REVIEW ARTICLE

PLATELET-RICH FIBRIN: A NOVEL APPROACH TO PERIODONTAL REGENERATION

Dr. Surbhi Gautam*, Dr. Sk Ejaz Ahamed*, Dr. Nurul Hasan Mollah**
Dr. Somen Bagchi***, Dr. Ashit Kumar Pal****

ABSTRACT

Platelet concentrates are the growth factors releasing substances having excellent healing characteristics. Although Platelet Rich Fibrin (PRF) is the choice of autologous regenerative material in the defects yet advancements in platelet concentrates are being made till now. PRF is an autologous biological platelet concentrate which are rich in various growth factors. These growth factors help in wound healing and hence are very useful in regenerative dentistry. PRF has wider applications in the field of periodontics as well as other medical fields. Over the years, advancement has been made for its easy use and for less discomfort to the patient. This review is constructed by selecting more than 25 articles on the topic.

KEY WORDS

Platelet Concentrates (PC), Platelet Rich Fibrin (PRF), Growth Factors, Advancement

ABOUT THE AUTHORS

*2nd Year PGT, ** 3rd Year PGT, ***Professor and Head ,****Professor Department of Periodontics and Implanatology
Dr. R. Ahmed Dental College & Hospital
Kolkata, West Bengal

CORRESPONDING AUTHOR

Dr. Sk Ejaz Ahamed
MDS, 2nd Year, Department Of Periodontics And Implanatology,
Dr.R. Ahmed Dental College& Hospital
114, A.J.C Bose Road Kolkata West Bengal,700014
e-mail:drejazahamed@gmail.com
Ph no:8584868682

1. INTRODUCTION

Periodontal therapy is aimed at suppressing the inflammatory process, preventing further development of periodontal diseases and also at regenerating the lost periodontal structures. Periodontal regeneration is a dynamic multifactorial path in a structured sequence comprising biological series such as cell adhesion, migration, proliferation, and differentiation. The regenerating materials currently available have the minimal ability to recover the damaged periodontal tissue entirely, but just a fraction of the original total volume of tissue.[1,2] Regeneration is the expected healing result during periodontal treatment. Ross et al in 1974 found the regenerative potential of platelets.[3] Due to its capacity to secrete numerous growth factors and cytokines, the role of platelets in wound healing is a known fact.[4] Platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and epidermal growth factor are different growth factors secreted by platelets (EGF).[5] These growth factors are responsible for neovascularization, collagen synthesis, cell division, cell differentiation, induction, and migration of other cells to the injured site.[6] Fibrin, vitronectin and fibronectin are also released by platelets, serving as a matrix for connective tissue and as adhesion molecules for more appropriate cell migration.[7] This developed the notion of using the platelet concentrate (PC) which was introduced over 20 years ago, following periodontal therapies to improve the regeneration of various periodontal tissues. The theory is based on the idea that the supply of blood is a requirement for tissue regeneration, with the aim to use human blood proteins as a source of growth factors successful in promoting angiogenesis and tissue ingrowth.[8]

Platelet Rich Plasma (PRP) was originally developed and is known as a platelet concentrate of the first generation. The primary target of PRP was to isolate the highest quantity of platelets and ultimately growth factors associated with their collection and re-use them during surgery.[9] Platelet Rich Fibrin (PRF) was later introduced due to certain inferior PRP properties like the lengthy centrifugation protocols. Hence, PRF is one of the new promising regenerative materials in the field of periodontics.

2. History of Platelet Concentrates (PC):

Various researchers have worked over the years keeping in view the wound healing property of platelets. Over the last two decades, platelet concentrate is being used as regenerative tool in various periodontal therapies. Evolution of the PC has been described in **Fig:1** from the past years till recently.

