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Platelet-rich fibrin: Evolution of a second-generation platelet concentrate

V Sunitha Raja[1], E Munirathnam Naidu[2],
[1] Department of Periodontics, Meenakshiammal Dental College and Hospital, Alapakkam Road, Maduravoyal, Chennai - 600 095, India
[2] Department of Prosthodontics, Meenakshiammal Dental College and Hospital, Alapakkam Road, Maduravoyal, Chennai - 600 095, India

Correspondence Address:
V Sunitha Raja
Department of Periodontics, Meenakshiammal Dental College and Hospital, Alapakkam Road, Maduravoyal, Chennai - 600 095
India

Abstract

Platelet-rich plasma (PRP) is a platelet concentrate that has been used widely to accelerate soft-tissue and hard-tissue healing. The preparation of PRP has been described by several authors. Platelet-rich fibrin (PRF) was first described by Choukroun *et al.* in France. It has been referred to as a second-generation platelet concentrate, which has been shown to have several advantages over traditionally prepared PRP. Its chief advantages include ease of preparation and lack of biochemical handling of blood, which makes this preparation strictly autologous. This article describes the evolution of this novel platelet concentrate, referred to as PRF.

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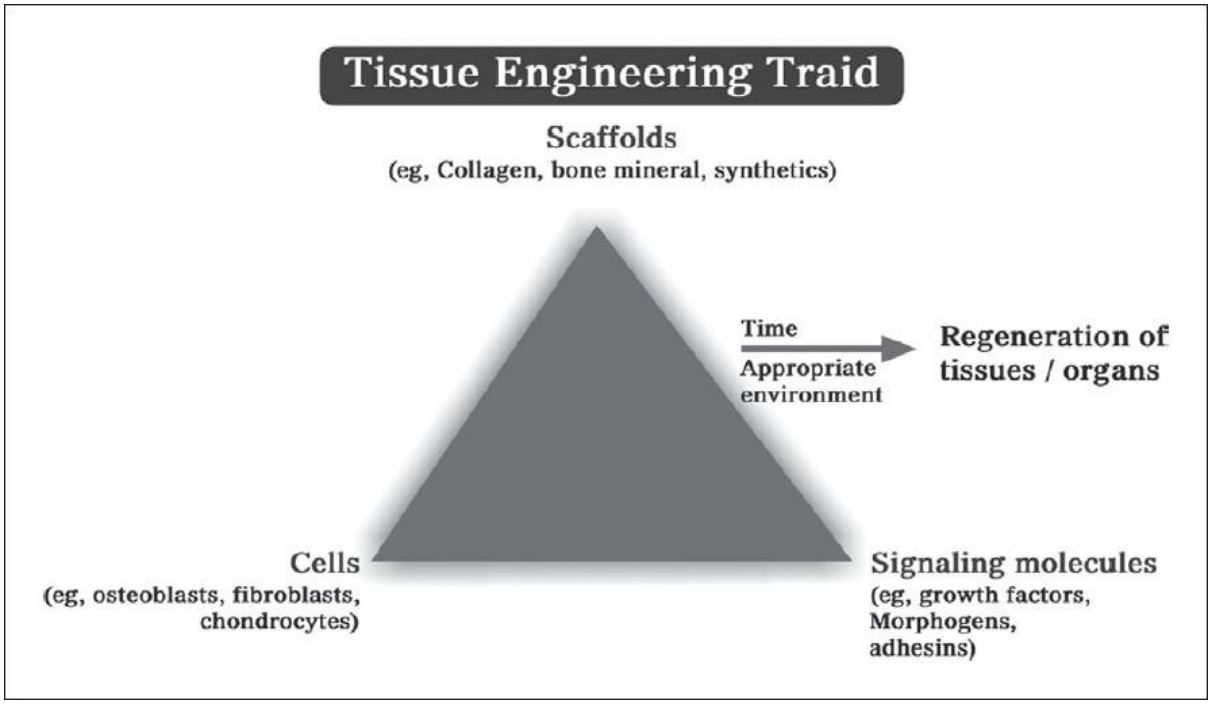


