REVIEW ARTICLE

INJECTABLE PLATELET RICH FIBRIN - A REVIEW

ABSTRACT

Platelet concentration [PC] has been utilized in regenerative dentistry as a supra-physiological concentrate of autologous growth factors capable of stimulating tissue regeneration. The objective of all these technologies is to extract all the elements from a blood sample that could be used to improve healing and promote tissue regeneration. Although leukocyte rich and leukocyte poor PC have their place in literature, the importance of non-platelet components in a platelet concentrate remains a mystery. PC has come a long way since its first appearance in 1970s to the T-PRF, A-PRF and i-PRF introduced recently. Platelet concentration has almost replaced platelet-rich plasma, owing to its advantages such as being 100% autogenous, ease of technique and cost-effectiveness with superior and prolonged growth factor release. These Platelet concentrates are frequently used for surgical procedures in dentistry. It has various application in periodontics, for treating gingival recession, guided bone and periodontal regeneration and in the management of peri-implant defects. It is also widely experimented for pulpal regenerative therapy. Hence the aim of this article is to review the biological properties of platelet-rich fibrin and the advancement in the PRF technologies since its inception

Key words: platelet concentrates, growth factors, platelet rich fibrin.

J Ind Dent Assoc Kochi 2020;2(1):16-22.

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INTRODUCTION

Favourable wound healing has always been a major quest in dental surgery. It is a complex biological process where many cellular events are taking place simultaneously leading to the repair or regeneration of damaged tissues. Many attempts have been made in the field of tissue regeneration to predictably repair, regenerate, or restore damaged and diseased tissues. These include strategies with foreign materials often derived from allografts, xenografts, or synthetically produced alloplasts to regenerate host tissues. While many of these materials have shown promise in various aspects of regenerative medicine, it is important to note that most of these materials may create a "foreign body reaction"[1].

Platelet concentrates collected from whole blood was first introduced over 20 years ago. The concept was developed to utilize human blood proteins as a source of growth factors capable of supporting angiogenesis and tissue ingrowth based on the notion that blood supply is a prerequisite for tissue regeneration[1].

In the past two decades, the use of autologous platelet concentrates (PCs) has gained great popularity in a variety of medical fields such as dentistry, oral surgery, orthopaedics, dermatology, ophthalmology, cosmetic and plastic surgery[2].

The rationale behind its regenerative potential is the presence of various growth factors in the alpha-granules of platelets which are released at the local site on their activation. Besides this, they also impart anti-inflammatory properties, thereby reducing postoperative pain and swelling. Also, few studies have explored its antibacterial potential although its mechanism is controversial. In the past few years, various platelet concentrates have evolved depending on the technique employed which vary in their centrifugation protocols[3].

Initially platelet concentrates were developed with anticoagulants to prevent the rapid coagulation of blood before centrifugation. Original protocols were designed utilizing a two-step centrifugation procedure, which produced what was later termed platelet-rich plasma (PRP), and many fields of medicine and dentistry have benefited from the ability of these protocols to induce a 6- to 8-fold increase in blood-derived growth factors. The main concern regarding the use of anticoagulants was its negative impact on wound healing by preventing clot formation, which is an essential step during the natural wound healing process[4].

For these reasons, in 2001 Choukroun et al. pioneered new research aimed at utilizing platelet concentrates without incorporating anti-coagulants within their preparations. This novel formulation, later termed platelet-rich fibrin (PRF), was the first strategy utilizing platelet concentrates without anti-coagulants. Two main advantages reported were the fact that the wound healing cascade was not inhibited by the anti-coagulants and that natural clot formation occurred. Furthermore, PRF contains a high concentration of host immune cells (namely leukocytes), which act to promote local wound healing and fight infection[4].

