# Platelet-rich fibrin (PRF): a growth factor-rich biomaterial. Part 1 – the platelet concentrates milieu & review of the literature

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## Introduction

The use of platelet concentrates in healing and augmentation has been reported on for more than forty years.[1-4] Their preparations have evolved yet all methods involve the centrifugation of a patient's own blood, with or without additional preparation steps and additives, to derive an autogenous biomaterial.[5] Whilst their use is widely reported in the literature in the treatment of sports tendon injuries, surface wound treatment, and plastic surgery, their most widespread application are as intraoral graft materials for tissue regeneration and pre-implant surgery. Of the more widely published among these platelet concentrates as an autogenous graft material used in oral surgery and implantology, is Choukroun's PRF – a leukocyte and platelet-rich fibrin.[6] Choukroun's PRF is derived from centrifuging a sample of the recipient patient's own blood and separating it into platelet-poor plasma, a platelet-rich fibrin clot, and red blood cells.[7] PRF is autogenous since the blood sample is derived from the patient requiring the augmentation procedure, processed externally, free of additives, and then as with autografts it is re-implanted to the surgical site in the same patient. However, there currently is no gold standard as to which platelet concentrate supersedes all others as an intraoral graft material or as a biomaterial to accelerate healing. PRF in implant surgery has extensively been reported on over the last two decades, yet there is a paucity of literature reporting objective, histological evaluation of tissue resulting from PRF grafted intraoral sites.[8-13] Aside from the hype generated in recent years around this biomaterial, it is pertinent for the clinician treating patients with PRF to have a sound understanding of its place among the platelet concentrates, understand its biology and applications, have a knowledge of the current and available literature, and be aware of the material's limitations as well its potential.

## Intraoral augmentation

Augmentation of an intraoral site in pre-implant / pre-restorative surgery requires selection among a host of techniques and materials.[14] Whatever the technique, a graft material is generally required and the implications of each are to be well understood by the clinician. Selecting allograft, xenograft, or alloplastic material is at an increased treatment cost, may be contraindicated by the patient's religious beliefs, and requires the materials to be available on hand. Autogenous bone remains the literature supported gold standard of care due to its superior osteogenic, osteoinductive, osteoconductive, and non-immunogenic properties, despite its faster resorption in comparison to xenogeneic or alloplastic materials.[15] In recent years platelet concentrates have widely been proposed as viable autogenous materials for use in periodontal, oral and implant surgery. The literature is abundant predominantly with Anitua's PRGF and Choukroun's

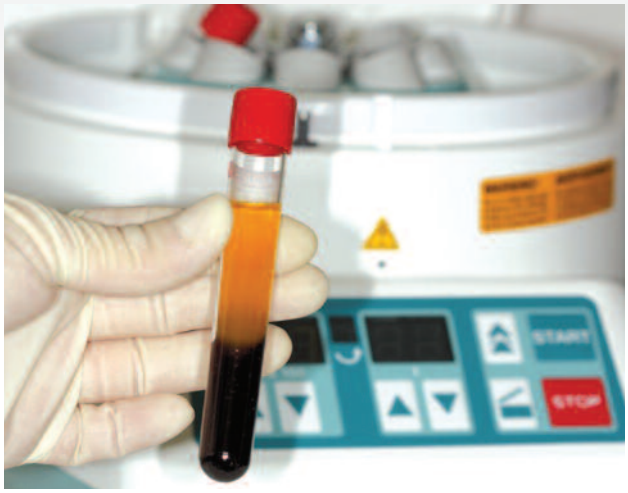


*Figure 1: Centrifuge for the production of PRF (Process, France) and blood tube immediately after centrifugation*


*Figure 2: Blood tubes immediately after centrifugation with identifiable strata; A) platelet-poor plasma, B) platelet-rich fibrin, C) erythrocytes.*

*Figure 3: PRF clot being withdrawn from the blood tube.*

PRF materials.[6, 16] PRF has been proposed for use in sinus augmentation, used alone or as a combination with autogenous bone, allograft, or with alloplastic materials.[9] It has extensively been reported on and proposed for use in ridge preservation – prepared as condensed plugs filling extraction sockets.[6] Case reports demonstrate how this biomaterial when thinned and extruded of its liquid content forms pliable and resilient barrier membranes.[17] Access to PRF is generally easy and draws from the benefits of selecting an autogenous material and at minimal additional cost, at minimal clinical invasiveness. The nomenclature, indications, and classifications of the platelet concentrates may however elude the clinician.[5]

