Factors affecting the preparation, constituents, and clinical efficacy of leukocyte- and platelet- rich fibrin (L-PRF).

SADJ August 2016, Vol 71 no 7 p298 - p302

MT Peck[1], D Hiss[2], L Stephen[3]

ABSTRACT

Platelet-rich fibrin (PRF) was first introduced by Choukroun *et al*, in 2001 as a method of concentrating autologous human leukocytes, platelets and fibrin for autotransplantation into surgical wound sites to accelerate healing. Even though several clinical reports have documented the use of L-PRF, controversy still exists with regards to many aspects of this biomaterial. Diverse publications report the use of non-standardised methods to prepare L-PRF, resulting in variable clinical results. The impact of the type of centrifuge, as well as of the growth factor release kinetics, have recently been studied and have yielded new insights into the structure and function of L-PRF. The presence of bone morphogenetic proteins as well as stem cells has also been documented. In this report we analyse various factors affecting L-PRF preparation and its constituents and highlight some of the controversies surrounding the biomaterial.

INTRODUCTION

Platelet-rich fibrin (PRF) was first introduced by Choukroun *et al*. in 2001 as a method of concentrating autologous human leukocytes, platelets and fibrin for autotransplantation into surgical wound sites to accelerate healing.[1] This method of concentrating blood platelets was different to previous techniques in that it centrifuged the collected blood only once, no anticoagulant agents were added, and leukocytes and fibrin were deliberately included in the final product. Previous similar techniques had sought to concentrate platelets only, with little consideration for the other constituents.[2,3] Choukroun's protocol (Process protocol, Nice, France) was simple and essentially consisted of collecting venous blood into dry glass tubes, after which the tubes would be spun at a low centrifuge speed to allow the blood to separate into the constituents.[4] This resulted in three distinct layers forming in the blood collecting tube, i.e. a red blood cell layer at the bottom of the tube, an acellular layer at the uppermost part of the tube, and a leukocyte- and platelet-rich fibrin (L-PRF) layer formed in the middle of the tube.[4] The L-PRF layer was considered as the active biomaterial and has, since its development, been promoted as an agent that accelerates wound healing and tissue regeneration.[5] Even though several clinical reports have documented the use of L-PRF in oral and extra-oral surgical procedures, controversy still exists with regards to several aspects of this biomaterial. In this report we set out to highlight some of these debates.

1. **Mogammad Thabit Peck:** Department of Oral Medicine and Periodontology, University of the Western Cape.
2. **Donavon Hiss:** Department of Medical Biosciences, University of the Western Cape.
3. **Lawrence Stephen:** Department of Oral Medicine and Periodontology, University of the Western Cape.

Corresponding author

Mogammad Thabit Peck:
Department of Oral Medicine and Periodontology, Faculty of Dentistry, University of the Western Cape, Private Bag X1 Tygerberg, 7505, South Africa. Tel: 021 9373186. Fax: 086 618 7560.
E-mail: mpeck@uwc.ac.za

ACRONYMS

BMPs:	bone morphogenic proteins
G-CSF:	granulocyte colony-stimulating factor
HUVEC:	human umbilical vein endothelial cells
IGF-1:	insulin-like growth factor-1
MSC:	mesenchymal stem cells
PRF:	platelet-rich fibrin
PDGF-AB:	platelet derived growth factor AB
RCF:	relative centrifugal force
RPM:	revolutions per minute
VEGF:	vascular endothelial growth factor

The terminology and classification of L-PRF

In an attempt to distinguish various platelet concentrates from each other, Dohan Ehrenfest *et al*. used three key parameters i.e; the preparation process, the pharmacological properties, and the characteristics of the final material to establish a functional classification.[6] By applying specific criteria to these parameters, the authors were able to classify platelet concentrates into four distinct categories (Table 1).[6]

Table 1: Categories of platelet concentrates as proposed by Dohan Ehrenfest *et al*,[6]

1. Pure platelet-rich plasma (P-PRP)
2. Leucocyte- and platelet-rich plasma (L-PRP)
3. Pure platelet-rich fibrin (P-PRF)
4. Leukocyte- and platelet-rich fibrin (L-PRF)

Although this classification elucidates and simplifies the distinction of various platelet concentrates, it is not the only existing proposed system to classify platelet concentrates.[7] However, Dohan Ehrenfest's classification is widely quoted in the literature and at the time of its publication, in 2009, Choukroun's PRF was the only platelet concentrate included in the category of L-PRF.

