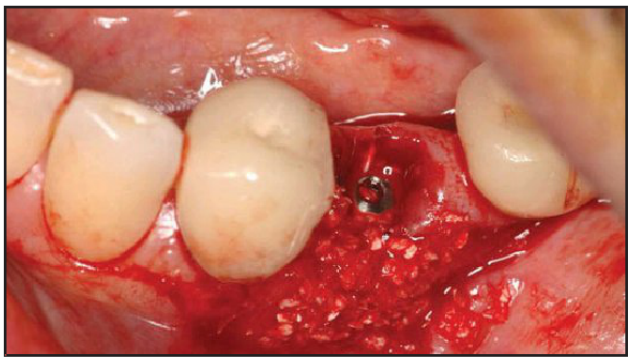
Use of an Autologous Leukocyte and Platelet-Rich Fibrin (L-PRF) Membrane in Post-Avulsion Sites: An Overview of Choukroun's PRF

Marco Del Corso, DDS, DIU[1] • Michael Toffler, DDS[2]
David M. Dohan Ehrenfest, DDS, MS, PhD[3]

Abstract



Choukroun's Platelet-Rich Fibrin (PRF) can be considered an autologous healing biomaterial, incorporating leucocytes, platelets and a wide range of key healing proteins within a dense fibrin matrix. With its strong fibrin architecture and slow release of growth factors and glycoproteins over several days, this natural bioactive membrane can enhance soft/hard tissues healing while protecting both surgical sites and grafted materials from external aggressions. In this article, we propose an overview of the use of PRF in post-avulsion sockets or defects. PRF can be used as a filling material in avulsion (or extraction) sockets alone or mixed with a bone substitute. Used as a covering membrane for guided bone regeneration (GBR), PRF both protects the grafted material and accelerates wound closure, particularly when contiguous suture of the wound margins is not possible. The range of clinical applications of PRF is wide, but an accurate knowledge of the biomaterial, its biology, efficiency and limits is necessary to optimize its systematic use in daily practice.

KEY WORDS: Platelet rich fibrin, platelet rich plasma, autologous growth factors

1. Private Practice, Turin, Italy.
2. Private Practice, New York, NY, USA.
3. Researcher, Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden.

INTRODUCTION

The research and development of protocols promoting haemostasis and healing is a recurrent issue in all surgical disciplines. Fibrin is the first matrix in all wound healing processes,[1,2] and the use of fibrin-based surgical additives, mainly fibrin glues, has a long history in oral and maxillofacial surgery.[3-5] The evolution of these techniques resulted in the development of autologous fibrin glues and, since platelets contain a large amount of fibrinogen (the precursor of fibrin), to the concept of platelet concentrate for surgical use. Whitman et al[6] first described the use of these platelets gels. Since the report by Marx et al,[7] these products have been referred to as platelet-rich plasma (PRP), like the transfusional platelet concentrates from blood banks. Most authors refer to these products as a source of autologous growth factors. Unfortunately, the passion for growth factors has lead to an underestimation of the function of leukocyte content and fibrin architecture in the healing equation.[8]

Since these early publications, a variety of autologous platelet concentrate techniques and devices have been developed, marketed and tested in a large number of clinical situations.[9] In periodontal, implant and maxillofacial surgery, platelet concentrates were first used for their release of growth factors to stimulate the healing process. However, the clinical benefit is difficult to evaluate, and the literature is quite controversial on the subject.[8] This is mainly due to the large number of techniques available, and to the absence of clear classification of the different products. Moreover, the PRP's were both expensive and time consuming protocols and their development in private practice remains quite limited. In 2001, a new protocol was suggested in France by Choukroun at al[10] to concentrate platelets and fibrin in a simpler way without blood modification: Platelet-Rich Fibrin (PRF).

DEFINITION OF PRF

PRF can be considered as an autologous healing biomaterial, incorporating in a matrix of autologous fibrin most leukocytes, platelets and growth factors harvested from a simple blood sample.[11-13] At the present time, the PRF protocol is both the most simple and inexpensive way to produce a platelet concentrate.[8] The blood sample is drawn from the patient at the time of the surgical procedure and is treated with a single centrifugation, with a specific centrifuge (figure 1) and collection kit (Process, Nice, France), without blood manipulation: no anticoagulant during blood collection and no bovine thrombin or calcium chloride for fibrin polymerization.[14] At the end of the centrifugation process, three distinct fractions are produced: 1) at the bottom of the tube, red cells are concentrated (and easily discarded); 2) the superficial layer is a liquid serum called platelet-poor plasma; 3) the intermediate fraction is a dense PRF clot, which can then be used clinically in the form of a membrane. The protocol requires a special tool (PRF box, Process, Nice, France) to prepare standardized membranes and to harvest PRF exudate, in a sterile environment (figures 2, 3).

