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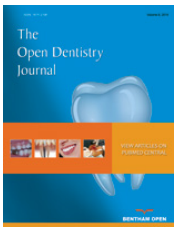

Platelet Rich Fibrin in Periodontal Regeneration

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Abstract: Periodontitis is a chronic bacterial infection resulting in destruction of the supporting structures of the teeth. Regeneration of the lost tissues has faced difficulties primarily due to the lack of support during the intricate healing processes. A surgical additive which can 'jump start' the healing process to a more predictable regenerative process is always on the wish list of any periodontist. Platelet-rich fibrin (PRF) is a second generation platelet concentrate that has been considered to be an important, easy to obtain, predictable surgical additive for periodontal regeneration. This autologous scaffold provides the much needed bio-chemical mediators which has the potential for enhancing reconstruction of the periodontium. This review article tries to understand as to why PRF would be an important link to reach predictable periodontal regeneration.

Keywords: Alveolar bone, Chronic bacterial infection, Periodontal regeneration, Platelet concentrate.

INTRODUCTION

The ultimate goal of periodontal therapy includes arrest of periodontal disease progression and the regeneration of structures lost due to pre-existing disease process.

Conventional surgical techniques offer only limited potential towards recovering the lost periodontal structures. Successful periodontal reconstruction comprises of regeneration of multiple tissues of the periodontium which regenerates at differential rates. It is a complex biological process in itself which is intricately regulated between cells, locally acting growth factors and the extracellular matrix components. The key to periodontal regeneration is to stimulate the progenitor cells to re-occupy the defect [1].

Earlier attempts to achieve regeneration includes denudation of interdental bone to treat intrabony defects and use of autografts to fill the surgical site. Also, favorable results have been gained in treatment of such defects using a combination of graft material and collagen membranes [2]. However, recently, the attention has shifted to the use of growth factors which are the biologic mediators that regulate the proliferation, chemotaxis and differentiation of the locally derived progenitor cells in the defect site [3].

Among the rich sources of autologous growth factors are various generations of platelet concentrates that are currently in use. Platelet Rich Plasma, (PRP) the first generation concentrate, has been used alone and in combination with grafting materials and barrier membranes in the management of periodontal and surgical defects [4, 5]. However, the effects of Platelet rich plasma on bone regeneration have been limited. The second and latest generation of platelet concentrates is Platelet Rich Fibrin (PRF). It is a promising, completely autologous leukocyte and platelet concentrate which is being successfully used in various fields of dentistry and medicine. PRF has shown successful results when used as a sole agent in the treatment of periodontal intrabony defects.

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REGENERATION OF PERIODONTAL TISSUES

Periodontitis is an inflammatory disease characterized by destruction of alveolar bone, root cementum, periodontal ligament and gingiva as a response to insults elicited by microbial accumulations on tooth surfaces [6]. These responses can result in a variety of intraosseous defects of various architectures. Periodontal regeneration refers to complete restoration of functional supporting tissues, including alveolar bone, cementum and periodontal ligament. It is defined as the reproduction or reconstruction of lost or injured part with form and function of lost structures restored [7].

Melcher *et al.* [8] proposed the type specific repopulation theory, which was further established by Gotlow *et al.* [9]. The theory states that, different periodontal connective tissues compete for the root surface during healing each resulting in a selected cell population occupying the periodontal wound and resulting in a specific type of repair or regeneration. Trombelli *et al.* [10] in 2002, in their systematic review reported various grafting modalities and bone substitutes that have been in use over the years for regenerative purposes. They compared results of open flap debridement alone and in combination with graft materials and concluded implantation of graft materials provided favorable results such as gain in clinical attachment levels, reduction in pocket probing depths and gain in defect fills.

Needleman [11] in 2002 and Giannobile *et al.* [12] in 2003, in their respective systematic reviews on application of guided tissue regeneration and enamel matrix derivatives reported significant increase in clinical attachment levels (CAL), however the magnitude of the observed additional benefits was modest.

