-rich plasma and bone morphogenetic protein. *Alpha Omegan* 2000; 93:26-30.
- 13) 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.
- 14) 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.
- 15) Uggeri J, Belletti S, Guizzardi S, Poli T, Cantarelli S, Scandroglio R, *et al.* Dose-dependent effects of platelets on activities of human osteoblasts. *J Periodontol* 2007; 78:1985-91.
- 16) 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: Leucocytes activation: A new feature for platelet concentrates? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006; 101:E51-5.
- 17) Vinazzer H. Fibrin sealing: Physiologic and biochemical background. *Fac Plast Surg* 1985; 2:291-5.
- 18) 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.
- 19) Kawamura M, Urist MR. Human fibrin is a physiologic delivery system for bone morphogenetic protein. *ClinOrthopRelat Res* 1988; 235:302-10.
- 20) Pierce GF, Mustoe TA, Altrock BW, Deuel TF, Thomason A. Role of platelet-derived growth factor in wound healing. *J Cell Biochem* 1991; 45:319-26.
- 21) Bolander ME. Regulation of fracture repair by growth factors. *ProcSocExpBiol Med* 1992; 200:165-70.
- 22) Cromack DT, Porras-Reyes B, Mustoe TA. Current concepts in wound healing: Growth factor and macrophage interaction. *J Trauma* 1990;30:S129-33.
- 23) Loike JD, Sodeik B, Cao L, Leucona S, Weitz JI, Detemers PA, *et al.* CD11c/CD18 on neutrophils recognizes a domain at the N Terminus of the A alpha chain of fibrinogen. *Proc Natl Acad Sci USA* 1991; 88:1044-8.