

Platelet rich fibrin – its structural design and composition

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Abstract

Platelets have a very essential role in periodontal regeneration as they are pond of cytokines and growth factors which are the key source for accelerating hard and soft tissue healing. Autologous platelet concentrate i.e. Platelet-rich plasma (1st Generation platelet concentrate) and platelet-rich fibrin (2nd Generation platelet concentrate) are formulated from patient’s own venous blood. Present day studies are being concentrated on the evolution of alternatives which are non-toxic, easy to prepare, biocompatible and cost effective that lead in the local release of growth factors for regeneration and maturation of the bone and soft tissue. PRF is a natural autologous fibrin-based biomaterial derived from an anticoagulant-free venous blood, with no artificial biochemical modifier that ushers to procure fibrin membranes enriched with platelets & growth factors. During centrifugation, slow polymerization forms PRF i.e a fibrin-based structure healing biomaterial better than 1st Generation platelet concentrate and other fibrin adhesives.

Keywords: Platelet rich fibrin, Platelet concentrate, Growth factors, Regeneration, Guided tissue regeneration.

Introduction

Choukroun et al. in France developed a new family of platelet concentrate in 2001,¹ which was neither a fibrin glue nor a classical platelet concentrate.²

PRF i.e. Choukroun’s PRF (L-PRF), is an autologous biomaterial rich in leukocyte and platelet³ with a peculiar configuration and three-dimensional architecture. PRF, an autologous platelet concentrate is formulated as a natural biomaterial without incorporation of any blood modifiers⁸ to terminate the menace connected with usage of anticoagulants (bovine thrombin).³

PRF preparation is very simple and cost effective: blood is obtained in dry tubes or glass-coated plastic tubes and instantly softly centrifuged at 3000 rpm for 10 minutes. Three layers are obtained (Fig. 1)

1. At the lowest part- red blood cell(RBC) base,
2. PRF clot (buffy coat) in the centre
3. Acellular plasma (platelet-poor plasma PPP) at the top.⁴

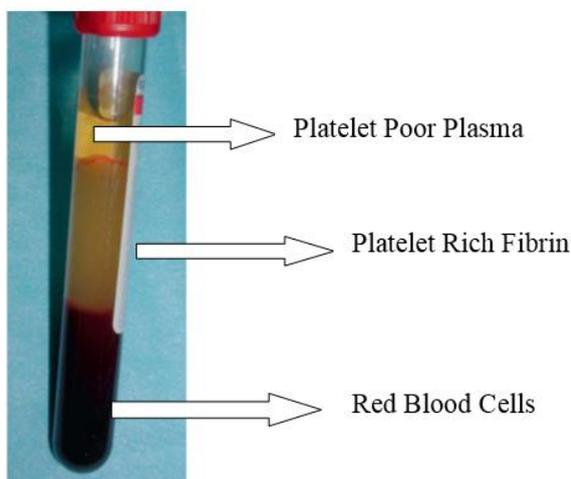


Fig. 1

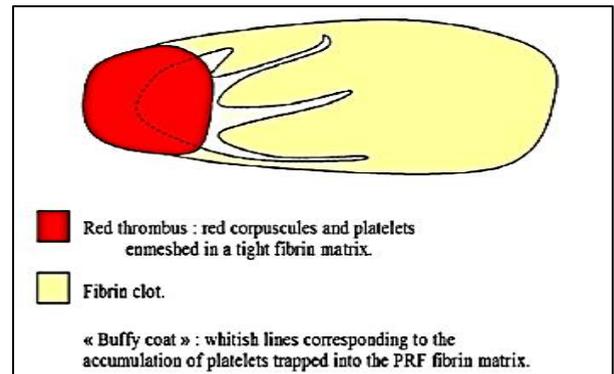


Fig. 2

Histologic assessment showed (Fig. 2) that the platelets fetched at the lower part of the fibrin clot, that is the juncture between the fibrin clot and RBCs.^{5,6}

The maximum platelet/leukocyte concentration (97%) exists in the first millimeter of the yellow clot, just after the red clot. The platelet/ leukocyte concentration become progressively less as move farther from the red clot, platelets or leukocytes concentration beyond the first half of the yellow clot is almost insignificant. In the first 2mm located away from the yellow/red border, the platelet/leukocyte allotment is homogenous throughout the clot width.⁷

Table 1: Comparison of platelet concentrates⁶

Sample	RBC(%)	Platelets (%)	WBC (%)
Blood clot	95	5	1
PRF	2	97	1
PRP	4	95	1

Three Dimensional Structural Design and Cell Composition of PRF

The slow fibrin polymerization manner of PRF and cicatricial capacity forms a physiologic structural design

which foster wound healing.⁸ Equilateral junctions (Connected trimolecular) allows the organization of a flexible and fine fibrin network to hold up cytokines enmeshment and cellular migration. This 3-dimensional structure gives great elasticity to the fibrin matrix which is seen in elastic, flexible and strong PRF membrane (Fig. 3).⁵

