Platelet Rich Fibrin(PRF)-A Novel Generation of Regeneration in Endodontics: A Review

Manali Nath[1], Shashirekha Govind[2] , Siba Prasad Jena[3]

[1]Post Graduate Trainee, [2]Professor & Head, [3]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Institute of Dental Science, Siksha O Anusandhan (Deemed to be University) Bhubaneswar

Abstract

In the field of regenerative medicine, PRP/PRF whichwas will be is used as a surgical adjuvant became the new glorified approach. For the Regenerative endodontic treatment based on the tissue engineering concept, creating a favourable environment free of pathogens, for preservation and migration of stem cells, pathogen eradication, stem cell preservation, presence of scaffold facilitating spatial orientation and signal molecules released by cells is of utmost importance. In tooth revascularisation and revitalization, successful application of platelet concentrates as scaffolds have been reported. Being autologous, comprising of high growth factor concentrates and relatively hassle-free preparation in dental setup, platelet concentrates are preferred. The advancement in the regenerative endodontics field, with the introduction of the different generation of platelet concentrates, is a breakthrough.

Keywords*: Regenerative endodontics, PRF, Platelet concentrates, Tissue engineering.*

Introduction

Blood coagulation, that leads to fibrin/platelet clot and matrix formation acts as the initiator of the natural healing process. Platelet concentrate that was introduced, reinforced the process of wound healing, that ultimately progressed to tissue engineering, explicit in its way. All the essential and primary components involved in the process of wound healing, namely platelets, altruistic in growth factors, leukocytes, fibrin as the supporting matrix, growth factors and some other cells when combined, form a kind of engineered tissue.[1]

In the field of regenerative medicine, PRP/PRF which was used as a surgical adjuvant became the new glorified approach. For the regenerative endodontic treatment based on the tissue engineering concept, creating a favourable environment free of pathogens, for preservation and migration of stem cells, pathogen eradication, stem cell preservation, presence of scaffold facilitating spatial orientation and signal molecules released by cells is of utmost importance.[2]

Corresponding author:
Dr. Shashirekha Govind
Professor and Head, Department of Conservative Dentistry and Endodontics Institute of Dental Sciences, Siksha O Anusandhan (Deemed to be University), Bhubaneshwar, Odisha, India.
Email shashirekha123@yahoo.com

In tooth revascularisation and revitalization, successful application of platelet concentrates as scaffolds have been reported. Being autologous, comprising of high growth factor concentrates and relatively hassle-free preparation in dental setup, platelet concentrates are preferred. The second generation of platelet concentrate (PRF) is preferred over the first generation (PRP) as the addition of exogenous agents not required, formation of organized fibrin network, circulating immune cells that act against infection. All these make PRF an optimal bioscaffold for tooth revascularization and revitalization.[3]

Platelets And Platelets Concentrates:

Thrombocytes, also known as platelets, derived from bone marrow megakeratocytes, are discoidal anucleate cytoplasmic fragments with an average diameter of 2-3 μm primarily causes the hemostasis at the site of injury. The average lifespan of platelet being approximately 8-10days, 1.5-4 thousand per μL in peripheral blood is the normal platelet count for an adult human.

During tissue repair, two different types of dense granules, having serotonin, adenosine triphosphate and calcium, are released from activated platelets. Platelets also contain α-granules, rich in growth factors that exhibit chemotactic and mitogenic properties. These growth factors assist in promoting and modulating cell proliferation and function that leads to tissue healing and regeneration

Table 1: Compilation of some major research work done in the evolution of platelet concentrates.