Figure 1: Tissue engineering triad that includes cells, scaffolds, and signalling molecules. This triad is essential for regeneration[1]

The term tissue engineering was originally coined to denote the construction in the laboratory of a device containing viable cells and biologic mediators (e.g., growth factors and adhesins) in a synthetic or biologic matrix, which could be implanted in patients to facilitate regeneration of particular tissues. The role of tissue oxygenation in wound healing became the focal point in the 1980s. Tissue oxygenation enhances phagocytic and bactericidal ability of host immune cells and supports collagen as well as other protein synthetic events. The importance of growth factors in enhancing wound healing has become the focus of research in the present day. In addition, a link has been established between tissue oxygenation and growth factors. Macrophage stimulation causes the release of angiogenic and other growth factors that support wound healing and resist infection. [1] In general, tissue engineering combines three key elements, namely scaffolds (collagen, bone mineral), signaling molecules (growth factors), and cells (osteoblasts, fibroblasts) [Figure 1]. Tissue engineering has been redefined presently as the relatively new, highly promising field of reconstructive biology, which draws on the recent advances in medicine and surgery, molecular and cellular biology, polymer chemistry, and physiology.

These principles of tissue engineering have found widespread application in several branches of dentistry, such as periodontics, oral and maxillofacial surgery, and oral implantology. In the field of implant dentistry, the most frequently encountered problems at the implantation site are lack of adequate

bone and proximity to anatomic structures, such as the maxillary sinus and the inferior alveolar nerve canal. Advanced surgical procedures that act as an adjunct in dental implants consist of sinus grafting and guided bone regeneration. These procedures are quite predictable when proper surgical protocols are established and followed.

Bone graft materials commonly used for these procedures are demineralized freeze-dried bone allograft (DFDBA) and freeze-dried bone allograft (FDBA). The osteoinductive properties of DFDBA have made it the grafting material of choice as compared to FDBA, xenografts, and alloplasts. However, the osteoinductive potential of DFDBA procured from different bone banks or from different batches of the same bank may vary highly. The bioactivity of DFDBA seems to be dependent on the age of the donor; the younger the donor the more osteoinductive the graft material. [2] This controversy as well as concerns about disease transmission has pushed clinicians toward using xenografts and alloplastic materials. Although these materials are biocompatible and are osteoconductive in nature, clinical outcomes are unpredictable. The problem that arises next is how to improve clinical outcomes by improving the properties of these grafts.

Platelets isolated from peripheral blood are an autologous source of growth factors. When platelets in a concentrated form are added to graft materials, a more predictable outcome is derived. Platelet-rich plasma (PRP) is an easily accessible source of growth factors to support bone- and soft-tissue healing. It is derived by methods that concentrate autologous platelets and is added to surgical wounds or grafts and to other injuries in need of supported or accelerated healing. A blood clot is the center focus of initiating any soft-tissue healing and bone regeneration. In all natural wounds, a blood clot forms and starts the healing process. PRP is a simple strategy to concentrate platelets or enrich natural blood clot, which forms in normal surgical wounds, to initiate a more rapid and complete healing process. A natural blood clot contains 95% red blood cells, 5% platelets, less than 1% white blood cells, and numerous amounts of fibrin strands. A PRP blood clot contains 4% red blood cells, 95% platelets, and 1% white blood cells.

