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RESEARCH ARTICLE

PLATELET CONCENTRATION - A NEWER CONCEPT FOR PERIODONTAL REGENERATION

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Abstract

Platelet concentrates are the derivatives of blood which helps in haemostasis and wound healing after periodontal regenerative 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. This review highlights various types of platelet concentrates, and their clinical applications within the treatment of periodontal diseases.

Introduction:-

Periodontitis is an inflammatory disease of the periodontium that leads to loss of tooth supporting tissues. Following periodontal therapy, healing occurs by regeneration, repair or the combination of both. Healing depends on the availability of cell types and signalling cascades that regulate stimulation of these cells. The natural wound healing cascade is commenced by the clot formation accompanied by proliferative and maturation phases. Thus, there is fibrin formation, platelet aggregation and release of several growth factors into tissues from platelets. Growth factors also helps in wound healing by favouring the mitogenesis, chemotaxis, and angiogenesis of the cells. [1,2]

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).[4,5]

As the platelets contain biologically active proteins, they create a chemotactic gradient for recruitment of stem cells which undergoes the differentiation andpromote healing by regeneration. Hence, autologous platelet concentrates have a promising scope in periodontal regeneration. In this review,the different platelet concentrates and their application in periodontal regenerative therapy have been discussed.

Evolution of platelet concentrates

In 1954, Kingsley[6]was the first to use the term Platelet rich plasma(PRP) to earmark thrombocyte concentrate during experiments related to blood coagulation.

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The first platelet product used as a surgical adjuvant was the "Fibrin glue" by Matras[7] in 1970, which improved skin wound healing in rat models. The regenerative potential of platelets was initially introduced in 1974 by Ross et al[8] and in 1986, Knighton et al[9], 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[10] introduced the first generation of platelet concentrates known as platelet rich plasma (PRP) and in 2000, Choukroun et al [11] introduced the "second generation" platelet concentrate known as platelet rich fibrin (PRF). Bielecki et al [12] and Cieslik-Bielecka et al [13] 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[14]. The first classification on platelet concentrates was proposed by Dohan Ehrenfest et al[15] 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 [16]. Recently, certain modifications of platelet rich fibrin were introduced. These were the advanced platelet rich fibrin (A-PRF) introduced by Choukroun [17] in 2014, Titanium prepared platelet rich fibrin (T-PRF) by Tunali et al [18] and injectable PRF (i-PRF) by Mourão et al [19] in 2015.

Generations of Platelet Concentrates and their clinical application for periodontal regeneration

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 eliminates the risk of disease transmission due to its procurement from autologous blood.

Preparation

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 [20].

Figure 1(a):- Centrifuge Machine.

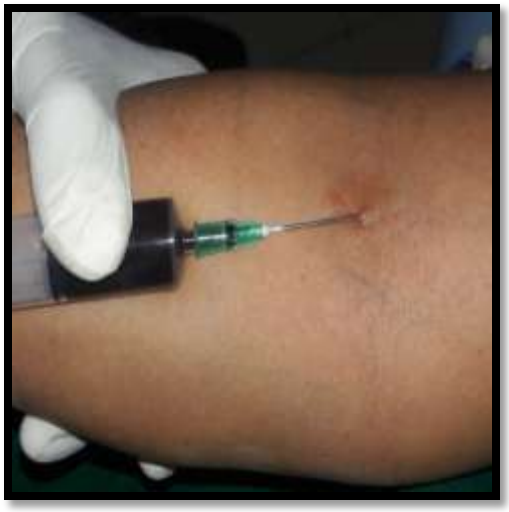

Figure 1(b):- Drawing blood from ante-cubetal fossa.

Platelet rich fibrin (PRF) – second generation platelet concentrates

The PRF clot is obtained by the natural polymerization process during centrifugation. The fibrin architecture has a distinctive property of slow release of important growth factors such as the transforming growth factor β, platelet-derived growth factor, vascular endothelial growth factor and matrix glycoproteins such as thrombospondin-1 and cytokines for a period of about 28 days. It enhances strong reparative and regenerative processes. It also forms a trimolecular fibrin meshwork which makes it flexible to support cytokine function and cellular migration.

Preparation

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. [21,22]

Properties of PRF

1. It contains cytokines, structural glycoproteins meshed between slowly polymerized fibrin network
2. It is considered as an immune node which stimulates the defense mechanism
3. There is significant inflammatory response at surgical site after PRF placement due to cytokines enmeshed within the matrix.

Advantages of PRF over PRP

1. No bovine thrombin or anticoagulant is used for its preparation.
2. Single centrifugation cycle is required.
3. It is easy to handle.
4. It acts as a vehicle for tissue engineering.