3. Classification of Platelet Concentrate:

In 2009, Dohan Ehrenfest et al provided the first PC classification. Different researchers have given different classifications to date, but the most widely accepted category is that suggested by Dohan[20] It is a basic description focused on the presence or absence of leukocytes and the platelet concentrate density of the fibrin architecture. According to this classification, there are four types of platelet concentrate described in **Fig:2**

CHART-1

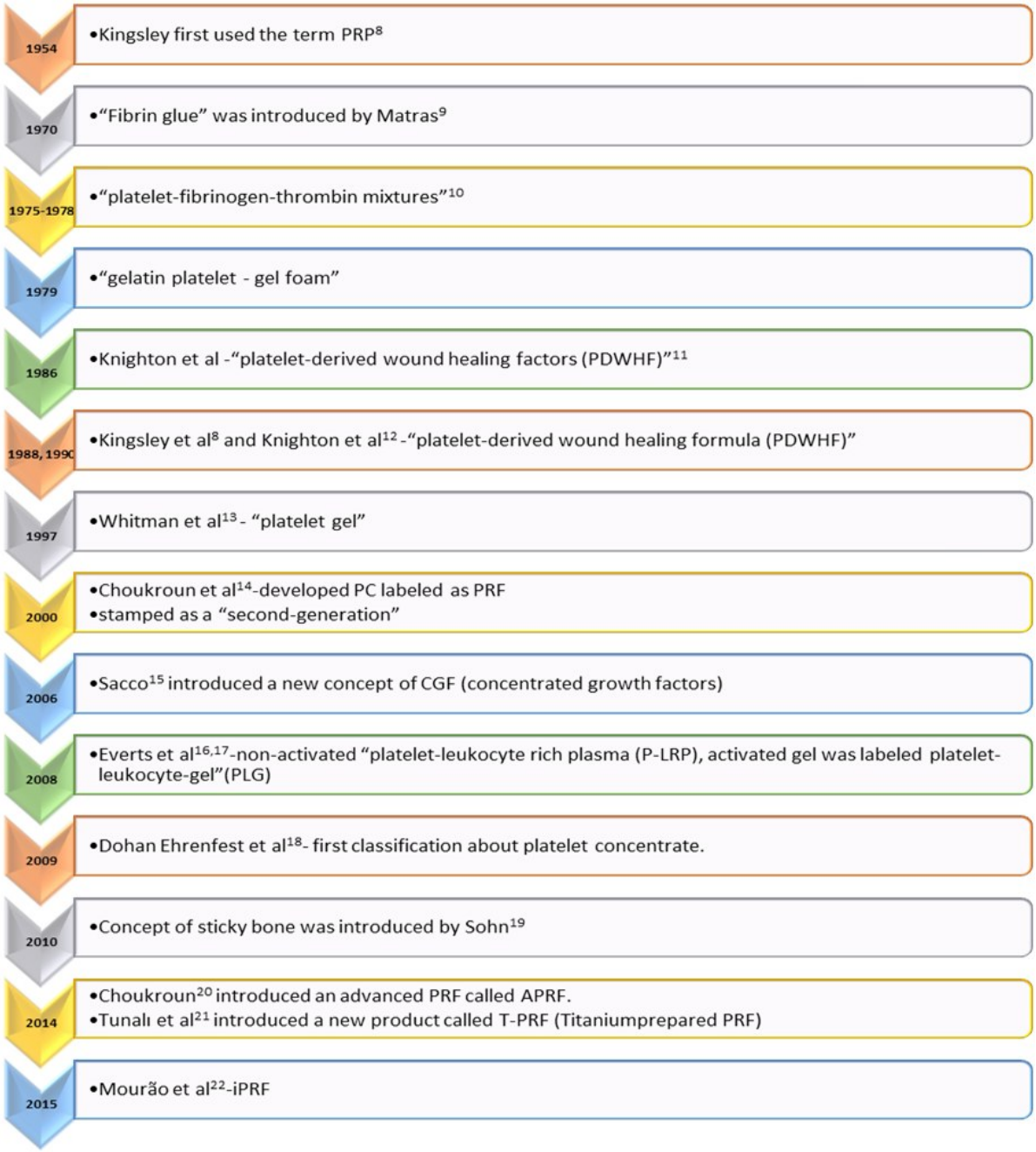

Fig:1 History of platelet concentrates

CHART-2

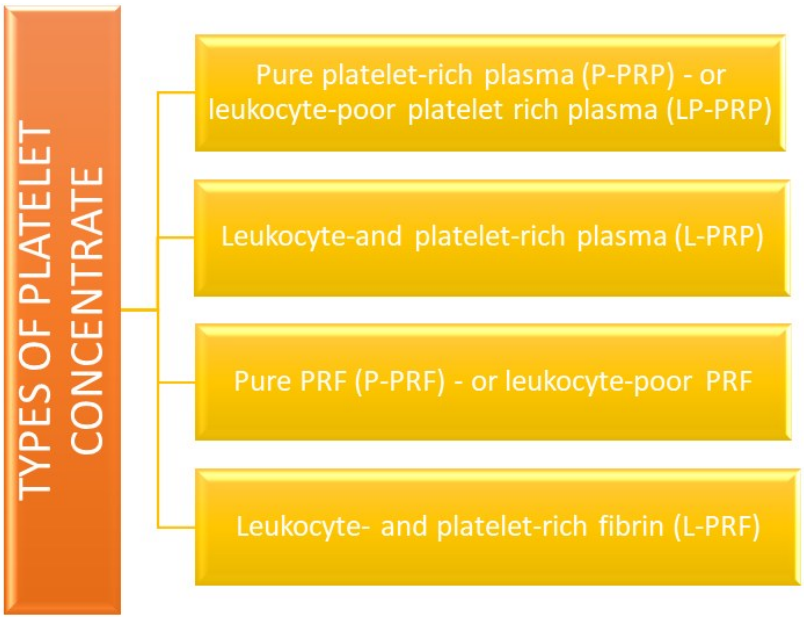

Fig:2 Classification of PRF as given by Dohan Ehrenfest et al

4. PRF:

Platelet Rich Fibrin was first developed by Dr. Joseph Choukroun in France in 2000.[16] Platelet-rich fibrin (PRF) from Choukroun is a biomaterial of leukocyte and platelet rich fibrin with a particular structure and three-dimensional architecture.[20] It is known as a platelet concentrate of the second generation. It has gained worldwide popularity as PRF improves soft and hard tissue healing. The PRF clot consists of a solid natural fibrin matrix that focuses on both platelets and blood growth factors and displays a convoluted architecture as a healing matrix with special mechanical properties that makes it unique from other concentrated platelets.[25,26]

4.1 Advantage of PRF over PRP:

PRF has various advantages over its predecessor PRP by several criteria: it does not involve complex preparatory methods. Low cost and less time of preparation as PRF does not necessitate the direct activation with additional factors such as bovine thrombin or extrinsic anticoagulants as in PRP.[27] Due to its fibrous nature, PRF carries a large number of growth factors and cytokines in a supportive three-dimensional fibrin scaffold for cell migration.[28] PRF dissolves more gradually in tissues than PRP, organizing into a solid fibrin matrix and slowly rebuilding itself in the manner of a natural blood clot. Platelets and cytokines are then slowly released for a certain time period. While PRP has been seen to release most of its growth factors and cytokine within the first day itself, over a period of 10 days, PRF scaffold allows a continuous gradual release of growth factors and cytokines.[29] Therefore, the migrating cells in the fibrin matrix and growth factors are close to the vicinity of the PRF scaffold during the integral growth cycle of the PRF.[30]

4.2 PRF Processing Protocol:

To achieve the correct quantity and consistency for the fibrin matrix, leukocytes, platelets, and growth factors, a standard procedure for PRF processing should be followed. Autologous blood is used for the processing of PRF. Without any addition of anticoagulants and bovine thrombin in the glass-coated plastic test tube, 10mL of blood is obtained from the subject. It is then immediately centrifuged for 10 minutes at the velocity of 3000 rpm .[26] Many researchers have used for 12 minutes at the speed 2700 rpm and got the same results.[6] The fibrin scaffold is formed in the middle layer as a result of centrifugation. A normal and progressive polymerization that happens during centrifugation results from PRF.[31]