The use of PRP in place of recombinant growth factors has several advantages, in that growth factors obtained from platelets not only have their own specific action on tissues but also interact with other growth factors, resulting in the activation of gene expression and protein production. [3] Therefore, the properties of PRP are based on the production and release of multiple growth and differentiation factors upon platelet activation. These factors are critical in the regulation and stimulation of the wound healing process, and they play an

important role in regulating cellular processes such as mitogenesis, chemotaxis, differentiation, and metabolism. [4]

Platelet Concentrates: Evolution

In general, platelet concentrates are blood-derived products used for the prevention and treatment of hemorrhages due to serious thrombopenia of the central origin. The development of platelet concentrates as bioactive surgical additives that are applied locally to promote wound healing stems from the use of fibrin adhesives. Since 1990, medical science has recognized several components in blood, which are a part of the natural healing process; when added to wounded tissues or surgical sites, they have the potential to accelerate healing. Fibrin glue was originally 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 were low.

These adhesives can be obtained autologously from the patient or can be obtained commercially (Tisseel, Baxter Healthcare). These products are heat-treated, thus immensely reducing, but not entirely eliminating, the risk of disease transmission. Therefore, the commercially available adhesives constitute an infinitely small risk of disease transmission. PRP is an autologous modification of fibrin glue, which has been described and used in various applications with apparent clinical success. PRP obtained from autologous blood is used to deliver growth factors in high concentrations to the site of bone defect or a region requiring augmentation. [3]

The purpose of this article is to describe the preparation of a novel second-generation platelet concentrate called PRF, which is an improvement over the traditionally prepared PRP. The preparation and use of PRF is prevalent in France, and is not yet popular in the rest of the world for unknown reasons. This article aims to highlight the simplicity of the preparation of PRF as compared to PRP.

Platelet-Rich Plasma: Preparation

Platelets are small, irregularly shaped anuclear cells, 2–4 mm in diameter, which are derived from fragmentation of precursor megakaryocytes. The average life span of a platelet is between 8 and 12 days. Platelets play a fundamental role in hemostasis and are a natural source of growth factors. 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 [Table 1]. The release of growth factors is triggered by the activation of platelets, which may be initiated by a variety of substances or stimuli, such as thrombin, calcium chloride, collagen or adenosine 5c-diphosphate. [5] In addition to these growth factors, PRP contains fibrinogen and a number of adhesive glycoproteins that support cell migration.

PRP can be prepared by two techniques. The techniques differ in their technical aspects and are divided into:

General-purpose cell separatorsPlatelet-concentrating cell separators

Table 1: Growth factors released from platelets and their biologic actions

Growth factor	Source cells	Target	Biologic action
Platelet-derived growth factor	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	Fibroblasts, smooth muscle cells, glial cells, macrophages, neutrophils	Stimulates DNA and protein synthesis in osseous tissues; mitogenic effects on mesenchymal cells; angiogenic effect on endothelial cells
Transforming growth factor β	Platelets, T-lymphocytes, macrophages/monocytes, neutrophils	Fibroblasts, marrow stem cells, endothelial cells, epithelial cells, preosteoblasts	Stimulates angiogenesis; enhanced woven bone formation; stimulate matrix synthesis in most culture systems; chemotactic effect on osteoblastic cells; stimulates endothelial chemotaxis; stimulates bone formation by inhibitory effect on osteoclasts
Platelet-derived angiogenesis factor	Platelets, endothelial cells	Endothelial cells	Mitogenic effect on endothelial cells; increased angiogenesis and vessel permeability
Insulin-like growth factor 1	Osteoblasts, macrophages, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondroblasts	Stimulates proliferation of osteoblasts and matrix synthesis; increases expression of bone matrix proteins, such as osteocalcin; in combination with PDGF it enhances the rate and quality of wound healing
Platelet factor 4	Platelets	Fibroblasts, neutrophils	Chemoattractant for neutrophils and fibroblasts

PDGF - platelet-derived growth factor, TGF-β - transforming growth factor β, PDAF - platelet-derived angiogenesis factor, IGF-1 - insulin-like growth factor -1, PF-4 - platelet factor - 4

General-purpose cell separators require large quantities of blood (450 ml) and generally require to be operated in a hospital setting. Blood is drawn into a collection bag containing citrate-phosphate-dextrose anticoagulant. It is first centrifuged at 5,600 rpm to separate RBCs from platelet-poor plasma (PPP) and PRP. The centrifugation speed is then reduced to 2,400 rpm to get a final separation of about 30 ml of PRP from the RBCs. With this technique, the remaining PPP and RBCs can be returned to the patient's circulation or can be discarded. The ELMD-500 (Medtronic Electromedic, Auto Transfusion System, Parker, CO, USA) cell separator is widely used for this technique.