Although, periodontal regeneration is a possible objective of several periodontal therapeutic modalities, outcomes of such modalities are not always predictable. Complete regeneration may be an unrealistic goal for many situations due in part to the complexity of the biological events and cells underlying successful periodontal regeneration. Wang *et al.* [13] in 2006 concluded that various factors which determined the predictability of bone regeneration include primary wound closure, blood supply, defect architecture, space maintenance and wound stability. All these factors play a significant role in deciding the amount and extent of achievable regeneration *via* various grafting modalities.

PLATELETS & PLATELET CONCENTRATES

Platelets are un-nucleated fragments of bone marrow megakaryocytes which circulate in blood for 8-10 days [14]. Historically, platelets are thought to contribute to the hemostatic process, where they adhere together to form a platelet plug in a severed vessel and actively extrude several initiators of the coagulation cascade.

Ross *et al.* [15] in 1974 introduced the regenerative potential of platelets by discussing their role in wound healing. The alpha granules of platelets contain various mitogenic factors such as Platelet Derived Growth Factor, Vascular endothelial growth factor and transforming growth factor -β. This storage pool of growth factors proteins is vital to initial wound healing. Upon connective tissue contact, as occurs in injury or surgery, the cell membrane of the platelet is "activated" to release these alpha granules. Active proteins are thus secreted which bind to transmembrane receptors of the target cells to activate intracellular signaling proteins. This results in expression of a gene sequence that directs cellular proliferation, collagen synthesis and osteoid production [16].

Platelet Concentrates

Application of fibrin adhesives in surgical management of hemostasis is well documented since early 1900s. These correspond to a natural biologic mechanism of fibrin polymerization, amplified in an artificial way. Thus yesteryear fibrin adhesives paved the way for the present day platelet concentrates. Concentrating blood components *via* centrifugation provides with an opportunity to amplify the rich and advantageous components of patients own blood. Platelet rich plasma and platelet rich fibrin are two such emerging platelet concentrates. These are basically fibrin matrices enmeshed with morphogenic proteins (Growth factors) and leukocytes.

Fabbro *et al.* [17] summarized the ideal role of platelet concentrates as:

1. Augmentation of tissue healing: By increased proliferation of connective tissue progenitors that stimulate fibroblast and osteoblast activity and enhance osteogenesis [18].
2. Anti-microbial activity: Against bacterial species involved in oral infections [19, 20].
3. Modification of host defense mechanism: By delivery of signaling peptides that attract macrophage cells [21].
4. Modification of immune reaction: By releasing leukocytes that synthesize interleukins [14].

Platelet Rich Plasma

The first generation of platelet concentrate, which consists of a limited volume of plasma enriched with platelets obtained from the patient, was called platelet rich plasma (PRP). If a normal human blood clot contains 5% platelets, according to Sunitha *et al.* [22], a PRP blood clot contains 95% platelets. PRP is known to contain growth factors such as PDGF and TGF – β that may influence the regenerative process. Also in-vitro studies by Creeper *et al.* [23] have reported proliferation of PDL and osteblastic cells under the influence of PRP. Although PRP contains growth factors, their release in wound site tends to be rapid and for a short duration of time. Also, complex production protocol involving use of bovine thrombin and other biochemical agents has limited the benefits of platelet rich plasma [24].

The potential benefits of PRP have been variable in literature. Although some authors reported significant improvements in tissue healing and bone formation using PRP [25 - 27], others failed to observe improvement [28, 29]. Benefits of treating intrabony periodontal defects with PRP combined with bone mineral were reported. However, the final consensus of PRP on bone grafts remains questionable. Thus, the technical and regenerative limitations of platelet rich plasma led to the discovery of a better, completely autologous fibrin matrix called Platelet Rich Fibrin.

Platelet Rich Fibrin

A second generation platelet concentrate, developed in France in 2001 by Choukroun *et al.* [30], is an autologous growth factor reservoir which attempts to accumulate platelets and cytokines in a physiologic fibrin clot. PRF clot concentrates 97 % of platelets and >50 % of leukocytes in a specific three dimensional distribution. It consists of intimate assembly of cytokines, glycanic chains and structural glycoproteins enmeshed within a slowly polymerized fibrin network [14].

