ARTICLES SCIENTIFIQUES

Post extraction socket management using L-PRF and xenografts: a case report

Gestion de l'alvéole post-extractionnelle à l'aide de L-PRF et de xénogreffes : un rapport de cas

Hamed Harbi, Mohamed Tlili, Raki Selmi, Mootaz Mlouka, Faten Khanfir, Mohamed Salah Khalfi, Faten ben Amor

Faculty of Dentistry, Oral and Facial Rehabilitation Research Laboratory, Lr12es11, 5000 - Monastir (Tunisia)

Abstract

After tooth extraction, a marked resorption of the alveolar ridge occurs due to the tooth-bundle bone dependent relationship (1*), both horizontally and vertically. A more recent study (2*), with analysis of the alveolar ridge after tooth avulsion via cone beam computed tomography (CBCT) , reported even 3.5 times more severe bone resorption than the findings described in the existing literature. These changes in the global volume of the bone have to be taken into account when implants are planned .So the indication of a filling materiel to preserve the bone is imposing .Leukocyte- and platelet-rich fibrin (L-PRF) is an autologous platelet concentrate rich in growth factors and plasma proteins, obtained by centrifugation of patient whole blood without the addition of any additive or anticoagulants, and widely used in oral surgery and it can be exploited to improve the results obtained by BSMs only . This report describes two cases of alveolar ridge preservation (ARP) by using a L-PRF then a mixture between L-prf and BSMs as a filling materiel to a post extraction socket . two patients requiring tooth extraction and subsequent implant rehabilitation was treated with atraumatic extraction (piezosurgery) and socket filling. Within 3 months, a radiographic control (CBCT) and implant placement were performed.

Key words

Leukocyte- and platelet-rich fibrin (L-PRF) , bone substitute materials (BSMs) , alveolar socket, ridge preservation, piezo surgery

Résumé

Après l'extraction dentaire, une résorption marquée de la crête alvéolaire se produit en raison de la relation de dépendance entre la dent et l'os alvéolaire (1*), à la fois horizontalement et verticalement. Une étude plus récente (2*), avec une analyse de la crête alvéolaire après l'avulsion dentaire par tomographie à faisceau conique (CBCT), a rapporté une résorption osseuse 3,5 fois plus importante que les résultats décrits dans la littérature existante. La fibrine riche en leucocytes et en plaquettes (L-PRF) est un concentré plaquettaire autologue riche en facteurs de croissance et en protéines plasmatiques, obtenu par centrifugation du sang total du patient sans ajout d'additif ou d'anticoagulant, largement utilisé en chirurgie buccale et qui peut être exploité pour améliorer les résultats obtenus par les BSM uniquement. Ce rapport décrit deux cas de préservation de la crête alvéolaire par l'utilisation d'un L-PRF puis d'un mélange de L-PRF et de matériau de substitution osseuse (MSO) comme un matériau de comblement alvéolaire post- extractionnel. Deux patients nécessitant une extraction dentaire et une réhabilitation implantaire ont été traités par extraction atraumatique (piézochirurgie) et comblement alvéolaire. Après 3 mois, un contrôle radiographique (CBCT) et la pose d'un implant ont été effectués.

Mots clés

Fibrine riche en leucocytes et en plaquettes (L-PRF), matériaux de substitution osseuse (MSO), alvéole dentaire, préservation de la crête, la piézoéchirurgie.

INTRODUCTION

The avulsion of a tooth is systematically accompanied by an alveolar bone resorption. In order to preserve this bone this will allow us to use endosseous implants (1*, 2*), several techniques have been described. In particular, bone substitute materials (BSMs) (auto grafts, allografts, xenografts, or alloplastic materials) have been tested combined with resorbable or non resorbable membranes (3*, 4*). Despite their height potential to maintain bone volume, the quality of the bone after

healing, the risks of exposure of resorbable or non resorbable membranes and the financial conditions of the patient are not to be neglected when implants are planned.