Platelet-concentrating cell separators are more widely used since this equipment can be accommodated in a dental clinic setup. These technologies permit the procurement of PRP using smaller quantities of blood. Currently, two such systems are approved by FDA and commercially available: Harvest SmartPrep Platelet Concentrate System (HSPCS; Harvest Technologies, Plymouth, MA, USA) and the 3i Platelet Concentrate Collection System (3i PCCS; 3i Implant Innovations, Palm Beach Gardens, FL, USA). Several studies have been performed to compare the efficacy of these systems. [6],[7],[8] Although traditionally a double-spin technique has been used, authors such as Eby et al . [9] have proposed the use of a single spin technique. The preparation and processing of PRP is quite similar in most of the platelet-concentrating systems although the anticoagulant used and the speed and

duration of centrifugation may differ with different systems.

Venous blood is drawn into a tube containing an anticoagulant to avoid platelet activation and degranulation.The first centrifugation is called "soft spin", which allows blood separation into three layers, namely bottom-most RBC layer (55% of total volume), topmost acellular plasma layer called PPP (40% of total volume), and an intermediate PRP layer (5% of total volume) called the "buffy coat".Using a sterile syringe, the operator transfers PPP, PRP and some RBCs into another tube without an anticoagulant.This tube will now undergo a second centrifugation, which is longer and faster than the first, called "hard spin". This allows the platelets (PRP) to settle at the bottom of the tube with a very few RBCs, which explains the red tinge of the final PRP preparation. The acellular plasma, PPP (80% of the volume), is found at the top.Most of the PPP is removed with a syringe and discarded, and the remaining PRP is shaken well.This PRP is then mixed with bovine thrombin and calcium chloride at the time of application. This results in gelling of the platelet concentrate. Calcium chloride nullifies the effect of the citrate anticoagulant used, and thrombin helps in activating the fibrinogen, which is converted to fibrin and cross-linked. [10]

Potential Risks of Using PRP

Sanchez et al . [11] have elaborated on the potential risks associated with the use of PRP. The preparation of PRP involves the isolation of PRP after which gel formation is accelerated using calcium chloride and bovine thrombin. It has been discovered that the use of bovine thrombin may be associated with the development of antibodies to the factors V, XI and thrombin, resulting in the risk of life-threatening coagulopathies. Bovine thrombin preparations have been shown to contain factor V, which could result in the stimulation of the immune system when challenged with a foreign protein. Other methods for safer preparation of PRP include the utilization of recombinant human thrombin, autologous thrombin or perhaps extra-purified thrombin. Landesberg et al . [12] have suggested that alternative methods of activating PRP need to be studied and made available to the dental community.

