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Platelet Concentrate – A Boon In Periodontal Regeneration

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Abstract
Platelets play an important role in coagulation and wound healing. They also help in repair and regeneration of tissues. Centrifugation of whole blood at various speeds and time to form different types of platelet concentrates with or without anti-coagulantincludes platelet rich plasma (PRP), platelet rich fibrin (PRF), etc. They are widely used in various fields of medicine and dentistry to assist in initial phase of wound healing, tissue regeneration, andalso used as an alternative to bone graft or incombination with bone graft. This article provides detailed knowledgeabout evolution of platelet concentrate, its preparation technique and its application in periodonticsincluding recent advances.

Key Words: *Platelet, Growth Factors, Regeneration, Platelet Rich Plasma, Platelet Rich Fibrin*

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I. Introduction

Periodontitis is an immunoinflammatory disease caused by specific or group of microorganisms leading to destruction of periodontium,which if untreated leads to tooth loss. The main focus of periodontal therapy is to eliminate the inflammatory process, prevent progression of the disease, and also regenerate the lost tissues [1]. Periodontal regeneration is a complex process which includes biological cell events like cell adhesion, migration, proliferation and differentiation in a unifiedmanner. Periodontal regenerative procedures include bone grafts, soft tissue grafts, root biomodifications, guided tissue regeneration, and combinations of these procedures [2]. The current perspective is that regenerative periodontal therapies till date can only restore a fraction of the original tissue volume and have a limited potential in attaining complete periodontal restoration[3,4]. Diverse treatment modalities are available for periodontal regenerative therapy, including bone grafting, bone replacement, guided tissue regeneration, growth factors, tissue engineering applications, or a combination of two or more methodslisted above. Periodontal wound healing requires a series of interactions between epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. Disruption of the vasculature during wound healing leads to fibrin formation, platelet aggregation, and the release of certain growth factors from platelets into the tissue through molecular signaling primarily mediated by cytokines and growth factors[5]. There are evidences that the presence of growth factors and cytokines in platelets play a key role in inflammation and wound healing. Platelets also secrete fibrin, fibronectin, and vitronectin, which act as a matrix for connective tissue and as adhesion moleculefor more efficient cell migration. This led to the idea of using platelets as therapeutic tools to enhance tissue repair, especially in periodontal wound healing[6].

II. Platelets

Histologically, platelet diameter ranges from 1 to 4 micrometres; they are colourless having a moderately refractive body but no nucleus. Platelet precursor cells, the megakaryocytes are extremely large hematopoietic cells found in the bone marrow. These megakaryocytes fragment into small disc-shaped structures called platelets, either in the blood or in the bone marrow itself from where they are squeezed into the

capillaries. The normal concentration of platelets in the blood is from 150,000 to 300,000/mm^3.The average lifespan of a platelet is about 5 to 10 days.The spleen acts as a reservoir for platelets, which are then released when needed by sympathetic contractions of the splenic muscle [7,8].

III. Role Of Platelet In Wound Healing

A proinflammatory biochemical environment impairs the wound healing process associated with increased protease activity, which reduces the concentration of various Growth Factors (GFs). Rich source of Growth Factors, Platelet concentrates are used as alternative treatment for wounds as they have mitogenic, angiogenic and chemotactic properties. Platelets exert their effects mainly through three different granules, namely (alpha) granules, lysosomes and dense granules. These granules release lot of growth factors which includes Platelet Derived Growth Factors (PDGF), Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor β (TGFβ), Insulin like Growth Factor (IGF),Epidermal Growth Factors (EGF) Angiogenesis Factor (AF), basic Fibroblast Growth Factor (bFGF), Platelet Factor 4 (PF4)[9]**(Table no 1).** Platelets release these growth factors within 10 min after clotting and approximately more than 95% of these pre-synthesized growth factors are released within the first hour[10,11]. When direct action of platelets diminishes, macrophages arrive through vascular ingrowth stimulated by the platelets and then they become responsible for wound healing regulation by secreting their own factors[9]. So, the platelets play a key role in wound healing.