Also all BSMs are processed non-cellularly and therefore contain only osteoconductive properties, whereas autologous bone (with no antigenic properties) is loaded with cells and growth factors that stimulate new blood vessel formation and trigger osteoinduction (5*)

The first generation of platelet concentrates, which included platelet rich plasma (PRP) (6*) and plasma rich in growth factors (PRGF) (7*), were developed. However, they had some disadvantages: expensive, operator dependent and extended production time. The second generation of platelet concentrates appeared to improve and ease the use of this technique (8*, 9*). Leukocyte- and platelet rich fibrin (L-PRF) belongs to the 2nd generation of platelet concentrates (9*) today the use of L-PRF allowed to define a new therapeutic concept called the Natural Bone Regeneration (NBR) for the reconstruction of the alveolar ridges at the gingival and bone levels after teeth extractions. The standard protocol to obtain it is that 9-10 ml blood is withdrawn from the patient in plastic/glass coated tubes through venepuncture. No anticoagulants or additives are used. The blood is immediately centrifuged at 400 g during 10-12 minutes. After centrifugation, 3 layers are obtained: at the bottom, red blood corpuscles (RBC); at the top, platelet-poor plasma (PPP); and in the middle, a fibrin clot (L-PRF). Once the clot is separated from the supernatant (PPP) and the red blood cells, it can be compressed into membranes by extracting injectable PRF (I-PRF). L-PRF contains a dense fibrin fibre network where platelets and leucocytes are enmeshed and it can serve as scaffold for other type of cells due to its favourable mechanical properties (10*, 11*). Its content in leucocytes and platelets results in a constant release of growth factors such as PDGF, transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF) for 7-14 days (11*, 12*). Those factors modulate reparative inflammatory process, increase tissue regeneration, angiogenesis, and neo vascularization; and reduce postoperative pain and oedema (13*).

L-PRF has been also used with as a mixture with BSMs. It was evaluated if the combination of PRF with BSMs in different clinical approaches not only functions as a signalling protein for osteoinduction but also allows the bone graft particles to stick together for better clinical handling (14*).

So, the aim of this work is to report tow cases of atraumatic extraction using piezosurgery in the first one the post extraction socket was grafted with L-PRF membranes and in the second it was filled by a combination: L-PRF and xenograft (bio-oss).

CLINICAL OBSERVATIONS

Case 1

B.N a 32-year-old non smoker systemically healthy patient was referred to the outpatient Department of monastir dental clinic, faculty of dental medicine of monastir (Tunisia).

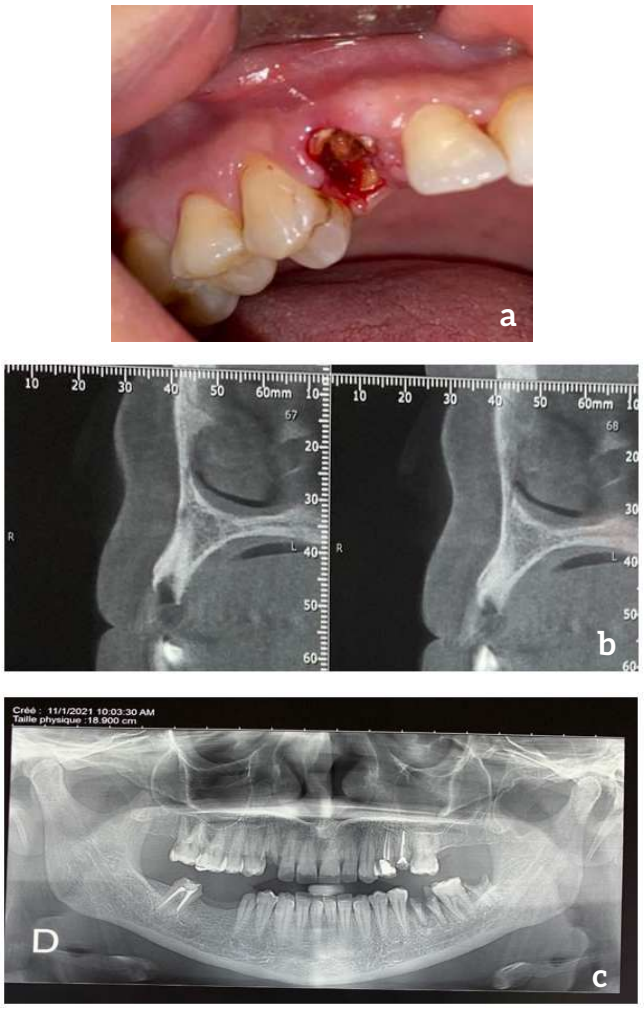

Figure 1 (a) Preoperative occlusal view; (b) Pre operative (CBCT); (c) Orthopantomography