## Classification of platelet concentrates

The invention of platelet concentrates originated from the treatment of haemorrhagic disorders, by the separation of whole blood to harvest platelets for transfusion to treat leukaemias, thrombocytopenias, etc.[18-21] Matras first introduced into the literature the use of 'fibrin glues' as a surgical haemostatic agent in the 1970s.[1] Fibrin glues were thought to possibly stimulate healing, and later with the discovery of growth factors contained within platelet granules, such as transforming growth factor (TGF-β1), did it spur the pursuit of platelet concentrates' use in tissue and bone regeneration.[22, 23] Platelet-rich plasma (PRP) was first introduced into the literature for use in oral surgery by Whitman in 1997.[24] Anitua in 1999 introduced another variation of these concentrates, naming it Platelet Rich in Growth Factors (PRGF).[25] Choukroun thereafter introduced platelet-rich fibrin (PRF) in 2001.[26] Clinicians have since invented their own protocols for platelet concentrates and so too have commercial organizations introduced products and equipment to purpose their own versions of these materials.

Among the diverse variations in the literature, Choukroun's PRF is possibly one of the more widely published techniques. Nevertheless, not only is the platelet concentrate nomenclature complex, but also their preparation protocols. They differ in their biology, in their concentrate constituent, as well as in their applications.[27] Moreover, little to no literature concisely reports on their exact constitutions.[28] Clinical applications of these by case series and reports may be abundant, but not a single systematic review or meta-analysis of their ultrastructural morphology and composition

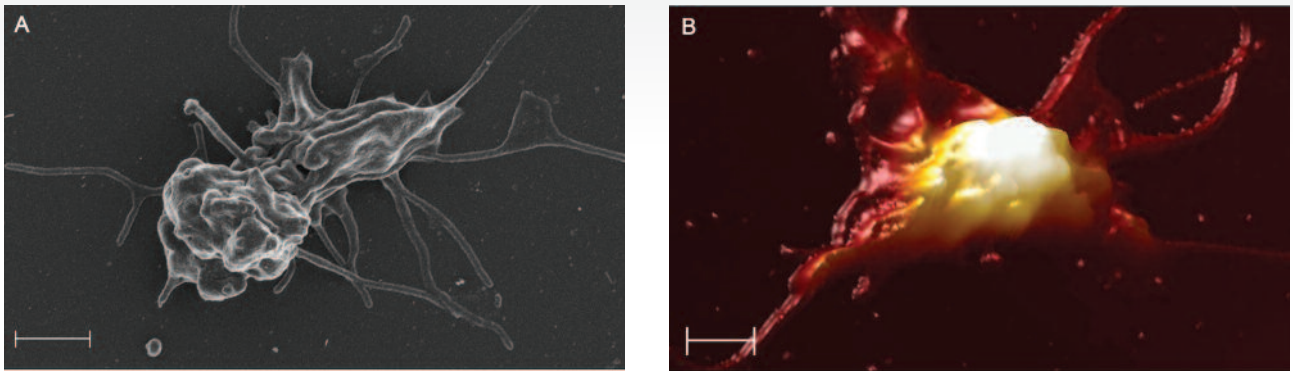


*Figure 4: Platelet morphology A) SEM view, scale 1µm and B) autofluorescence microscopy (AFM), scale 1µm*

exists. This platelet concentrate milieu may well confuse the clinician. Whilst not exhausting the inquiry, a classification derived from a review of the literature identifies four main types of platelet concentrate products based on their fibrin architecture and cell content, namely:

i. Pure platelet-rich plasma (P-PRP)
ii. Leukocyte and platelet-rich plasma (L-PRP)
iii. Pure platelet-rich fibrin (P-PRF)
iv. Leukocyte and platelet-rich fibrin (L-PRF)

