

# Platelet rich fibrin: a new paradigm in periodontal regeneration

R. Vinaya Kumar · N. Shubhashini

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**Abstract** Among the great challenges facing clinical research is the development of bioactive surgical additives regulating inflammation and increasing healing. Although the use of fibrin adhesives and platelet-rich plasma (PRP) is well documented, they have their own limitations. Hence, reconstructive dental surgeons are looking for an “edge” that jump starts the healing process to maximize predictability as well as the volume of regenerated bone. Overcoming the restrictions related to the reimplantation of blood-derived products, a new family of platelet concentrate, which is neither a fibrin glue nor a classical platelet concentrate, was developed in France. This second generation platelet concentrate called platelet-rich fibrin (PRF), has been widely used to accelerate soft and hard tissue healing. Its advantages over the better known PRP include ease of preparation/application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required). This article serves as an introduction to the PRF

“concept” and its potential clinical applications with emphasis on periodontal regeneration.

**Keywords** Platelet-rich fibrin · Cytokines  
Fibrin fibrillae · Root coverage · Regeneration · Healing

## Abbreviations

ALP	Alkaline phosphatase
CCE	Crestal core elevation
CD	Cluster of Differentiation
cPRP	Concentrated platelet-rich plasma
CR3	Complement Receptor 3
FDBA	Freeze-dried bone allograft
FDPs	Fibrinogen degradation products
GBR	Guided bone regeneration
IGF	Insulin-like growth factor
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
L-PRF	Leucocyte- and platelet-rich fibrin
MCAF	Modified coronally advanced flap
OFD	Open flap debridement
OMSFE	Osteotome-mediated sinus floor elevation
OPG	Osteoprotegerin
PDGF	Platelet-derived growth factor
p-ERK	Phosphorylated extracellular signal-regulated protein kinase
PPP	Platelet-poor plasma
PRF	Platelet-rich fibrin
PRFe	PRF extract
PRFM	Platelet-rich fibrin matrix

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R. Vinaya Kumar (✉)  
Department of Periodontics, Rajarajeswari Dental College and Hospital, Mysore Road, Ramohalli Cross, Bangalore 560060, India  
e-mail: drrvinayakumar@gmail.com

N. Shubhashini  
Department of Conservative Dentistry and Endodontics, Rajarajeswari Dental College and Hospital, Mysore Road, Ramohalli Cross, Bangalore 560060, India

PRP	Platelet-rich plasma
RBCs	Red blood cells
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
WBCs	White blood cells

## Introduction

Among the great challenges facing clinical research is the development of bioactive surgical additives regulating inflammation and increasing healing. In fact, after every intervention, surgeons face complex tissue remodeling phenomena and the consequences on healing and tissue survival (Dohan et al. 2006a). Hence, reconstructive dental surgeons are constantly looking for an “edge” that jump starts the healing process to maximize predictability as well as the volume of regenerated bone (Toffler et al. 2009).

## Evolution of platelet concentrates

Platelet concentrates were initially used for the treatment and prevention of hemorrhage due to severe thrombopenia. The use of these blood-derived products to seal wounds and stimulate healing started with the use of fibrin glues, which were first described 40 years ago and are constituted of concentrated fibrinogen. These adhesives can be obtained from the patient or procured commercially (Tisseel, Baxter Healthcare). Their use though remains limited owing to the complexity and cost of their production protocols as well as the risk of disease transmission (Raja and Naidu 2008; Dohan Ehrenfest et al. 2009).

Hence, the use of platelet concentrates to improve healing and replace fibrin glues has been greatly explored during the last two decades. Platelet-rich plasma (PRP) was first identified in the early 1990s through the use of plasmapheresis and PRP sequestration (Jameson 2007). It is an autologous concentration of human platelets in a small volume of plasma (Marx 2004). A PRP blood clot contains 4 % RBCs, 95 % platelets and 1 % WBCs (Toffler et al. 2009). Furthermore, it is a concentration of the fundamental protein growth factors actively secreted by platelets to initiate wound healing as well as the proteins in blood

known to act as cell adhesion molecules for osteoconduction and as a matrix for bone, connective tissue, and epithelial migration (Marx 2004). Thus, PRP is a safe, cost effective, autologous product. It is readily available at the point-of-care, provides antibacterial protection, while eliminating the risk of disease transmission and immunogenic reactions (Jameson 2007). Once developed, PRP is stable and remains sterile in the anticoagulated state for 8 h and hence is effective in longer surgeries (Marx 2001). However, rare reactions to bovine thrombin have led to lower dose use of thrombin and to better purification processing (Jameson 2007). Besides, its poor mechanical properties make conventional PRP difficult to handle when used in clinical settings and requires secure implantation in a specific site (Lucarelli et al. 2010). PRP has limited potential to stimulate bone regeneration as it releases growth factors quickly, just before the cell outgrowth from the surrounding tissue (Saluja et al. 2011).