Growth factors	Target cell	Function
Pdgf	Fibroblasts, smooth muscle cells,macrophages/neutrophils	Stimulate neutrophils/ macrophages, collagen synthesis & collagenase secretion
Tgfβ	Fibroblasts, endothelial cell, marrow stem cells, epithelial cells, pre-osteoblasts	Collagen synthesis & collagenase secretion, stimulates osteoblast, fibroblast.
Igf	Fibroblast, osteoblast, chrondrocytes	Stimulates cartilage growth, bone matrix formation, and replication of pre-osteoblasts and osteoblasts
Vegf	Endothelial cells macrophages/ neutrophils	Stimulate angiogenesis; migration and mitosis of endothelial cells; creation of blood vessel lumen; chemotactic for macrophages and granulocytes; vasodilation indirectly by release of nitrous oxide
Pf4	Fibroblast, neutrophils	Attracts fibroblast & neutrophils, potent anti-heparin agent.
Af	Endothelial cells increases angiogenesis and vessel permeability	Stimulates mitogenesis for endothelial cells by direct or indirect actions; upregulate several cytokines and growth factors, including igf-1, tgf, pdgf, bfgf, egf, and il-1β.
Egf	Fibroblasts, endothelial cells, epithelial cells	Epithelial/mesenchymal mitogenesis, stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion.
Bfgf	Fibroblasts, endothelial cells, epithelial cells	Increasesangiogenesis, epithelization and fibroblastic mitogenesis.

Table no 1: growth factors their target cell and functions

IV. History And Classification Of Platelet Concentrate

Kinsley first coined the term PRP for thrombolytic concentrate in 1954[12]. In the late 1970s, Matras introduced "fibrin glue", also known as fibrin sealants or fibrin tissue adhesive. Homologous and autologous are two types of fibrin sealants. A Homologous/commercial variant was prepared by mixing two components, namely a fibrinogen component containing factor XIII and thrombin component containing calcium ions. Homologous fibrinogen concentrates were prepared from plasma cryoprecipitate or Cohn fraction. However, it carries a risk of transmitting infections. Therefore, autogenous fibrin is preferred, which includes fibrinogen and clotted serum containing thrombin as the final product[13]. In 1986, Knighton et al, first demonstrated that platelet concentrate (PC) promotes successful wound healing which they termed it as "platelet-derived wound - healing factors (PDWHF)", which was successfully tested for the treatment of skin ulcers[14]. Whitman et al (1997) coined their PRP as "platelet gel"[15]. All of these products were designated as PRP without knowing the quality of their content or architecture, and this lack of terminology persisted for many years. Some commercial companies, instead of being more recognizable, began to label their products with separate trade names, for example: P-PRP was marketed under the name Vivostat PRF (Alleroed, Denmark). However, as the name suggests, it is not a PRF but produces a PRP product. Choukroun J et al, in France (2006) developed another form of autologus platelet concentrates which was called "Platelet-Rich Fibrin (PRF)" due to the strong polymerization of fibrin gel[6]. AlthoughPRP ("First Generation" of platelet concentrates) require an external coagulation factor to initiate the clotting process, the present PRF ("second generation" of platelet concentrates) preparation does not require the extrinsic clotting factors to initiate the clotting process. The development of the new terminology came when Dohan Ehrenfest et al, pointed out that the PC also involves various types of circulating cells, especially leukocytes, and named it as L-PRP (Leukocyte rich platelet rich plasma)[16].Sacco (2006) introduced "Concentrated growth factors (CGFs)". Medifuge (Italy), a special centrifuge was used to prepare CGF[17]. The idea of producing bone graft matrix with rich in growth factors known as "sticky bone"

using autologous fibrin glue has been demonstrated since 2010. Sticky bone provides stability to the bone graft in the defect area and thus, accelerates tissue healing and minimizes bone loss during the healing period[18]. Mourão et al (2015) described a technique to obtain an injectable form of PRF called i-PRF. I-prf could be injected or mixed with bone graft to give a well agglutinated "steak" for bone grafting[19]. Tunali et al, introduced a new product called T-PRF (Titanium prepared PRF) in 2014. The use of titanium tubes for collection and centrifugation instead of glass tubes was introduced based on the hypothesis that titanium may be a more effective platelet activator than silica for the preparation of L-PRF[20].