As the popularity of Choukroun's protocol for the production of L-PRF grew, publications appeared describing processes that purported to produce L-PRF. However, none had applied the exact criteria as described by Choukroun (Process protocol, Nice, France).[8-11] It is unclear whether L-PRF produced by other than the Choukroun method can be classified as a true L-PRF.[4] Publications incorrectly use the terms L-PRF and Choukroun's PRF interchangeably, even though the exact method as described by Choukroun has not been used to produce the platelet concentrate.[12] This has lead to incorrect assumptions and a clear, unequivocal, classification of platelet concentrates that is universally accepted is therefore sought. For the purposes of clarity, the following proposed terminology will be used throughout this article:

- L-PRF – Leukocyte- and platelet-rich fibrin. Defined as a broad and all-inclusive category that is used to describe a mixed platelet, leukocyte and fibrin concentrate prepared using no-anticoagulants and a single spin centrifuge technique.
- L-PRF (C) – Leukocyte- and platelet-rich fibrin (Choukroun type). Defined as a specific leukocyte and platelet-rich fibrin prepared using Choukroun's protocol i.e. (*the equipment and the preparation method follows the exact recommended protocol as outlined by Choukroun*[1]).
- L- PRF (I/E) – Leukocyte- and platelet-rich fibrin (Intraspin/ EBA 20 type). Defined as a specific leukocyte and platelet-rich fibrin prepared using either an Intraspin (Intra-Lock International, Boca-Raton, FL, USA), or EBA 20 (Andreas Hettich GmbH & Co KG, Tuttlingen, Germany) centrifuge and following the recommended protocol as outlined by Dohan Ehrenfest *et al*.[4]
- L-PRF (O) – Leukocyte- and platelet-rich fibrin (Other). Defined as a leukocyte- and platelet-rich fibrin prepared in a manner similar to L-PRF (I/E) and L-PRF(C) production, but using a non-purpose-built centrifuge.

TECHNIQUES AND METHODS OF PRODUCING L-PRF

Choukroun's method of producing L-PRF was intended to be a simple technique that would allow for the production of high quality platelet and leukocyte concentrates, which could be prepared easily and used in everyday healthcare facilities.[4] This method specified the use of a PC-02 table centrifuge and a collection kit from Process (Nice, France).[4] Further, the blood sample was to be taken without anticoagulant in 10-mL blood collecting tubes which were then immediately centrifuged at 3000 revolutions per minute (RPM) (approximately 400g of relative centrifugal force (RCF)) for 10 minutes. The formed L-PRF clot could then be removed from the blood collecting tube and used as required.

The influence of centrifuge type and RCF on L-PRF

Even though Choukroun's protocol was clearly outlined, a number of publications were subsequently produced that reported on procedures which did not follow the prescribed methods.[13-15] Key to these differences was the failure to use a specific centrifuge (PC-O2, Process, Nice, France) and a specific RCF (400g). In many publications, the RCF was not reported, and instead the centrifuge speed and time was quoted.[13-15] This is a significant deviation from the protocol since the influence of the RCF is underestimated and not considered.

Relative centrifugal force (RCF) can be defined as the amount of accelerative force applied to a sample in a centrifuge.[16] It is not equivalent to revolutions per minute (RPM) and the terms cannot be used interchangeably. Centrifuges work by putting samples in rotation around a fixed axis, thereby applying an accelerative force perpendicular to the axis.[16] This resultant force causes the separation of various elements in the sample based on the individual weight of the sample elements and is the basis for blood separation techniques carried out by laboratory centrifuges. RCF is measured in multiples of the standard acceleration due to gravity at the Earth's surface (x g) and is based on two specific variables i.e. how wide the rotor is and how fast it is moving.[16] The radius of the centrifuge or rotor is as critical to the process of producing a specific RCF as is the RPM. Only those processes where the RPM and the rotor radius are identical are comparable and any deviations from these criteria may result in inaccuracies. Consequently, RCF will only be constant for centrifuges with the same rotor radii. Results derived from investigations using centrifuges with different radii will produce differing RCF's.[16] Therefore one cannot assume that all centrifuges used for producing L-PRF and spinning at 3000 RPM will produce an RCF of 400g. This is a significant parameter that is often misunderstood. RCF is critical to the production of L-PRF and must be calculated for each centrifuge used, especially if this parameter is not pre-set on the machine.