Overcoming the restrictions in the French law related to the reimplantation of blood-derived products, a new family of platelet concentrate, which is neither a fibrin glue nor a classical platelet concentrate, was developed. This new biomaterial, called platelet-rich fibrin (PRF), looks like an autologous cicatricial matrix (Dohan et al. 2006a; Raja and Naidu 2008). This article serves as an introduction to the PRF “concept” and its potential clinical applications with emphasis on periodontal regeneration.

## Platelet rich fibrin

PRF, developed in France by Choukroun et al in 2001, is a second generation platelet concentrate widely used to accelerate soft and hard tissue healing. It has been defined as an autologous leukocyte and platelet-rich fibrin biomaterial (Toffler et al. 2009; Dohan et al. 2010).

Its advantages over PRP include ease of preparation/application, minimal expense, and lack of biochemical modification as no bovine thrombin or anticoagulant is required (Toffler et al. 2009; Table 1).

The PRF production protocol attempts to accumulate platelets and released cytokines in a fibrin clot. This clot combines many healing and immunity promoters present in the initial blood harvest. It can be used directly as a clot or after compression as a strong membrane (Toffler et al. 2009; Dohan et al. 2010).

**Table 1** Advantages and disadvantages of platelet-rich fibrin

Advantages of platelet-rich fibrin	Disadvantages of platelet-rich fibrin
Ease of preparation/application	Quick handling is the only way to obtain a clinically usable PRF clot
Lack of biochemical modification	
Minimal expense	
Long term effect	
Able to support cytokines enmeshment and cellular migration	
Increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines)	
It is an immune organizing node	
Supports and accelerates the healing process	

## Types of PRF

### Leucocyte-poor or pure platelet-rich fibrin (P-PRF) concentrates

The Fibrinet Platelet-Rich Fibrin Matrix (PRFM) kit by Cascade Medical (New Jersey, USA) contains two tubes, one for blood collection and another for PRFM clotting, together with a transfer device. About 9 ml of blood is drawn into the collection tube containing tri-sodium citrate (anticoagulant) and a proprietary separator gel, and centrifuged at high speed for 6 min. The three typical layers of RBCs, buffy coat and PPP are obtained. Buffy coat and PPP are transferred to a second tube containing calcium chloride ( $\text{CaCl}_2$ ) through a specifically designed tube connection system.

The clotting process is triggered by  $\text{CaCl}_2$  and the tube is immediately centrifuged for 15 min, after which a stable PRFM clot can be collected. Very low amounts of leucocytes are collected owing to the specific separator gel. However, the platelet collection efficiency is high and the preservation of the platelets during the procedure seems acceptable. The fibrin matrix in Fibrinet PRFM is denser and more stable than that in PRPs, probably due to the dynamic clotting during the second centrifugation step, which is more efficient than a static PRP polymerization. The

simultaneous processing of a large number of samples with this method remains difficult and is expensive in daily practice. In addition, studies demonstrating the efficiency of Fibrinet PRFM are not yet available (Dohan Ehrenfest et al. 2009).

### Leucocyte- and platelet-rich fibrin (L-PRF) concentrates: Choukroun's PRF

Choukroun's PRF protocol, on the other hand is a simple and free technique. PRF preparation requires a centrifuge and collection kit including a 24 gauge butterfly needle and 9 ml blood collection tubes. Whole blood is drawn into the tubes without anticoagulant and is immediately centrifuged. The different centrifugation protocols proposed are listed in Table 2.

