Review Article

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Journey of Platelet concentrates in Periodontal Regeneration – A review

Abstract:

Platelet concentratesare the derivatives of blood which helps in haemostasis and wound healing after periodontal regenerative procedures and are enriched with growth factors are well-known to boost the healing process and have started to be in trend and utilized clinically in periodontal surgical procedures. These are prepared from the patient's own blood throughout which the activated platelets become close and form a fibrin matrix scaffold that releases growth factors and cytokines which plays a key role in tissue regeneration; including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis. Its autologous nature provides an advantage to the patients as it reduces treatment cost and minimizes the risk of cross-infection. Despite its exclusive application in promoting healing, there is data paucity on the role of platelet concentrates on bone regeneration. Therefore, this review aims to explore the potential bone regenerative effect of platelet concentrates that would be beneficial as one of the alternative options in periodontal regenerative procedures. Even though the application of platelet concentrates has shown promising outcomes, there is a need for further studies to discover the potential of platelet concentrates in bone regeneration.

This review highlights various types of platelet concentrates, and their clinical applications within the treatment of periodontal diseases.

Key-words: Platelet concentrates, Platelet rich fibrin, Concentrated growth factor, Sticky bone, Injectable platelet rich fibrin, sticky bone

Introduction:

Periodontal repair and regeneration is the ultimate goal in treatment of periodontal defects. Various biomaterials, natural as well as synthetic have been tried since many years to accelerate wound healing of both soft and hard tissue. Naturally occurring materials also known as "autologous biomaterials" are present in the body and provides signals for repair, regeneration and healing. Similarly, synthetically generated alloplastic materials have also shown promising result in many fields of regenerative dentistry but the drawback is that they may exhibit foreign body reaction. One of greatest disadvantage of synthetic materials considered for regeneration is that most of them are avascular in nature, to improve the acceptance the concentration of autologous biomaterials is increased to the wound site for enhancing healing. Two important autologous biomaterials, platelets and fibrin are believed to play important role in promoting the healing of wound and regeneration. Platelet concentrates the concept was first started in the field of hematology. In 1970s the term PRP was coined to describe plasma with a platelet count many folds above that of peripheral blood count, which was earlier used to treat patients with thrombocytopenia by transfusion products. Later in same era Fibrin glue was made by polymerizing fibrinogen with thrombin and calcium. Fibrin sealants are human plasma derivatives that mimic the final stages of blood coagulation, forming a fibrin clot. In 1986 Knighton *et al*18was the first to demonstrate that platelet concentrate successfully promote healing and termed it as "Platelet Derived Wound Healing Factors (PDWHF), which were successfully used initially for the treatment of ulcers of skin.

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Platelets:

Platelets are small irregularly shaped cells derived from the precursor megakaryocytes. They are approximately 2–3 μm in diameter, and consists of the granules, few mitochondria, and prominent membrane structures. The canalicular system and a well stacked tubular system on the cell surface helps in expulsion of growth factors upon platelet activation.3 The substances located in the αgranules, dense granules, and lysosomes of platelets modulate its activation. The most abundant ones are α-granules that contains many bioactive mediators. During tissue injury, the platelets get activated and release wound healing factors like platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and epidermal growth factor (EGF).1,2 As the platelets contain biologically active proteins, they create a chemotactic gradient for recruitment of stem cells which undergoes the differentiation and promote healing by regeneration.

Evolution of platelet concentrates:

In 1954, Kingsley[3] was the first to use the term Platelet rich plasma(PRP) to earmark thrombocyte concentrate during experiments related to blood coagulation.

The first platelet product used as a surgical adjuvant was the "Fibrin glue" by Matras4 in 1970, which improved skin wound healing in rat models. The regenerative potential of platelets was initially introduced in 1974 by Ross et al[5] and in 1986, Knighton et al[6], termed platelet concentrates as "platelet derived wound healing factors" as they promoted healing when used for the treatment of the skin ulcers.

