PLATELET RICH FIBRIN (PRF) IN PERIODONTAL REGENERATION

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Abstract

The ultimate goal of periodontal therapy is to restore the periodontal health by complete regeneration of the attachment apparatus. Though numerous methods of regeneration are available, researches are directed towards search for autologous materials considering the safety and availability issues. Among the various autologous material options, use of platelet concentrates have found to be more promising due to ease of procurement, handling and biochemical properties. This article throws limelight on the role of platelets on wound healing, preparation, mechanical properties, biochemical properties and clinical application of PRF in periodontal regeneration.

Key Words : periodontal regeneration, platelet rich fibrin, platelet rich plasma, tissue engineering

Introduction

Periodontitis is an inflammatory disease of bacterial origin, affecting the periodontal tissues, resulting in loss of attachment apparatus.[1] The goal of periodontal therapy is to improve periodontal health and thereby to satisfy the patient's esthetic and functional demands. To achieve this complete re-establishment of the lost attachment is necessary. [2] Histological analysis of periodontal new attachment procedures revealed that periodontal healing occurs with process of repair rather than regeneration.[3]

Over the years numerous periodontal regenerative therapies have been developed from simple periodontal debridement to use of various biomaterials like guided tissue regeneration membranes, enamel matrix proteins and bone graft materials. Studies have shown that combination therapies resulted in better results as compared to stand-alone treatment modalities.[4,5] But the availability and cost are other important factors to be considered. Studies have also shown substantial variation in clinical predictability, degree of efficacy, and histological outcomes.

Use of autologous materials is considered a promising alternative. Autologous soft tissue grafts, buccal pad of fat,[6] periosteum,[7] and bone grafts have been tried, but again they were technique sensitive and required a second surgical site. So with the advent of platelet concentrates, the direction of periodontal regenerative therapies took a turn as they were simple to procure and easy to use.

Why Platelet Concentrates?

Platelet concentrates are blood derived products obtained by centrifugation of autologous blood and are widely used as surgical additive biomaterial to aid tissue healing. [8,9] The intracellular and extracellular events mediated by various signalling proteins during tissue healing are a complex cascade of events that still needs complete understanding. Platelets play a dominant role in every phase of healing of hard and soft tissues. Immediately following tissue injury, platelets are activated and start a sequence of events that result in formation of platelet plug and fibrin clot. This causes hemostasis and secretion of biologically active proteins.[9]

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These proteins, initiate tissue healing responses, starting with the initiation, differentiation and chemotaxix of the desired cells, angiogenesis, formation of extracellular matrix and remodelling to the desired tissue. [10] Studies have also found a dose–response relationship between platelet concentration and the proliferation of human adult mesenchymal stem cells, fibroblasts, and production of collagen by them.[11]

Platelets contain numerous growth factors within α granules that has a strong influence on the wound healing events. Among them growth factors of periodontal importance are platelet-derived growth factor (PDGF), transforming growth factor (TGF α), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), platelet-derived endothelial growth factor (PDEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin (TSP). [12] The role of individual growth factors in stages of wound healing is summarized in Table 1.

Classification of Platelet Concentrates

Dohan et al in 2009 classified platelet concentrates according to the composition of the centrifuged product. Based on this the platelet concentrates were classified into four categories [13]

P-PRP - Pure Platelet Rich Plasma
L-PRP - Leucocyte and Platelet Rich Plasma
P-PRF - Pure Platelet Rich Fibrin
L-PRF - Leucocyte and Platelet RichFibrin

Platelet Rich Pasma

Platelet rich plasma (PRP) is considered as the first generation platelet concentrate preparation. It is obtained by double step centrifugation of autologous blood thereby concentrating platelets in a gel. Hence they are also termed as 'platelet pellet' or 'platelet gel'.[14] But the disadvantages associated with PRP is additional use of calcium chloride and bovine thrombin. The use of bovine thrombin is found to be associated with risk of development of coagulopathies.[15]