Within a few minutes, the absence of anticoagulant allows activation of the majority of platelets in the sample to start the coagulation cascade. Initially, fibrinogen is concentrated in the upper part of the tube, until the circulating thrombin transforms it into a fibrin network. The outcome is a fibrin clot containing platelets in the middle of the tube, between the red blood cell layer at the bottom and acellular plasma at the top. This clot is removed from the tube and the attached red blood cells scraped off and discarded. The PRF clot is then placed on the grid in the PRF Box (Process Ltd., Nice, France), and covered with the compressor and lid. This produces an inexpensive autologous fibrin membrane in approximately 1 min. The PRF Box produces membranes of constant thickness that remain hydrated for several hours and recovers the serum exudate expressed from the fibrin clots which is rich in the proteins vitronectin and fibronectin. The exudate collected at the bottom of the box may be used to hydrate graft materials, rinse

**Table 2** Different Centrifugation Protocols

Centrifuged at (rpm)	Time (min)	Reference
3,000	10	Dohan et al. (2010)
2,700	12	Raja and Naidu (2008)
2,500	10	Choukroun et al. (2006b)
3,000	12	Tsai et al. (2009)

surgical sites, and store autologous grafts (Toffler et al. 2009).

However, another alternative to obtain a PRF membrane is by pressing the clot between two gauzes thereby squeezing out the fluids in the fibrin clot (Raja and Naidu 2008).

The PRF clot can also be placed into the cylinder in the PRF Box and slowly compressed with the piston which results in “plugs” or thick small discs of PRF measuring 1 cm in diameter. These are useful in protecting extraction sites (Toffler et al. 2009).

Unlike the PRPs, Choukroun’s PRF does not dissolve quickly after application; instead, the strong fibrin matrix is slowly remodeled similar to a natural blood clot. Platelets and leucocytes are collected with high efficiency in this method and leucocytes are preserved throughout. This method allows the production of a high quantity of L-PRF clots simultaneously, making it suitable for larger surgeries. Another advantage of this technique is its low cost and simplicity, which allows the production of many concentrates quickly and by natural means. Therefore, this method seems to be most suitable for widespread use in daily practice (Dohan Ehrenfest et al. 2009).

### Different polymerizations, different biologies

One of the main differences between fibrin adhesives, cPRP (concentrated platelet-rich plasma) and PRF is attributable from the gelling mode.

Fibrin adhesives and cPRP use a bovine thrombin and  $\text{CaCl}_2$  alliance to initiate the last stages of coagulation and sudden fibrin polymerization. The speed of this reaction is dictated by the use of these surgical additives, and their hemostatic function implies a quasi-immediate setting and therefore significant quantities of thrombin.

PRF, on the other hand, has the characteristic of polymerizing naturally and slowly during centrifugation. The thrombin concentrations acting on the collected autologous fibrinogen are almost physiologic because there is no bovine thrombin addition.

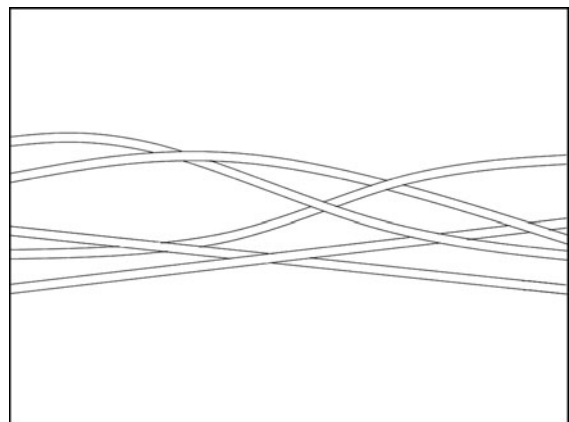
The mode of polymerization will significantly influence the mechanical and biologic properties of the final fibrin matrix. During gelling, the fibrin fibrillae can be assembled among them in 2 different biochemical architectures: condensed tetramolecular or bilateral junctions and connected trimolecular or

equilateral junctions. Bilateral junctions are constituted with strong thrombin concentrations and allow the thickening of fibrin polymers leading to the constitution of a rigid network, unfavorable to cytokine enmeshment and cellular migration (Fig. 1).