Both PRF exudate and platelet-poor plasma contain significant amounts of growth factors (Transforming Growth Factors TGFβ-1, Platelet-Derived Growth Factors PDGF-AB, Vascular Endothelial Growth Factors VEGF, etc)[12,13] and matrix glycoproteins, particularly fibronectin and vitronectin. Fibronectin and vitronectin are two key proteins for cell-matrix contact; therefore,

Figure 1: PRF specific centrifuge.

using this exudate for biomaterial impregnation may be beneficial. With the PRF Box®, PRF fibrin membranes are obtained with consistent size and thickness (figure 3). This tool is essential to guarantee objective and reproducible results.[15]

The PRF fibrin membrane is more elastic and consistent than the fibrin bulk sometimes obtained with some PRP protocols. PRP's are enhanced fibrin glues, and PRF is a true fibrin-based biomaterial[8] which may be employed in many clinical situations.[16-18] For example, its elasticity allows it to function as a suturable membrane. This biomaterial is both very easy and inexpensive to produce; therefore, its systematic use during oral and maxillofacial surgery must be considered a relevant clinical option. Moreover, it is completely autologous, so there is no ethical limitation or toxicity concerns related to this natural optimized blood clot.[14]

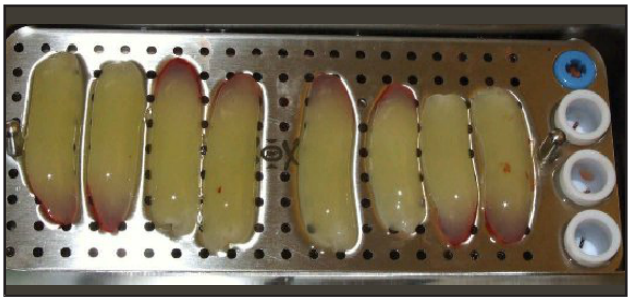


Figure 2: Using the PRF Box, PRF clots are collected and standardized.

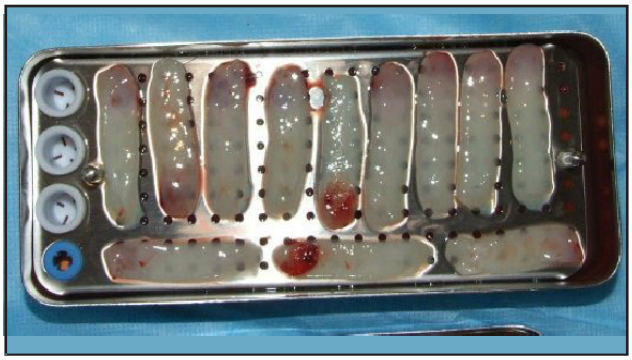


Figure 3: After compression in the PRF Box, uniform PRF membranes are obtained.

PRF: AN AUTOLOGOUS BIOACTIVE MEMBRANE

Recently, a global classification of platelet concentrates was published, and these products are now classified in 4 families related to their leucocytes and fibrin contents.[8] Choukroun's PRF is currently the sole product in the L-PRF class (Leukocyte and Platelet-Rich Fibrin), with both high leukocyte content and strong fibrin architecture. In addition, PRF membranes release high amounts of growth factors such as TGFβ1, PDGF-AB, VEGF and matrix glycoproteins (such as thrombospondin-1) during 7 days *in vitro*.[19] The fibrin matrix with its intrinsic factors and leukocyte content contains the key ingredients for an enhanced healing of superficial

and bone tissues, particularly through the stimulation of neoangiogenesis. It was recently demonstrated in vitro that PRF enhances proliferation of many different cell types such as fibroblasts, osteoblasts, adipocytes, and keratinocytes.[20,21] PRF also stimulates osteoblastic differentiation.[21] The influence of leukocytes was already pointed out in this study, as these cells are true regulation turntables and produce large amounts of VEGF involved in angiogenesis.[19] The PRF fibrin matrix as a filling biomaterial has produced consistently favorable clinical results.[22-24] PRF, as an optimized blood clot, has also been shown to be a very efficient osteoconductive material in sinus-lifts.[25,26]

The PRF protocol is finally a way to transform a natural blood clot into a clinically usable bioactive membrane. The synergetic effects of the fibrin matrix and its growth factor content lead to a natural and enhanced healing of soft and hard tissues. The platelet and leukocyte cytokines are gradually released during fibrin matrix physiological resorption,[19] and matrix glycoproteins allow quick cell migration and proliferation within the PRF tissue-like architecture. This gradual release of cytokines appears to play a regulatory role in the inflammatory phenomena within the wounded tissues. However, the mechanical function of PRF must also be considered since the PRF membranes allow early wound protection and aid in primary soft tissue closure.[17,18,27] This technique, which mimics the natural coagulation process, produces an inexpensive and simple bioactive membrane. Many researchers have tried to develop such membranes in artificial ways by incorporating growth factors in collagen membranes for example. This simple PRF technique produces the most natural bioactive product currently available.