The substance obtained consists of three layers: the upper layer of acellular plasma (platelet weak plasma), the center of the PRF clot, and the bottom of the red corpuscle base. This clot is then detached from the below red corpuscle cells by using a sterile scissors and tweezers and immediately transferred to a metal PRF compression box and condensed to form the PRF membrane. The clot can also be squeezed between two sterile gauze pieces for obtaining the inexpensive PRF membrane.[28] After compression of the PRF clot, the lower house of the box absorbs the exudate that may be used for hydrating other regenerative materials.

CHART-3

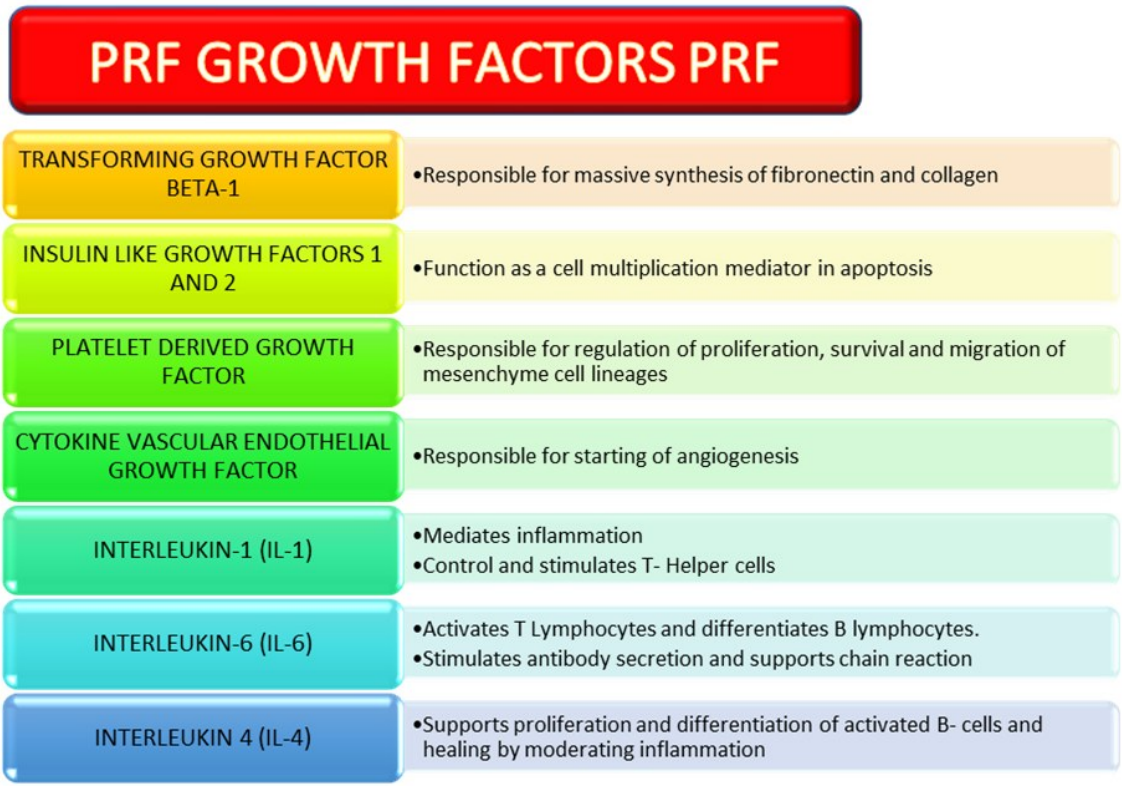

Fig:3 Growth Factors present in PRF

The PRP membrane structure consists of equilateral junctions that are trimolecularly linked to a network of fine and elastic fibrin promoting cytokine enmeshment and cell migration. This three-dimensional structure of the PRF membrane gives strength.