In 1998, Marx et al[7] introduced the first generation of platelet concentrates known as platelet rich plasma (PRP) and in 2000, Choukroun et al 8introduced the "second generation" platelet concentrate known as platelet rich fibrin (PRF). Bielecki et al[9] and Cieslik-Bielecka et al 10 defined PRP as an inactive substance and Platelet Rich Gel (PRG) as a biologically activated fibrin matrix in 2006. The concept of concentrated growth factors (CGF) was introduced by Sacco in 2006[11]. The first classification on platelet concentrates was proposed by Dohan Ehrenfest et al [12] in 2009 and was based on the two key parameters which is the presence of cell content (mostly leukocytes) and the fibrin architecture. This classification included: Pure platelet-rich plasma(P-PRP) or leukocyte-poor platelet rich plasma, Leukocyte and platelet-rich plasma (LPRP), Pure PRF (P-PRF) or leukocyte-poor PRF and Leukocyte and platelet-rich fibrin (L-PRF). Sohn introduced the concept of sticky bone in 2010 [13]. Recently, certain modifications of platelet rich fibrin were introduced. These were the advanced platelet rich fibrin (A-PRF) introduced by Choukroun[14] in 2014, Titanium prepared platelet rich fibrin (T-PRF) by Tunali et al[15] and injectable PRF (i-PRF) by Mourão et al [16] in 2015.

Generations of Platelet Concentrates and their clinical application for periodontal regeneration:

1. Platelet rich plasma (PPP) – first generation platelet concentrates

PRP is the terminal stage of coagulation cascade, that is fibrin clot formation. The release of growth factors through the α granules increases early wound strength by cell proliferation and angiogenesis. It promotes collagen synthesis and angiogenes is leading to increased early wound strength. It is proven that PRP "jumps tarts" the regenerative cascade after trauma leading to quality tissue healing and patient care. PRP is a simple strategy to concentrate platelets or enrichment of the natural blood clot that forms in normal surgical wounds for accelerated complete healing. A natural bloodclot contains 95% red blood cells (RBCs), 5% platelets and 1% white blood cells (WBCs)and many fibrin strands. A PRP blood clot is composed of 4% RBCs, 95% platelets, and 1% WBCs. The specific components of PRPare: platele tderived growth factor (PDGF), transforming growth factors (TGFs). Both of the searecontained inthealpha-granules of platelets i.e. fibronect in and vitronectin.

Procurementofprp:

5 ml of blood is withdrawn from the ante cubital fossa and then centrifuged in the centrifugation machine (Figure 1(a) and (b)). Generally, PRP is developed via a two-step centrifugation preparation of anticoagulated blood sample. In the first step (Soft Spin) of centrifugation (300g for 5 min at 12°C or 240g for 8 min at 16°C), three layers are distinguished: platelet poor plasma (PPP) plasma on top, buffy coat (BC) middle layer that contains platelets and leukocyte and red blood cells (RBCs) on the bottom. For production of Pure PRP (P-PRP), PPP and superficial BC are transferred to another tube, then centrifuged for a second time (Hard Spin) to make sure proper plasma separation (700g for 17 min at 12°C), most of the PPP layer is threw away. The final P-PRP concentrate comprised of an undetermined section of BC (containing a large number of platelets) put up in some fibrin-rich plasma. For production of Leukocyte-rich PRP (L-PRP), PPP, the whole BC layer and some residual RBCs are shifted to another tube. After hard spin centrifugation, the PPP is threw away. The final L-PRP made up of the entire BC, which comprises most of the platelets and leukocytes, and residual RBCs put up in some fibrin-rich plasma [17].

Figure 1(a) - Centrifuge Machine

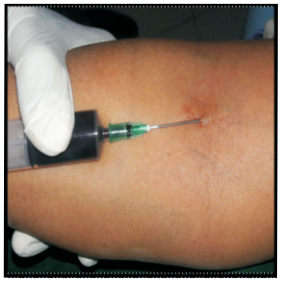

Figure 1(b) - Drawing blood from ante-cubetal fossa

Roleofprpin Periodontal Regeneration:

During a bone grafting procedure, platelets become entrapped in a graft clot and degranulate within hours, releasing PDGF and TGF-β.PDG Fbinds with endothelial cells to initiate capillary ingrowth, and TGF-β binds to osteoblasts and stemcells to mitosis and to stimulate osteoid production. Initially, macrophages are attracted tothe graft site with the help of an oxygen gradient of 30-40 mm Hg. These macrophages then drive the remaining bone regeneration and healing process. Bone regeneration is extended by two mechanisms. The first mechanism is the stem cell increase in to osteoblasts, which can then produce TGF-β. The second mechanism for bone regeneration extension is from macrophage replacement of platelets. By day 14, complete vascularization of the graft is seen. Stem cells have differentiated into osteoblasts and osteoid is being laid down.