In comparison, platelet rich plasma shows abrupt fibrin polymerization- depending on the amount of surgical additives (calcium chloride and thrombin) and bilateral junctions (Condensed tetramolecular) are created with strong thrombin concentrations and allow the thickening of fibrin polymers leading to the development of a inflexible network, critical to cellular migration and cytokine enmeshment (Fig. 4).⁵

The biochemical study of the PRF composition shows that this biomaterial is formed by assembly of glycanic chains, cytokines, and structural glycoproteins (fibronectin) enmeshed within a slowly polymerized fibrin network. (Fig. 5).⁵

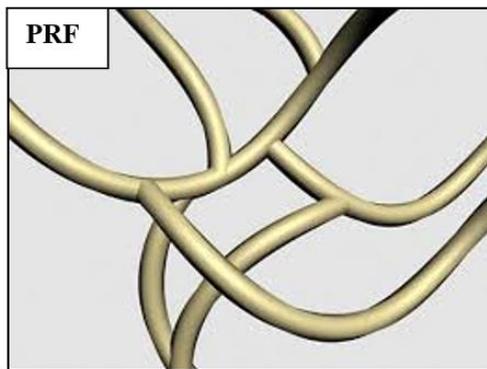


Fig. 3: Theoretical computer modeling of trimolecular or equilateral fibrin branch junctions. Note the flexibility of this net architecture.

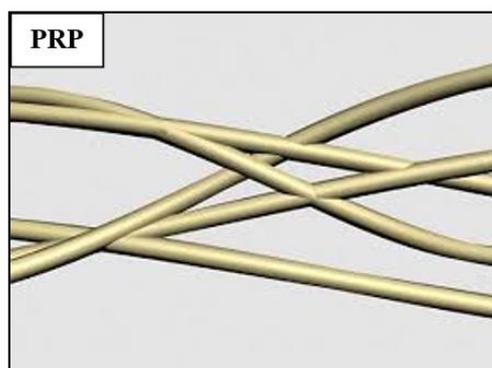


Fig. 4: Theoretical computer modeling of tetramolecular or bilateral fibrin branch junctions. Note the rigidity of this architecture

PRF has a dense fibrin network with leukocytes, cytokines, growth factors such as Platelet derived growth factor (PDGF), Transforming growth factor (TGF β 1), Vascular endothelial growth factor (VEGF) and glycoproteins such as thrombospondin-1. Leukocytes that are more in number in PRF scaffold have a fundamental role

in growth factor release, anti-infectious activities, immune regulation, and matrix remodeling during wound healing.

The L-PRF contains the vast number of the platelets and half the count of leukocytes present in the initial blood collection. Platelets are by and large activated and perform as a cement to sustain the strongly polymerized fibrin matrix. The platelets GF are entrapped within the fibrin network. With this structural design, L-PRF is the resource of growth factors which are released slowly in more than 7 days in vitro.

In the migration and adhesion of the platelets, Vitronectin and Fibronectin both are main proteins that take part in a very important function. Initially there is slow liberation of the fibronectin over a period of 1 week, i.e. the free fibronectin from the exudates and afterward is replaced by the fibronectin from the PRF membrane further, during the first four hours vitronectin is released from the PRF membrane, followed by almost a insignificant release over the next week.⁶

Role of Fibrin Matrix

1. Natural guide of angiogenesis.
2. Natural support to immunity.
3. Guides the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts.²

Cell composition of PRF

Platelet Cytokines⁵

1. Transforming growth factor- β (TGF β -1)
2. Platelet derived growth factor (PDGFs)
3. Insulin like growth factor (IGF)

Leucocyte and Cytokines

Inflammatory Cytokines⁵

1. Interleukin-1 β (IL-1 β)
2. Interleukin-6 (IL-6)
3. Tumour necrosis factor - α (TNF- α)

Healing Cytokines⁵

1. Interleukin-4 (IL-4)
2. VEGF

TGF β -1

1. TGF β -1 is the chiefly produced isoform of TGF- β . It represents the most powerful fibrosis agent among all cytokines (Dohan et al. 2006).
2. It stimulates fibroblast chemotaxis as well as the production of collagen and fibronectin by fibroblast.
3. It inhibits collagen degradation by decreasing proteases and increasing protease inhibitors, all of which favour fibrogenesis (Carlson and Roach 2002).
4. Further, TGF- β brings about chemotaxis and mitogenesis of osteoblast precursors while also stimulating osteoblast deposition.
5. It inhibits osteoclast development and bone resorption, thus favor bone formation over resorption (Marx et al. 1998).