The effect of varying RCF's during platelet concentrate preparation was recently reported.[17] Amable, Carias, Teixeira *et al*. analysed various factors affecting the preparation of Platelet-rich plasma (PRP), and showed that changes in RCF significantly influenced the platelet yield even though other parameters such as period of centrifuge time as well as temperature remained constant. Dhurat and Sukesh reviewed several PRP preparation methods.[18] Based on their analysis, it was shown that the use by authors of different RCF parameters resulted in variations in the platelet yields of the PRP produced. More pertinently, scrutiny of the literature reveals that although PRP has been clinically used for several years, no standardised preparation protocol has yet been documented. With regards to the preparation methods of L-PRF that are published in the literature, similar inconsistencies exist.

Other centrifuge parameters that may influence L-PRF preparation.

A series of articles recently published by a team of authors reported on investigations into the effect of various parameters on the quality of the resultant L-PRF's.[19-21] Using the same centrifugal force (400g) as well as the same type of blood collecting tubes, the authors tested four different commercially available L-PRF centrifuges. The results indicated that centrifuge vibration as well as centrifuge type significantly affect the quality and quantity

of the L-PRF clot produced. Under scanning electron microscope (SEM) analysis, the L-PRF clots produced from the different centrifuges showed variations in cell morphology and fibrin architecture, with some cells showing signs of significant damage. These differences were attributed to the type of centrifuge used.[19-21] The Intra-Spin L-PRF centrifuge (Intra-Lock International, Boca-Raton, FL, USA) produced clots displaying cells with the most stable and normal shape.[20] It is therefore critical that identical processes should be followed if the biomaterial product is to be standardised. Researchers cannot simply recreate the biomaterial by using any centrifuge with a setting of 400g RCF even at the appropriate spin time.

Growth factors and their release kinetics

The preferred use of L-PRF in clinical practice is largely due to its reported release of autogenous growth factors. It is assumed that the high concentration of these growth factors results in reduced healing time as well as the stimulation of tissue regeneration.[8] These growth factors have been well documented in the literature.[22] Recently however, the release kinetics of these growth factors has been questioned.[23] Schär *et al*. prepared L-PRF (I/E) with a single spin protocol at 400g for 12 minutes using an EBA 20 (Andreas Hettich GmbH & Co KG, Tuttlingen, Germany) centrifuge.[23] This is the same machine, recently upgraded, as the Intra-Spin L-PRF centrifuge (Intra-Lock International, Boca-Raton, FL, USA).[24-26] The authors compared the release of various growth factors from L-PRF, L-PRP and a coagulated blood clot. The results demonstrated that the total growth factor release of vascular endothelial growth factor (VEGF) as well as of interleukin-1β (IL-1β) was higher from the blood clot than from any of the platelet concentrates. Furthermore, no statistically significant differences could be established between the blood clot and the various platelet concentrates as regards the amounts of insulin-like growth factor-1 (IGF-1) and of platelet-derived growth factor AB (PDGF-AB) that were released. The L-PRF (I/E) clot released the highest concentrations of transforming growth factor β1 (TGF-β1). When the release kinetics of L-PRF (I/E), L-PRP and the blood clot were investigated, the researchers found that the various growth factors were released at different times as well as for different lengths of time. An examination of this effect on the migration on human bone marrow-derived mesenchymal stem cells (MSC) and human umbilical vein endothelial cells (HUVEC) found no significant differences in the overall patterns of migration for any of the groups tested. However it was reported that IGF-1 had a positive correlation with the migration of both cell types whereas PDGF-AB had a negative correlation with both cell types i.e. MSC and HUVEC. It may be of relevance that IGF-1 had the highest concentration in the blood clot and that there were no differences in the release kinetics of this growth factor when compared with those of L-PRF and L-PRP.[23]

In a similar study, in which the release of growth factors as well as the effect of platelet concentrates on tendon cells were compared with those of a whole blood clot, it was shown that the platelet concentrates had the ability to significantly increase cell proliferation as compared with that of the blood clot.[27] However, it must be pointed out that the techniques of preparing these platelet concentrates were completely different between the two studies, thereby influencing the final architecture and possible biological properties of the various concentrates.