Early bone formation is occurring at 4-6weeks leads to woven bone is formed. During phase two modeling, lamellar bone is formed, representing a more organized bone, which in turn matures via functional loading with stresses placed on it. Through the application of PRP in the bone graft wound site, a substantial increase in the platelet count is offered. This increases the availability for platelets to create the cascadesystem via PDGF and TGF-β. The average platelet count in an individual's blood is between 111,000 and 523,000. By concentrating the platelets to PRP, the average platelet count increases to arrange of 595,000-11000,000, with an average increase of 33.8%. Platelets, which are in the graft site for <5 days, allow the body to efficiently begin acascade of reactions using growth factors. It can be considered that PRP jump-starts the casc ade of regenerative events leading to the formation of a mature graft site.[18]

In a systematic review and meta-analysis conducted by Rosello et al[19] in 2015to assess the influence of platelet-richplasma (PRP) onthe regeneration of periodontal intrabony defects by means of evaluating clinical and radiographic outcomes in prospective humanclinical trials. It was concluded that PRP might offer some beneficial effects on clinical and radiographic out comes for regeneration of periodontal intrabony defects.

Shukla et al[20] in 2016 conducted a study to clinically and radiographically evaluate the use of calcium hosphosilicate (CPS) puttyaloneandin combination with PRP in the treatment of periodontal intrabony defects. Twenty patients each with at least two defect slocated in different quadrants were enrolled. It was concluded that addition of PRP to CPS putty does not seem to provide any additive benefit to treatment.

1. Platelet Rich Fibrin (PRF)- Platelet Rich Fibrin (PRF) is a second-generation platelet concentrate widely used to accelerate soft and hard tissue healing and is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines. PRF was developed in France by Choukroun et al. in 2001, it is a second-generation platelet derivative because, unlike other platelet concentrates like PRP, this technique does not require anticoagulantsn or bovine thrombinoranyo the rjellifying agent.[21]

Procurement of PRF:

The ideal technique for PRF preparation was first put forward by Choukroun et al. 5-ml of venous blood is collected in two separate 6-ml tubes that are not coated with an anticoagulant and centrifuged at 3000 rpm at 400 g for a period of 12 mins. The end product consists of 3 layers: cellular PPP as the topmost layer, middle layer of PRF clot and bottom layer of red blood cells. The main setback of this technique involves rapid coagulation of blood initiated on contact with the wall of the test tube. Therefore, it is important to speed up the centrifugation process giving less working time for the clinician. It can also be used as a membrane by squeezing out fluids present in the fibrin clot. [21] (Figure 2)

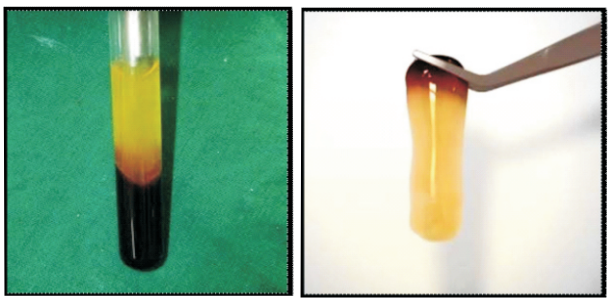

Figure 2 – Platelet rich Fibrin

Clinical implications of PRF:

1. PRF and PRF membrane have been use dincombination with bone graftstohasten the healing in lateral sinus floor elevation procedures (Choukran 2006).[21]

2.Protectionandstabilizationofgraftmaterialsduringridgeaugmentationprocedures.[22]

3. Socket preservation after tooth extraction or avulsion.[23]
4. PRF enhances palatal wound healing after free gingival graft.
5. Filling of cystic cavity.
6. PRF promotes dentinogenes is by stimulating cell proliferation and differentiation of Dental Pulp Cells[24]