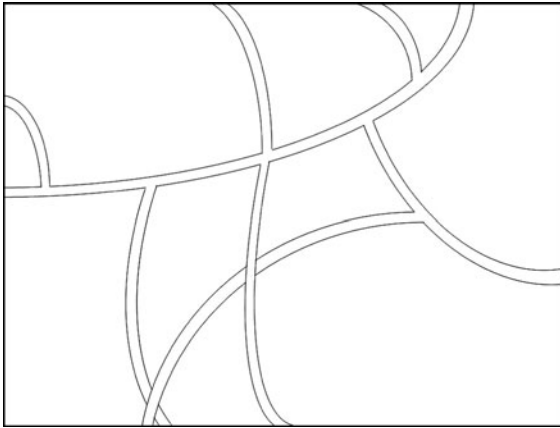
In contrast, weak thrombin concentrations imply a very significant percentage of equilateral junctions. These connected junctions allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration. This 3-dimensional organization gives great elasticity to the fibrin matrix which is observed in a flexible, elastic, and very strong PRF membrane (Fig. 2).

The brutal polymerization mode of cPRP and fibrin adhesives makes intimate incorporation of the cytokines in the fibrin matrix difficult. Thus the released platelet cytokines will be extrinsic, i.e., trapped in the colloidal suspension between the fibrin network meshes during gelling. Their physiologic elimination will therefore be fast and a great share of the theoretical cytokines/fibrin synergies will be lost.

A progressive polymerization mode signifies increased incorporation of the circulating cytokines in the fibrin meshes (intrinsic cytokines). Such a configuration implies an increased lifespan for these cytokines, because they will be released and used only at the time of initial cicatricial matrix remodeling (long term effect). The cytokines are thus maintained available in situ for a convenient period, when the cells start cicatricial matrix remodeling, i.e., when they have to be stimulated to launch injured site reconstruction.



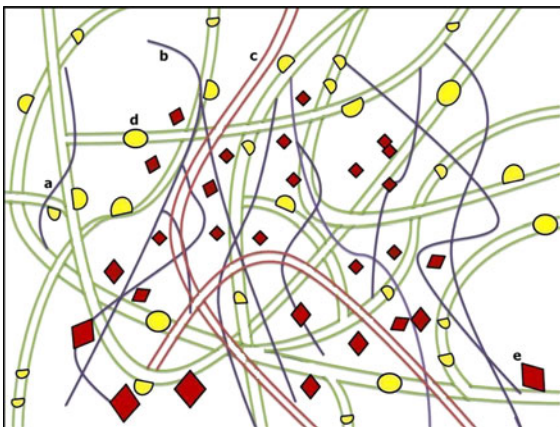
**Fig. 1** Bilateral junctions



**Fig. 2** Equilateral junctions

The biochemical analysis of the PRF composition indicates that this biomaterial consists of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins (fibronectin) enmeshed within a slowly polymerized fibrin network (Fig. 3). These biochemical components have well known synergetic effects on healing processes. For example, fibronectin, as cell proliferation and migration guide, potentiates the stimulative effects from PDGF-BB.

Therefore, these 3 fibrin biotechnologies employ different polymerization modes which involve very different biologic integration mechanisms.



**Fig. 3** Diagrammatic representation of a PRF clot: *a* Fibrin fibrillae associated with glycanic chains and intrinsic cytokines *b* Fibrin-associated glycanic chains *c* Circulating glycoproteins (fibronectin) *d* Cytokine intrinsically retained within fibrin fibrillae *e* Platelet cytokine in solution (extrinsically associated with fibrin polymers)

All these comparative parameters make it possible to consider PRF as a healing biomaterial rather than a new kind of fibrin biological adhesive (Dohan et al. 2006a, b).

## Platelets and PRF

### Platelet distribution in PRF

Initial hematologic studies have revealed that platelets accumulate in the lower part of the fibrin clot, mainly at the junction between the red corpuscles (red thrombus) and the PRF clot itself. This observation emphasizes the idea that the PRF red extremity would be of interest for clinical use and even more effective than the higher part of the fibrin clot.

It is of interest to note that the PRF matrix enmeshes glycosaminoglycans (heparin, hyaluronic acid) from blood and platelets i.e., these glycanic links are incorporated within fibrin polymers. Glycosaminoglycans have a strong affinity with small circulating peptides (such as platelet cytokines) and a great capacity to support cell migrations and healing processes (Dohan et al. 2006b).