Platelet-rich plasma (PRP) was established in the literature in the 1970s in the directives of the American Association of Blood Banks, the American National Red Cross Blood program, and the Canadian Red Cross Blood Transfusion Program.[18-21] PRP was used in replacement therapy following blood loss. It was derived by centrifugation, with authors reporting in succession on modifications of centrifugation speed and duration to obtain higher platelet yield.[29] This period notes the first mention of the term 'buffy coat', what we now know to be platelet-rich fibrin (PRF) today. Nearly 40 years later, clinicians and scientists continue to proffer new centrifugation techniques. P-PRP was first mentioned in the literature for its wound healing and therapeutic use in 1950. [30] Niekisch was the first to report on its application in managing haemorrhaging disorders in maxillofacial and oral surgery, in 1980.[31] Only in 1997 again did Whitman an co-workers report on P-PRP as a 'fibrin glue' and haemostatic agent in oral surgery, though proposing for the first time its potential to improve healing following oral surgery.[24] Marx and co-workers then in 1998 reported an increase in both bone formation and density as a result of platelets releasing growth factors, thus reinforcing P-PRP use in intraoral grafting, augmentation, regenerative therapy.[32]

### i. Pure platelet-rich plasma (P-PRP)

P-PRP are products low in or without leukocyte content, and have a low-density fibrin network after preparation. All products within this group are a liquid or gel that can be injected or administered topically. As P-PRP preparation techniques developed to the present day, from the literature they may be subdivided into automated and manual. The automated P-PRP variants are typically commercial products. One of the first among such automated P-PRP was the Curasan kit introduced in 2001, though the product has long since been discontinued.[28] Similarly, the Platelet Concentrate Collection System (PCCS) for P-PRP was launched in 2002 (3i Implant Innovations). This product too has since been discontinued.[33] The Vivostat PRF system (Vivolution, Denmark) was later introduced in 2005 as a fully automated production of autogenous fibrin sealant.[34] The confusion in nomenclature becomes evident. Whilst the manufacturer names the concentrate a PRF, as well as an 'autogenous fibrin sealant', by being leukocyte poor and having a low density fibrin content it is classified a P-PRP. The Vivostat PRF system is probably one of the more prominent and commercially used P-PRP products available. Its large processing unit acts as a cell separator of a patient's whole blood, applying multiple centrifugations, and incrementally introducing additives such as an acetate buffer. Whilst the temperature is maintained at 36 ° C, the plasma is separated out and fibrinogen activator is added. Fibrin and serum are separated and the steps are repeated whilst the acetate buffer is added. The buffer dissolves the fibrin, and the final product is ready for application. Supporting its categorization as a P-PRP is this final step of removing the active fibrin component.

Of the manual preparations of P-PRP, Anitua's technique

introduced in 1999 is possibly the most widely published.[16] Anitua named the platelet concentrate product of his technique: Platelet Rich in Growth Factors (PRGF). This platelet concentrate has since been commercialized into a product, PRGF-ENDORET, by Anitua's company: BTI Biotechnology.[35] Like other platelet concentrates, the preparation of PRGF involves the centrifugation of whole blood, but in several smaller blood tubes, to form three layers – red blood cells, a buffy coat, and acellular plasma. Anitua considers the upper portion of the acellular plasma as being platelet poor. This plasma is pipetted off and discarded. The lower portion is considered platelet rich, and thus the PRGF. Manually pipetting this plasma off relies on the operator's discretion. PRGF from several tubes are then combined and fibrin polymerization is induced by adding 10 % calcium chloride. After ± 20 minutes the PRGF gel is formed and ready for use.

### ii. Leukocyte and platelet-rich plasma (L-PRP)

L-PRP comprises the largest group of commercially available products among the four main platelet concentrate types.[36] L-PRP products contain leukocytes and have a low-density fibrin network, and all products within this group are also used as a liquid or gel administered by injection or topically. An example of this system is Harvest SmartPrep (Harvest Technologies).[37] It is essentially a modified centrifuge that instead of receiving blood tubes, separates whole blood in a dual-chamber capsule containing a "floating shelf". Another very similar L-PRP product that centrifuges a capsule containing a "floating buoy" is the GPS III (Biomet).[38] These floating devices claim to adapt to the cellular density that is patient specific to separate plasma, platelets, and leukocytes, from red blood cells.