Preparation and Properties of PRF

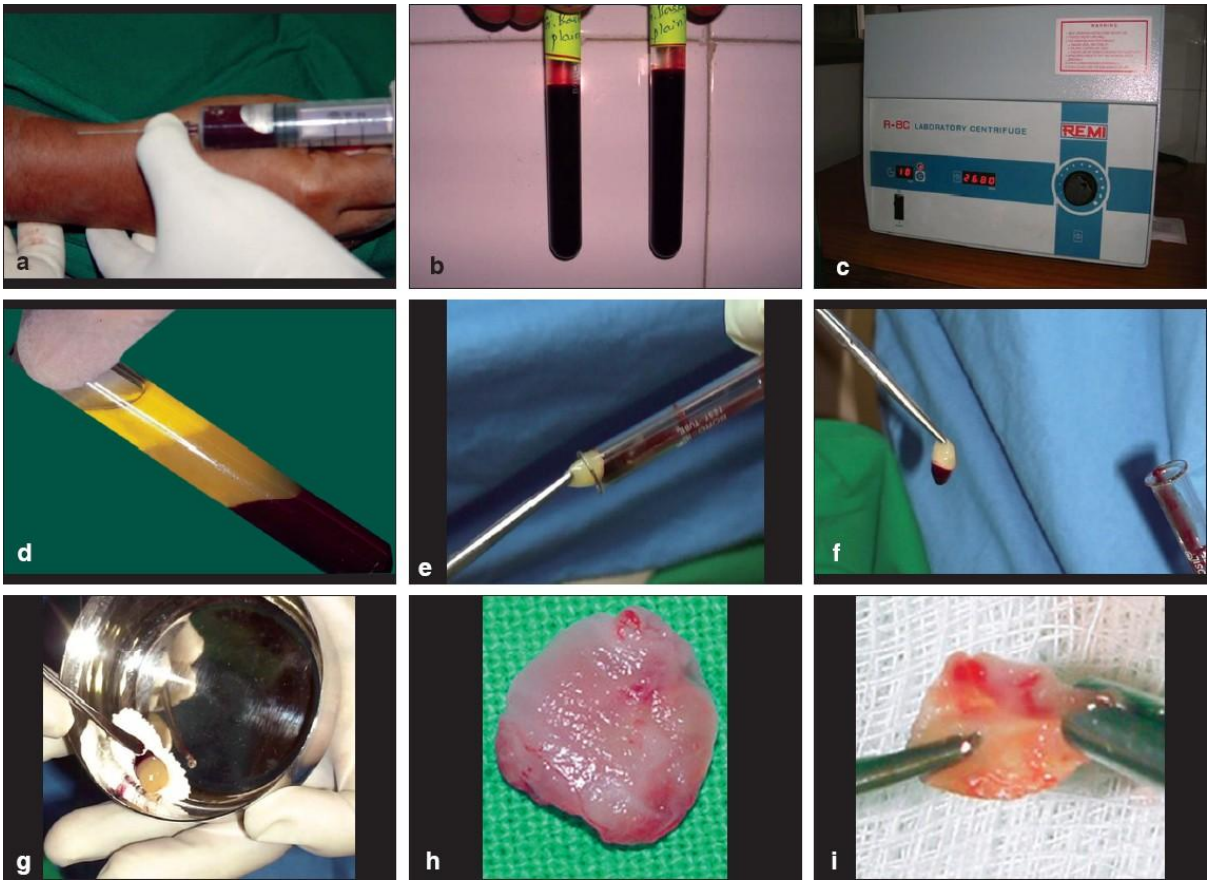
Overcoming the restrictions in the French law related to the reimplantation of blood-derived products, PRF was first developed in France by Choukroun et al . This second-generation platelet concentrate eliminated the risks associated with the use of bovine thrombin. A report of clinical trials comparing the growth

factor content of PRF and PRP was presented by Wiltfang et al . at the Second International Symposium on Growth Factors held in May 2005. [13]

Preparation

The advantages of PRF over PRP are its simplified preparation and lack of biochemical handling of the blood. The required quantity of blood is drawn into 10-ml test tubes without an anticoagulant and centrifuged immediately. Blood is centrifuged using a tabletop centrifuge (REMY Laboratories) for 12 min at 2,700 rpm [Figure 2].

Figure 2: (a) and (b) Blood being drawn into test tubes. (c) Centrifuge used for preparation of PRF (Remi Laboratories). (d) Prepared PRF. (e) and (f) PRF clot being retrieved, (g) PRF being mixed with bone graft. (h) and (i) PRF membrane



The resultant product consists of the following three layers:
Topmost layer consisting of acellular PPPPRF clot in the middleRBCs at the bottom

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. [14] PRF can be obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.