The clinical exam revealed the remaining of multiple root tips which will be extracted. On the maxillary bone. The intraoral exam revealed a non restorable carious lesion in the upper right canine (Figure 1(a)), confirmed with orthopantomography (Figure 1(c)) and a CBCT (Figure 1(b)), in which a periapical periodontitis (granuloma) was highlighted. Before proceeding with tooth extraction, alternative therapeutic solutions, such

as orthodontic extrusion or crown lengthening followed by endodontic therapy and prosthetic rehabilitation, were explained to the patient. Considering the advantages and disadvantages of these therapies, in agreement with the patient, the treatment plan included tooth extraction and socket grafting with L-PRF membranes from blood centrifugation, followed by single unit implant rehabilitation. The patient was treated in accordance with the Declaration of Helsinki of 1975, as revised in 2013 .A written informed consent was obtained from the patient before the treatment. Seven days before tooth extraction, the patient was under gone scaling and root planning and trained to correct hygiene procedures.

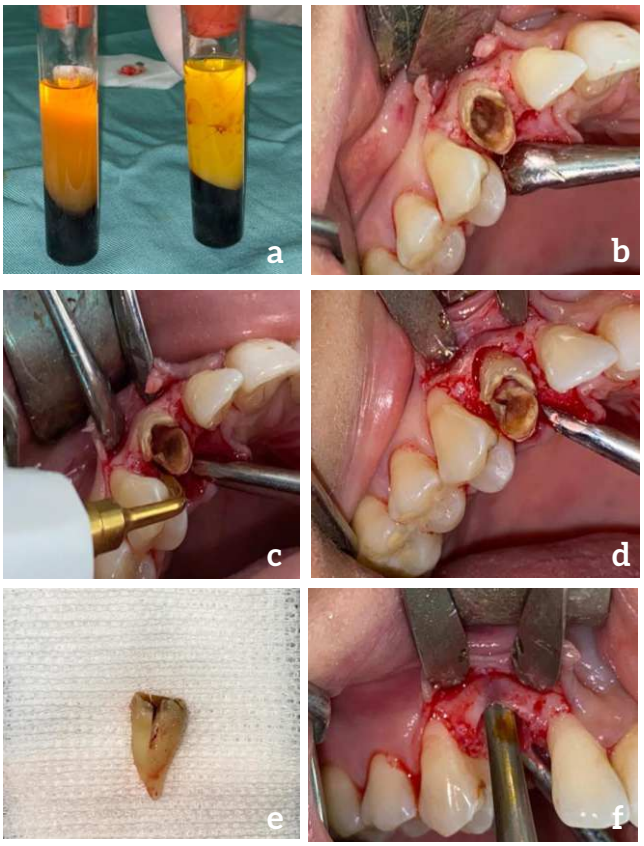

Figure 2
(a) Result of the centrifugation of the collected blood
(b) Per operative view of the root
(c) Insertion of the piezo device to dislocate the root
(d) Extrusion of the root tip
(e) Root tip after extraction
(f) Evaluation of the buccal cortical

Figure 3
(a) filling the alveolar socket with 2 membranes of L-PRF ; (b) post operative view after suture