USING PRF IN DAILY PRACTICE FOR POST-AVULSION SITES

The management of avulsion or extraction[28] sites is a daily issue since bone resorption following tooth removal can compromise both implantation and aesthetic results. For this reason, it is often recommended to insert a filling material inside the residual avulsion socket to maintain adequate bone volume. Many bone substitutes function primarily as a space-maintainer. However, these materials are often quite slow to resorb and remodel, and their use often delays the vascularization and bone regeneration at the site. In addition, the management of soft tissue over the graft requires flap release, extensive dissection, and vertical incisions in order to cover the grafted volume, reducing microvascularization at the margins. Used in this indication, PRF acts in the following ways as an optimized blood clot to enhance the natural healing process:

As a filling material in avulsion sockets,[22] PRF will act as a stable blood clot for neovascularisation and an accelerated tissue reconstruction (figures 4-8),[2] particularly in infected sites or in patients with medical conditions that may delay healing (eg. diabetes, immunosuppression). PRF stimulates both coagulation (with thrombospondin-1) and wound closure, making it a useful adjuvant in patients under anticoagulant therapies.

As a membrane for guided bone regeneration (GBR), the PRF dense matrix architecture covers, protects, and stabilizes the bone graft material and the operative site in general.[17] Particularly, the elasticity and strength of the PRF fibrin membrane makes it easy to suture. When the socket is too wide for primary closure, the PRF fibrin matrix can be used as a covering and protective membrane that promotes re-

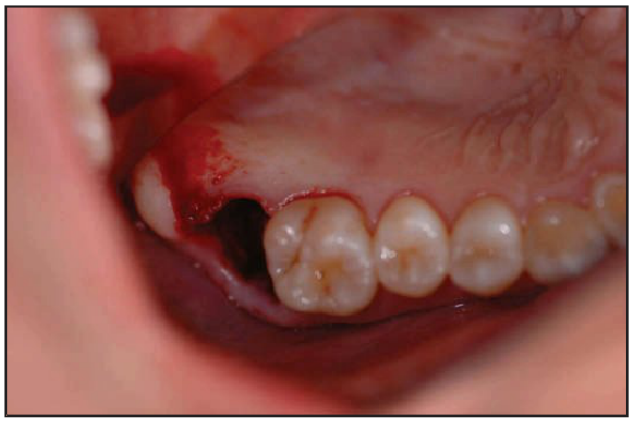


Figure 4: Avulsion/Extraction of tooth 15.

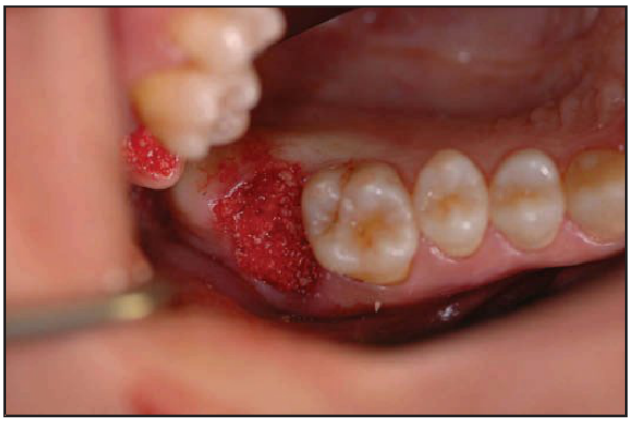


Figure 5: Preservation of site 15 with allograft.

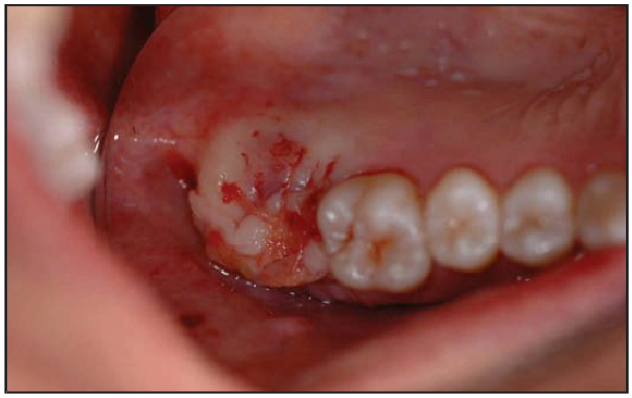


Figure 6: Placement of PRF membrane. Primary closure not required.