Discussion

PRF is in the form of a platelet gel and can be used in conjunction with bone grafts, which offers several advantages including promoting wound healing, bone growth and maturation, graft stabilization, wound sealing and hemostasis, and improving the handling properties of graft materials. PRF can also be used as a membrane. Clinical trials suggest that the combination of bone grafts and growth factors contained in PRP and PRF may be suitable to enhance bone density. In an experimental trial, the growth factor content in PRP and PRF aliquots was measured using Elisa kits. The results suggest that the growth factor content (PDGF and TGF-β) was comparable in both. Another experimental study used osteoblast cell cultures to investigate the influence of PRP and PRF on proliferation and differentiation of osteoblasts. In this study, the affinity of osteoblasts to the PRF membrane appeared to be superior. [11]

PRF has many advantages over PRP. It eliminates the redundant process of adding anticoagulant as well as the need to neutralize it. The addition of bovine-derived thrombin to promote conversion of fibrinogen to fibrin in PRP is also eliminated. The elimination of these steps considerably reduces biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin. The conversion of fibrinogen into fibrin takes place slowly with small quantities of physiologically available thrombin present in the blood sample itself. Thus, a physiologic architecture that is very favorable to the healing process is obtained due to this slow polymerization process.

Literature pertaining to PRF is found in French, and the material is being used widely in France. The popularity of this material should increase considering its many advantages. The findings of Wiltfang et al . [13] from a series of clinical trials are encouraging, in that they show improved properties of PRF as compared with PRP. In future, more histologic evaluations from other parts of the world are required to understand the benefits of this second-generation platelet concentrate.

References

1 Marx ER. Platelet-rich plasma: A source of multiple autologous growth factors for bone grafts. *In* : Lynch SE, Genco RJ, Marx RE, editors. Tissue engineering, applications in maxillofacial surgery and periodontics,

Quintessence Publishing Co, Inc: Illinois; 1999. p. 71-82.
2 Schwartz Z, Somers A, Mellonig TJ, Carnes DL Jr, Dean DD, Cochran DL, *et al* . Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation is dependent on donor age but not gender. J Periodontol 1998;69:470-8.
3 Carlson ER. Bone grafting the jaws in the 21 st century: The use of platelet-rich plasma and bone morphogenetic protein. Alpha Omegan 2000;93:26-30.
4 Garg AK. The use of platelet-rich plasma to enhance the success of bone grafts around dental implants. Dental Implantol Update 2000;11:17-21.
5 Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:638-46.
6 Appel TR, Potzsch B, Muller J, von Lindern JJ, Bergı SJ, Reich RH. Comparison of three different preparations of platelet concentrates for growth factor enrichment. Clin Oral Implants Res 2002;13:522-8.
7 Weibrich G, Kleis WK. Curasan PRP kit vs. PCCS PRP system: Collection efficiency and platlet counts of two different methods for the preparation of platelet-rich plasma. Clin Oral Implants Res 2002;13:437-43.
8 Weibrich G, Kleis WK, Buch R, Hitzler WE, Hafner G. The Harvest Smart PreP system versus the Friadent-Schutze platelet-rich plasma kit. Clin Oral Implants Res 2003;14:233-9.
9 Eby BW. Platelet-rich plasma: Harvesting with a single-spin centrifuge. J Oral Implantol 2002;28:297-301.
10 Sonnleitner D, Huemer P, Sullivan DY. A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: A technical note. Int J Oral Maxillofac Implants 2000;15:879-82.
11 Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. Int J Oral Maxillofac Implants 2003;18:93-103.
12 Landesberg R, Moses M, Karpatkin M. Risk of using platelet-rich plasma gel. J Oral Maxillofac Surg 1998;56:1116-7.
13 Par Wiltfang J, Terheyden H, Gassling V, Acyl A. Platelet rich plasma (PRP) vs. platelet rich fibrin (PRF): Comparison of growth factor content and osteoblast proliferation and differentiation in the cell culture. *In:* Report of the 2nd International Symposium on growth Factors (SyFac 2005).
14 Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, *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.