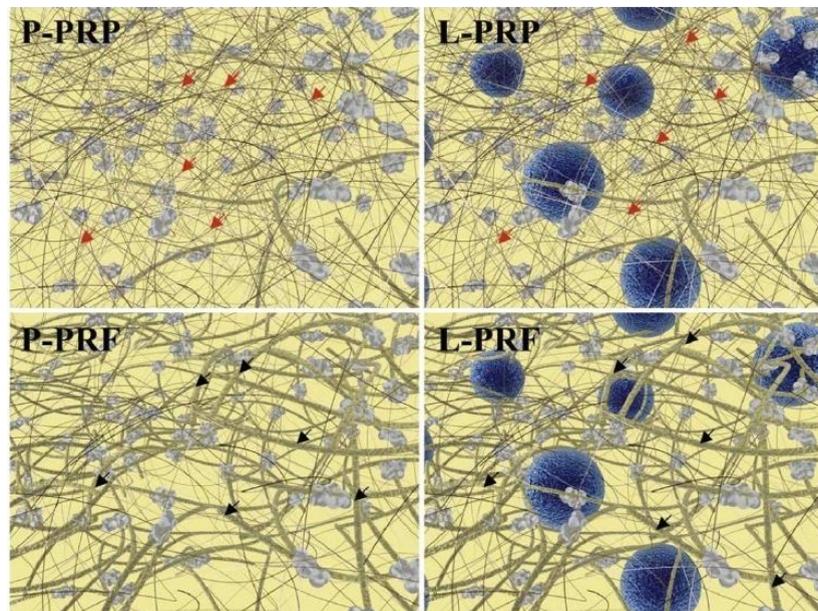


Fig. 5: Matrix and cell structure of the four categories of platelet concentrates

PDGFs

1. PDGF the first growth factor present in a wound and it starts connective tissue healing, including bone repair and regeneration (Marx et al. 1998).
2. PDGFs are crucial regulators for the migration, proliferation and survival of mesenchymatous cell lineages (Dohan et al. 2006).
3. PDGF activities are mitogenesis (increase number 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).
4. Every single platelet has 1,200 molecules of PDGF in it. 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 1999)

IGF

1. IGFs I and II are positive regulators of most cell types for proliferation and differentiation, including tumor cells. They have major role in apoptosis regulation. (Dohan et al. 2006).

IL-1 β

1. IL-1 β remains the prevalent isoform and is a key mediator of inflammation control. Its main activity is the stimulation of T helper lymphocytes.
2. In combination with TNF- α , IL-1 leads to osteolysis, i.e. it activates osteoclasts and inhibits osteoblasts. (Dohan et al. 2006).

IL-6

1. IL-6 was initially originally recognized as a B cell differentiation factor which induced the 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. 2006).

2. In addition, IL-6 is an essential accessory factor for T cell activation and proliferation. IL-6 induced not only proliferation but also induce differentiation of cytotoxic T cells in the presence of IL-2 from murine and human thymocytes and splenic T cells (Kishimoto 1989).
3. IL-6 has positive effect on hematopoiesis and was described by Ikebuchi et al in 1987. IL-6 and IL-3 act in a synergistic way to increase hematopoietic stem cell proliferation in vitro where IL-6 activates cells at the G0 stage to enter into the G1 phase (Kishimoto 1989; Dohan et al. 2006).
4. IL-6 functions as a hepatocyte stimulating factor and induces expression of various acute phase genes (Kishimoto et al. 1995).

TNF- α

1. TNF derives its name from the ability to stimulate tumor necrosis and regression (Ikram et al. 2004). TNF- α is one of the initial cytokines seen during the inflammatory response to bacterial endotoxin. TNF- α stimulates the remodeling capacities of fibroblasts and activates monocytes. It also promotes phagocytosis, neutrophil cytotoxicity and modulates the expression of key mediators such as IL-1 and IL-6 (Dohan et al. 2006).

IL-4

- IL-4 induces differentiation of naive helper T cells into TH2 cells (Goldsby et al. 2003). IL-4 also leads to proliferation and differentiation of the activated B cells. During inflammatory phenomena, it supports healing by moderating inflammation (Dohan et al. 2006). Moreover, IL4 is a potent inducer of Interleukin1 receptor antagonist (ILRa), which contributes to its anti-inflammatory actions by neutralizing the biological effects of IL1 (Tilg et al. 1994).

VEGF

1. VEGF is considered as a master regulatory molecule for angiogenesis related processes. Factors like IGFI and IL1 β regulate angiogenesis by upregulating the expression of VEGF (Mattuella et al. 2007). It plays a straight job in the control of endothelial cell behaviors, such as proliferation, migration, specialization or just survival (Dohan et al. 2006).

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