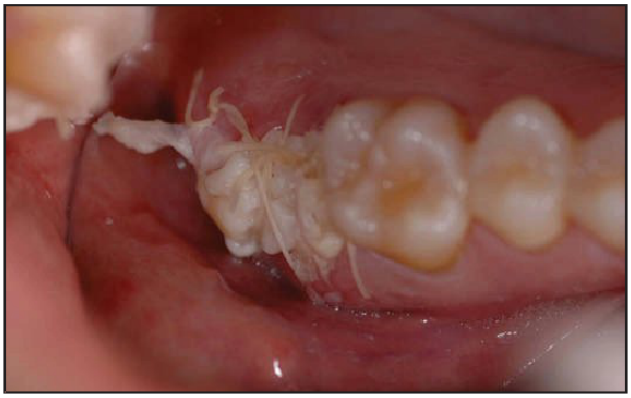


Figure 7: Healing at 24 hours. The PRF membrane protects the socket and stimulates wound healing.

epithelialization of the site and accelerates the merging of the gingival margins (figures 9-22). However, in such circumstances, several PRF layers are required to adequately protect the grafted material and achieve the desired effect.

The mechanisms of these 2 common applications are in fact similar. Epithelial and connective tissue healing on PRF membranes is related both to the growth factors and the fibrin matrix.[1] Gingival fibroblasts easily migrate into this matrix and remodel it. The acceleration of the healing process makes the surgical site less sensitive to

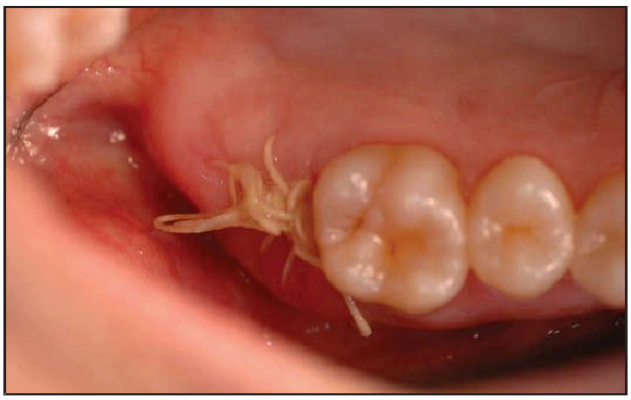


Figure 8: Healing of site 15 at 15 days post-op.

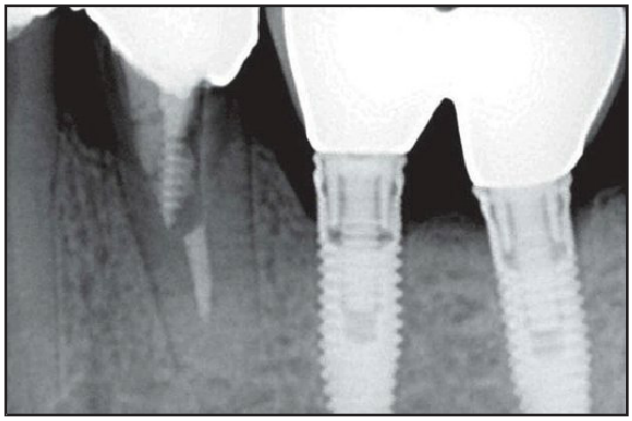


Figure 9: Radiograph of fractured tooth 20.

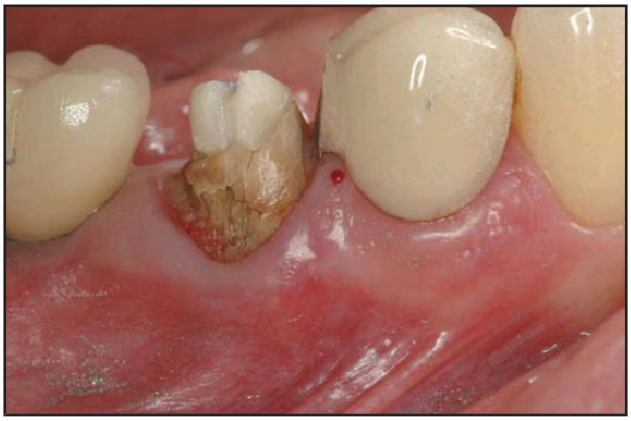


Figure 10: Intraoral view of fractured tooth 20.