TABLE 1 – Role of individual growth factors in periodontal regeneration

S.no	Growth factor	Role in wound healing
1	Transforming growth factor-β (TGF-β)	Stimulates proliferation and chemotaxix of osteoblasts thereby enhancing the woven bone formation.
2	Platelet-derived growth factor (PDGF	Stimulates migration and proliferation of mesenchymal lineage cells
3	Vascular endothelial growth factor (VEGF)	Initiates angiogenesis
4	Insulin growth factor-1 (IGF-1)	Acts in synergy with the TGF. Stimulates osteoblast proliferation. Stimulates proliferation and chemotaxix of osteoblasts thereby enhancing the woven bone formation.
5	Fibroblast growth factor (FGF)	Enhances fibroblast proliferation, migration and differentiation.
6	Epidermal growth factor (EGF)	Stimulation of cell proliferation and extracellular matrix turnover

Platelet Rich Fibrin

It is a second generation autologous platelet concentrate biomaterial harvested from a simple blood sample. The main component is a fibrin matrix which incorporates leucocytes, platelets and growth factors in it. PRF was first developed in France in 2000 by Choukroun et al. This protocol proposed by him eliminated the risk associated with use of bovine thrombin.[16]

Preparation

The armamentarium includes, blood collection kit, 10-mL dry glass test tube (without anticoagulant), and a table centrifuge. Blood sample must be collected aseptically from the patient by venipuncture and immediately transferred into the test tube and centrifuged at 3000 rpm or 10 minutes.[17,18] One another protocol suggested to use 2700 rpm for 12 minutes and found similar results.[19]

Mechanism

Absence of anticoagulant triggers platelet activation and fibrin polymerization immediately after it contacts the glass surface. Centrifugation splits the components of blood and as a result three layers are formed: the RBC base layer, acellular plasma top layer and a PRF clot in the middle.(Fig 1a,b,c) The PRF clot basically is a strong fibrin mesh with a complex three dimensional structure in which concentrates the platelets and leucocytes.[20]

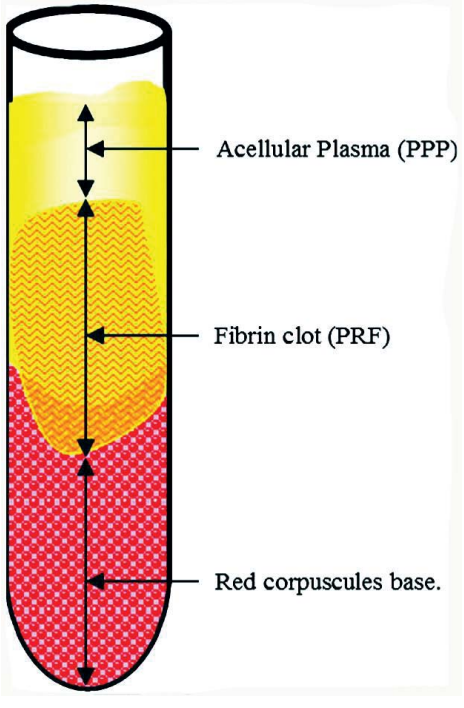

Figure 1 a – Schematic representation of the centrifugation strata. [22]

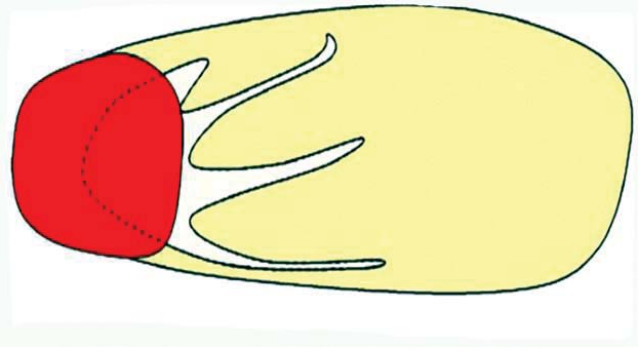

Figure 1 b – The PRF clot (Schematic) [22]

Figure 1 c – PRF clot

The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. Quick handling is the only way to obtain a clinically usable PRF clot. Without the anticoagulant, the sample starts to coagulate immediately on contact with the glass. If delay in centrifugation occurs, the fibrin will polymerize in

a diffuse way and a clot without any firm structure and a small amount of fibrin gel will result.