On the day of the surgery, blood samples were collected from the patient in two tubes of 9mL for a few minutes and immediately centrifuged. Centrifugation (LC-04R centrifuge, scientific system) was performed for 12 minutes at 2700 rpm. Tubes did not contain any additive or anticoagulants. After centrifugation, L-PRF clots were collected from each tube (figure 2 (a)) and separated from the red thrombus (composed of red cells), obtaining a fibrin clot with a small red portion in order to include the intermediate portion rich in leucocytes and platelets (15*). L-PRF clots were then placed in a sterile box and slightly compressed for about 5 minutes under a stainless steel plate to form 2 membranes. Before surgery, the patient rinsed with 0.2% chlorhexidine for 1 minute and local anaesthesia (2% mepivacaine with 1 : 100000 adrenaline) was administered. Atraumatic extraction was performed using a piezoelectric device (figure 2 (b) and (c)) to preserve the buccal bone which was evaluated through CBCT (figure 1(b)) to be so fragile (thickness< 1mm). The root tip was extracted (figure 2 (d) and (e)), followed by alveolar debridement and irrigation with sterile saline solution. After tooth extraction, the buccal and palatal bone walls were both preserved (figure 2(f)) . The socket was then filled with one L-PRF membranes previously folded and condensed with sterile gauze. Finally, one membrane folded in triple layer was placed to cover the socket (figure 3 (a)). Soft tissues were sutured (3/0 polyglactin suture, 19mm needle) at the mesial, median, and distal aspects of the socket (figure 3 (b)).

The patient was prescribed an antibiotic /analgesic therapy (amoxicillin 3000mg/day; paracetamol 2000mg/day), anti septic mouth rinses (0.2% chlorhexidine) twice daily for 7 days), and post operative recommendations. The patient was advised to follow a soft and liquid diet, avoiding hot and spicy food for the following hours .Sutures were removed at 7 days, and the patient underwent a follow-up monitoring the wound healing.

Figure 4 CBCT of the alveolar ridge after 3 months

After 3 months a CBCT was realized to evaluate the Alveolar ridge width in the buccal-palatal direction which was 9mm immediately after extraction (figure 1 (b)) and 8 mm at 3 months(figure 3) showing a minimal reduction.

Case 2

M.K a 28 years old male, non smoker and systemically healthy was also referred to the outpatient Department of monastir dental clinic, faculty of dental medicine of monastir (Tunisia) in order to restore the upper left central incisor.

Figure 5 (A) Preoperative Occlusal view; (B) Preoperative buccal view

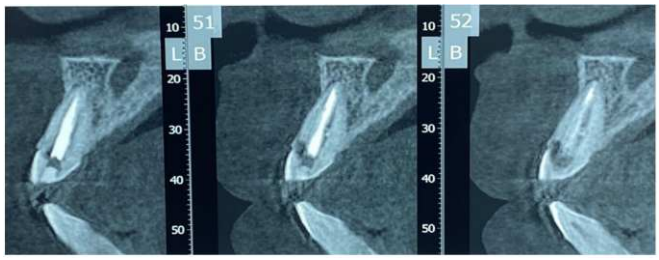

Figure 6 Preoperative CBCT scan

The clinical exam revealed an ancient trauma on the upper left central incisor with a fracture line in the upper third of the crown (figure 5). The tooth was repeatedly repaired using adhesive materials after endodontic treatment but it always fall down. The CBCT scan (figure 6) revealed an external radicular resorption which may affect the buccal cortical bone . Under those circumstances and with the agreement of the patient, the therapeutic solution was an atraumatic extraction of the root tip, alveolar ridge preservation with filling materials and finally implant rehabilitation after healing.

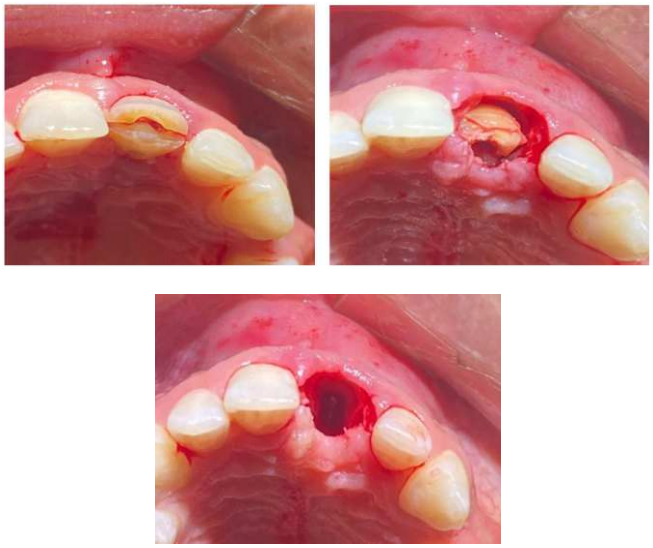

Figure 7 Extraction protocol (A) ; Misodistal Separation ; (B) Luxation; (C) Post extraction view