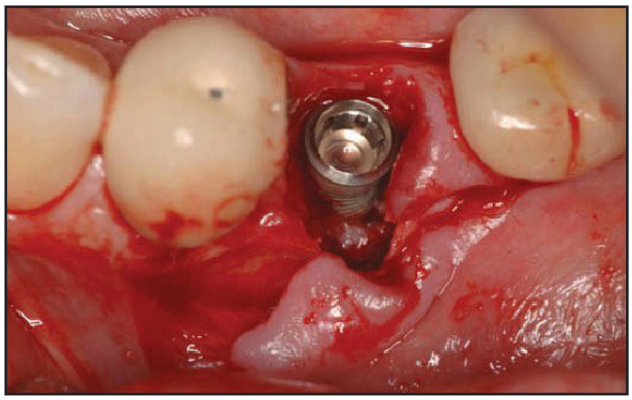


Figure 11: Tooth 20 was avulsed/extracted and immediately replaced with an implant (Intra-Lock, Boca Raton, FL, USA).

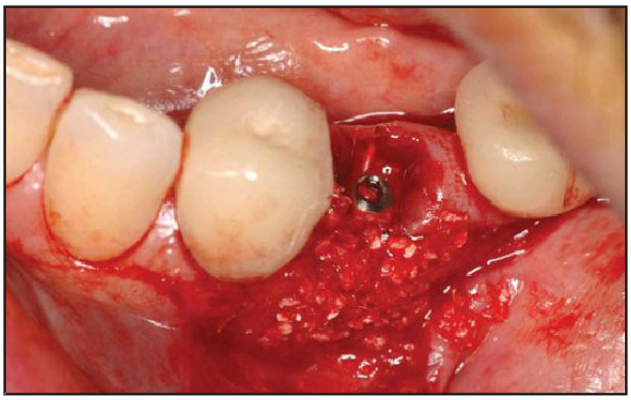


Figure 12: A cortico-spongious porcine bone (GenOs, Osteobiol, Tecnoss Dental, Turin Italy) mixed with PRF was grafted in the defect.

aggressions (mechanical, bacterial and chemical) and thus positively influences both the aesthetic result and postoperative sensitivity. At a deeper level, PRF increases the cohesion between the graft materials as fibrin acts as a physiological glue between wounded tissues. Natural blood coagulation leads to the formation of a fibrin matrix that biologically links wounded tissues together allowing cell proliferation, cell migration, neomatrix apposition and remodelling. Therefore, the combination of PRF with different kinds of filling materials should improve the integration of the grafted material, since PRF is an optimized blood clot.[25]

However, even though these mechanisms are quite well known, the ideal application of PRF must still be accurately defined.[15] The filling of avulsion sockets with PRF leads to very favourable results when the bony walls are intact. A combina-

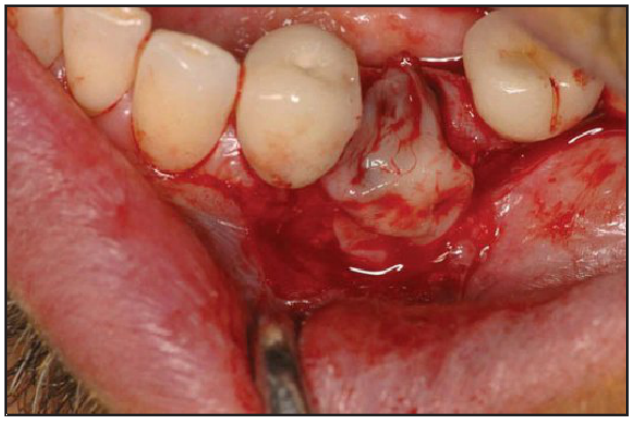


Figure 13: PRF membrane used to cover graft.

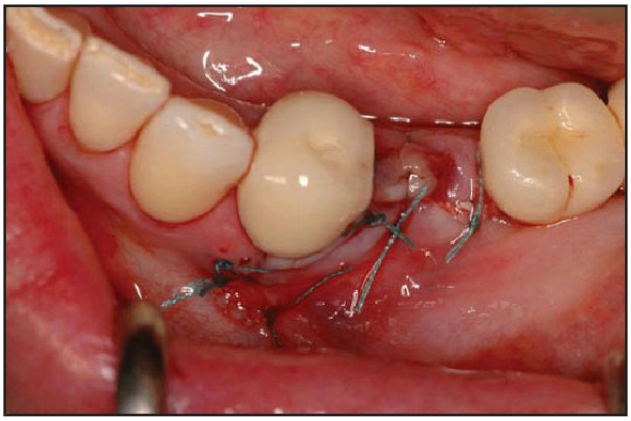


Figure 14: Closure of surgical site with some PRF membrane exposed.