### iii. Pure platelet-rich fibrin (P-PRF)

P-PRF are products without leukocytes and have a high-density fibrin network. Thus, these are applied as dense gels and are not injectable. Only one commercially available product represents this group: Fibrinet PRFM (Cascade Medical).[39] It consists of a wholly disposable kit, including an anticoagulant containing blood tube. The process involves centrifugation followed by multiple transfer steps before its administration.

### iv. Leukocyte and platelet-rich fibrin (L-PRF)

L-PRF products contain leukocytes within a high-density fibrin network. They are mostly solid or dense gels and cannot be injected (although Choukroun has developed an injectable variant, i-PRF which remains in the testing stages[40]). Of the L-PRF products, Choukroun's PRF (Process, France) is possibly the most widely reported on. A near identical product is Intra-Spin L-PRF (Intra-Lock Inc.).[41] L-PRF products are discernible from other platelet concentrates as being entirely autogenous, have neither anticoagulant nor other additives, and consist of only a one step centrifugation process.[7] Production of PRF does not strictly require any commercial materials. Any centrifuge calibrated to ± 400 g of force and set for 10 minutes can derive PRF from a sample of a patient's whole blood collected in an additive free blood tube (Fig. 1). The clinician is to note that Choukroun has updated his protocol, proffering a newer "Advanced PRF" or A-PRF protocol, with a lower centrifugation RPM for a longer cycle duration.[42]

## Platelet-rich fibrin biology

The basic purpose of manipulating a sample of a patient's whole blood to derive a platelet concentrate, is to segregate the blood's constituents, retain a portion and discard others, promote activation of the retained portion, and then therapeutically deliver this to a treatment site within a patient. Centrifugation or a variation thereof is integral to all the techniques. The separating of whole blood is done chemically, mechanically, or as a combination. All the L-PRP and P-PRP

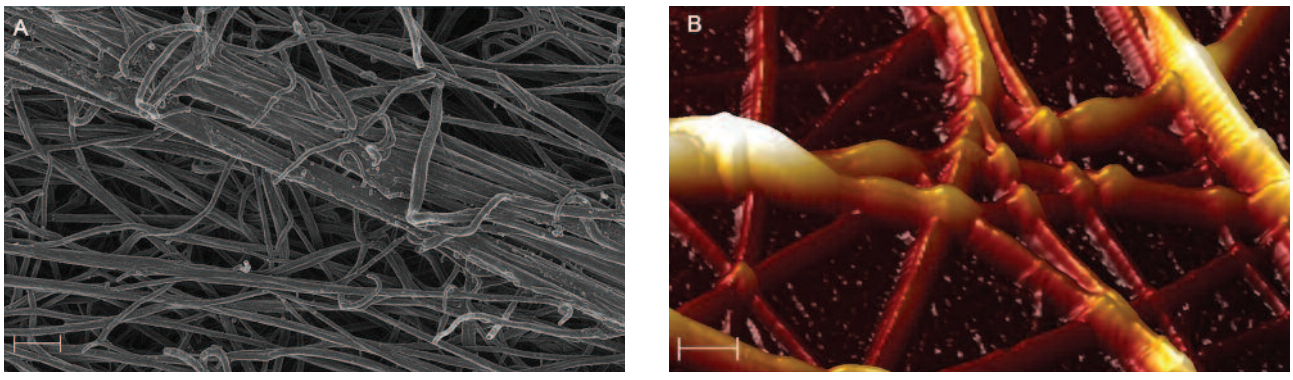


*Figure 5: Fibrin network arrangement and morphology. A) SEM, scale 1µm. B) AFM, scale 1µm.*