Bone morphogenic proteins

Bone morphogenic proteins (BMPs) are low molecular weight glycoproteins that are responsible for ectopic bone formation.[28] First described in the 1960's, these proteins play a critical role in various aspects of cell function, differentiation and tissue repair. More significantly, they are crucial in the maintenance of skeletal integrity and bone fracture healing. BMPs are released and synthesised by a number of cells including osteoblasts, osteoprogenitor cells, chondrocytes, platelets and macrophages.[28,29] It is therefore clear that the synthesis of these key proteins is not restricted to bone forming cells.

It has recently been shown that L-PRF (I/E) releases BMP-2 over a period of seven days, but that the amounts released are relatively small.[21] Dohan Ehrenfest *et al*. found it difficult to explain the exact origin of these BMPs, but suggested it was related to the presence of leukocytes in the platelet concentrate.[21] However it had been shown previously that the platelets themselves contain significant amounts of BMP-2 and that the release of these proteins is pH dependent.[30] As a result, it has been suggested that the release of BMP-2 by platelets may play a significant role in the initial stages of bone fracture healing, since the pH in this environment is optimal for platelet activation.[30]

Other researchers have found that other BMPs such as BMP-6, BMP-7 and BMP-4 are also released by platelets, and, further, that the concentration of BMPs contained in platelets is patient dependent.[31,32] It has also been shown, by genome-wide micro analysis, that lysed platelets have the ability to upregulate proliferative pathways of osteoblast like cells *in-vitro*.[33]

The potentially ground-breaking findings from various studies investigating the BMP potential of L-PRF as well as its variants must be seen in the light of patient variation as well as the pH of the test environment.[21,28,30,32] Further research into these factors may have clinical implications and could explain the reasons for the inconsistent clinical outcomes experienced when using platelet-rich concentrates for bone grafting or regeneration. By implication then, it would currently be difficult to control the amounts of BMP's released from platelets when used in a clinical setting.

Stem cells

Stem cells are undifferentiated cells that can differentiate into specialized cells, including more stem cells or other cell types during development.[34] Recently, a variant of L-PRF(C) has been analysed, thought to contain haematopoetic stem cells (HSC).[35] The presence of these HSC cells is mostly determined using immunohistochemical analysis for the detection of specific cell markers, in this case, CD34. This is a transmembrane phosphoglycoprotein that is predominantly used as a marker for HSC as well as haematopoetic progenitor cells.[36] Although traditionally linked to cells of haematopoetic cell origin, CD34 has recently been linked to other non-haematopoetic cells types such as mesenchymal stem cells (MSC), endothelial progenitor cells and interstitial dendritic cells.[36] Therefore, the mere presence of CD34 positive cells does not allow the assumption that a specific cell type such as HSC, exists. In order to verify the existence of HSC, the cells should, in addition to the proven presence of CD34, display other traits such as a low expression of CD90, a lack of expression of CD38 and human leukocyte antigen-DR (HLA-DR), as well as a panel of mature lineage markers (lin-).[36]

The potential of CD34 positive cell types in L-PRF(C) and its variants appears promising, but requires further investigation due to the variation in CD34 detection methods. Almost all CD34 detection methods use antigen-antibody interactions. These interactions are non-covalent and are reversible, with the potential of affecting the detection of the CD34 marker. Because of this, it is suggested that multiple methods be used to verify the presence of CD34.[36]

Although peripheral blood has been used as a source for CD34 positive cells in many forms of therapy, the baseline concentration of these cells in peripheral blood is relatively low.[37,38] As such, most therapies that require the use of CD34 positive cells enrich the presence of these cells in the vasculature by using granulocyte colony-stimulating factor (G-CSF).[39] This allows for an adequate amount of cells to be harvested for local or systemic transplantation. Whether the levels of CD34 positive cells in L-PRF and its variants are at therapeutic concentrations, requires further analysis.