A systematic review and meta-analysis considering four clinical trials to evaluate the additive effect of PRF in treatment of intra bony defects when used along with otherregenerative procedures in terms of clinical and radiological outcome was proposed by Saurav Pand, Sankari M, VargheseS (2013).[25] It was concluded that PRF canimprove to be effective as a sole regenerative material for treatment of intrabony defect sincombinationto pen flap debridement. Zeba et al[26] in 2016 conducted a study to evaluate clinically and radiographically theefficacy of platelet rich fibrin versus β-tricalcium phosphate (β-TCP) in the treatment ofgrade II mandibular furcation defects. 45 grade II furcation defects in mandibular molarswere assigned to open flap debridement with PRF group I, OFD with β-TCP group II andtoOFD alone ingroupIII. Rehan et al in 2018 aimed to evaluate the effectiveness of coronally advanced flap (CAF)with either PRF membrane or bioresorbable amniotic membrane (AM) in treatment oflocalized gingival recession defects. Sixteen healthy adult patients presenting with Miller Class I recession defects were treated surgically with CAF along with AM (Group I) or PRF (Group II) for coverage of the recession defects. It was concluded that both CAF +PRF and CAF + AM are equally effective in providing clinically significant outcomes with respect to root coverage with AM showing the better percentage of root coverage ascompared to PRF.

3. Advanced Platelet Rich Fibrin (A-PRF)

A-PRF is a modified form of pure platelet rich fibrin obtained by decreasing the rpm and increasing the centrifugation time that is 1300 rpm for 14 minutes (Figure 3a,3b). The resultant clot has an increased number of neutrophils and macrophages. Also it permits cell migration into the defected area but also provides important biological factors that accelerates wound-healing such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), platelet factor 4 (PF4), IL-1, vascular endothelial growth factor (VEGF), epidermal growth factor, endothelial cell growth factor (ECGF), platelet-derived endothelial growth factor (PDEGF), insulin-like growth factor, osteocalc in, osteonect in, fibrinogen, vitronectin, fibronectin, and thrombospondin[28]. It has a greater concentrate and more homogenous distribution of monocytes which are believed to playakeyrolein bone formation. Clark et al[29] in 2018 conducted a multi-arm parallel randomized controlled clinical trial to evaluate the efficacy of A-PRF alone or with freeze-dried bone allograft (FDBA) in improving vital bone for mation and alveolar dimensional stability during ridge preservation and concluded that A-PRFalone or augmented with FDBA is a suitable biomaterial for ridge preservation.

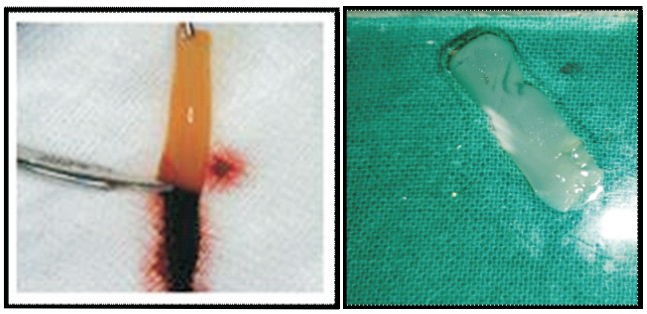

Figure 3 – Advanced Platelet Rich Fibrin used as a membrane

4Titanium Platelet Rich Fibrin (T-PRF) - It is a new platelet concentrate the method of preparation of which is based on the hypothes is that titanium tubes may be more effective at activating platelets than the glass tubesusedin Choukroun' smethod. This method is used to avoid any adverse effects in the short or long term or both of dry glass or glass-coated plastic tubes and to eliminate any speculations about silica. In the initials trials, it was found titanium-induced platelet aggregation similar to that glass tubes, and the cloth produced in titanium tubes was clinically identical to that produced in glass tubes.[30] Activation of platelets with titanium compared with activation with silica particles provides the distinctive characteristics of T-PRF, including its increased biocompatibility. 10 ml of blood sample is collected without anticoagulant in 10 ml titanium tubes, which are immediately centrifuged at 2800 rpm for12 min.[30] The absence of anticoagulant implies that most platelets in the blood sample will be activated within a few minutes after contact with the wall of titanium tube, which initiates the coagulation cascade. A study conducted by Olgunet al[31] in2018 evaluated the clinical, radiographic, and histological comparisons of completely autologous titanium-prepared platelet rich fibrin(T-PRF) orallograft insinus-lifting procedures. It was concluded that use of T-PRF aloneinsinus-liftingoperations has successful clinical and histomorphometric results.