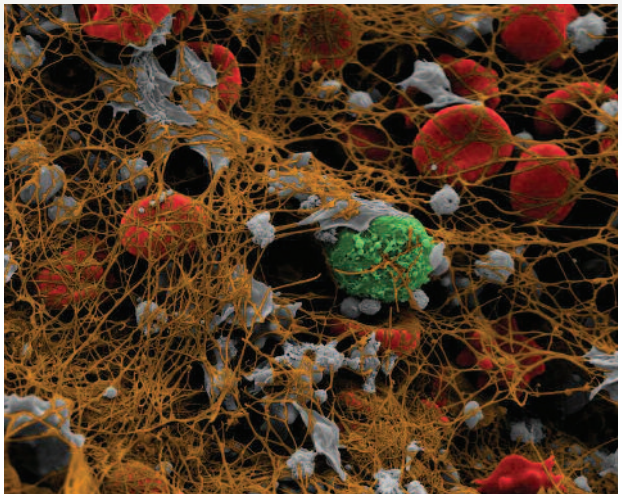

*Figure 6: SEM view of activated platelets (white) enmeshed in a fibrin network (orange), with erythrocytes (red), and a leukocyte (green).*

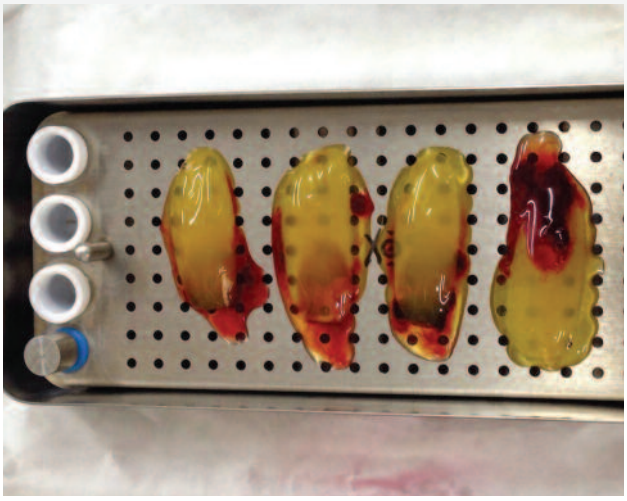

*Figure 7: PRF clots placed within the box, the lid when placed on top extrudes serum and forms membranes, (Process, France)*

products include an anticoagulant in the preparation process to prevent activation of fibrin.

The Vivostat P-PRP system additionally includes an acetate buffer to dissolve fibrin as a final step in its preparation.34 In contrast to these processes, preparation of L-PRF such as Intra-lock and Choukroun's PRF solely uses mechanical means to separate whole blood.[7, 41] The products can be considered natural and wholly autogenous since the blood tubes are additive free. In preparing Choukroun's PRF, venous blood is drawn and immediately centrifuged in a standard table-top centrifuge for 10 minutes (Choukroun's original protocol[6]) or 14 minutes (Choukroun's revised A-PRF protocol[42]).

When applying continuous centrifugal force, the blood components with higher density in the tube migrate away from the axis of rotation and lighter components migrate toward it. This causes red blood cells to sediment in the bottom portion of the tube. The PRF clot collects in the middle, and plasma (also known as supernatant) collects in the upper portion (Fig. 2).[6] Anitua's PRGF protocol would pipette this layer off. Choukroun's PRF protocol disregards it. The PRF clot is manually withdrawn from the tube with sterile tweezer forceps (Fig. 3), and the red blood cells if still attached in part to the distal portion can be cut free or scraped from the PRF clot.

This process of blood manipulation and its separation is synchronous with the constituents' activation processes. When whole blood is drawn into an anticoagulant free tube, the coagulation pathway is initiated – sequentially activating coagulation factor proteins, arriving at activated thrombin converting fibrinogen into fibrin.[43] In vivo, coagulation is among other things the result of tissue factor release from injured endothelium and from activated platelets bound to exposed collagen and each other.[44] In vitro when whole blood is centrifuged, centrifugal forces expose platelets to the tube wall. This contact of platelets with each other and the tube wall results in their activation.