Clinical results from studies using L-PRF

Several randomised controlled trials have been published that involve the use of L-PRF in the clinical management of a variety of disorders.[40-43] These trials have contradictory results, which may be related to a variation in the techniques and equipment used to prepare L-PRF.[41-45] Nevertheless, several articles report positive clinical outcomes even when standardised preparation techniques have not been used.[46-49] Whilst most of these publications are in fact case reports, nevertheless even randomised controlled trials where the RCF has not been verified, have shown positive clinical outcomes.[43] This is similar to reports about the use of PRP, which show a variety of clinical results based on the various methods of producing PRP.[18] Further research is therefore required to verify clinical differences which may be associated with the various methods of preparing L-PRF.

Does generic L-PRF exist?

L-PRF (O) is prepared using standard blood collecting tubes and a non-purpose built table top centrifuge delivering an RCF of 400g with a centrifuge time of 12 minutes. This method allows for the preparation of a platelet and leukocyte concentrate without the need for specialised equipment. Several case reports have demonstrated positive clinical results when using this preparation method.[50,51] However one cannot assume that this generic type of L-PRF has properties similar to those of L-PRF(C) or to L-PRF(I/E) since it has previously been shown that the centrifuge type may play a significant role in the final morphological features of the end product.[19,20] Further analysis of this biomaterial is therefore required to determine equivalence to the established protocols for L-PRF preparation.

CONCLUSIONS

The concept of L-PRF as a bioactive material with possible regenerative properties has resulted in it being adopted for use in various clinical procedures. However, the widespread use of this material has resulted in the production of several variants. Whether the biological properties of all these variants are similar, is unknown. Contradictory clinical results are reported in the literature with several generic types of L-PRF showing diverse results. In order to minimise the controversies associated with L-PRF, further research is required to determine which factors affect the biological properties of the material and whether these factors are clinically beneficial and relevant.

Disclosure policy

The authors declare no conflict of interest regarding the publication of this paper. This paper forms part of the requirements for partial fulfilment of the specifications for the degree PhD.

References

1. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: Le PRF. Implantodontie 2001; 42: 55–62.
2. Carlson, NE, Roach Jr, RB. Platelet-rich plasma: clinical applications in dentistry. Journal of the American Dental Association 2002; 133 (10); 1383.
3. Jameson,C. Autologous platelet concentrate for the production of platelet gel. Laboratory Medicine 2007; 38 (1): 39-42.
4. Dohan, DM, Choukroun, J., Diss, A., *et al*. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2006; 101 (3): 37–44.
5. Choukroun, J, Diss, A, Simonpieri, A, *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 2006; 101 (3): 56-60.
6. Dohan Ehrenfest, DM, Rassmusson, L, Albrektsson, T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends in Biotechnology 2009; 27(3):158-67.
7. Burnouf, T, Goubran, HA, Chen, TM, OU, KL, *et al*. Blood-derived biomaterials and platelet growth factors in regenerative medicine. Blood Reviews 2013; 27(2): 77-89.
8. Sunitha Raja, V, Munirathnam Naidu, E. Platelet-rich fibrin: evolution of a second-generation platelet concentrate. Indian Journal of Dental Research 2008; 19(1): 42-6.
9. Karunakar, P, Prasanna, JS, Jayadev, M, Shravani, GS. Platelet-rich fibrin, "a faster healing aid" in the treatment of combined lesions: A report of two cases. Journal of the Indian Society of Periodontology 2014; 18:651-5.
10. Baslarli, O, Tumer, C, Ugur, O, Vatankulu, B. Evaluation of osteoblastic activity in extraction sockets treated with platelet-rich fibrin. Med Oral Patol Oral Cir Bucal. 2015 1; 20(1): e111-6.
11. Sam, G, Vadakkekuttical, RJ, Amol, NV. *In vitro* evaluation of mechanical properties of platelet-rich fibrin membrane and scanning electron microscopic examination of its surface characteristics. Journal of the Indian Society of Periodontology 2015; 19(1): 32-6. doi: 10.4103/0972-124X.145821
12. Wang, Z, Weng, Y, Lu, S, Zong, C, Qiu, J, Liu, Y, Liu, B. Osteoblastic mesenchymal stem cell sheet combined with Choukroun platelet-rich fibrin induces bone formation at an ectopic site. Journal of Biomedical Materials Research B: Applied Biomaterials 2015; 103(6):1204-16.
13. Singh, J, Bharti, V. Laterally positioned flap-revised technique along with platelet rich fibrin in the management of Miller class II gingival recession. Dental Research Journal (Isfahan) 2013;10(2): 268-73.
14. Anilkumar, K, Geetha, A, Umasudhakar, Ramakrishnan, T, Vijayalakshmi, R, Pameela, E. Platelet-rich-fibrin: A novel root coverage approach. Journal of Indian Society of Periodontology 2009; 13(1): 50-4.
15. Agarwal, K, Chandra, C, Agarwal, K, Kumar, N. Lateral sliding bridge flap technique along with platelet rich fibrin and guided tissue regeneration for root coverage. Journal of Indian Society of Periodontology 2013; 17(6):801-5.
16. Oswald, N. RPM does not equal RCF. (11 May 2009). Retrieved from http://bitesizebio.com/1777/rpm-does-not-equal-rcf/
17. Amable, PR, Carias, RB, Teixeira MV, da Cruz Pacheco, I, Corrêa do Amaral, RJ, Granjeiro, JM, Borojevic, R. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. Stem Cell Research and Therapy 2013 7; 4(3); 67. doi: 10.1186/scrt218.
18. Dhurat, R, Sukesh, M. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. Journal of Cutaneous and Aesthetic Surgery 2014; 7(4): 189-97.