5. Injectable Platelet Rich fibrin (i-PRF) - Introduced by Joseph Choukronin 2014, injectable platelet-richfibrin (PRF) morecommonly referred as i-PRF™. It was introduced based on the similar concept as that of PRF with added advantage of it being in injectable form. This injectable form of PRF can be utilize daloneor combined easily with various biomaterials. Its protocol is based on the concept that slower and shorter centrifugation spin results in a higher presence of regenerative cells with higher concentrations of growth factors. It requires neither any anticoagulant nor any additive. It is obtained by centrifuging blood at low-speed 700 rpm. This results in PRF for use in liquid (injectable) form.[32](Figure 4)Karde et al [32] in 2017 conducted a study to evaluate the antimicrobial property, and platelet countofi-PRF in comparison toot her platelet concentrates, i.e., PRF, platelet-richplasma (PRP), and control (whole blood). I-PRF has maximum antimicrobial efficacy and higher platelet count in comparison to other platelet concentrates, the reby in dicating to have a better regenerative potential then others.

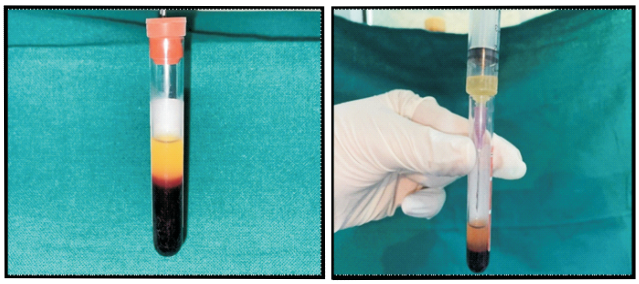

Figure 4 – Injectable Rich Fibrin (i-PRF)

6. Concentrated Growth Factor (CGF) – It is a new regeneration platelet aggregate which is used widely in periodontal and oral surgeries. It was first developed by Sacco (2006), is a relatively new technology within the area of regenerative medicine. It is adopted without the use of chemicals which grades it more eco-friendly. It contains various growth factors which enhances its action and promotes wound healing. CGF is currently used along with autologous bone particles to induce bone regeneration and connective tissue attachment that shows excellent results. It is an excellent biomaterial which showcases back to normal periodontium .Concentrated growth factor (CGF) is a newer second generation platelet concentrate that is prepared by centrifuging blood samples at alternating and controlled speeds using a special centrifuge. Differential centrifugation results in the formation of a denser fibrin matrix richer in growth factors than those observed in the PRF and PRP.[33]

Procurement of Cgf:

A standard, disposable, two 10 mL nonanticoagulant glass tubes and a matching centrifuge device is used. 20 mL of intravenous blood sample from the patient is placed in centrifuge tubes without anticoagulants and accelerated for 30 s, centrifuged at 2700 rpm for 2 min, 2400 rpm for 4 min, 2700 rpm for 4 min, and 3000 rpm for 3 min, and decelerated for 36 s to stop. All of these processes are adjusted automatically by "preprogramming" in the centrifuge machine. From the three layers formed, the uppermost platelet-deprived fraction was removed with a sterile syringe. The layer in the form of a fibrin gel containing the CGF was separated from the red blood cell layer [33] (Figure 5)

Figure 5 – Concentrated Growth Factor (CGF)

CGF is used as a barrier membrane to facilitate tissue healing and results in obtaining the attached gingival width in root coverage procedures like sliding flap technique. CGF membrane provides dual coverage to the exposed root surface. It is an excellent bioactive protein which enhances bone healing due to its stimulatory effect on epithelialization and angiogenesis. CGF is mixed with autologous bone particles or biomaterials to fill the bone defects to induce bone regeneration. The advantages of CGF over platelet-rich plasma are lack of biochemical modification, easy method of preparation, application with minimal expense. It also serves as a resorbable interpositional membrane. CGF contains more growth factors than other platelet preparation thus has favorable effects on healing period of the implant. It accelerates the osseointegration of the implant and affected the stabilization values positively. [34]