### Platelet cytokines

#### *Transforming growth factor $\beta$ -1 (TGF $\beta$ -1)*

TGF $\beta$ -1 is the chiefly produced isoform of TGF  $\beta$ . It represents the most powerful fibrosis agent among all cytokines (Dohan et al. 2006b).

It stimulates fibroblast chemotaxis as well as the production of collagen and fibronectin by cells, while inhibiting collagen degradation by decreasing proteases and increasing protease inhibitors, all of which favour fibrogenesis (Carlson and Roach 2002). Further, TGF- $\beta$  brings about chemotaxis and mitogenesis of osteoblast precursors while also stimulating osteoblast deposition. In addition, it inhibits osteoclast formation and bone resorption, thus favouring bone formation over resorption (Marx et al. 1998).

#### *Platelet derived growth factors (PDGFs)*

PDGF seems to be the first growth factor present in a wound and it initiates connective tissue healing, including bone regeneration and repair (Marx et al.

1998). PDGFs are crucial regulators for the migration, proliferation and survival of mesenchymatous cell lineages (Dohan et al. 2006b). Important activities of PDGF include mitogenesis (increase in cell population of healing cells), angiogenesis (endothelial mitoses into functioning capillaries), and macrophage activation (debridement of the wound site and a secondary phase source of growth factors for continued repair and bone regeneration). There are about 1,200 molecules of PDGF in every individual platelet. Therefore, a greater concentration of platelets as seen in PRF can be expected to have a profound effect on wound healing enhancement and bone regeneration (Marx et al. 1998).

#### *The Insulin-like growth factor (IGF) axis*

IGFs I and II are positive regulators of proliferation and differentiation for most cell types, including tumor cells. They form the major axis of programmed cell death (apoptosis) regulation, by inducing survival signals protecting cells from many matricial apoptotic stimuli (Dohan et al. 2006b).

### **Leucocytes and cytokines**

#### Inflammatory cytokines

##### *Interleukin-1 $\beta$ (IL-1 $\beta$ )*

IL-1 $\beta$  remains the prevalent isoform and is a key mediator of inflammation control.

Its main activity is the stimulation of T helper lymphocytes. In combination with TNF- $\alpha$ , IL-1 would be implied in osteolysis, where it activates osteoclasts and inhibits bone formation (Dohan et al. 2006c).

##### *Interleukin 6 (IL-6)*

IL-6 is a multifunctional cytokine that was originally identified as a B cell differentiation factor which induced the final maturation of B cells into antibody-producing cells (Kishimoto et al. 1995). Within the B lymphocyte populations, IL-6 significantly stimulates the secretion of antibodies by 120–400 times (Dohan et al. 2006c).

In addition, IL-6 is an essential accessory factor for T cell activation and proliferation. IL-6 induced not

only proliferation but also differentiation of cytotoxic T cells (CTL) in the presence of IL-2 from murine as well as human thymocytes and splenic T cells (Kishimoto 1989).

The positive effect of IL-6 on hematopoiesis was first described by Ikebuchi et al in 1987. IL-6 and IL-3 act in a synergistic way to promote hematopoietic stem cell proliferation in vitro where IL-6 activates cells at the G<sub>0</sub> stage to enter into the G<sub>1</sub> phase (Kishimoto 1989; Dohan et al. 2006c).

Lastly, IL-6 functions as a hepatocyte-stimulating factor and induces expression of various acute phase genes (Kishimoto et al. 1995).

IL-6 therefore constitutes a major amplification pathway for signals transmitted to immune cells. Thus, it supports the reaction chains leading to inflammation, destruction and remodeling (Dohan et al. 2006c).

##### *Tumor necrosis factor $\alpha$ (TNF- $\alpha$ )*

TNF derives its name from the ability to stimulate tumor necrosis and regression (Ikram et al. 2004).

TNF- $\alpha$  is one of the first cytokines released during the inflammatory response to bacterial endotoxin aggression. TNF- $\alpha$  activates monocytes and stimulates the remodeling capacities of fibroblasts. In addition, it increases phagocytosis, neutrophil cytotoxicity and modulates the expression of key mediators such as IL-1 and IL-6 (Dohan et al. 2006c).