19. Dohan Ehrenfest, DM, Kang, B, Del Corso, M, Nally, M, Marc Quirynen, M, Wang, H, Pinto, NR. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a Leukocyte- and Platelet-Rich Fibrin (L-PRF) clot and membrane. Part 1: Evaluation of the vibration shocks of four models of table centrifuges for L-PRF. POSEIDO 2014 June; 2(2): 129-39.
20. Pinto, NR, Pereda, A, Jiménez, P, Del Corso, M, Kang, B, Wang, H, Quirynen, M, Dohan Ehrenfest, DM. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a Leukocyte- and Platelet-Rich Fibrin (L-PRF) clot and membrane. Part 2: Macroscopic, photonic microscopy and scanning electron microscopy analysis of four kinds of L-PRF clots and membranes. POSEIDO 2014; 2 (2): 141-54.
21. Dohan Ehrenfest, DM, Del Corso, M, Kang, B, Lanata, L, Quirynen, M, Wang, H, Pinto, NR. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a Leukocyte- and Platelet-Rich Fibrin (L-PRF) clot and membrane. Part 3: Comparison of the growth factors content and slow release between the original L-PRF and the modified A-PRF (Advanced Platelet-Rich Fibrin) membranes. POSEIDO 2014; 2(2): 155-66.
22. Kang, YH, Jeon, SH, Park, JY, *et al*. Platelet-rich fibrin (PRF) is a bio-scaffold and reservoir of growth factors for tissue regeneration. Tissue Engineering 2011;17 (3-4): 349-59.
23. Schär, MO, Diaz-Romero, J, Kohl, S, Zumstein, MA., Nesic, D. Platelet-rich concentrates differentially release growth factors and induce cell migration *in vitro*. Clinical Orthopaedics and Related Research 2015; 473(5): 1635-43.
24. Miller, RJ. PRF Protocol: centrifuge speed to use? (27 May 2012). Retrieved from http://www.osseonews.com/prf-protocol-centrifuge-speed-to-use/
25. L-PRF – IntraSpin system. Retrieved from http://intra-lock.com/intraspin.html
26. Another check in the spin column — Mikro 185 Centrifuge. Retrieved from http://www.hettweb.com/mikro185
27. Visser, LC, Arnoczky, SP, Caballero, O, Egerbacher, M. Platelet-rich fibrin constructs elute higher concentrations of transforming growth factor-β1 and increase tendon cell proliferation over time when compared to blood clots: a comparative *in vitro* analysis. Veterinary Surgery: VS 2010; 39(7): 811-7.
28. Carreira, AC, Lojudice, FH, Halcsik, E, Navarro, RD, Sogayar, MC, Granjeiro, JM. Bone morphogenetic proteins: facts, challenges, and future perspectives. Journal of Dental Research 2014; 93(4): 335-45.
29. Bhat, A, Wooten, RM, Jayasuriya, AC. Secretion of growth factors from macrophages when cultured with microparticles. Journal of Biomedical Materials Research. Part A 2013; 101(11): 3170-80.
30. Wahlström, O, Linder, C, Kalén, A, Magnusson, P. Acidic preparations of platelet concentrates release bone morphogenetic protein-2. Acta Orthopaedica 2008 ;79(3):433-7.
31. Sipe, JB, Zhang, J, Waits, C, Skikne, B, Garimella, R, Anderson, HC. Localization of bone morphogenetic proteins (BMPs)-2, -4, and -6 within megakaryocytes and platelets. Bone 2004; 35(6): 1316-22.