One such recently introduced platelet concentrate by Joseph Choukron in 2014 is injectable platelet-rich fibrin (PRF) more commonly referred to as i-PRF. It requires neither any anticoagulant nor any additive. It is obtained by centrifuging blood at low-speed. This results in PRF for use in the liquid (injectable) form. It coagulates within few minutes after the injection and is believed to contain not only white cells and platelets but also circulating stem cells and endothelial cells. Hence, it is considered as a "blood concentrate" and not just a platelet concentrate.[3]

BACKGROUND[5]

The evolution of the first and second generation of platelet concentrates (platelet-rich plasma and platelet-rich fibrin respectively) from their fore runner-fibrin sealants. The following tables outlines the various techniques in chronological order as Platelet concentrates evolved.

	Name	Proposed by	Technique	Drawbacks
1.	Platelet concentrates	1970's	Donor plasma which was then mixed with thrombin and calcium which led to polymerization of fibrinogen	Poor stability or risk of disease transmission in case of commercially available products
2.	Autologous fibrin glue	Tayapongsak 1994	Pre-operative (one to three weeks before the procedure) collection of blood followed by around 30 minutes (ammonium sulphate precipitation technique) to 48 hours (cryoprecipitate technique) of handling.	The technique was long and complex The amount of concentrate obtained was quite less as compared to the amount of blood collected
3.	Platelet-rich plasma	Whitman 1997	Double centrifugation of autologous blood with anticoagulant. It consisted of a soft spin followed by which the blood would separate into the red corpuscular base, buffy coat and the platelet-poor plasma. The last two components were aspirated and re centrifuged at a hard spin after which PRP was collected in the bottom of the tube.	Bovine thrombin which could give rise to life-threatening coagulopathies in rare cases
4.	Plasma rich in growth factors	Anitua & co-workers 1999	Autologous blood with anticoagulant was centrifuged at 460G for 8 mins and this resulted in the collection of plasma rich in growth factors (PRGF) at the bottom of the tube. This PRGF was then taken from the bottom of the tube and cacl2 was added (0.05ml/ ml of PRGF). This led to coagulation in around 10 minutes and a gelatinous PRGF was obtained.	Led to incomplete activation of platelets and low levels of growth factors release.
5.	Platelet-rich fibrin (PRF)	Choukroun et al. in 2001	The basic protocol of producing PRF requires around 10 ml of blood to be collected from the patient without anticoagulant in a glass tube. After collection, the blood is quickly subjected to centrifugation at 2700 rpm for 12 minutes.	

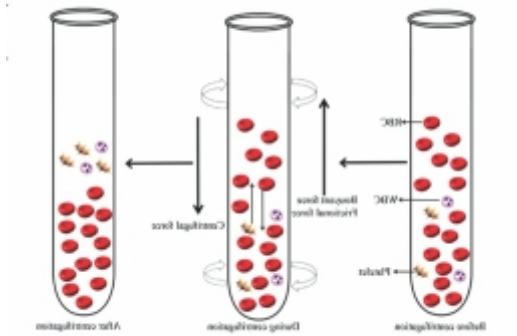
Action of centrifugal force on blood[5]

Principle - Is to allow the blood to clot as it would physiologically. Under normal circumstances, the blood would coagulate to form a blood clot.

In the centrifuge, two processes are occurring simultaneously-

1. Separation of blood elements under the centrifugation force

- The force of centrifugation exerted is directly proportional to the mass of the individual particle.



- under the centrifugation force, RBC's, which have relatively higher mass settle towards the bottom of the tube. Whereas WBC's, platelets and plasma along with its clotting factors which have comparatively lower mass are pushed towards the top of the tube.
- This occurs in the early part of the centrifugation cycle (before approximately 2-3 mins)