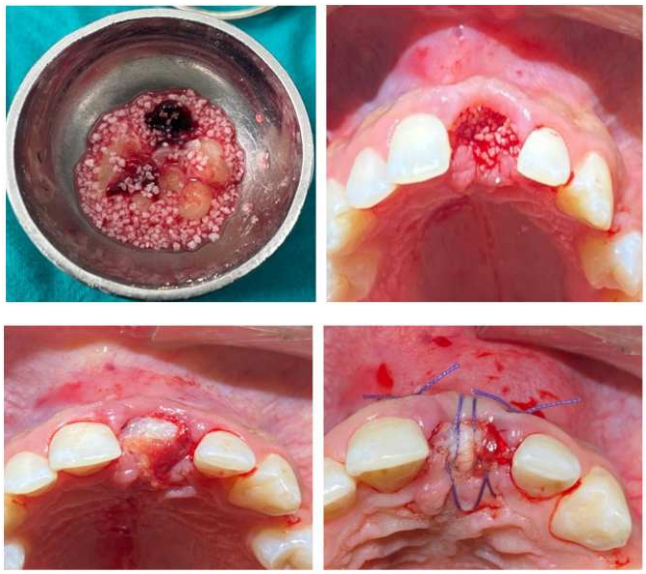

Figure 8 (A) Preparation of bone substitutes and L_PRF ; (B) Alveolar ridge filling ; (C) Using a second L_PRF; (D) Post operative view

On the day of the surgery the same protocol was followed to prepare 3 L-prf membranes.
A piezo device was used to atraumatically extract the root tip (figure 7). Then two L-prf membranes was mixed with 1g of xenograft (figure 8 (A)), the mixture was placed into the gap (figure 8 (B)) and a third membrane was used as a shield to cover it (figure 8 (C)). Finally, a tensionless suture was performed to protect the wound (figure 8 (D)).

DISCUSSION

The healing of alveolar socket is characterised by a reducing in bone height and width which are the most common limitations for implant placement. To overcome these limitations the management of the alveolar socket is unavoidable to preserve this volume. The study from Temmerman et al. reported

a statistically significant difference in the reduction of total width of the alveolar ridge when L-PRF is used in comparison with natural healing. Likewise, the radiographic evaluation reported a significant difference in the socket fill (16*, 17*).

Alveolar ridge preservation (ARP) is a relatively new surgical procedure aimed at retaining maximum bone and soft tissue after a tooth has been removed. It includes several techniques which have been proposed (18*). Though graft materials (auto grafts, allograft, xenografts, or alloplastic materials) were proven to reduce alveolar bone resorption, they often have poor vascularization and less vital bone formation compared to spontaneous healing because it´s not totally incorporated into the newly formed bone so when compared to sites without graft material, they show less vital bone formation (19*) but those points could be overcame by addition of L-prf to BSMs . In some cases ARP requires the use of collagen membranes. In these cases a 25% membrane exposure rate has been reported, and this directly affects the amount of bone fill that takes place within the socket (18*). Finally the high costs and the risk of disease transmission often limit the use of biomaterials in the clinical practice. Leukocyte- and platelet-rich fibrin (L-PRF) was first described by Dohan et al. in 2006 (11*). It is considered a second-generation platelet concentrate obtained by centrifugation of patient whole blood without the addition of any additive or anticoagulants, thereby eliminating the possibility of disease transmission or foreign body reactions