1. Autologous fibrin glue and sticky bone:

The concept of mixing autologous fibrin glue with bone graft to obtain sticky bone was introduced by Sohn in the 2010 [35]. Autologous fibrin glue (AFG) was obtained by centrifuging 20 -60 CC of blood in non-coated tubes at 2400-2700 rpm for 2 mins to obtain two layers. The RBC's form the bottom layer and autologous fibrin glue forms the superficial layer. Then AFG was extracted using a syringe and mixed with particulate bone powder. It was allowed to rest for 5-10 mins for polymerization and this resulted in a yellow coloured mass called sticky bone [36] (Figure 6). It can used for space maintenance, angiogenesis and tension free primary suture in guided bone regeneration. The use of "sticky bone" preparation was found to be useful for the alveolar ridge augmentation as the bone graft trapped within the cross-linked fibrin meshwork prevented any undesirable movement of graft particles during the healing phase. This stabilized the bone graft onto the defect without the need of using any bone tacks or titanium mesh and this promoted tissue healing. The fibrin inter connection prevents the ingrowth of soft tissue into the sticky bone graft.

Figure 6 – Sticky bone

Conclusion:

With PRF being more user friendly and economic, this arsenal is finding wider applications in surgical field. Looking at the current trends, PRP and PRF are most commonly used and have been researched upon. Newer advances suchas A-PRF,i-PRF,t-PRFconcepthave been report edinsingle or few cases but no long term or controlled trial have been done to prove the advantage of their advancement over conventional PRP and PRF. The introduction of i-PRF will also find suitable applications, where injectable form of platelet concentrate is required. So clinicians should use the advancements with caution. Further long term, multicenter studies with biochemical assays and histological evaluation for the regenerated tissues should be performed that would advocate and support the out comes of these newer advancements.

References:

1. Marx, RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004;62:489–496.
2. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRPfi Implant Dent. 2001;10:225-28.
3. Kingsley CS. Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma. *Nature.* 1954;173:723–724.
4. Matras H. [Effect of various fibrin preparations on reimplantations in the rat skin] *Osterr Z Stomatol.* 1970;67:338–359.
5. Ross R, Glomset J, Kariya B, Harker L. A platelet dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc Natl Acad Sci USA 1974; 71:1207-10.
6. Knighton DR, Ciresi KF, FiegelVD, Austin LL, Butler EL. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg 1986; 204: 322-330.
7. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 1998; 85: 638-646.
8. Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: The PRF. Implantodontie 2001; 42:55-62.
9. Bielecki T, Gazdzik TS, Szczepanski T. Re: "The effects of local platelet rich plasma delivery on diabetic fracture healing". What do we use: Platelet-rich plasma or platelet-rich gelfi Bone 2006; 39: 1388-1389.
10. Cieslik-Bielecka A, Gazdzik TS, Bielecki TM, Cieslik T. Why the platelet-rich gel has antimicrobial activityfi Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2007; 103: 303-305.
11. Sacco L. Lecture, International academy of implant prosthesis and osteoconnection. Lecture 2006; 12: 4.
12. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's