PRF Preparation [14, 30]

Platelet rich fibrin (PRF) preparation protocol developed by Choukroun *et al.* [30]. Just prior to surgery approximately 5-6 ml of intravenous blood was drawn from the cubital fossa of the patient and collected in a 10-ml sterile glass tube without anticoagulant and immediately centrifuged at 3000 rpm for 10 minutes. Blood centrifugation results in separation of blood into a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma (Platelet-poor plasma) at the top [27]. After removal of PPP, PRF can be easily separated from red corpuscles base [preserving a small red blood cell (RBC) layer] using sterile tweezers and scissors.

Significance of PRF

Role of Fibrin Matrix

The three dimensional structure of the matrix resembles that of physiologic fibrin [31]. The enmeshed cytokines influence the extracellular matrix which allows migration, division and phenotypic change of endothelial cells, thus leading to angiogenesis.

Role of Platelets and Growth Factors

Periodontal regeneration is a multi-factorial and requires an orchestrated sequence of biological events including cell adhesion, migration, multiplication and differentiation [32]. The scientific rationale behind the use of platelet concentrates lies in the fact that the platelet α granules are a reservoir of many growth factors (GFs) that play a crucial role in hard and soft tissue repair mechanism [33, 34]. Platelet growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, cell proliferation and regeneration [35].

The growth factors released by α granule encompass a group of cytokine polypeptides with relatively low molecular weight ranging from 6-45kDa. PRF growth factors include Platelet derived growth factors (PDGFs), Transforming growth factor –β (TGF-β), Vascular endothelial growth factor (VEGF), Epidermal growth factor (EGF) and Insulin-like growth factor -1(IGF-1) [36].

PDGF

PDGF plays role in regulation, migration, proliferation and survival of mesenchymal cell lineages. It has mitogenic effects on stem cells and osteoblasts, stimulates pre-mitotic partially differentiated osteoprogenitor cells, stimulates cell replication of endothelial cells and promotes angiogenesis [37]. It modulates the effects of other growth factors and

promotes perivascular healing of the wound.

TGF –β

Of the three isoforms TGF-β1 is the most significant. It is an inflammatory regulator and the most powerful fibrosis agent amongst all cytokines [37]. TGF-β1 and TGF-β2 activate fibroblasts, which undergo cell division and produce collagen [38]. They control cellular differentiation and proliferation of cementoblasts and activate osteoprogenitor cells and further differentiates them to produce bone matrix; activate endothelial cells to produce new capillaries.

VEGF

It is the most powerful and omnipresent known vascular growth factor. The main role is includes initiation of angiogenesis.

IGF -1

Although present mainly in plasma it exerts chemotactic effects towards human osteoblasts, regulates cell migration, proliferation, differentiation and matrix synthesis. Acts as cell multiplication mediators in apoptosis by inducing survival signals protecting cells.

Role of Leukocytes

Fibrin mesh provides natural immunity under the influence of fibrinogen degradation products (FDP) that stimulate the migration of neutrophils, modulates phagocytosis and enzymatic degradation of the neutrophils. Also chemotactic agents trapped in fibrin control wound colonization by macrophages [39]. Leukocytes trapped in PRF have anti-infectious effect and act as an immune regulation node. PRF contains all key immune cytokines like IL 1β,

IL 6, IL 4 and TNF [39]. They have the ability to control the inflammatory response at the wound site.

Activation

Activation and degranulation of platelets are important to initiate and support their aggregation at the healing site. Given the absence of anticoagulant, activation of platelets in contact with silica of glass tube walls starts the coagulation cascade. Fibrinogen forms fibrin in the presence of physiologic thrombin. Post centrifugation fibrin is obtained in the middle of the tube with massively concentrated platelets Activation of platelets, thus releases the cytokines (IL-1 beta, IL-6, TNFalpha) and growth factors (TGF beta 1, PDGF, VEGF, EGF) that stimulates cell migration and proliferation within the fibrin matrix and thus begins the first stage of healing [39].