4.3 Role of PRF in Regeneration:

In a tetra molecular system in which platelets, leukocytes, cytokines and flowing stem cells are integrated, PRF forms a fibrin matrix network that is polymerized.[32] Stimulation of osteoblasts, gingival fibroblasts, and proliferation of periodontal ligament cells as a mitogen is done by PRF. Its low thrombin concentration along with molecular structure acts as an optimal matrix in which migration of endothelial cells and fibroblasts occur.[31] It allows a quick angiogenesis and smooth remodelling of fibrin. PRF plays a role of immune regulator because of the leucocytes and other crucial immune cytokines such as IL 1β, IL 6, IL 4 and TNF α that are trapped in PRF giving it the anti-infectious effect.[31]

PRF consists of condensed growth factors suspended within the platelets.[28,34-36] These growth factors act as tissue regenerative promoter and play a role in wound healing.[25,27]

4.4 Applications of PRF in Periodontics:

In the area of periodontics, PRF is a potent autogenous living biomaterial with an extensive applications. PRF is being used in surgical environments where healing and regeneration safety and relaxation are important and in cases where there is no tissue regeneration scaffold and growth factors due to which tissue repair prognosis may be potentially affected. Continuous research is being done for using PRF and its membrane in further more regenerative procedures. Some of its application include:

I. EXTRACTION SOCKET AUGMENTATION-

II. GUIDED BONE REGENRATION (GBR)

III. SINUS ELEVATION PROCEDURES

IV. IN INTRABONY AND FURCATION DEFECTS

V. ROOT COVERAGE OF GINGIVAL RECESSIONS

VI. INTERDENTAL PAPILLA AUGMENTATION

VII. PALATAL BANDAGE

VIII. TISSUE ENGINEERING

5. Advancements in Platelet Concentrates:

1) Concentrated Growth Factors (CGF):

It is a novel platelet concentrate of the second generation, produced by Sacco in 2006.[38,39] CGF, an upgraded version of PRF, is a fibrin-rich organic matrix containing growth factors, platelets, leukocytes and CD34+ stem cells that helps in the process of regeneration.[40,41] The fibrin network consists of thin and thick fibrillary components that are trapped within the collagen matrix by multiple elements. A strong regenerative ability and flexibility tend to be acquired by CGF.[42]

The tubes are centrifuged (Medifuge, Silfradent, Sofia, Italy) after blood collection in sterile vacuette tubes lacking anticoagulant solutions, with a single-step centrifugation protocol: 30sec speeding up, 2min at 2700 rpm, 4min at 2400 rpm, 4min at 2700 rpm, 3min at 3000 rpm, 36sec slowdown and stop. This leads to four separate stages that are as follows:43

1. upper layer – Serum

2. Interim layer – Fibrin buffy coat

3. Liquid layer – Growth factors

4. Inferior layer – Red blood cells

2) Titanium Platelet-Rich Fibrin (T-PRF):

Adopted in 2014 by Tunali et al, it is a new product focused on the premise that titanium can be a more dynamic platelet activator when used with glass tubes than silica activators in the leukocyte and platelet-rich fibrin (L-PRF) system of Choukroun. Tunalı et al concluded that T-PRF has immensely formulated network along with a continuous integrity and the fibrin network covered more area and was thicker after analysing through light, scanning electron and fluorescence microscopy analysis.[44] Silica has been used to avoid the harmful effects of plastic tubes filled with dry glass or glass.