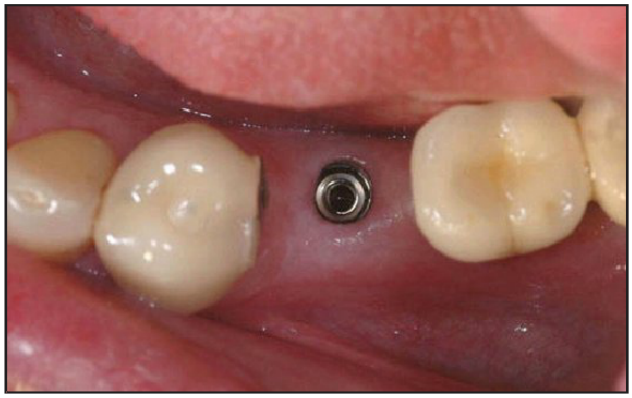


Figure 15: Healing at 3 months post-op.

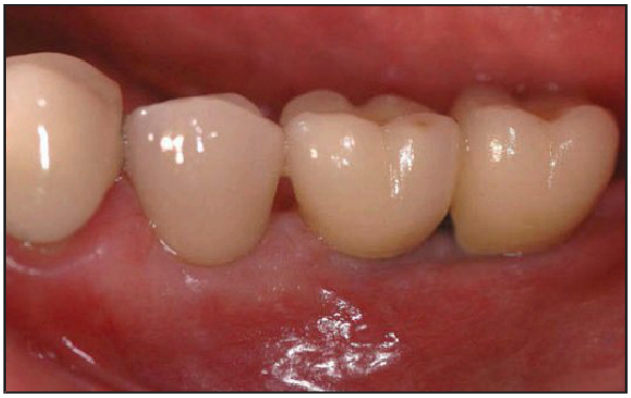


Figure 16: Final implant supported prosthesis.

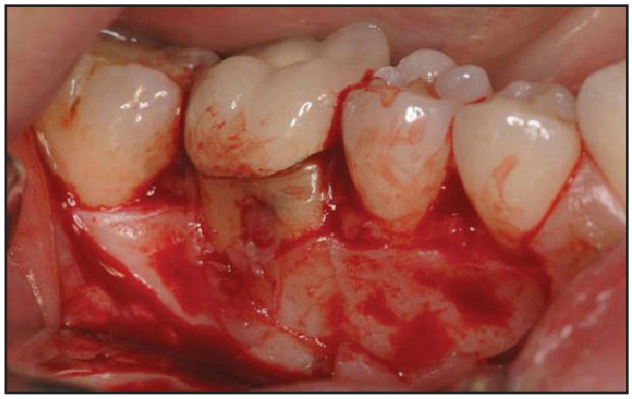


Figure 17: Pre-op view of hopeless tooth #30.

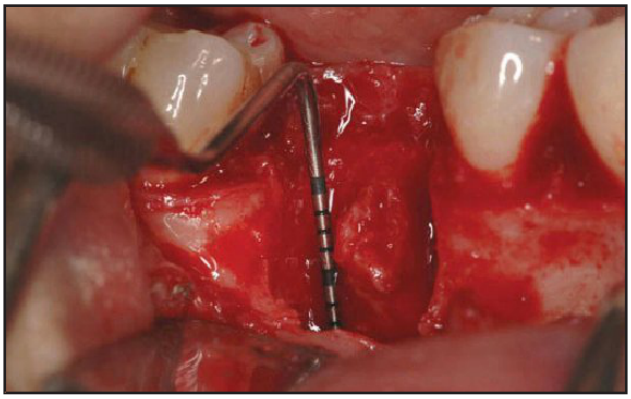


Figure 18: Resulting periodontal defect following removal of tooth #30.

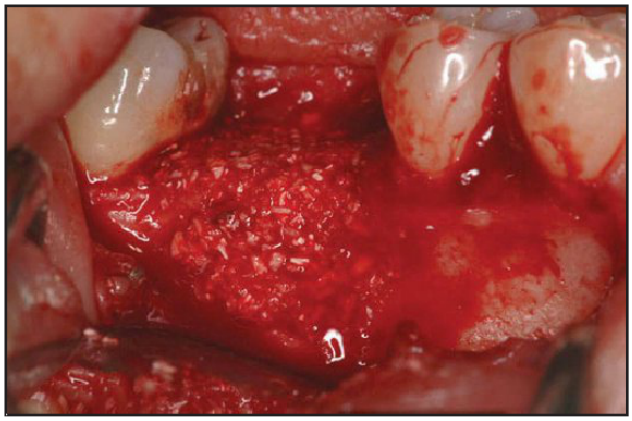


Figure 19: Bone graft + PRF mixture added to site #30.