. With this kind of preparation technique, at least 95% of platelets are embedded into the fibrin network of L-PRF (20*). High concentrations of platelets allow the slow release of growth factors from their alpha granules (21*). These growth factors include Platelet-Derived Growth Factor (PDGF), Vascular Endothelium Growth Factor (VEGF), Transforming Growth Factor-beta (TGF-beta), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Insulin-like Growth Factor (IGF), and Hepatocyte Growth Factor (HGF). All of these factors have an important role in wound healing (soft and hard tissues). It modulate reparative inflammatory process, increase tissue regeneration, angiogenesis, and neo vascularization; and reduce postoperative pain and oedema (9*). As a result L-PRF has been used in different surgical procedures, such as oral and maxilla facial surgery and periodontal surgery such as root coverage procedures (22*), single and multiple gingival recession defects (23*) and sinus augmentation procedures (24*), in order to enhance wound healing process. The results of this report are consistent with those found in other studies (24*, 25*), where L-PRF showed to be effective in preserving hard and soft tissues without interfering with physiological bone healing process. Ridge preservation techniques are always desirable when functional and aesthetic results are demanded. The beneficial effects of L-PRF in reducing dimensional changes may derive from its capacity to promote tissue regeneration. The dimensional remodelling observed in the first case is comparable to those reported with other techniques (26*) where bone substitutes were used. However, a recent randomized controlled trial (27*)has suggested that PRF may not significantly enhance bone formation after tooth extraction as compared to spontaneous healing even though studies with larger population will be required to reach valid conclusions.

The uses of L-PRF is growing day after day due to its simple procedure of preparation, affordable cost ,and minimum risk of infection, In addition, as an autologous product, there is no risk of disease transmission or graft infection.

It has been also used to overcome the limitations of using BSMs previously mentioned.

the pro-angiogenic effect of the PRF has been demonstrated to mainly impact soft tissue regeneration procedures (28*,29*), but the complex interplay of different cytokines and growth factors leads to an increased proliferation and differentiation of different cell lines, inter alia, osteoblasts (30*,31*)

A biological connector between the different parts of the bone graft material is obtained when it is mixed with A-PRF and i-PRF. PRF assists angiogenesis, engagement of the stem cells and migration of osteogeneous cells in the central part of the graft.(32*,33*)

Mixing of bone graft material with pieces of PRF and its infiltration with i-PRF leads to delivery of growth factors inside the wound, which helps the migration of osteopromotor cells and attracts circulating stem cells to the wound (fast angiogenesis).Adding PRF to the bone graft reduces the required bone graft material volume and improves its manipulative qualities. The use of PRF accelerates the healing of hard and soft tissues, and the use of PRF membrane instead of another barrier membrane reduces the cost of the procedure.

CONCLUSION

PRF can be administered in different applications in oral surgery and can promote adequate soft tissue healing and bone regeneration in association or with BSMs. Most of the included studies showed superior outcome results in PRF-groups over non-PRF groups at extraction sockets. The use of L_PRF membranes to fill the socket after tooth extraction led to improved alveolar bone healing with a better preservation of the alveolar crest width and work in synergy with BSMs. Of note, the surgical procedure seems to be as important as the grafting material. These data could have a major impact on the recommended surgical procedure for tooth extraction before implant placement given the important role played by the surrounding bone micro architecture in the process of implant osseointegration.

REFERENCES

1 Araujo , M. G. &Lindhe, J. (2005). Dimensional ridge alterations following tooth extraction. An experimental study in the dog. Journal of Clinical Periodontology, 32, 212-218.*

2 Chappuis, V., Engel, O., Reyes, M., Shahim, K., Nolte, L. P. & Buser, D. (2013). Ridge alterations post-extraction in the esthetic zone: a 3D analysis with CBCT. Journal of Dental Research, 92, 195S-201S.*

3 D. Laurito, M. Lollobrigida, F. Gianno, S. Bosco, L. Lamazza, and A. De Biase, "Alveolar ridge preservation withn c-HA and d-PTFE membrane: a clinical, histologic, and histo morphometric study," International Journal of Periodontics and Restorative Dentistry, vol. 37, no. 2, pp. 283–290, 2017.*