In the absence of anticoagulant this platelet activation is immense. Activated platelets up-regulate the tissue factor expression of leukocytes in the tube.[45] As platelets become activated, their granules, specifically α-granules burst, releasing cytokines and tissue factors, β-thromboglobulin, fibronectin, thrombospondin, fibrinogen, coagulation factors, growth promotors, fibrinolysis inhibitors, and immunoglobulins (Fig. 4).[46] Each of these has a highly specific function in tissue healing, in haemostasis, inflammation and proliferation. Degranulation releases cytokines that promote cell migration and proliferation in the clot's fibrin matrix, promoting the early stages of healing. Of these, transforming growth factor $\beta_1$ (TGF-$\beta_1$), platelet derived growth factor (PDGF), and insulin-like growth factors I and II (IGF-I, II) are the most prominent.[47] TGF-$\beta_1$ has extremely varied effects. In bone healing it may either stimulate osteoblasts or cause their inhibition, depending on its concentration, on the matrix environment, and the cell types present.[48] Regardless, it is a powerful fibrosis agent for most cell types and induces rapid and substantial endochondral bone (in primates).[49] TGF-$\beta_1$ will greatly induce collagen I and fibronectin synthesis from fibroblasts and osteoblasts.[50] It is considered an inflammatory mediator by its capacity to induce fibrous healing, though its regulatory mechanisms are complex.

Insulin-like growth factors (IGFs) I and II positively regulate

both proliferation and differentiation of a majority of cell types by acting as tissue growth factors and hormones of energy metabolism.[51] They stimulate cellular proliferation during healing over the long term. Whilst not only platelets but many tissues release IGF-I, it has been shown to increase serum levels of alkaline phosphatase, and serum procollagen – both which stimulate osteoblastic activity.[52]

Platelet-derived growth factors (PDGF) are essential regulators of the migration and proliferation of mesenchymal cells.[53] PDGF induce tissue repair mechanisms and promote fibroblast proliferation, collagen production, and are powerful mitogens for angioneogenesis.[54] Similar to transforming growth factors, they may also stimulate or inhibit. This regulation is central to tissue remodelling.

Whilst forming the PRF clot, the activated fibrin has been shown to enmesh glycosaminoglycans, namely heparin and hyaluronic acid (Fig. 5).[55] These have a strong affinity for binding cells and cytokines – supporting cell migrations during the healing phase, and wholly incorporating the cytokines into their fibrils in high concentrations.[55, 56] PRP is low in fibrin density, and as such cytokines are more freely available within the material. These cytokines' release and activity are measurably high in PRP, intense and short-lived over the first days of healing. By contrast the cytokines entrapped within the fibrin matrix of a PRF clot allow for a slower and long term release.[55]

The net result of this PRF biology is the preparation of a biomaterial, dense and resilient in its activated fibrin structure, and richly incorporated by leukocytes and platelets (Fig. 6). The implantation of such a biomaterial may deliver the slow release of growth promoting factors and cytokines to an area of healing (Fig. 7).

## Conclusion

PRF has in recent years become popularized as a biomaterial rich in growth factors valuable to pre-implant and pre-restorative surgery. In theory the biology behind the technique is literature based, the material does contain growth factors, and growth factors do aid healing. Definitive, histological evidence of its healing promoting effect on either bone or soft tissue is not conclusive. The material's potential remains significant and yet additional histological evidence and clinical trials are needed to further validate.

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## References

1. Matras H. Effect of various fibrin preparations on reimplantations in the rat skin, Osterr. Z, Stomatol. 1970; 67(9):338-359.

2. Childs CB, Proper JA, Tucker RF. Serum contains a platelet derived transforming growth factor. Proc Natl Acad Sci USA. 1982;79(17):5312-6.

3. Assoian RK, Komoriya A, Meyers CA. Transforming growth factor-beta in human platelets: Identification of a major storage site, purification, and characterization. J Biol Chem. 1983;258(11):7155-60.

4. Whitman DH, Berry RL, Green DM. Platelet gel: an autogenous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg. 1997;55(11):1294-9.

5. Ehrenfest DMD, Sammartino G, Shibli JA, Wang HL, Zou DR, Bernard JP. Guidelines for the publication of articles related to platelet concentrates (Platelet-Rich Plasma - PRP, or Platelet-Rich Fibrin - PRF): the international classification of the POSEIDO. POSEIDO. 2013;1(1):17-26.

6. Platelet rich fibrin PRF search results [online]. PubMed NCBI. US: National Institutes of Health, US National Library of Medicine; [updated 2014 August; cited 2012 November]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/?term= platelet +rich+fibrin+PRF

7. Dohan D.M., Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology. 2006;101(3):37-44.

8. Bölükbaşı N, Yeniyol S, Tekkesin MS, Altunatmaz K. The use of platelet-rich fibrin in combination with biphasic calcium phosphate in the treatment of bone defects: a histologic and histomorphometric study.