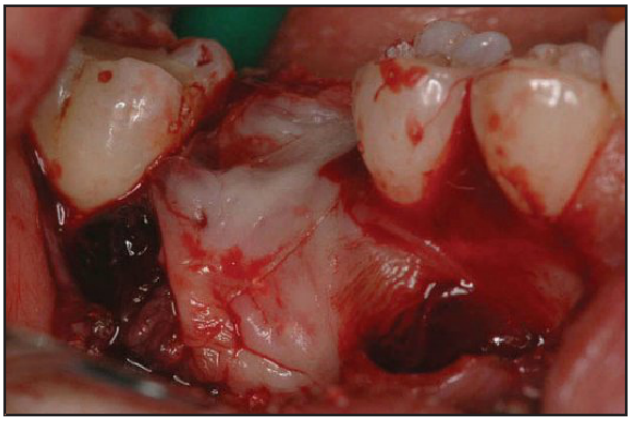


Figure 20: Graft covered with PRF membranes.

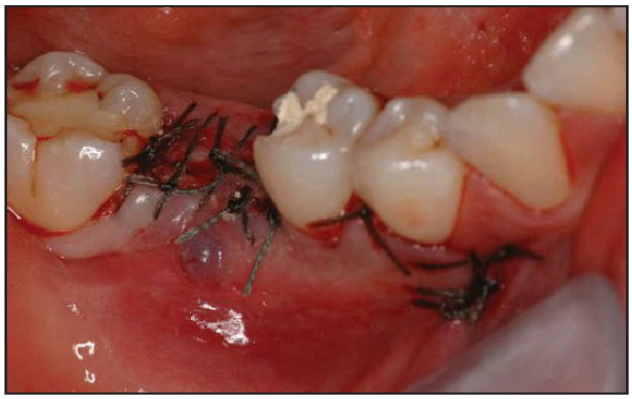


Figure 21: Mucoperiosteal flap closure.

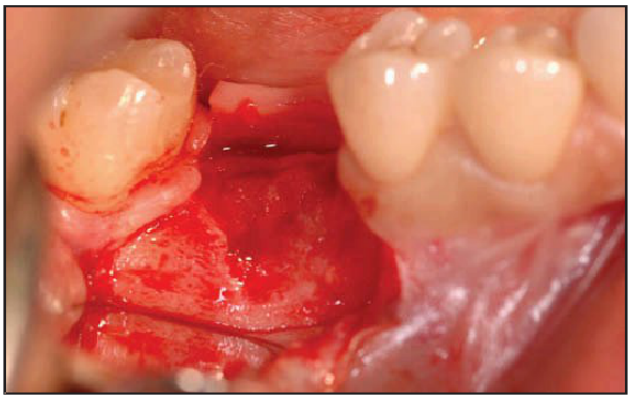


Figure 22: Bone healing at 3 months.

tion of PRF with bone substitutes and other adjuvants may be necessary in residual defects where one or several walls are missing or damaged in order to predictably provide an adequate reconstitution of bone volume.[17,18,29] In all cases, the systematic use of PRF leads, in our experience, to optimized gingival and bone regeneration which is particularly useful in implant site development.

CONCLUSIONS

The clinical benefits for the systematic use of PRF in daily practice are many. Inexpensive and simple to handle, this technique leads to the production of a large quantity of bioactive autologous membranes with a powerful healing potential on both soft and hard tissues. Its range of clinical applications in oral and maxillofacial surgery is wide; as a filling material or protective membrane, and often as both. Used as a covering membrane, PRF accelerates healing and closure of the wound margins, stabilizes graft materials, and protects the surgical site from external aggressions. It generally provides a perceptible reduction in superficial tissue healing time, and patients often declare reduced postoperative pain. Mixed with graft material,

PRF will serve as biological cement between the particles and enhance neoangiogenesis and bone regeneration, particularly in stimulating osteoblastic proliferation and differentiation. However, this material is only an optimized and usable blood clot. Its potential applications are broad, but an accurate working knowledge of the biomaterial, its biology, efficiency and limits are necessary to optimize its use in daily practice. Therefore, additional studies evaluating the use and performance of PRF are warranted.