V. Classification Of Platelet Concentrate

Classification given by	Nomenclature
Dohan ehrenfest et	1) pure platelet-rich plasma (p-prp) - or leukocyte-poor platelet-rich plasma (l-prp); (2) leukocyte-and platelet-rich plasma (l-prp); (3) pure prf (p-prf) - or leukocyte-poor prf; and (4) leukocyte- and platelet-rich fibrin (l-prf)
Mishra et al	Type 1: l-prp solution; Type 2: l-prp gel; Type 3: p-prp solution; Type 4: p-prp gel.
Delong et al	Similar to mishra et al

Table no 2: classification of platelet concentrates

The first classification about platelet concentrate was proposed by Dohan Ehrenfest et al in 2009**(Tableno 2).** This classification was based on the cellular (mainly leukocytes) and fibrin architecture[21].

Mishra et al in 2012 proposed another classification which was limited to PRP and applicable to sports medicine only[22]. Based on presence or absence of leukocytes and whether or not the PRP is activated, all types can fall into 2 sub-types: A: Platelets > 5 × baseline or B: Platelets < 5 × baseline. In all the following types "solution" means non-activated PRP and gel means activated PRP**(Table no 2).**

DeLong et al in 2012 introduced another classification system called PAW (Platelets quantity, Activation mode, White cells presence)[23] [Table-2]. However, it is again limited to PRP families and it was similar to classification by Mishra et al. Difference between PRF and PRP **(Table no 3).**

FEATURES	PRF	PRP
FORM	FIBRIN MATRIX	PLASMA
Technique	1. Simple 2. No bovine thrombin 3. No anticoagulant 4. Economical (cost wise) 5. More quantity achieved	1. Complex 2. Bovine thrombin required 3. Anticoagulant added 4. Expensive 5. Less quantity achieved
Properties	Mechanical Properties: Strong Growth Factors: More Leucocytes: >65%, >75% IN L-PRF	Mechanical Properties: Weak Growth Factors: Less Leucocytes: 0-50%

Table no 3: Difference between PRF and PRP

VI. Preparation Protocol For Various Platelet Concentrate

Platelet Rich Plasma (PRP) is prepared through a two-step centrifugation preparation of anticoagulated blood samples, usually using EDTA or 1.0 ml of acid citrate dextrose –ACD- solution. In the first centrifugation step (Soft Spin) (300g for 5 minutes at 12°C or 240g for 8 minutes at 16°C), three layers are formed namely red blood cells (RBCs) at the bottom, 'buffy coat' (BC) middle layer containing platelets and leukocyte and platelet poor plasma (PPP) on upper layer.

To produce Pure PRP (P-PRP), the PPP and superficial BC were transferred to another tube, then centrifuged for a second time (hard spin) to ensure good plasma separation (700 g for 17 minutes at 12°C), most of the PPP layer is removed. The final P-PRP concentrate consists of an undetermined portion of BC (containing a large number of platelets) suspended in some fibrin-rich plasma.

On the other hand, to produce Leukocyte-rich PRP (L-PRP), PPP, the entire BC layer and some remaining red blood cells are transferred to another tube. After hard spin centrifugation, the PPP is discarded. The final L-PRP consists of the entire BC, which contains most of the platelets and leukocytes, and residual RBCs suspended in some fibrin-rich plasma. Coagulation cascade then activated by adding thrombin to any of these products, permitted to clot at 37°C before injecting into patient[24].