Material Aspect

The fact that the polymerization occurs naturally is that main advantage of this material. Since no thrombin is added, the thrombin concentration action on the fibrinogen is almost physiologic. This determines the structure of the fibrin mesh. During the gelling process, the fibrin is assembled in two architecture - condensed tetramolecular or bilateral junctions and connected tri molecular or equilateral junctions.[18] In PRF process the centrifugation results in formation of equilateral junctions, result in weak thrombin concentration that ensures fine, flexible fibrin network. This fine mesh entraps cytokines and allows cell migration. Moreover, this structure will give great elasticity to the fibrin matrix. [19] (Fig 2) But in PRP, the two step centrifugation

Figure 2 – Model of condensed tetra molecular fibrin structure [22]

results in formation of bilateral junctions result in thickened fibrin polymers, which are not favorable for cytokine enmeshment and cellular migration, but

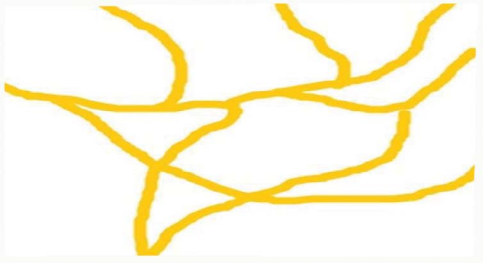

Figure 3 - Model of condensed bilateral fibrin structure [22]

can act as an effective biologic glue to seal tissues due to greater rigidity.(Fig 3)[12]

Biologic Properties

Unlike other fibrin adhesives the PRF results from progressive polymerization during centrifugation. Thus it forms a three dimensional organization, coherent than natural clots. As polymerization progresses, the fibrin mesh becomes more intricate and traps the circulating platelets and intrinsic cytokines.(Fig 4)This ensures sustained release and action of cytokines throughout healing and remodeling. PRF promotes the production of phosphorylated extracellular signal-regulated protein kinase (p-ERK) and osteoprotegerin

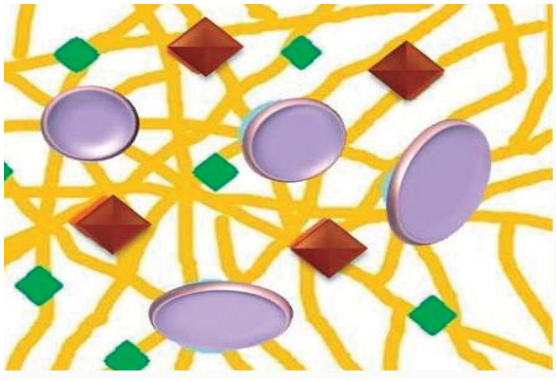

Figure 4 – Platelets and cytokines trapped in fibrin mesh [22]

(OPG). This inturn induces proliferation of the osteoblasts.[20] This upregulation of osteoprotegerin and alkaline phosphatise was also found to stimulate the differentiation of cells in human dental pulp.[21,22]

Effects on Tissue Healing

Angiogenesis, immunity and epithelial cover are considered as the key elements of any tissue healing. The PRF and the PRF membrane is found to support all the three phenomena.

Angiogenesis

The intricate fibrin mesh entraps the platelets which eventually release growth factors that promote angiogenesis like fibroblast growth factorbasic (FGFb), vascular endothelial growth factor (VEGF), angiopointein and platelet-derived growth factor (PDGF). Moreover, the fibrin in the clot regulates