Correspondence:
David M. Dohan Ehrenfest
Department of Biomaterials,
Institute for Clinical Sciences,
The Sahlgrenska Academy at
University of Gothenburg,
Medicinaregatan 8B, 41390
Gothenburg, Sweden
e-mail: LoB5@mac.com

Disclosure
The authors report no conflict of interest with any products mentioned in this article.

References

1. Clark RA. Fibrin and wound healing. Ann NY Acad Sci 2001; 936: 355-367.
2. van Hinsbergh VW, Collen A, Koolwijk P. Role of fibrin matrix in angiogenesis. Ann NY Acad Sci 2001; 936: 426-437.
3. Matras H. Effect of various fibrin preparations on reimplantations in the rat skin. Osterr Z Stomatol 1970; 67(9): 338-359.
4. Matras H. The use of fibrin sealant in oral and maxillofacial surgery. J Oral Maxillofac Surg 1982; 40(10): 617-622.
5. Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant? Transfusion 1990; 30(8): 741-747.
6. 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(11): 1294-1299.
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 Radiol Endod 1998; 85(6): 638-646.
8. 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(3): 158-167.
9. Dohan DM, Choukroun J. PRP, cPRP, PRF, PRG, PRGF, FC . How to find your way in the jungle of platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103(3): 305-306.
10. Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: the PRF (french). Implantodontie 2001; 42: 55-62.
11. Dohan DM, 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 Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101(3): e37-44.
12. Dohan DM, 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 Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101(3): e45-50.
13. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. 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(3): e51-55.
14. Dohan DM, Del Corso M, Charrier JB. Cytotoxicity analyses of Choukroun's platelet-rich fibrin (PRF) on a wide range of human cells: The answer to a commercial controversy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103(5): 587-593.
15. Del Corso M, Sammartino G, Dohan Ehrenfest DM. Choukroun's Platelet-Rich Fibrin (PRF) membranes in periodontal surgery: understanding the biomaterial or believing into the magic of growth factors ? J Periodontol 2009: In Press.
16. Diss A, Dohan DM, Mouhyi J, Mahler P. Osteotome sinus floor elevation using Choukroun's platelet-rich fibrin as grafting material: a 1-year prospective pilot study with microthreaded implants. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105(5): 572-579.
17. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part I: a new grafting protocol. Implant Dent 2009; 18(2): 102-111.
18. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of Choukroun's Platelet-Rich Fibrin (PRF) and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: implant surgery, prosthodontics and survival. Implant Dent 2009; 18(3): 220-229.
19. 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(1): 63-69.
20. Choukroun J, Braccini F, Diss A, Giordano G, Doglioli P, Dohan DM. Influence of platelet rich fibrin (PRF) on proliferation of human preadipocytes and tympanic keratinocytes: A new opportunity in facial lipostructure (Coleman's technique) and tympanoplasty? Rev Laryngol Otol Rhinol (Bord) 2007; 128(1-2): 27-32.
21. Dohan Ehrenfest DM, Diss A, Odin G, Doglioli P, Hippolyte MP, Charrier JB. In vitro effects of Choukroun's PRF (Platelet-Rich Fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes and maxillofacial osteoblasts in primary cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009: In Press.
22. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM. 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(3): e56-60.
23. Braccini F, Dohan DM. The relevance of Choukroun's platelet rich fibrin (PRF) during facial aesthetic lipostructure (Coleman's technique): preliminary results. Rev Laryngol Otol Rhinol (Bord) 2007; 128(4): 255-260.
24. Charrier JB, Monteil JP, Albert S, Collon S, Bobin S, Dohan Ehrenfest DM. Relevance of Choukroun's Platelet-Rich Fibrin (PRF) and SMAS flap in primary reconstruction after superficial or subtotal parotidectomy in patients with focal pleiomorphic adenoma: a new technique. Rev Laryngol Otol Rhinol (Bord) 2008; 129(5): 313-318.
25. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101(3): 299-303.
26. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. Sinus floor Augmentation With Simultaneous Implant Placement Using Choukroun's PRF (Platelet-Rich Fibrin) as sole grafting material: a radiological and histological study at 6 months. J Periodontol 2009: In Press.
27. Saadoun AP, Touati B. Soft tissue recession around implants: is it still unavoidable?--Part II. Pract Proced Aesthet Dent 2007; 19(2): 81-87.
28. Dohan Ehrenfest DM, Vazquez L. Pulling out, extraction or avulsion? Implant Dent 2008; 17(1): 4.
29. Choukroun J, Simonpieri A, Del Corso M, Mazor Z, Sammartino G, Dohan Ehrenfest DM. Controlling systematic perioperative anaerobic contamination during sinus-lift procedures by using metronidazole: an innovative approach. Implant Dent 2008; 17(3): 257-270.