4 D. Laurito, R. Cugnetto, M. Lollobrigida et al.,"Socket preservation with d-PTFE membrane: histological analysis of the newly formed matrix at membrane removal," International Journal of Periodontics and Restorative Dentistry, vol. 36, no. 6, pp. 877–883, 2016.*

5 Titsinides, S.; Agrogiannis, G.; Karatzas, T. Bone grafting materials in dentoalveolar reconstruction: A comprehensive review. JPN. Dent. Sci. Rev. 2019, 55, 26–32. [CrossRef] [PubMed]*

6 Marx, R. E. (2001). Platelet -rich plasma (PRP): what is PRP and what is not PRP? Implant Dentistry, 10, 225 -228*

7 Anitua, E. (1999). Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. International Journal of Oral and Maxillofacial Implants, 4, 529-535*

8 Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., Dohan, A. J., Mouhyi, J. &Dohan, D. M. (2006). Platelet-richfibrin (PRF): a second-generation platelet concentrate. Part V:histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 101, 299-303.*

9 Dohan, D. M., Andia, I., Zumstein, MA., Zhang, C. Q., Pinto, N. R. &Bielecki, T. (2014). Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopaedic and sports medicine: current consensus, clinical implications and perspectives. Muscles Ligaments Tendons Journal, 4, 3-9.*

10 Khorshidi, H., Raoofi, S., Bagheri, R. &Banihashemi, H. (2016). Comparison of the Mechanical Properties of Early Leukocyte- and Platelet-Rich Fibrin versus PRGF/Endoret Membranes. International Journal of Dentistry, doi: 10.1155/2016/1849207.*

11 Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J. &Gogly, B. (2006b). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II:platelet-related biologic features. Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics, 101, e45-50.*

12 Schär, M. O., Diaz -Romero, J., Kohl, S., Zumstein, M. A. & Nesic, D. (2015). Platelet - rich concentrates differentially relase growth factors and induce cell migration in vitro. Clinical Orthopaedics and Related Research, 5, 1635 - 1643*

13 J. Choukroun, A. Diss, A. Simonpieri et al., "Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing," Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology, vol. 101, no. 3, pp. e56–e60, 2006*

14 Thanasrisuebwong, P.; Kiattavorncharoen, S.; Surarit, R.; Phruksaniyom, C.; Ruangsawasdi, N. Red and Yellow Injectable PlateletRich Fibrin Demonstrated Differential Effects on Periodontal Ligament Stem Cell Proliferation, Migration, and Osteogenic Differentiation. Int. J. Mol. Sci. 2020, 21, 5153. [CrossRef] [PubMed]

15 D. M. Dohan Ehrenfest, G. M. de Peppo, P. Doglioli, and G. Sammartino, "Slow release of growth factors and thrombospondin-1 in Choukroun'splatelet-richfibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies," Growth Factors, vol. 27, no. 1, pp. 63–69, 2009.*

16 Temmerman A, Vandessel J, Castro A, Jacobs R, Teughels W, Pinto N, Quirynen M. The use of leucocyte and platelet-rich fi brin in socket management and ridge preservation: a split-mouth, randomized, controlled clinical trial. J Clin Periodontol 2016;43:990-9.*

17 Marenzi G, Riccitiello F, Tia M, di Lauro A, Sammartino G. Infl uence of leukocyte- and platelet-rich fi brin (l-prf) in the healing of simple postextraction sockets: a split-mouth study. Biomed Res Int 2015;2015:369273.*

18 I. Darby, S. T. Chen, and D. Buser, "Ridge preservation techniques for implant therapy," The International Journal of Oral & Maxillofacial Implants, vol. 24, pp. 260–271, 2009.*

19 J. M. Iasella, H. Greenwell, R. L. Miller et al., "Ridge preservation with freeze-dried bone allograft and a collagen membrane compared to extraction alone for implant site development: a clinical and histologic study in humans," Journal of Periodontology, vol. 74, no. 7, pp. 990–999, 200*