Blood processing was carried out in titanium coated grade IV tubes without any anticoagulant. Then the tubes were instantly centrifuged for 12 minutes at 2800 rpm. The findings obtained were: T-basic PRF's histological structure is comparable to L-PRF; however, T-PRF fibrin strands were more intricately intertwined and thicker than typical L-PRF fibrin strands.[45]

3) Advanced -Platelet Rich Fibrin (A-PRF):

Modified form of the PRF produced by Choukron in 2014 containing larger number of white blood cells especially neutrophills.[46] They found that the increased appearance of neutrophilic granulocytes in the outer portion of the clot, prolonged and enhanced release of growth factors, while raising the centrifugation time (1300 rpm, 14 minutes) and decreasing the rpm and centrifugal g-force, gave an improved appearance. Owing to the involvement of monocytes/macrophages and their growth factors, this could affect the differentiation of host macrophages and macrophages inside the clot after implantation, thereby affecting the regeneration of bone and soft tissue.[47]

4) Advanced -Platelet Rich Fibrin (A-PRF+):

Another modification of A-PRF as proposed by Kobayashi & co-workers in 2016. The centrifugation time has been reduced to 1300 rpm for 8 minutes. They proposed that by decreasing the time would result in decreasing the amount of forces that the blood cells would be disclosed to, thus increasing the number of cells containing in the PRF matrix.[48] Comparing with other types of platelet concentrates like Advanced PRF and L-PRF, the release of different growth factors such as TGF-β1, PDGF, EGF, and IGF are greater in A-PRF+.[47]

5) Injectable Platelet-Rich Fibrin (i-PRF):

To overcome the limitation of gel form of PRF, i-PRF is introduced. This has made it possible for clinicians to employ platelet concentrate in liquid formulation, which can be utilized alone or in conjunction with different biomaterials. In contrast to other PRF formulations, a larger percentage of regenerative cells having a greater concentration of growth factors are seen with slower and reduced centrifugation speeds.

Blood obtained in clear vacuum tubes in absence of anticoagulant was instantly centrifuged for 3 min at 700 rpm. On addition of particulate bone to i-PRF results in polymerization within 15 minutes to produce red coloured sticky bone. In a report, i-PRF resulted in additional growth factors release even after 10 days, while PRP was essentially completely dissolved after 10 days. Mourão et al in 2015 also obtained the i-PRF by increasing the rpm by 3300 and reducing the time by 2 minutes.[49]

6) Autologous Fibrin Glue (AFG) and Sticky Bone:

In 2010, Sohn et al demonstrated the understanding of the use of autologous fibrin glue (AFG) for the development of bone graft matrix enhanced with growth factors (called 'Sticky bone'). In non-coated tubes without anticoagulants, blood is collected and centrifuged for 2 minutes at 2400-2700 rpm to acquire autologous fibrin glue. Two layers

are obtained out of which the superficial layer is AFG and deeper layer consists of red blood cells. The AFG thus attained is extracted using syringe and blended with particulate bone powder and left for 5-10 minutes for polymerization resulting in a yellow coloured mass known as sticky bone.[50] The sticky bone thus acquired is normal, moldable, entangles in its fibrin network platelets and leukocytes, prevents micro and macro motion of grafted bone, prevents soft tissue from developing in the graft.

5. Future Scope:

To discover the biological properties of PRF and its broader applications in the area of periodontics and implant dentistry, PRF is increasingly being investigated. Positive growth and improved healing results and less patient pain have been seen in current studies.

6. Conclusion:

PRF is the future of modern regenerative dentistry that has wider applications not only in the periodontics field and implant dentistry but also in oral surgery, endodontics, tissue engineering and other medical field including orthopaedics and plastic surgery. Its accelerated wound healing property along with its anti-bacterial and anti-haemorrhagic are beneficial for the patients which attracts more clinicians to adopt this technology.

Reference:

1. Sander L, Karring T. Healing of periodontal lesions in monkeys following the guided tissue regeneration procedure. A histological study. J Clin Periodontol 1995;22:332–7.

2. Greenwell H. Committee on research, science and therapy, American Academy of Periodontology. Position paper: guidelines for periodontal therapy. J Periodontol 2001;72:1624–8.

3. 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;71:1207-1.