Disadvantages:

1. The relatively low quantity of PRF obtained from samples.
2. The working time is less and it should be immediately used after preparation as it can lose the structural integrity by shrinkage due to dehydration.
3. Leucocyte platelet richfibrin (L-PRF)

These are the modified PRF clot or membrane that contains most of the platelets and leukocytes from the initial blood harvest plus platelet growth factors and stem cells entrapped within the fibrin network with

enhanced strength. It is obtained by modification of technique used for preparation of pure platelet rich fibrin. The Blood should be collected in less than 20 seconds in 9ml glass-coated plastic tubes. It is then immediately centrifuged at 2700 rpm for 12 minutes to produce L-PRF clots.The clots are then collected into a sterile surgical box and are compressed into membranes.

It can be used as a fibrin plug and for sticky bone preparation by mixing with particulate bone.[23] A randomized controlled pilot clinical trial by Temmerman A, Cleeren GJ, Castro AB, Teughels W, Quirynen M, 2018 has shown good results when L-PRF membrane was used for increasing the width of keratinized mucosa around implants.[24]

Advanced platelet rich fibrin (A-PRF)

A-PRF is a modified form of pure platelet rich fibrin obtained bydecreasing the rpm and increasing the centrifugation time that is 1300 rpm for 14 minutes.The resultant clot has an increased number of neutrophils and macrophages [25].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, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin [26].

Injectable platelet rich fibrin (i-PRF)

Injectable form of PRF is a platelet concentrate in liquid formulation which can be used alone or in combination with other biomaterials. It was observed to havea higher presenceof regenerative cells and growth factors as compared to other forms of PRF. It clots and attains a gel form after about 10-15 minutes for the sustained release of growth factors in the tissue and induces expression of transforming growth factor-β and collagen-1 mRNA [27]. I-PRF is prepared by collecting blood in a 9 mL tube without anticoagulants and centrifuging it for 2 mins at 3300 rpm.The resultant product obtained in the tube is an orange coloured fluid called i-PRF. Recently a randomized controlled clinical trial observed that the application of i- PRF by a non- surgical method influenced the increase in gingival thickness in individuals with thin gingival biotype [28].

Concentrated growth factors (CGF)

The preparation protocol used for CGF yields a much denser and larger fibrin matrix rich in growth factors. Intravenous blood samples are drawn from the patient into 10-ml non-anticoagulant centrifuge tubes. It is then accelerated for 30 s, centrifuged at 2700 rpm for 4 min, 2400 rpm for 4 min, 2700 rpm for 4 min, and 3000 rpm for 3 min, and then decelerated for 36 s to stop.Three layers are observed in the tube: a bottom layer of red blood cells, a middle layer of fibrin gel with concentrated growth factor and platelet aggregation and a top layer of plateletdeprived plasma. First, the uppermost platelet-deprived fraction is removed with a sterile syringe. Then the layer containing concentrated growth factor is separated from the other two layers and pressed into a membrane [29] (Figure 2(a) (b)).

Figure 2(a):- Prepared CGF with three layers.

Figure 2(b):- Separated buffy coat layer of CGF.

It also acts as a fibrin tissue adhesive and an effective haemostatic agent. It helps in wound healing and accelerates the process of osteogenesis. It enhances attachment of a new connective tissue to the root surface thus improving wound stability. It acts as a scaffold that helps in attachment of cytokines and cellular migration, promotes epithelial, endothelial and epidermal regeneration. It also possesses an antimicrobial as well as an anti-angiogenic property on chronic non healing wounds [30]. Clinical trials have shown that CGFs can act as an alternative to bone grafting and could be used for bone regeneration for sinus augmentation [31] and as a membrane support in implants to accelerate the bone integration [32].

Autologous fibrin glue (AFG) and sticky bone

The concept of mixing autologous fibrin glue with bone graft to obtain sticky bone was introduced by Sohn in the 2010 [33]. 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 [32](Figure 3). It can used for space maintenance, angiogenesis and tension free primary suture in guided bone regeneration [34, 35].

Figure 3:- Sticky bone.

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 theingrowth of soft tissue into the sticky bone graft [32].

Conclusion:-

Platelet rich plasma areconsidered as improved fibrin glues without consistency and Platelet rich fibrin is regarded as the dense fibrin biomaterial with biomechanical properties. A dense fibrin clot can serve as a biological healing matrix which supports the cell migration and release of cytokine. PRF have many advantages and prepared in less time with no complicated method of preparation of PRF. It is simple to handle which makes it a better choice for periodontal regeneration. Newer advances such as A-PRF, i-PRF, CGF and sticky bone concept have been used for the regeneration of periodontium but no long term or controlled trial have been done to prove the advantage of their advancement over conventional PRP and PRF.

