Review article

Platelet rich fibrin- a boon to periodontics

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Abstract:
Periodontal diseases are characterised by loss of connective tissue attachment and other tissues of the periodontium. These periodontal diseases are multifactorial and can be widely complex, which ultimately might accelerate as periodontal breakdown. Hence periodontal therapy always aims for preventing further progression of the worsening condition of the periodontium and possible regeneration of the lost periodontal tissues. PRF is being considered as a very mighty biomaterial and can be used in various procedures of periodontal regeneration like periodontal diseases with intrabony defects, guided bone regeneration, socket preservation, mucoginigival surgeries, recession coverage procedures, connective tissue augmentation at different sites including implant sites, sinus floor elevation etc because of its inherent regenerative ability, which is perceived by release of various useful cytokines which are present in the PRF because of the degranulation that takes place in the platelet cells. Thus, this review article will further elaborate about PRF and its various uses in the field of periodontics.

Keywords: PRF, Platelet concentrates, Platelet rich fibrin, Cytokines, Regeneration.

Introduction:

Platelets are anucleate cytoplasmic fragments containing alpha granules that are spherical or oval structures with diameters ranging from 200-500 nm.[1] They are vital in the initiation of wound healing as they contain biologically active proteins that bind on to a developing fibrin mesh or to the extracellular matrix. Thus, creating a chemotactic gradient for recruitment of stem cells, which ultimately undergo differentiation, cellular organisation, remodelling, migration and promote healing via regeneration. They form an intracellular storage pool of proteins vital to wound healing, including platelet derived growth factor (PDGF), transforming growth factor (TGF beta), insulin like growth factor (IGF-I)[2]. Hence, autologous platelet concentrates (APC) do act as a propitious treatment modality in periodontal regeneration field. Various techniques of autologous platelet concentrates have been developed the first been platelet rich plasma and the second-generation APC is platelet rich fibrin (PRF).

WHAT IS PRF?

PRF is a second-generation platelet derivative developed by Choukroun et al.in 2001 in France. It is known as a second-generation platelet derivative because unlike the first generation the technique used to obtain PRF does not require anticoagulants, bovine thrombin nor any other form of gelifying agent ensuring absence of any artificial biochemical modification. The essence of PRF synthesis lies in the attempt to accumulate platelets and release cytokines in a fibrin clot which is succumbed during centrifugation which is a natural polymerization process.[3,4,5]

Platelets and cytokines play a vital role in the biology of PRF, degranulation of platelets entails the release of cytokines, the functions of which are mentioned in the table below.[Table 1]

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Cytokines present in PRF	Functions
Transforming growth factor- beta	Released from alpha granules of platelets[2]
	Stimulates proliferation of osteoblasts [6]
	Synthesis of type I collagen and fibronectin
	Enhanced woven bone formation
	Enhanced chemotaxis of osteoblast cells
	Stimulates angiogenesis
Platelet derived growth factor	Migration and proliferation of mesenchymal lineage cells[7]
	Angiogenic effect on endothelial cells[8]
Vascular endothelial growth factor	Angiogenesis [9]
Insulin growth factor- 1 and Fibroblast growth factor	Stimulates proliferation of osteoblasts[8]
	Chemotactic effects towards human osteoblasts
	Osteocalcin expression is increased
	Wound healing is accelerated
Epidermal growth factor	Stimulation of cell proliferation and extracellular matrix turnover[10]
	Periodontal fibroblasts show chemotactic effect

Table 1: Various Cytokines and their functions in the PRF.

Fibrin being an activated form of fibrinogen molecule present in plasma as well as the alpha granules of platelet plays a major role in platelet aggregation finally achieving haemostasis by transformation of soluble fibrinogen into insoluble fibrin that polymerizes to a cicatricial matrix.[11] During the centrifuge performed in PRF preparation a homogenous 3-dimensional organization or matrix is formed due to slow and natural polymerisation of fibrin, which is flexible, elastic and strong.[11,12] PRF also consists of a weak thrombin concentration which entails equilateral junctions, which support cytokines and promotes cell migration that occurs. The increase in this cell migration increases the life span of the cytokines hence serving the period required by the cells to initiate healing.[12]

Preparation of PRF:

The indigenous intent is to spin at high centrifugation speeds in order to phase separate the layers between the red corpuscle cell base and the overlaying clear liquid accommodating the leukocytes and plasma. Preparation of PRF follows the protocol developed by Choukroun et al. in nice, France.[13] Though procedure followed in the preparation of PRF is very straightforward, it has to be fabricated just before its use in the clinic.