Year	Research work
1954	While conducting experiments pertinent to coagulation of blood, Kingsley contrived the term PRP attributed to thrombocyte concentrate.5
1970	Fibrin glue, introduced by Matras.6 The polymerisation of fibrinogen with thrombin and calcium leads to the development of fibrin glue. But, the low concentration of fibrinogen in donor plasma, resulted in suboptimal quality and stability of fibrin glue.
1975	platelet-fibrinogen-thrombin mixtures.7
1979	A newer proposition, "gelatin platelet - gel foam", that was mainly used for the quality of gluey-effect, ensured platelet performance and showed superlative results in disciplines of general surgery, ophthalmology and neurosurgery
1986	Knighton et al originally coined the term "platelet-derived wound healing factors (PDWHF)", where the platelet concentrates promoted the successful healing of the wound.8
1988 1990	The term platelet-derived wound healing formula (PDWHF)", was coined by Kingsley et al5 and Knighton et al9
1997	Initially, during preparation, Whitman et al used the term PRP to name their product.10 But then the final product had more of a fibrin gel consistency and so it got labelled under the name of "platelet gel"
1998	The craze for the newer techniques started after the publication of an article by Marx et al.11 Before that, these techniques developed at a slow pace.
1993	Plasma rich in growth factors (PRGF), by Endoret(Victoria, Biotechnology Institute BTI, Spain) was commercialized and advertised on a large scale. But there was some major disadvantage associated with this technique, due to lack of ergonomics and specific pipetting techniques.4Apart from this, another product named Vivostat PRF (Alleroed, Denmark) was commercialized, even though it was nothing but one PRP product.
2000	A form of PC, based on a strong fibrin gel polymerisation, was developed by Choukroun et al in France and was marked as PRF. As PRF was different from other PRPs, its development was an important milestone and so it was imprinted as "second-generation" platelet concentrate.11
2006	Bielecki et al and Cieslik-Bielecka et al proposed that when compared with PRG (platelet-rich gel), PRP was not an active substance. On the other hand, PRG was biologically activated, with a fibrin matrix rich in active molecules.12-14 A newer notion of GCF (concentrated growth factors) was established by Sacco. The resultant fibrin rich blocks were larger, richer and denser too.15
2008	The focus of Everts et al was on the leucocyte component of PC and its forms; inactivated/non-activated form["platelet-leukocyte rich plasma (P-LRP)] and activated gel form platelet-leukocyte-gel".16,17
2009	Pioneer classification of platelet concentrate by Dohan Ehrenfest et al[4].
2010	Sohn in 2010 unveiled the concept of autologous fibrin glue mixed with bone graft, the sticky bone concept.18
2014	A-PRF, an advanced PRF claiming to contain a comparatively greater number of monocytes was introduced by Choukroun. A newer type, Titanium-prepared PRF(T-PRF) was introduced by Tunalı et al.19,20
2015	A technical note, with elaborate detail on the preparation of i-PRF, was given by Mourão et al.21

PRF- Platelet Rich Fibrin

Platelet-rich fibrin, a platelet concentrate of the second generation, can stimulate the proliferation of osteoblasts, gingival fibroblast and PDL cells. Also, it contains VEGF, which is a potent stimulator of angiogenesis.

This generation of platelet concentrate was developed in France in the year 2001 by Choukroun et. Al. After the name of its inventor, PRF is also called as Choukroun's PRF. The procedure of PRF preparation involves instantaneous centrifugation of the blood (at 3000rpm for 10mins) that is collected into test tubes, without an anticoagulant. After centrifugation, three layers are seen in the resultant product, viz, at the base level the Red portion of blood, PRF clot at the intermediate level and straw-coloured acellular platelet-poor plasma at the topmost level.[22]

Use of PRF in dentistry:

· In the procedures involving lateral sinus elevation, PRF when used along with bone grafts, hastens the healing process.

· In augmentation procedures, PRF in addition to the graft material protects and stabilizes the graft material.

· PRF used after tooth extraction preserves the socket.

· Regenerative procedures in the treatment of 3wall defect.

· Treatment of periodontic endodontic lesion

· Treatment involving the furcation defect.

· PRF enhances palatal wound healing graft.

· For filling of the cystic cavity

PRF act as an immune regulator. The α-granules, present in the PRF, release cytokines interleukin (IL)-1β, IL-6, TNF-α and growth factors TGF β1, PDGF, VEGF, EGF, on activation and degranulation, that initiates the healing process through stimulation of migration of cells and proliferation of cells. The function of the growth factors found in PRF is listed in table-2.