#### Healing cytokines

##### *Interleukin 4 (IL-4)*

IL-4 induces differentiation of naive helper T cells into T<sub>H</sub>2 cells (Goldsby et al. 2003). This cytokine also supports proliferation and differentiation of the activated B cells. During inflammatory phenomena, it supports healing by moderating inflammation (Dohan et al. 2006c). Moreover, IL-4 is a potent inducer of Interleukin-1 receptor antagonist (IL-Ra), which contributes to its anti-inflammatory actions by neutralizing the biological effects of IL-1 (Tilg et al. 1994).

##### *Vascular endothelial growth factor (VEGF)*

VEGF is considered as a master regulatory molecule for angiogenesis-related processes. Factors like IGF-I and IL-1 $\beta$  regulate angiogenesis by upregulating the



expression of VEGF (Mattuella et al. 2007). It plays a direct role in the control of endothelial cell behaviors, such as proliferation, migration, specialization or just survival (Dohan et al. 2006c).

### Effects on healing

Wound healing of injured tissues is fundamental for survival. Platelets are a rich source of polypeptidic growth factors that can promote wound healing (Danielsen 2008). Although platelet and leukocyte cytokines play an important part in the biology of PRF, the fibrin matrix supporting them constitutes the determining element responsible for the real therapeutic potential of this biomaterial (Toffler et al. 2009).

The keys to healing and soft tissue maturation are angiogenesis, immune control, harnessing the circulating stem cells and wound protection by epithelial cover. The angiogenesis property is explained by the 3-dimensional structure of the fibrin gel, the simultaneous action of cytokines trapped in the meshes and the presence of angiogenesis soluble factors in it. Fibrin stimulates  $\alpha v \beta 3$  integrin expression allowing the cells to bind to fibrin, fibronectin, and vitronectin, an important phase in angiogenesis (Choukroun et al. 2006a).

Fibrin and fibrinogen degradation products (FDP) provide the stimulus for directed neutrophil migration while also increasing CD11/CD18 expression on the neutrophil surface. These complexes mediated adherence of neutrophils to the blood vessel endothelium and thereby facilitate transmigration. Neutrophil activation by FDPs also stimulates release of neutrophil proteases. These enzymes facilitate cell penetration through blood vessel basement membranes as well as degradation of the fibrin clot. Neutrophils at the wound site destroy contaminating bacteria via phagocytosis coupled with toxic oxygen radical generation and enzyme digestion (Clark 2001). Monocytes arrive at the injury site later than neutrophils. It has been demonstrated that the wound colonization by macrophages is controlled by fibronectin via the chemical and physical properties of fibrin and by chemotactic agents trapped in its meshes (Choukroun et al. 2006a). Further, fibrin interaction with the monocyte/macrophages modulates phagocytosis through the intergrin receptor Mac-1 (CD11b/CD18; CR3). The fibrin

fragment D-dimer induces secretion of proteases which facilitates tissue debridement. Thus, macrophages appear to play a pivotal role in the transition between wound inflammation and repair which is modulated by fibrin (Clark 2001). Thereby, fibrin constitutes a natural support to immunity (Choukroun et al. 2006a).

The fibrin clot matrix traps circulating stem cells brought to the injured site by initial neovascularization. These cells converge on a secretory phenotype, allowing vascular and tissue restoration. The fibrin matrix guides the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts. Around the wound's margins, epithelial cells lose their basal and apical polarity and produce basal and lateral extensions toward the wound side. The cells subsequently migrate on the transitory matrix made by fibrinogen, fibronectin, tenascin and vitronectin. Growth factors, particularly PDGF and TGF- $\beta$ , in concert with the clot matrix proteins fibrin and fibronectin, presumably stimulate fibroblasts of the periwound tissue to proliferate, express appropriate integrin receptors and migrate into the wound space (Clark 2001). After migration and degradation of fibrin, fibroblasts start the collagen synthesis (Choukroun et al. 2006a).

### Clinical applications

Some applications of this autologous biomaterial have been described in oral, maxillofacial, ENT (ear, nose and throat) and plastic surgery.

In plastic surgery, PRF clots are often directly used to fill cavities (Charrier et al. 2008) or mixed with an adipocyte graft during a liposuction (Braccini and Dohan 2007). Membranes could also be useful for small otologic surgery (Choukroun et al. 2007; Braccini et al. 2009).

Concerning specific procedures with regards to dentistry, PRF has widespread applications.