Technical Significance

According to the study by Su *et al.* [40] in 2009, platelet rich fibrin allows continuous release of growth factors for over 300 minutes following its preparation. Hence, it must be used immediately after preparing. The progressive release of cytokines and leukocytes continues for a period of 7-11 days, as the fibrin network disintegrates [41]. Slow and natural polymerization of PRF in the presence of physiologic thrombin gives it the crucial three dimensional organization of fibrin network. This characteristic fibrin network provides it with great elasticity, thus forming a very strong PRF membrane. Waiting for more than a minute or two may cause the fibrin to polymerize in a diffuse way, leaving behind only a small poorly formed clot in the test tube [42].

The various advantages of PRF include (Dohan *et al.*) [14, 36, 39, 42]:

1. Completely autogenous
2. Extended growth factor release for 7 days
3. Simple and faster technique
4. In-expensive
5. No requirement of any additive constituent such as bovine thrombin
6. No biochemical handling involved
7. No associated immune reactions
8. No associated infections
9. Acts as an ‘immune regulation node’
10. Has anti- inflammatory effects

Limitations

Connell *et al.*. [43] in 2007 raised concern regarding the safety issue of PRF methodology. He commented on the types of tubes to be used to produce PRF and the possible hazards of silica containing glass tubes. However, Dohan *et al.* [44] in the same year conducted a cytotoxicity analysis of PRF on wide range of human cells and concluded that silica microparticles coating these tubes are not cytotoxic for the tested human cells. They also reported improved mitotic proliferation and suggested that contact with silica is necessary to start the polymerisation process as silica behaves as clot activator. Thus, to produce PRF either dried glass tubes or glass coated plastic tubes must be used. Other sensitive issues not yet revealed, that may influence the nature of PRF include variation in quantity and quality of PRF with aging, influence of systemic diseases (thrombocytopenia, bleeding disorders, diabetes, leukocyte adhesion syndromes *etc*.), nutrition, environmental or racial differences, blood profile, autoimmunity and genetic predisposition.

PRP *vs.* PRF

According to Mosesson *et al.* [45], who described the structural and biological features of fibrinogen and fibrin in detail, the 3-dimensional organization of fibrin network depends on activation mechanism.

1. Strong concentration of thrombin leads to condensed tetramolecular or bilateral junction's in turn causing thickening of fibrin polymer. This rigid network is not very favorable for cytokine enmeshment and cellular migration. However, it can seal biologic tissues well. Such a structural organization is observed in platelet rich plasma (PRP).
2. Weak concentration of thrombin leads to trimolecular or equilateral junctions which result in a fibrin matrix that is flexible and can support cytokine and cellular migration. Such a flexible elastic and very strong network is seen in platelet rich fibrin (PRF).31 Unlike PRP, PRF results from a natural and progressive polymerization that occurs during the centrifugation process.

In an *in- vitro* comparison of PRF with PRP, He *et al.* [46] in 2009 demonstrated gradual extended release of autologous growth factors and better induction of osteoblastic differentiation and proliferation by PRF. In a clinical trial by Pradeep *et al.*. [47] in 2012 comparative evaluation of autologous PRF and PRP in intrabony defects demonstrated equally favorable clinical and radiographic results in both groups when compared to open flap debridement alone.

Applications

The vast benefits of PRF have led to its applications in different fields of medicine and dentistry:

1. Ear, nose, throat and plastic surgery [48]
2. Oral and maxillofacial surgery, [21]
3. Pre-implant and implant surgery [49].

CONCLUSION

Platelet-rich fibrin has been studied through clinical and histological studies. In addition to being autologous, there is real interest in PRF due to its regenerative potential in periodontal micro environment. The role it has in enhancing periodontal regeneration is being elucidated. However the mechanism of PRF in mediating the inflammatory and healing processes are yet to be studied on cell based models. Also the effect of regeneration and its stability has not been researched through long term clinical studies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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