Plasma Rich in Growth Factors (PRGF) is a type of (P-PRP) using special single spin protocol (PRGF-Endoret technology) first described by Dr. Anitua. Blood samples (~9.6 mL per tube) were collected using 18G needles in citrated tubes (contained 0.2 ml sodium citrate). The tubes were then centrifuged at 2270 rpm for 8 min at room temperature. After centrifugation, the blood sample was formed into four separate layers: -

1) 0.5 ml of Plasma Poor in Growth Factors (PPGFs) in the upper part of the tube,

2) 0.5 ml of Plasma with Growth Factors (PGFs),
3) 0.5 mL of (PRGF) located JUST above the RBC portion in the tube,
4) Concentrated RBC layer.

The PPGF was removed and the PRGF was separated with a 500 µL pipette and transported into an independent tube then activated using 50 µL of 10 % calcium chloride to be used as liquid or incubated for 20 minutes in 37°C to produce easy to handle gelatinous layer (PRGF) in the form of a fibrin scaffold or membrane, Anitua therefore simplified the PRP preparation protocol and replaced animal-derived thrombin with calcium for blood coagulation[25].

Platelet Rich Fibrin (PRF) Preparations: Choukroun's Pure Platelet Rich Fibrin (P-PRF) belongs to a new generation of platelet concentrates, with simplified processing and no need for biochemical manipulation in the blood. Standard PRF preparation procedure should be followed to obtain the appropriate quantity and quality of the fibrin matrix, platelets, and growth factors; A 24-gauge butterfly needle is used to collect 9 ml of blood into sterile glass coated plastic tubes without anticoagulant which are immediately centrifuged at 3000 rpm for 10 minutes[26]. During centrifugation, when the blood comes into contact with the wall of the test tube, platelets are activated, leading to the initiation of blood clotting. After centrifugation, the obtained product consists of three layers. The top layer consisting of acellular PPP (platelet poor plasma), middle layer consists of PRF clot and erythrocytes at the bottom of the test tube. The fibrin clot obtained after centrifugation is removed from the tube and the attached red blood cells scraped off from it and discarded. PRF can also be prepared as a membrane by removing the fluid contained in the fibrin clot.

Leukocyte- Platelet Rich Fibrin (L-PRF): Preparation of Choukroun's PRF - the clot or membrane of L-PRF is a modified PRF to contain most of the platelets and leukocytes present in the initially drawn blood as well as platelet growth factors and stem cells that are also trapped within the fibrin network with enhanced strength[27]. It is produced by modifying the original technique of (P-PRF); Blood should be collected rapidly into 9 ml glass-coated plastic tubes (less than 20 seconds per tube) and immediately (within 1 minute) centrifuged in an Intra-Spin centrifuge at room temperature (2700 rpm for 12 minutes) to form L-PRF clots. The clots can be carefully collected in asuitable sterile surgical container and pressed against membrane, left as a fibrin plug, or mixed with bone graft to form a sticky bone[28].

Advanced - Platelet Rich Fibrin (A-PRF): this is another modification from of (P-PRF) in which they found that reducing RPM while increasing the centrifugation time (1300 rpm, 14 minutes) increased the presence of neutrophils in the distal part of the clot and the release of growth factors was prolonged.As a result, this might be able to influence the differentiation of host macrophages and macrophages in the blood clot after implantation [29]. Therefore, they postulated that this would influence bone and soft tissue regeneration, specifically through the presence of monocytes/macrophages and their growth factors.

Injectable-Platelet Rich Fibrin (i-PRF): The development of injectable PRF formulation was intended to provide clinicians with an easy-to-use platelet concentrate formulation in liquid which can be utilized alone or combined with various biomaterials. By taking advantage of slower and shorter centrifugation speeds, a higher presence of regenerating cells with higher growth factors concentrations can be observed compared to other PRF formulations.The preparation protocol for i-PRF - 10 mL of whole blood collected in plain vacuum tubes without anticoagulant was immediately centrifuged at 700 rpm for 3 min. The 1 mL upper plasma layer was then collected with a 21-gauge needle and designated as i-PRF. Adding i-PRF to bone particles causes polymerization within 15 minutes to produce sticky bone[30].