PRF membranes may be utilized in combination with graft materials to expedite healing in lateral sinus floor elevation. Choukroun et al. (2006b) evaluated the potential of PRF in combination with freeze-dried bone allograft (FDBA) to enhance bone regeneration in lateral sinus floor elevation. The use of PRF in combination with FDBA to perform sinus floor augmentation seemed to accelerate bone regeneration.

When performing ridge augmentation, PRF membranes are used to protect and stabilize the graft materials. The membranes act as fibrin bandages, hastening the healing of the soft tissues, aiding rapid closure of the incision despite adding a substantial volume of bone (Toffler et al. 2009).

A new technique for maxillary reconstruction using FDBA, PRF membranes and 0.5 % metronidazole solution has shown a high degree of gingival maturation after healing with a thickening of keratinized gingival tissues that improved the esthetic integration and final result of the prosthetic rehabilitations. In addition, the use of PRF seemed to reduce postoperative pain and edema, and limited even minor infectious phenomena (Toffler et al. 2009).

As a membrane for guided bone regeneration (GBR), the PRF dense matrix architecture covers, protects, and stabilizes the bone graft material and the operative site in general. The elasticity and strength of the PRF fibrin membrane makes it easy to suture (Del Corso et al. 2010).

Zhao et al. (2012) in their case report have shown that the combination of PRF membrane and bioactive glass is an effective modality of regenerative treatment for radicular cysts.

Simon et al. (2011) quantified ridge changes associated with the healing of extraction sites using PRFM alone as a graft. The grafted sites displayed rapid clinical healing, minimal flap reopening and excellent bone density. The authors concluded that compared to GBR procedures, PRFM may have advantages like less surgical time, elimination of techniques and potential healing difficulties associated with membranes and less resorption during healing.

Another study evaluated the clinical and histologic parameters in an extraction socket filled with PRF prior to implant placement. At the time of placement, there were no untoward clinical symptoms and histological examination revealed new bone formation. Thus, the authors proposed PRF as a viable therapeutic alternative for implant site preparation (Zhao et al. 2011). Small PRF discs are easily inserted into residual extraction defects to expedite soft tissue healing in site preservation procedures permitting ideal prosthetic implant placement. PRF plugs are also positioned in the implant osteotomy to facilitate sinus floor elevation using a crestal core elevation (CCE) procedure or osteotome-mediated sinus floor elevation

(OMSFE) with simultaneous implant placement (Toffler et al. 2009).

Mazor et al. (2009) found that the use of PRF as the sole filling material during a simultaneous sinus lift and implantation stabilized a high volume of natural regenerated bone in the subsinus cavity up to the tip of the implants following 6 months after surgery. They inferred that Choukroun's PRF is a simple and inexpensive biomaterial, and its systematic use during a sinus lift seems a relevant option.

Not only can PRF be used for particulate grafting to predictably elevate the sinus floor using a crestal approach, the PRF membrane can provide protection for the sinus membrane during the use of an osteotome, and in case of perforation, the fibrin matrix can aid in wound closure. PRF membranes can be utilized in the lateral window osteotomy procedure to line the membrane prior to grafting as "membrane insurance" possibly sealing an undetected perforation which can lead to serious postoperative sequelae (Toffler et al. 2009).

Another study assessed the implant osteointegration as well as the course of bone regeneration and healing processes using PRF as a filling material in association with Bio-Oss (Deproteinized bovine bone mineral) in maxillary sinus lift cases. The authors observed a successful implant-prosthetic rehabilitation in all cases (Inchingolo et al. 2010).

PRF membranes have been used in conjunction with different root coverage techniques for the treatment of gingival recession. Anilkumar et al. (2009) used PRF membrane along with laterally displaced flap technique for the treatment of an isolated recession defect and reported complete root coverage with excellent gingival tissue status after 6 months. Aroca et al. (2009), on the other hand, reported that addition of a PRF membrane positioned under the modified coronally advanced flap (MCAF) provided inferior root coverage but an additional gain in gingival/mucosal thickness at 6 months compared to MCAF alone.