4. Gassling VL, Açil Y, Springer IN, Hubert N, Wiltfang J. Platelet-rich plasma and platelet-rich fibrin in human cell culture. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:48–55.

5. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. invitro release of growth factors from platelet rich fibrin (PRF): a proposal to optimize the clinical applications of PRF. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;10856–61.

6. Kiran NK, Mukunda KS, Tilak Raj TN. Platelet concentrates: A promising innovation in dentistry. J Dent Sci Res. 2011;2:50–61.

7. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate, part I: technological concept and evolution. Oral Surg Oral Med Oral Path Oral Radiol Endod 2006;101:E37–44.

8. Jung RE et al. Long-term outcome of implants placed with guided bone regeneration (GBR) using resorbable and non-resorbable membranes after 12–14 years. Clinical Oral Implants Research. 2013;24(10):1065-1073

9. Upputuri PK, Sivasubramanian K, Mark CS, Pramanik M. Recent developments in vascular imaging techniques in tissue engineering and regenerative medicine. BioMed research international. 2015; 2015:783983

10. Kingsley CS. Blood coagulation; evidence of an antagonist to factor VI in platelet-rich human plasma. Nature 1954; 173: 723-724 [PMID: 13165629 DOI: 10.1038/173723a0]

11 Matras H. [Effect of various fibrin preparations on reimplantations in the rat skin]. Osterr Z Stomatol 1970; 67: 338-359 [PMID: 4917644]

12. Rosenthal AR, Egbert PR, Harbury C, Hopkins JL, Rubenstein E. Use of platelet-fibrinogen-thrombin mixture to seal experimental penetrating corneal wounds. Albrecht Von Graefes Arch Klin Exp Ophthalmol 1978; 207: 111-115 [PMID: 308778 DOI: 10.1007/ BF00414308]

13. 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 [PMID: 3753059 DOI: 10.1097/00000658-198609000-00011]

14. Knighton DR, Doucette M, Fiegel VD, Ciresi K, Butler E, Austin L. The use of platelet derived wound healing formula in human clinical trials. Prog Clin Biol Res 1988; 266: 319-329 [PMID: 3289047]

15. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg 1997; 55: 1294-1299 [PMID: 9371122 DOI: 10.1016/S0278-2391(97)90187-7]

16. Choukroun J, Adda F, Schoeffer C, Vervelle A. PRF: An opportunity in perio implantology. Implantodontie 2000; 42: 55-62

17. Sacco L. Lecture, International academy of implant prosthesis and osteoconnection. Lecture 2006; 12: 4

18 Everts PA, van Zundert A, Schönberger JP, Devilee RJ, Knape JT. What do we use: platelet-rich plasma or platelet-leukocyte gel? J Biomed Mater Res A 2008; 85: 1135-1136 [PMID: 17907242 DOI: 10.1002/jbm.a.31570]

19 Everts PA, Hoffmann J, Weibrich G, Mahoney

CB, Schönberger JP, van Zundert A, Knape JT. Differences in platelet growth factor release and leucocyte kinetics during autologous platelet gel formation. Transfus Med 2006; 16: 363-368 [PMID: 16999760 DOI: 10.1111/ j.1365-3148.2006.00708.x]

20 Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009; 27: 158-167 [PMID: 19187989 DOI: 10.1016 /j.tibtech. 2008.11.009]

21. Sohn DS. Lecture titled with sinus and ridge augmentation with CGF and AFG, Symposium on CGF and AFG. Tokyo, June 6, 2010

22. Choukroun J. Advanced PRF and i-PRF: Platelet concentrate or blood concentrate? J Periodontal Med Clin Pract 2014; 1: 3

23 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 [PMID: 22951383 DOI: 10.1016 /j.bjoms.2012.08.003]

24. 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 [PMID: 26814997 DOI: 10.1590/0100-69912015006013]

25. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:E45e50.

26. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:E51e55.