Titanium – Platelet Rich Fibrin (T-PRF): Based on the hypothesis that titanium may be a more effective platelet activator than silica, to prepare L-PRF Tunali et al in 2014, introduced Titanium - prepared PRF in which the 9 ml of blood was quickly collected in grade IV titanium tubes, and the tubes were immediately centrifuged at 2800 rpm for 12 minutes results in Immensely organized network along with a continuous integrity and fibrin network was thicker that covered a larger area[20].

Concentrated Growth Factors (CGF): an alternative centrifugation procedure is used for this preparation. The patient's Intravenous blood samples is placed in a standard 10-ml centrifuge tubes without anticoagulant and accelerated for 30 seconds, centrifuge at 2700 rpm for 4 minutes, 2400 rpm for 4 minutes, 2700 rpm for 4 minutes, and 3000 rpm for 3 min, and decelerate for 36 seconds to stop according to automatic settings by centrifugal device.Denser, larger, and more growth factors rich fibrin matrix consisting of three layers are observed in the tube: a layer of red blood cells at the bottom, platelet-free plasma layer (without cell) at the top, and a fibrin gel containing concentrated growth factor and platelet aggregation in the middle. The upper layer platelet free fraction was removed using a sterile syringe. The layer in the form of a membrane containing the condensed growth membrane was held with the aid of a hemostatic forceps, separated from the red blood cell layer by cutting with a pair of scissors and then pressed to form a membrane[17].

Autologous Fibrin Glue (AFG) and Sticky Bone: A concept for producing growth factors rich bone graft matrix known as "sticky bone" using autologous fibrin glue (AFG) has been demonstrated since 2010; To obtain autologous fibrin glue, 20- 60 CC of blood in uncoated tube is centrifuged at 2400-2700 rpm for 2

minutes. among the two layers obtained,0 the deepest layer is the RBC's layer and the superficial layer is the AFG. This AFG is then extracted with a syringe and mixed with particulate bone powder and left for 5-10 minutes to polymerize, results in a yellow-coloured mass called sticky bone[31].

VII. Applications Of Platelet Concentrates In Periodontics

The application of PRP to bone graft materials has demonstrated the ability to regenerate bone and heal soft tissues faster[32]. PRP can also retard epithelial migration by imparting it to resorbable barrier membranes. This will also provide a local source of growth factors that will accelerate the maturation of soft tissue and hard tissue[33]. Agrawal and Gupta (2014)[34], in a split-mouth study, concluded that the combination of PRP with DFDBA was more effective than DFDBA with saline in the treatment of non-contained intrabody defects. In addition, the combination of PRP with bovine porous bone mineral and GTR membrane also showed good clinical response [35]. The Combination of PRF and bone graft has also showed exceptional results in furcation defect[36]. However, Choi et al [37] questioned the advantage of mixing PRP and bone graft material, expressing their concern that it interfered with new bone formation. According to the authors, growth factors when present in high concentrations at inappropriate times over long periods, can negatively affect the cell activity. They further stated that proliferation and viability of alveolar bone cells were limited by high PRP concentrations but are accelerated by low PRP concentrations.

PRF is a powerful healing biomaterial with inherent regenerative properties and can be used in a variety of procedures such as periodontal intraosseous defects [38], furcation defects [39], sinus lift procedures [40] and as application in the field of tissue engineering, it can be used as a framework for human periosteal cells in vitro.

Eren and Atilla in 2012 treated bilateral gingival recession with coronally advanced flap (CAF) and subepithelial connective tissue graft (SCTG) on one side and CAF with PRF on other side. They noted improvement in all parameters with both techniques, because use of PRF was practical and simple to perform and also eliminates the need of donor site wound, thus they suggested that CAF + PRF is a better alternative to CAF + SCTG [41]. Anilkumar et al, reported PRF as a probable but innovative method for root coverage in treatment of mandibular anterior gingival recession using a combination of PRF membrane and laterally positioned flap technique [42]. Aroca et al in a randomized clinical trial, concluded that the addition of a PRF membrane placed under the MCAF (modified coronally advanced flap) resulted in increased gingival/ mucosal thickness but inferior root coverage in 6-months follow-up period compared to the conventional method [43].