Curr Ther Res Clin Exp. 2013;75:15-21

9. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): A second- generation platelet concentrate. PartV: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:299–303.

10. Zhao JH, Tsai CH, Chang YC. Clinical and histologic evaluations of healing in an extraction socket filled with platelet-rich fibrin. Journal of Dental Sciences. 2011;(6):116-22.

11. Zhang Y, Tangl S, Huber CD, Lin Y, Qiu L, Rausch-Fan X.

Effects of Choukroun's platelet-rich fibrin on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation: A histological and histomorphometric study. J Craniomaxillofac Surg 2012;(40):321-28.

12. Tanaka H, Toyoshima T, Atsuta I, Ayukawa Y, Sasaki M, Matsushita Y et al. Additional Effects of Platelet-Rich Fibrin on Bone Regeneration in Sinus Augmentation With Deproteinized Bovine Bone Mineral: Preliminary Results. Implant Dent. 2015 Jul 22. [Epub ahead of print].

13. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD and Dohan Ehrenfest DM. Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: a radiologic and histologic study at 6 months. J Periodontol 2009; 80: 2056-2064.

14. Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. Int J Oral Maxillofac Implants. 2009;24 Suppl:237-59. Review.

15. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. J Orthop Surg Res. 2014;9(1):18.

16. Platelet rich in growth factors PRGF search results [online]. PubMed NCBI. US: National Institutes of Health, US National Library of Medicine; [updated 2014 August; cited 2012 November]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/?term=platelet +rich+in+growth+factors+PRGF

17. Gluckman H, Du Toit J, Salama M. Guided bone regeneration of a fenestration complication at immediate implant placement simultaneous to the socket-shield technique. Int Dent AE. 2015;5(4):58-66.

18. American Association of Blood Banks. Technical Methods and Procedures, 6th ed., 1974, p. 353.

19. American National Red Cross Blood Program Directive 6. 17,1972.

20. American National Red Cross Blood Program Directive 4. 12, 1971, p. 1.

21. Canadian Red Cross Blood Transfusion Service, Serological and Immunological Methods, 7th ed., 1972, p. 290.

22. Childs CB, Proper JA, Tucker RF. Serum contains a platelet derived transforming growth factor. Proc Natl Acad Sci USA. 1982;79(17):5312-6.

23. Assoian RK, Komoriya A, Meyers CA. Transforming growth factor-beta in human platelets: Identification of a major storage site, purification, and characterization. J Biol Chem. 1983;258(11):7155-60.

24. Whitman DH, Berry RL, Green DM. Platelet gel: an autogenous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg. 1997;55(11):1294-9

25. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants. 1999 14(4):529-35.

26. Choukroun J, Adda F, Schoeffler C. Vervelle A. An opportunity in perio-implantology: The PRF. Implantodontie. 2001;(42):55-62.

27. Bielecki T, Ehrenfest DMD. Platelet-rich plasma (PRP) and Platelet-Rich Fibrin (PRF): surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. Curr Pharm Biotechnol. 2012;13(7):1121-30.

28. Weibrich G, Buch RS, Kleis WK, Hafner G, Hitzler WE, Wagner W. Quantification of thrombocyte growth factors in platelet concentrates produced by discontinuous cell separation. Growth Factors. 2002;20(2):93-7.

29. Persidsky MD, Ling NS. Separation of platelet-rich plasma by modified centrifugal elutriation. J Clin Apher. 1982;1(1):18-24.

30. Tassman IS. Experimental studies with physiologic glue (autogenous plasma plus thrombin) for use in the eyes. Am J Ophthalmol. 1950;33(6):870-8.

31. Niekisch R. Application possibilities of fibrin glue in dentistry and maxillofacial surgery Zahn Mund Kieferheilkd Zentralbl. 1980;68(6):555-61.

32. 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(6):638-46.