platelet-rich fibrin clot and membrane. J Periodontol 2010; 81: 546-555.
13. Sohn DS. Lecture titled with sinus and ridge augmentation with CGF and AFG, Symposium on CGF and AFG. Tokyo, June 6, 2010.
14. ChoukrounJ.Advanced PRF and i-PRF: Platelet concentrate or blood concentratefi J Periodontal Med Clin Pract 2014; 1: 3.
15. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E. In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): a new platelet concentrate. Br J Oral Maxillofac Surg 2013; 51: 438-443.
16. Mourão CF, Valiense H, Melo ER, Mourão NB, Maia MD. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. Rev Col Bras Cir 2015; 42: 421-423.
17. Ahmed, Dare EV, Hincke M (2008): Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng Part B Rev 14(2): 199-215.
18. Markou N, Pepelassi E, Vavouraki H, Stamatakis HC, Nikolopoulos G, Vrotsos I, et al. Treatment of periodontal endosseous defects with platelet-rich plasma alone or in combination with demineralized freeze-dried bone allograft: a comparative clinical trial. J Periodontol. 2009;80(12):1911–9.
19. Roselló-Camps, Àlvar et al. Platelet-rich plasma for periodontal regeneration in the treatment of intrabony defects: a meta-analysis on prospective clinical trials. Oral Sur, oral med, oral pathology and oral rad. 2015,120(5): 562-74.
20. Shukla, Sagrika et al. Effect of Addition of Platelet-rich Plasma to Calcium Phosphosilicate Putty on Healing at 9 Months in Periodontal Intrabony Defects. J of contemp dent practice. 2016; 17(3):230-41.
21. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral RadiolEndod . 2006;101(3):e37-44.
22. Kassolis, J. D., Rosen, P. S., and Reynolds, M. A. (2000). Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. *J. Periodontol*. 71, 1654–1661.
23. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier J-B. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol. 2010;81(4):546–55.
24. Huang, F.-M., Yang, S.-F., Zhao, J.-H. and Chang, Y.-C. (2010) Platelet-Rich Fibrin Increases Proliferation and Differentiation of Human Dental Pulp Cells. J of Endodontol; 2010; 36 (10), 1628-1632.
25. Panda S, Doraiswamy J, Malaiappan S, Varghese SS, Del Fabbro M. Additive effect of autologous platelet concentrates in treatment of intrabony defects: a systematic review and meta-analysis. J of investigative and clin dent. 2016 Feb;7(1):13-26.
26. Siddiqui, Zeba Rahman et al. Comparative evaluation of platelet-rich fibrin versus beta-tri-calcium phosphate in the treatment of Grade II mandibular furcation defects using cone-beam computed tomography. *European J of dent.2016;*10(4): 496-506.
27.Rehan, Mohd et al. Comparative Evaluation of Coronally Advanced Flap Using Amniotic Membrane and Platelet-rich Fibrin Membrane in Gingival Recession: An 18-Month Clinical Study. *Contemp clinic dent.2018;* 9(2): 188-194.
28. Corso M Del, Toffler M, Dohan Ehrenfest DM (2010) Use of an Autologous Leukocyte and Platelet-Rich Fibrin (L-PRF) Membrane in Post-avulsion Sites: An overview of Choukroun's PRF. J Implant Adv Clin Dent 2010;1: 27-35.
29. Clark D, Rajendran Y, Paydar S, et al. Advanced platelet-rich fibrin and freeze-dried bone allograft for ridge preservation: A randomized controlled clinical trial. Journal of Periodontology. 2018 Apr;89(4):379-387.
30. Tunalı M, Özdemir H, Küçükodacı Z, et al. A novel platelet concentrate: titanium-prepared platelet-rich fibrin. Biomed Research International. 2014 ;2014:209548.
31. Olgun, Ebru et al. Comparison of the clinical, radiographic, and histological effects of titanium-prepared platelet rich fibrin to allograft materials in sinus-lifting procedures. J investigative and clin dent.2018; 9(4): e12347.
32. Karde P, Sethi K, Mahale S, Khedkar S, Patil A, Joshi C. Comparative evaluation of platelet count and antimicrobial efficacy of injectable platelet-rich fibrin with other platelet concentrates: An in vitro study. J Indian Soc Periodontol . 2017;21(2):97-101.
33. MalliSureshbabu N, Selvarasu K, V JK, Nandakumar M, Selvam D. Concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: A report of two cases. Case Rep Dent .2019:7046203.

34. Kumar Y P. Role of CGF (Concentrated Growth Factor) in periodontal regeneration. J Dent Health Oral DisordTher . 2018;9(5).
35. Sohn DS. Lecture titled with sinus and ridge augmentation with CGF and AFG, Symposium on CGF and AFG. Tokyo, June 6, 2010.
36. Sohn DS, Huang B, Kim J, Park WE, Park CC. Utilization of autologous concentrated growth factors (CGF) enriched bone graft matrix (Sticky bone) and CGF-enrichedfibrin membrane in Implant Dentistry. J. Implant Adv. Clin. Dent 2015;7:11-29.