2. Blood coagulation

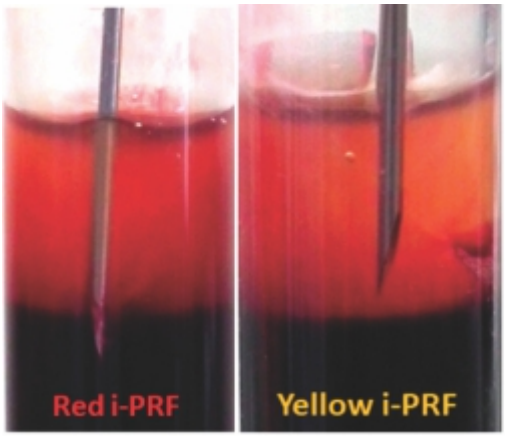
- By the time the final steps of coagulation cascade i.e. conversion of prothrombin to thrombin and fibrinogen to fibrin occurs, the factors required for coagulation are all present in the plasma, which is now located at the top of the tube near platelets under the force of centrifugation.
- Once this separation is achieved, the rest 6-8 minutes of the centrifugation cycle is to maintain the separation and let clotting proceed.
- Hence, RBC's which do not contribute significantly to the healing of a wound is effectively excluded from the blood clot under the centrifugation force, and the clot now consists mainly of platelets (1.5 to 3 lakh/ml in a blood clot to around 10 lakh/ml in PRF) and fibrin.

PRF PROTOCOLS

PRF	Described by (year)	RPM	TIME (Minutes)	TUBE
Leukocyte and Platelet-rich fibrin (L-PRF)	Chourkroun 2004	2700	12	Glass coated tube
Advanced -PRF	Ghanati 2014	1300	14	Sterile glass based vacuum tubes (A-PRF10 tubes)
A-PRF+	Fujioka-Kobayshimiron 2016	1300	8	Same as APRF
Injectable – PRF	Mourao 2015	700	3	Non coated
PRF Lysate		2700(after preparation it is incubated at 37°C in a humidified atmosphere of 5%CO2/95%air)	12	Glass coated tube
Titanium-PRF	Tunali & co-workers	2800	12	Medical grade titanium tube

Injectable PRF (IPRF)

- One of the latest developments in the PRF technology is the production of injectable PRF (i-PRF). As compared to PRP, one drawback that limits the applications of PRF is that PRF is obtained as a gel form which is not conducive to be injected.
- Thereby, according to the low-speed centrifugation concept, further reduction of the centrifugal force to 60 g and the use of plastic tubes allowed for the introduction of an injectable PRF matrix (i-PRF) without using anticoagulants.
- To avoid the need for external anticoagulants to generate an effective total autologous and fluid blood concentrate system, specific plastic tubes were developed to collect blood.
- In contrast to the glass tubes used in solid PRF matrices, the characteristics of the plastic surface do not activate the coagulation cascade during centrifugation.
- After centrifugation, the blood is separated into three main parts based on the buffy coat layer: A yellow upper part, a buffy coat middle part and a red blood cell containing the lower part[6].
- I-PRF is collected using an 18G hypodermic needle by controlled aspiration of the upper fluid part. The collected i-PRF maintains its fluid phase for up to 10 to 15 minutes after centrifugation.
- Remarkably, the reduction of the centrifugation force led to an enrichment of i-PRF with platelets and leukocytes. Consequently, flow cytometry showed that i-PRF includes the highest number of platelets, leukocytes and growth factors among all the solid PRF-based matrices.
- Also, I-PRF is devoid of the drawbacks related to bovine thrombin including the development of antibodies to the factors V, XI & thrombin and chances of life-threatening coagulopathies. Hence, an injectable variety of PRF theoretically would be a superior alternative to PRP for the abovementioned applications.



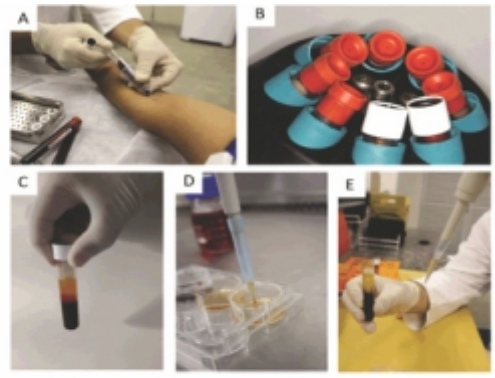


Figure 1: i-PRF preparation: (A) blood collection; (B) centrifugation machine; (C) aspect after centrifugation; (D,E) separation.