33. Implants: Trade News Focus [online]. Platelet Concentrate Collection System (PCCS). UK: British Dental Journal; [updated 2002 January 26, cited 2013 November 16]. Available from http://www.nature.com/bdj/journal/v192/n2/full/4801307a.html

34. Vivostat PRF [online]. Vivostat PRF (Platelet Rich Fibrin). US: Vivolution; (updated unknown, cited 2015 September 16). Available from http://www.vivostat.com/products/vivostat-prf

35. BTI [online]. Endoret PRGF Technology. Spain:BTI Technologies; [updated 2015, cited 2015 September 16]. Available from http://bti-biotechnologyinstitute.com/regenerative-medicine/

36. Everts PA, Hoogbergen MM, Weber TA, Devilee RJ, van Monftort G, de Hingh IH. Is the use of autogenous platelet-rich plasma gels in gynecologic, cardiac, and general, reconstructive surgery beneficial? Curr Pharm Biotechnol. 2012;13:1163-1172.

37. Harvest Smart-Prep [online]. Harvest Technologies. USA:Harvest Terumo;(updated 2014, cited 2013 November 16). Available from: http://www.harvesttech.com/harvest-smartprep-multicellular-processing-system

38. Biomet Inc [online]. Biomet GPS III. US:Biomet Inc.;[updated 2014, cited 2013 November 160]. Available

from: http://www.biomet.com/biologics/whyGPS.cfm

39. MTF Sports Medicine [online]. Cascade Autogenous Platelet-Rich Plasma. US: ConMed Linvatec; [updated 2014, cited 2013 November 16]. Available from: www.platelettherapy.com

40. Injectable PRF [online]. Process for PRF. France: Process; [updated 2014, cited 2014 August 12]. Available from http://www.a-prf.com/en/i-prf.html

41. Why L-PRF Leukocyte-Platelet Rich Fibrin [online]. Intra-lock International. US: Intra-lock; [updated 2014, cited 2013 November 28]. Available from http://intra-lock.com/index.php?option= com_wrapper&Item id=193

42. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40(6):679-89.

43. Bowen RA, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med. 2014;24(1):31-44.

44. van Hinsbergh VW. Endothelium–role in regulation of coagulation and inflammation. Semin Immunopathol. 2012;34(1):93-106.

45. Bouchard BA, Tracy PB. The participation of leukocytes in coagulant reactions. J Thromb Haemost. 2003;1(3):464-9.

46. Rivera J, Lozano ML, Navarro-Nunez L, Vicente V. Platelet receptors and signaling in the dynamics of thrombus formation. Haematologica. 2009;94(5):700-11.

47. Lee JW, Kwon OH, Kim TK, Cho YK, Choi KY, Chung HY, et al. Platelet-rich plasma: quantitative assessment of growth factor levels and comparative analysis of activated and inactivated groups. Arch Plast Surg. 2013;40(5):530-5.

48. Ripamonti U, Tsiridis E, Ferretti C, Kerawala CJ, Mantalaris A, Heliotis M. Perspectives in regenerative medicine and tissue engineering of bone. Br J Oral Maxillofac Surg. 2011;49(7):507-9.

49. Ripamonti U. Soluble and insoluble signals sculpt osteogenesis in angiogenesis. World J Biol Chem. 2010;1(5):109-32.

50. Ehrenfest DMD, Bielecki T, Jimbo R, Barbé G, Del Corso M, Inchingolo F, et al. Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-rich plasma (P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF). Curr Pharm Biotechnol. 2012;13(7):1145-52.

51. Smith TJ. Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? Pharmacol Rev. 2010;62(2):199-236.

52. Klinger B, Jensen LT, Silbergeld A, Laron Z. Insulin-like growth factor-I raises serum procollagen levels in children and adults with Laron syndrome. Clin Endocrinol. 1996;45(4):423-9.

53. Mantur M, Koper O. Platelet-derived growth factor: the construction, role and it's receptors. Pol Merkur Lekarski. 2008;24(140):173-6.

54. Mantur M, Koper O. Platelet-derived growth factor: the construction, role and it's receptors. Pol Merkur Lekarski. 2008;24(140):173-6.

55. Dohan D.M., Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology. 2006;101(3):45-50.

56. Taylor KR, Gallo RL. Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation. FASEB J. 2006;20(1):9-22.