References:-

1. Kanakamedala A, Ari G, Sudhakar U, Vijayalakshmi R, Ramakrishnan T, Emmad P. Treatment of a furcation defect with a combination of platelet-rich fibrin (PRF) and bone graft—A case report. ENDO (LondEngl) 2009; 3:127-35.
2. Deodhar AK, Rana RE. Surgical physiology of wound healing: a review. J Postgrad Med 1997; 43:52-6.
3.Harrison P. Platelet function analysis. Blood Rev 2005; 19:111-23.
4.Marx, RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004;62:489–496.
5.Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 2001;10:225-8.
6. Kingsley CS. Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma. Nature. 1954;173:723–724.
7. Matras H. [Effect of various fibrin preparations on reimplantations in the rat skin] Osterr Z Stomatol. 1970;67:338–359.
8. 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.
9.Knighton DR, Ciresi KF, Fiegel VD, 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.
10. 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 Radiol Endod 1998; 85: 638-646.
11.Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: The PRF. Implantodontie 2001; 42:55-62.
12.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 gel? Bone 2006; 39: 1388-1389.
13.Cieslik-Bielecka A, Gazdzik TS, Bielecki TM, Cieslik T. Why the platelet-rich gel has antimicrobial activity? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103: 303-305.
14. Sacco L. Lecture, International academy of implant prosthesis and osteoconnection. Lecture 2006; 12: 4.
15. 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.
16.Sohn DS. Lecture titled with sinus and ridge augmentation with CGF and AFG, Symposium on CGF and AFG. Tokyo, June 6, 2010.
17.Choukroun J.Advanced PRF and i-PRF: Platelet concentrate or blood concentrate? J Periodontal Med Clin Pract 2014; 1: 3.
18.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.
19.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.
20. Ahmed et al., Dare EV, Hincke M (2008): Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng Part B Rev 14(2): 199-215.

21. Dohan et al., Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J. (2006b): Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod.101:e45–e50.
22.Dohan et al., Choukroun J, Diss A (2006c): Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology 101(3): e51-e55.
23. 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.
24.Temmerman A, Cleeren GJ, Castro AB, Teughels W, Quirynen M. L-PRF for increasing the width of keratinized mucosa around implants: A split-mouth, randomized, controlled pilot clinical trial. J Periodontal Res. 2018;53:793-800.
25. Dohan Ehrenfest DM, Pinto NR, Pereda A, Jiménez P, Corso MD, Kang BS, Nally M, Lanata N, Wang HL, Quirynen M. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte-and platelet-rich fibrin (L-PRF) clot and membrane. Platelets. 2018;29:171-184.
26.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.
27. Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, GhanaatiS, Choukroun J. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? Clin Oral Investig. 2017;21:2619-2627.
28.Ozsagir ZB, Saglam E, Sen Yilmaz B, Choukroun J, Tunali M. Injectable platelet-rich fibrin and microneedling for gingival augmentation in thin periodontal phenotype: A randomized controlled clinical trial. Journal of Clinical Periodontology. 2020;47:489-499.
29. Borsani E, Bonazza V, Buffoli B, Cocchi MA, Castrezzati S, et al. Biological Characterization and In Vitro Effects of Human Concentrated Growth Factor Preparation: An Innovative Approach to Tissue Regeneration. Biol Med (Aligarh). 2015;7: 256.
30.Nityasri, Aromal S, Pradeep KY, et al. Role of CGF (Concentrated Growth Factor) in periodontal regeneration. J Dent Health Oral Disord Ther. 2018;9:350–352.
31.Sohn DS, Heo JU, Kwak DH et al. Bone regeneration in the maxillary sinus using an autologous fibrin-rich block with concentrated growth factors alone. Implant Dent. 2011; 20:389-395.
32.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.
33. Sohn DS. Lecture titled with sinus and ridge augmentation with CGF and AFG, Symposium on CGF and AFG. Tokyo, June 6, 2010.
34.Naag S, Savirmath A, Kalakonda BB, Uppada UK, Kamisetty S, Priyadarshini E. Platelet concentrates: Bioengineering dentistry's regenerative dreams. J Dent Res Rev 2015;2:86-90.
35.Agrawal AA. Evolution, current status and advances in application of platelet concentrate in periodontics and implantology. World J Clin Cases. 2017;5:159-171.