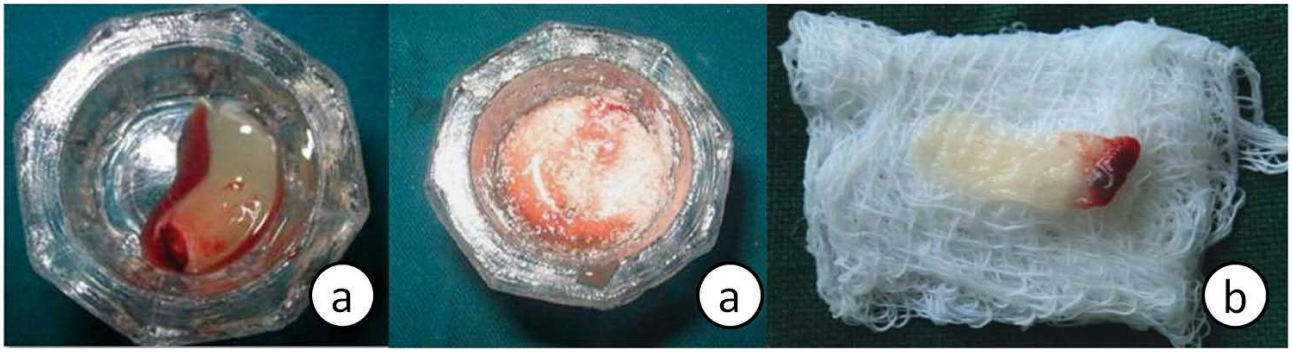

Figure 5 (a) PRF clot mixed with bone graft, (b) – PRF membrane

expression of integrin by endothelium that helps the cells to bind to fibronectin and vitronectin.[22,23,24,25]

Immune Modulation:

Migration of neutrophils and monocytes are regulated by fibronectin the chemical and physical properties of fibrin and by chemotactic agents trapped in its meshes.[26] The fibrin mesh and the entrapped fibrinogen degradation products increase the expression of CD18. This in turn stimulate the migration of neutrophils.[27] FDP in mesh also modulates phogocytosis and enzymatic degradation of neutrophils [28].

Epithelial Cover:

Growth factors like PDGF, TGF b, and epithelial growth factor, modulate the expression of receptors and integrins that aid in epithelial migration. Fibrin matrix acts as a guide for epithelial migration to cover the injured tissue.[29]

PRF And Tissue Engineering

Tissue engineering triad includes Scaffold, Stem cells and Growth factors. In the past few years, many researches have been conducted to use PRF for tissue engineering.

Gassling et al.[30] in his study concluded that PRF was superior to collagen when used as scaffold in tissue engineering. Moreover, PRF traps the circulating stem cells and also aids in their differentiation due to concentration of growth factors. PDGF acts as chemo attractant for fibroblasts that's aid regeneration.[31,32] In addition its immune functions, like regulation and activation of cytokines like IL-1, IL-4, IL-6 and TNF-β mediates tissue healing and regeneration.[18]

PRF in Periodontal Regenration

The obtained PRF clot can be mixed with the bone graft material and used for regeneration or the clot can be squeezed to form a membrane, which can be adapted over the root or bony surface. (Figure 5a, b)

For root coverage:

In a randomized controlled trial conducted by Janovich S et al, 2012 the results showed that PRF and CTG procedures were equally effective in treatment of gingival recessions.[33] PRF group showed enhanced wound healing and decreased postoperative discomfort. Martinez Zapata et al in his systematic review concluded that use of autologous PRF improved gingival recession.[34]

For regeneration in intrabony defects:

A randomized clinical trial was done bySharma et al.[35] for the treatment of three walled intrabony defects and they found that addition of PRF to the bone graft material resulted in greater bone fill than the controls. In another study by Thoratet al.[36]it was concluded that PRF with bone grafts resulted in more positive results than open flap debridement alone for intrabony defects. Chang et al,[37] found better radiographic bone fill following six months of treatment in the sites with bone graft and PRF as compared to bone grafts alone.

PRF in furcation defects:

Sharma et al studied the effects of PRF in grade II furcation defects and concluded that PRF with bone grafts are a better treatment option as compared to bone grafts alone.[38]

Disadvantages

The main disadvantage is the very little amount of material obtained which might be insufficient for periodontal regeneration. Another disadvantage is that it cannot be stored and used at a later point of time since it has to be used immediately after preparation. Storage also results in risk of bacterial contamination of the membrane. Delay in use will result in alteration of the structural and biological properties. Dehydration of the membrane will result in membrane shrinkage, decrease the concentration of growth factor and adversely affect the leukocyte viability.[9]

Conclusion

With these fundamental considerations, PRF is considered as a favorable biomaterial that can protect and glue the wound surface and accelerate tissue healing. Moreover the entrapment of leucocytes and the growth factors promotes true regeneration.

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