20 D. M. Dohan Ehrenfest, M. Del Corso, A. Diss, J. Mouhyi, and J. B. Charrier, "Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane," Journal of Periodontology, vol. 81, no. 4, pp. 546–555, 2010.*

*21 * C. Y. Su, Y. P. Kuo, Y. H. Tseng, C. H. Su, and T. Burnouf, "In vitro release of growth factors from platelet-rich fibrin (PRF): a proposal to optimize the clinical applications of PRF," Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology, vol. 108, no. 1, pp. 56–61, 2009.*

22 T. Ramakrishnan, R. Vijayalakshmi, E. Pameela, K. Anilkumar, A. Geetha, and Umasudhakar, "Platelet-rich-fibrin: a novel root coverage approach," Journal of Ind*

23 M. Del Corso, G. Sammartino, and D. M. Dohan Ehrenfest, "Re: clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study," Journal of Periodontology, vol. 80, no. 11, pp. 1694–1697, 2009, author replay pp. 1697–1699*

24 A. B. Castro, N. Meschi, A. Temmerman et al., "Regenerative potential of leucocyte- and platelet-rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review," Journal of Clinical Periodontology, vol. 44, no. 2, pp. 225–234, 2017*

25 M. Del Corso, A. Vervelle, A. Simonpieri et al., "Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 1: periodontal and dentoalveolar surgery," Current Pharmaceutical Biotechnology, vol. 13, no. 7, pp. 1207– 1230, 2012*

26 S. Das, R. Jhingran, V. K. Bains, R. Madan, R. Srivastava, and I. Rizvi, "Socket preservation by beta-tri-calcium phosphate with collagen compared to platelet-rich fibrin: a clinicoradiographic study," European Journal of Dentistry, vol. 10, no. 2, pp. 264–276, 2016.*

27 K. Areewong, M. Chantaramungkorn, and P. Khongkhunthian, "Platelet-rich fibrin to preserve alveolar bone sockets following tooth extraction: a randomized controlled trial," Clinical Implant Dentistry and Related Research, vol. 21, no. 6, pp. 1156–1163, 2019*

28 Ghanaati, S.; Herrera-Vizcaino, C.; Al-Maawi, S.; Lorenz, J.; Miron, R.J.; Nelson, K.; Schwarz, F.; Choukroun, J.; Sader, R. Fifteen Years of Platelet Rich Fibrin in Dentistry and Oromaxillofacial Surgery: How High is the Level of Scientific Evidence? J. Oral Implant. 2018, 44, 471–492. [CrossRef]

29 Blatt, S.; Burkhardt, V.; Kämmerer, P.W.; Pabst, A.M.; Sagheb, K.; Heller, M.; Al-Nawas, B.; Schiegnitz, E. Biofunctionalization of porcine-derived collagen matrices with platelet rich fibrin: Influence on angiogenesis in vitro and in vivo. Clin. Oral Investig. 2020, 24, 3425–3436. [CrossRef]

30 Miron, R.J.; Zucchelli, G.; Pikos, M.A.; Salama, M.; Lee, S.; Guillemette, V.; Fujioka-Kobayashi, M.; Bishara, M.; Zhang, Y.; Wang, H.-L.; et al. Use of platelet-rich fibrin in regenerative dentistry: A systematic review. Clin. Oral Investig. 2017, 21, 1913–1927. [CrossRef]

31. Dohle, E.; El Bagdadi, K.; Sader, R.; Choukroun, J.; Kirkpatrick, C.J.; Ghanaati, S. Platelet-rich fibrin-based matrices to improve angiogenesis in an in vitro co-culture model for bone tissue engineering. J. Tissue Eng. Regen. Med. 2018, 12, 598–610. [CrossRef]

32. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fi brin: a new concept for cell-based tissue engineering by means of infl ammatory cells. J Oral Implantol 2014;40(6):679-89.
33. Mourao C, Valiense H, Melo R, et al. Obtention of injectable platelets rich-fi brin (i-PRF) and its po- 366 I. Chenchev et al Folia Medica I 2017 I Vol. 59 I No. 3 lymerization with bone graft: technical note. Rev Col Bras Cir 2015;42(6):421-3.