VIII. Applications In Implantology

Choi et al conducted an animal study in 2006 to compare repair of sinus mucosal perforation using either the autologous fibrin glue (AFG) or the collagen membrane. Their histological evaluation showed that in repaired wounds, where AFG was used, newly regenerated continuous epithelium at the original perforation site.In the site treated with collagen membrane epithelium was absent and inflammatory infiltrates were observed along with extensive fibrosis even after 2 weeks of healing[44].

The literature showed various applications of PRP in continuity defects, sinus lift augmentation, vertical/horizontal ridge augmentation, ridge preservation, periodontal/ peri-implant defects. Several articles have reported the use of L-PRF membranes to stimulate bone and gingival healing during sub-antral sinus augmentations and global rehabilitations with dental implants. The effect of these membranes on the healing and maturation of soft tissue is particularly significant.

In yet another case report, Del Corso et al used L-PRF in immediate implant replacement of maxillary central incisor in 2012 and reported better healing and aesthetics[45]. Choukroun et al studied the effect of PRF with freeze-dried bone allograft (FDBA) to augment bone regeneration during direct sinus lift and found that bone regeneration was accelerated[46].

Simonpieri et al, in a two-part publication, reported a new technique for maxillary reconstruction using PRF membranes, FDBA and 0.5% metronidazole solution. An amount of 0.5% metronidazole solution (10 mg) provides an effective protection of the bone graft material against the bacterial contamination[47]. The membrane component of PRF was used to protect the surgical site and improves the soft tissue healing. However, the PRF fragments were mixed with the graft particles. They also proposed that the PRF membranes could be cut into pieces (millimetre size) and added to the graft material, acting as a "biological connector" between the different components of the graft, and would form a matrix that promote the migration of osteoprogenitor cells to the centre of the graft, new blood vessels formation and capture of stem cells. Using the procedure reported in the literature, they generally observed a higher level of gingival maturity after healing. They also noted thickening of the keratinized gingival tissues, which ultimately improved the aesthetic harmony and final result of their prosthesis. In addition, all their clinical experience showed that the use of PRF appears to reduce postoperative oedema and pain, and even the small risk of infections.

PRF can be condensed to form plugs that can be positioned at the implant osteotomy site to promote sinus floor elevation using a crestal approach or osteotome-mediated sinus floor elevation (OMSFE) with simultaneous implant placement[48]. PRF can not only be used as an alternative for particulate grafting to predictably elevate the sinus floor using a crestal approach, but PRF can also protect the sinus membrane when using an osteotome. Even when the sinus membrane is perforated, the fibrin matrix can facilitate wound closure. PRF plugs can also be used in the management of residual extraction sockets. A technique in which autologous PRF is used in the extracted socket after immediate bone grafting using a titanium membrane applied to the socket walls and allowing primary closure, has been shown to be feasible and safe with adequate bone filling after 8 weeks or above for implant placement.

Sohn et al compared CGF membrane and collagen membrane for alveolar ridge augmentation[49]. Their bone biopsy results revealsthat favourable new bone formation along mineral allograft with no sign of inflammation. They also evaluated three-dimensional ridge augmentation using sticky bone with or without the use of titanium mesh and found favourable ridge augmentation even without the use of titanium mesh[31].

IX. Conclusion

Even though PC has been a boon in tissue engineering, there are number of factors related to its success. Starting from the method of collection, handling of samples and viability of the cells post processing, all are crucial parameters in producing desired results. Furthermore, all the recent advances in PC like APRF, IPRF, TPRF have to be demonstrated with larger sample size and long-term studies in order to affirm the status of reliability in the field of periodontics.

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