BIOLOGICAL ACTION[5]

EFFECT	MEDIATED BY	ACTION
Angiogenesis	Vascular endothelial growth factor (VEGF), angiopoietin, platelet-derived growth factor (PDGF), basic-fibroblast growth factor (FGF-b)	• Cells in the wound vicinity to migrate, divide and change phenotype • stimulates expression of **α5β3** integrin on the endothelial cells which promote the binding of endothelial cells to fibrin, fibronectin & vitronectin.
Mitogenesis	TGF-**β** Fine & flexible trimolecular/ equilateral junctions	• Mitogen for cells including fibroblasts, marrow stem cells, endothelial cells, pre-osteoblasts, mesenchymal cells • Inhibitory effect on osteoclasts • Enhanced cytokine entrapment, promotes rapid cellular migration
Immunomodulatory effects	Fibrin and its degradation Products Fibronectin Leukocytes IL-4	• Stimulate migration, phagocytosis and enzymatic degradation by neutrophils • Increases the expression of CD11C/CD18 receptor on neutrophils which mediates adhesion to endothelium and fibrinogen • Releases certain chemotactic factors which regulate wound colonization by macrophages • Increased degranulation to release several molecules including IL-1, IL-4, IL-6 and TNF-**α** • Coherent healing without inflammatory excess
Wound recolonization	Fibrinogen, fibronectin, vitronectin and tenascin Fibrin	• Undergoes degradation and allows epithelial cell migration on wound margins • Binds to several molecules including fibronectin, PDGF & TGF-b through the **α**V**β**3 integrin • Promotes the migration of fibroblasts
Osteogenic effect		• May upregulate the expression of alkaline phosphatase and osteoprotegerin • Enhance the expression of phosphorylated extracellular signal-regulated protein kinase, osteoprotegerin and alkaline phosphatase activity
Entrapment of stem cells		Even though the intrinsic content of stem cells is quite low, it has been hypothesized that the fibrin clot may act as a trap for circulating stem cells which may converge to a secretory phenotype allowing vascular and tissue restoration

CLINICAL CONSIDERATIONS

- Currently, I- PRF has been used along with bone grafts, which on completion of the coagulation process forms a gel-putty consistency with the graft particles incorporated in the graft.
- The graft thus formed has a good workable consistency, leading to decreased leaching of the graft as it is tightly encapsulated in the fibrin matrix.
- Mixing the bone graft with i-PRF also gives the benefit of growth factor release at the recipient site which would otherwise be missing in a normal bone graft. This has the potential to convert any osteoconductive graft to osteopromotive (due to the presence of platelets & growth factors) which would translate into faster and better efficiency of bone formation
- As an adjunct it has been used for all grafting applications to increase their volume and bio-activity, including guided tissue regeneration in intra-bony defects and Grade II furcation involvements, guided bone regeneration in cases of socket preservation/ augmentation, for combined endodontic-periodontal lesions and hard and soft tissue augmentation around implants.
- Another kind of graft that has been obtained with i-PRF is the PRF block. For its preparation, i-PRF is mixed with a combination of bone graft and shredded PRF clot. This enhances the volume of the graft.
- Platelet-rich fibrin might serve as a potentially ideal scaffold in revascularization of immature permanent teeth with necrotic pulps as it is rich in growth factors, enhances cellular proliferation and differentiation, and acts as a matrix for tissue in growth; which can be used for the treatment of immature tooth with a necrotic pulp by revascularization procedure using PRF[7] and in procedures like Pulpotomy, PRF could be used as an alternate treatment to mineral trioxide aggregate or other materials in mature permanent teeth with pulpitis[8].

CONCLUSION

PRF as a biologic surgical additive has been successfully used for varied applications in dentistry. Technological advancements in the field of PRF such as i-PRF have paved way for the versatility in the applications of the platelet concentrates. With the increase in our understanding about the biology of PRF, in future, we can expect improved additives which will further enhance the wound healing experience.

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