Studies have also attempted to use PRF as a regenerative material in the treatment of periodontal defects. Chang et al. (2011) in their case report assessed the clinical and radiographic changes in periodontal intrabony defects treated with PRF. They concluded PRF to be an effective treatment modality as the results showed that its application exhibited pocket reduction and gain in clinical attachment along



with increased post-operative radiographic density in the treated defects. Pradeep and Sharma (2011b) found greater reduction in probing depth, greater gain in periodontal attachment level and greater bone fill in 3-wall intrabony defects treated with PRF and open flap debridement (OFD) when compared to OFD alone. Pradeep and Sharma (2011a), in another study on the treatment of mandibular Grade II furcation defects, showed statistically significant improvement at sites treated with PRF and OFD as compared to those with OFD alone. However, in a study by Pradeep et al. (2012) which explored the clinical and radiographical effectiveness of autologous platelet rich fibrin (PRF) and platelet rich plasma (PRP) in treatment of intrabony defects in chronic periodontitis subjects, no significant differences were found between the two platelet concentrates.

## Discussion

PRF is a matrix of autologous fibrin containing a large quantity of platelet and leukocyte cytokines during centrifugation. The intrinsic incorporation of cytokines within the fibrin mesh allows for their progressive release over time (7–11 days), as the network of fibrin disintegrates. The PRF membrane acts like a fibrin bandage, serving as a matrix to accelerate the healing of wound edges. It also provides a significant postoperative protection of the surgical site and seems to hasten the integration and remodeling of the grafted biomaterial (Toffler et al. 2009). Gassling et al. (2010) in their study concluded PRF membranes to be superior to collagen membranes as a scaffold for human periosteal cell proliferation.

Studies that have compared PRP and PRF have concluded that PRF has many distinct advantages over PRP. He et al. (2009) found PRF to be superior to PRP, in the expression of alkaline phosphatase (ALP) and induction of mineralization. They opined that PRF released autologous growth factors gradually and expressed stronger and more durable effect on proliferation and differentiation of rat osteoblasts than PRP *in vitro*.

Recent studies have recognized the importance of PRF in aiding regeneration. Tsai et al. (2009) investigated the biologic effects of PRF on human gingival fibroblasts, periodontal ligament cells, oral epithelial cells and osteoblasts. A culture revealed that PRF

stimulated cell proliferation of osteoblasts, periodontal ligament cells and gingival fibroblasts hinting that it may be beneficial for periodontal regeneration. Wu et al. (2012) conducted a study to determine the effects of PRF on cell attachment, proliferation, phosphorylated Akt, heat shock protein 47 and lysyl oxidase expression on human osteoblasts. It was concluded that PRF is capable of increasing osteoblast attachment, proliferation and simultaneously up-regulating collagen-related protein production all of which would effectively promote bone regeneration.

The findings of Kang et al. (2011) strongly support the distinctiveness of PRF as a bioscaffold and reservoir of growth factors for tissue regeneration. The PRF extract (PRFe) increased proliferation, migration, and promoted differentiation of the human alveolar bone marrow stem cells. In addition, transplantation of the fresh PRF into mouse calvaria enhanced regeneration of the critical-sized defect. In another study by Chang et al. (2010) PRF activation has shown to bring about expression of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and osteoprotegerin (OPG) signifying its benefits for bone regeneration. Furthermore, Huang et al. (2010) in their study demonstrated that PRF stimulates proliferation and differentiation of dental pulp cells by up-regulating OPG & ALP expression. A more recent study concluded that enhancement of p-ERK, OPG and ALP expression by PRF may provide benefits for periodontal regeneration (Chang and Zhao 2011).

The PRF clot can be considered as an immune organizing node. Its defense capacities against infections are significant due to the chemotactic properties of cytokines as well as by their capacity to facilitate access to the injured site (neovascularization). It is even likely that the significant inflammatory regulation observed on surgical sites treated with PRF is the outcome of retrocontrol effects from cytokines (particularly, IL-4) trapped in the fibrin network and released during the remodeling of this initial matrix (Dohan et al. 2006c).

## Conclusion

Early publications and clinical experience seem to indicate that PRF improves early wound closure, maturation of bone grafts and the final esthetic result of the peri-implant and periodontal soft tissues

(Toffler et al. 2009). However, it is now necessary to look further into the platelet and inflammatory features of this biomaterial. Only a perfect understanding of its components and their significance will enable us to understand the clinical results obtained and subsequently extend the fields of therapeutic application of this protocol (Dohan et al. 2006a). Further studies are required, underscoring the many clinical applications and healing benefits of this second generation platelet concentrate (Toffler et al. 2009).

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