Prerequisites:

(A) Table centrifuge
(B) 10-ml dry glass test tube (without any anticoagulant)
(C) Blood sample collection apparatus

The main advantages in PRF preparation are the single stage centrifugation and absence of bovine thrombin. The blood obtained from the subject is placed into the test tube and centrifuged immediately for 10 minutes at 3000 rpm[11]. Few others have also used 2700 rpm for 12 minutes with parallel findings.[14]

Following are the steps for fabricating PRF:

(1) Blood specimen is drawn from the patient

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(2) The blood specimen is placed in the centrifuge and is allowed to spin immediately for the stipulated time period
(3) Following this step the blood specimen settles into various layers.

The absence of any anticoagulant grants the activation of platelets to set off a coagulation cascade. Due to the absence of the anticoagulant, the blood coagulates immediately upon contact with the glass tube. Initially, fibrinogen occupies the upper part of the tube, only till the circulating thrombin transforms it into a fibrin network. [3]

The layers that are formed following centrifugation are as follows:
(a) The RBCs containing lower fraction
(b) The fibrin clot containing middle fraction
(c) and the upper fraction accommodating the straw-coloured acellular plasma.

The upper portion of the test tube containing the acellular plasma is removed. The middle portion containing the fibrin clot is then removed and is scrapped off from the lower part containing the red blood cells. The natural and progressive polymerization should result in a fibrin clot formation with considerable number of platelets and leukocyte growth factors inlayed into the fibrin matrix.[15]

To obtain a PRF membrane in order to place it on the surgical site, plant a autogenous graft or to just keep the hydration of graft material intact, the PRF can be squeezed between the 2 gauge pieces. This is considered as a very clever and inexpensive technique to acquire a autologous fibrin membrane.

CLINICAL IMPLICATIONS OF PRF IN PERIODONTICS:

- PRF as a plug during extraction socket healing and barrier membrane:
  The natural fibrin matrix of PRF makes it possible to be utilized alone thereby eliminating the necessity of bone grafts and at time barrier membranes in guided bone or tissue regeneration surgeries. Furthermore, management of extraction sockets has become very easy by using PRF as it ensures no risk of infection. Also, since PRF is purely autologous, it does not cause a foreign body reaction and thereby speeds the natural wound healing process without generating an immune response. It has been observed that within a 3-month healing period, the fibrin matrix is transformed into new tissue: bone in the socket with overlaying soft tissue.[16]

- PRF for soft-tissue root coverage:
  Management of root exposure with PRF has become promising in the recent years. Class I and II defects of miller's classification of recession with a thick biotype of gingiva can be successfully covered with PRF as it increases or rather improves vascularization, wound healing and patient morbidity. Nevertheless, one of the limitations of PRF is the reported stability of the keratinized mucosa when compared to CTG over time.[17]

- PRF as a sole material in sinus elevation procedures and for Schneiderian membrane repair:
  Along with its use in extraction socket management, PRF has also been used for sinus floor elevation procedures. In these indications, it may fulfil the task of being utilized as a sole grafting material, can be further used for the repair of the Schneiderian membrane, and has also been utilized to close the window during the lateral sinus approach. Few studies have shown additionally that PRF could be combined with a bone grafting material for sinus lift augmentation to decrease the overall healing time. [18,19,20,21]

- PRF for periodontal regeneration:

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Another group of clinical expertise is the use of PRF for periodontal regeneration of either intrabony or furcation defects. As PRF can be utilized being a safe and natural method to repair tissues inexpensively, many have attempted to use PRF for the regeneration of periodontal defects. Many reports have shown significant improvements in periodontal pocket depth reduction as well as clinical attachment level gains following regenerative periodontal therapy with PRF. Similar positive results have also been obtained for the treatment of furcation class ii involvement. [22,23]

- PRF for the regeneration of soft tissues around implants:
  PRF can be utilized quite conveniently as an inexpensive biomaterial able to initiate the early healing of soft tissues around implants. As a growing avenue of research use of PRF for both improved osseointegration and soft-tissue healing around dental implants limited to no available literature supporting its use exists, and the long-term evaluation of such protocols has seldom been investigated and need further venturing.