27. Choukroun J, Adda F, Schoeffler C, Vervelle A (2001) Une opportunité en paro-implantologie: le PRF. Implantodontie 42:e62

28. Toffler M, Toscano N, Holtzclaw D, Corso M, Dohan D (2009) Introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery milieu. J Implant Adv Clin Dent 1:22–31

29. Kobayashi E, Fluckiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, Miron RJ (2016) Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig. doi:10.1007/s00784-016-1719-1

30. Tsay RC, Vo J, Burke A, Eisig SB, Lu HH, Landesberg R (2005) Differential growth factor retention by platelet-rich plasma composites. J Oral Maxillofac Surg 63:521–528. doi:10.1016/j.joms. 2004.09.012

31. Malathi K, Muthukumaraswamy A, Beri S. Periodontal regeneration of an intrabony osseous defect with combination of platelet rich □brin and bovine derived demineralized bone matrix: A case report. IOSR-JDMS 2013; 4(2):20-26.

32. Singh S, Singh A, Singh S, Singh R. Application of PRF in surgical management of periapical lesions. Natl J MaxillofacSurg 2013; 4(1):94-99

33. Rudagi KB, Rudagi BM. One-step apexification in immature tooth using grey mineral trioxide aggregate as an apical barrier and autologus platelet rich fibrin membrane as an internal matrix. J Conserv Dent 2012; 15(2):196-99.

34. Cromack DT, Porras-Reyes B, Mustoe TA. Current concepts in wound healing: Growth factor and macrophage interaction. J Trauma. 1990;30:S129–33

35. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. In vitrorelease of growth factors from platelet-rich fibrin (PRF): A proposal to optimize the clinical applications of PRF. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:56–61.

36. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): A gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors. 2009;27:63–9

37. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101:e56–60

38. Anitua et al. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials, 2007; 28:4551.

39. Anitua et al. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost, 2004; 91:4.

40. De Boer HC et al. Activated platelets correlate with mobilization of naive CD34 (+) cells and generation of CD34 (+) /KDR (+) cells in the circulation. A metaregression analysis. J Thromb Haemost. 2013; 11:1583-92.

41. Yu B, Wang Z. Effect of concentrated growth factors on beagle periodontal ligament stem cells in vitro. Mol. Med. Rep. 2014; 9(1):235-242.

42. Padhayaya VU, Arora A, Goyal A. Bioactive Platelet Aggregates: Prp, Prgf, Prf, Cgf And Sticky Bone. J Dent Med Sci. 2017;16(5);05-11.

43. Jing Qiao, Jinyu Duan, Yong Zhang, Yi Chu1, Changzhou Sun. The effect of concentrated growth factors in the treatment of periodontal intrabony defects Future Science OA. 2016; 2:4.

44. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E, et al. (2013) In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): a new platelet concentrate. Br J Oral Maxillofac Surg 51(5): 438-443.

45. S. Takemoto, T. Yamamoto, K. Tsuru, S. Hayakawa, A. Osaka, and S. Takashima, "Platelet adhesion on titanium oxide gels: effect of surface oxidation," Biomaterials, vol. 25, no. 17, pp. 3485–3492, 2004.

46. Choukroun J. Advanced PRF, and i-PRF: Platelet concentrates or blood concentrates? J Periodont Med Clin Pract 2014;1:3.

47. Kobayashi E, Flückiger L, Fujioka Kobayashi M (2016) Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig 20(9): 2353-2360.

48. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. J Periodontol. 2017; 88(1):112–21.

49. Mourão CF de AB, Valiense H, Melo ER, Mourão NBMF, Maia MD C, et al. (2015) Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. Rev Col Bras Cir 42(6): 421-423.

50. Sohn DS, Huang B, Kim J, Park WE PC (2015) Utilization of autologous concentrated growth factors (CGF) enriched bone graft matrix (sticky bone) and CGF-enriched fibrin membrane in implant dentistry. J Implant Adv Clin Dent 7: 11-18.