Table 2: List of cytokine/growth factors present in PRF

IL-1	Stimulates T-helper cells, inflammatory mediator
IL-6	B cell differentiation, T cell activator, antibody secretion stimulation, inflammatory and remodelling mediator
IL-4	Proliferation and differentiation of activated B cells, moderates inflammation, increases fibroblast synthesis of fibrillary collagen
TNF-α	Monocyte activator, stimulate fibroblast remodelling, increase phagocytosis and neutrophil toxicity modulates IL-1 and IL 6 expression
VEGF	Initiates angiogenesis
TGF β1	Fibronectin and collagen synthesis
PDGF	Helps in the regulation of cell migration and further cell proliferation. Also renders help for the mesenchymal cell survival and cicatrisation
IGFs 1 and 2	Acts as a mediator in cellular apoptosis and has chemotactic effects towards human osteoblasts.

Prevailing Prominence of PRF in endodontics (current status):

For the revascularization of pulpal tissue, PRF serves as an ideal medium due to the fibrin mesh. This fibrin mesh entraps the cytokines, causes a slow and progressive release of cytokines over time around the surrounding tissues, as the degradation of fibrin happens. an additional function of PRF is that it accelerates the healing of wound edges by offering a function and also structural support mimicking fibrin bandage.

Even though when a tooth is classified as necrotic, there might be the presence of some pulpal tissue that survived in the apical region. Regenerative endodontics id based on the concept, that in the presence of the favourable condition, proliferation and regeneration of these pulpal tissues can be achieved. Due to the sustained key growth factor release, flexible trimolecular fibrin scaffold, the enmeshment of cytokine, for apexification and pulpal regeneration, PRF acts as a valuable adjunct and an ideal pulp capping agent.

PRF application: Use in avulsion and tooth transplantation

PRF is known to show a reduction in inflammation. In traumatized teeth, the inflammatory process may also cause ankylosis. In a study by Johns et al, when a 24month reevaluation was done following PRF placement (prepared via choukroun's method), reimplantation and splinting, it was observed that the tooth was vital to both percussion and thermal testing. Also, no radiographic signs of inflammation or replacement resorption were observed. As the pulp tissue in the canal assists in stimulating revascularization, so after a traumatic avulsion of dentition, PRF is quite a valuable adjunct for pulpal revascularization.[23]

PRF application: For the treatment of the chronic periapical lesion

Local application of growth factors maximizes the healing potential and ultimately promotes tissue regeneration and healing in the periapical region. As the stem cells that are present in the apical tissues attract growth factors, the PRF rich in growth factor is used to stimulate bone regeneration.[24] In a study by Wilting et al, reported that when PRP was added to tricalcium phosphate, there was an 8%-10% increase in bone formation. When compared to the first generation of platelet concentrate (PRP), the second generation (PRF) shows a stronger and more organized fibrin network. Apart from initiating bone growth, healing, maturation, β-tricalcium phosphate bone graft when mixed with PRF, causes an enhancement in the desired treatment outcome.[25] In a study by K.B.Jayalakshmi et. al, it was stated that when PRF was added to β-Tricalcium Phosphate, there was an acceleration in the capacity of bone regeneration and there was both clinical and radiographic evidence of bone formation.[26]

PRF application: For endodontic management of the open apex

The major difficulty faced in the case of an open apex is limiting the extrusion of the material into the periodontal tissue. This can be overcome by using a matrix, that avoids extrusion and causes a reduction in the leakage of the sealing substance. Out of the various materials used for the barrier formation at the apical end, PRF membrane is one of the preferable ones. In cases of single visit apexification, PRF membrane when combined with MTA (mineral trioxide aggregate) creates an artificial root-end barrier and results in faster healing in the periapical region.[27]

Conclusion

The advancement in the regenerative endodontics field, with the introduction of the different generation of platelet concentrates, is a breakthrough. The second generation of the platelet concentrates, the PRF was developed when the first generation (PRP) wasn't meeting all the desired qualities. The major drawback of PRP was wide variation in platelet concentration and also variation in the different centrifugation guidelines. The PRF that was developed became a boon. PRF with autologous growth factors and cytokines makes it a desirable treatment option in the field of regenerative endodontics. Further studies should be conducted for evaluating its effect and treatment outcome in a wide variety of clinical cases and setup.

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