- PRF in guided bone regeneration:
  PRF has also frequently been utilized in combination with bone augmentation procedures. Reported advantages include an increased vascular supply as well as excellent graft stability when PRF is utilized in combination with a bone grafting material.

❖ Advantages of PRF:

(i) No use of anticoagulants
(ii) Single step centrifugation.
(iii) Slow natural polymerization[2]
(iv) 3D fibrin network forming a matrix aiding in cytokine retention for extended periods
(v) Formulation of a PRF membrane that possesses elasticity and flexibility[2]
(vi) Simple and cost effective
(vii) An external addition of Bovine thrombin is not required, thus averting immunological reaction.
(viii) It is obtained from an autologous blood sample that requires minimal manipulation.

❖ Limitations of PRF:

(i) Rapid use of the PRF without delay or short handling time.[2]
(ii) PRF protocol has to be strictly followed hence becomes technique sensitive.
(iii) Owing to the fact that PRF is an autologous product, the availability of this biomaterial in larger amounts is a concern. Hence, its usage in surgical procedures should be well supervised. [24]
(iv) PRF possesses the circulating immune cells and antigenic molecules that prevent its use as an allogenic material. Also, there is an increased risk of transmitting infectious agents.[24]
(v) Experienced clinician is required for PRF manipulation.

RECENT ADVANCES:

Concentrated growth factors: The concept of concentrated growth factor was introduced by Sacco in 2006.A special centrifugation machine, Medifuge (Italy) is used. The process is similar to PRP but with different centrifugation speed the protocol of which is mentioned below. This newer technology allows it to separate a fibrin denser which is much denser, larger and richer in growth factors.[25]

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Preparation protocol sequence:

30sec acceleration.
2min at 2700rpm/735g,
4min at 2400rpm/580g
4min at 2700rpm/735g
3min at 3000rpm/905g
36sec deceleration and stop

- Titanium prepared platelets rich fibrin:

T -PRF was being introduced by Tunali et al in 2013. During the conventional PRP preparation procedure, researchers have pointed out that the possible health hazards caused by small particles which are small enough for a fraction to remain suspended colloidal in the buffy coat, fibrin and platelet poor layers of plasma.These particles might enter in the patient's body when the product is used for T-PRF is a newer method of preparation of platelets concentrate which is based on the hypothesis that titanium tubes may be more effective at activating platelets than the glass tubes used in Choukrouns method.[26]

- Advanced PRF (A-PRF)

Being introduced by Choukroun in 2014, it has a good source of VEGF and Bone morphogenic proteins. An attempt to incorporate the monocytes within PRF.as compared to the standard centrifugation protocol for the preparation of standard PRF, the centrifugation protocol for obtaining A -PRF is changed (1500rpm, 14min). In the lower concentrated protocol, the presence of the macrophages was improved in the PRF. Because of this reason it is called A-PRF from the new protocol.[26]

- Advanced PRF (A-PRF) +

In the newer low speed concept, newer form of PRF have been described. in A-PRF +, the centrifugation protocol was changed to 1300rpm for 8min. A-PRF + demonstrated significantly higher total growth factor release compared with A-PRF and L-PRF.[26]

- Injectable –PRF (i-PRF)

The injectable form of PRF is obtained by centrifugation of whole blood at 700 rpm for 3- 4 mins When the particulate bone graft is added to i-PRF, the result is the formulation of a well agglutinated red coloured Sticky bone. [26]

- Liquid PRF

Liquid PRF was developed with the goal of acting as a regenerative agent which could be delivered in liquid formulation by drawing blood rapidly in a centrifugation tube at a very low speed of 700 rpm (60 g) for an even shorter centrifugation time (3 to 4 minutes). The objective here was to centrifuge without anticoagulants or additives but yet to maintain the ability to separate two layers. This new formulation can be utilized for a variety of procedures, including mixing with bone.[27]

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