32. Kalén, A, Wahlström O, Linder CH, Magnusson P. The content of bone morphogenetic proteins in platelets varies greatly between different platelet donors. Biochemical Biophysical Research Communications 2008 17; 375(2): 261-4.
33. Wahlström, O, Linder, CH, Ansell, A, Kalén, A, Söderström, M, Magnusson, P. Acidic preparations of lysed platelets upregulate proliferative pathways in osteoblast-like cells as demonstrated by genome-wide microarray analysis. Platelets 2011; 22(6): 452-60.
34. Kobolak, J, Dinnyes, A, Memic, A, Khademhosseini, A, Mobasheri, A. Mesenchymal stem cells: Identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. Methods 2015 15. pii: S1046-2023(15)30092-X. doi: 10.1016/j.ymeth.2015.09.016. [Epub ahead of print]
35. Ghanaati, S, Booms, P, Orlowska, A, Kubesch, A, Lorenz, J, Rutkowski, J, Landes, C, Sader, R, Kirkpatrick, C, Choukroun, J. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. Journal of Oral Implantology. 2014; 40(6): 679-89.
36. Sidney, LE, Branch, MJ, Dunphy, SE, Dua, HS, Hopkinson, A. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cells. 2014; 32(6): 1380-9.
37. Philpott, NJ, Prue, RL, Marsh, JC, Gordon-Smith, EC, Gibson, FM. G-CSF-mobilized CD34 peripheral blood stem cells are significantly less apoptotic than unstimulated peripheral blood CD34 cells: role of G-CSF as survival factor. British Journal of Haematology. 1997; 97(1): 146-52.
38. Eidenschink, L, DiZerega, G, Rodgers, K, Bartlett, M, Wells, DA, Loken, MR. Basal levels of CD34 positive cells in peripheral blood differ between individuals and are stable for 18 months. Cytometry. Part B Clinical Cytometry 2012; 82(1): 18-25.
39. Herrmann, M, Verrier, S, Alini, M. Strategies to stimulate mobilization and homing of endogenous stem and progenitor cells for bone tissue repair. Frontiers in Bioengineering and Biotechnology 2015 2; 3:79. doi: 10.3389/fbioe.2015.00079. eCollection 2015.
40. Kumar, N, Prasad, K, Ramanujam, LKR, Dexith, J, Chauhan, A. Evaluation of treatment outcome after impacted mandibular third molar surgery with the use of autologous platelet-rich fibrin: a randomized controlled clinical study. Journal of Oral Maxillofacial Surgery 2015; 73(6): 1042-9.
41. Eshghpour, M, Dastmalchi, P, Nekooei, AH, Nejat, A. Effect of platelet-rich fibrin on frequency of alveolar osteitis following mandibular third molar surgery: a double-blinded randomized clinical trial. Journal of Oral Maxillofacial Surgery 2014; 72(8): 1463-7.
42. Zumstein, MA, Rumian, A, Lesbats, V, Schaer, M. Boileau, P. Increased vascularization during early healing after biologic augmentation in repair of chronic rotator cuff tears using autologous leukocyte- and platelet-rich fibrin (L-PRF): a prospective randomized controlled pilot trial. Journal of Shoulder and Elbow Surgery 2014; 23(1): 3-12.
43. Sharma, A, Pradeep, AR. Autologous platelet-rich fibrin in the treatment of mandibular degree II furcation defects: a randomized clinical trial. Journal of Periodontology 2011; 82(10): 1396-403.
44. Suttapreyasri, S, Leepong, N. Influence of platelet-rich fibrin on alveolar ridge preservation. Journal of Craniofacial Surgery 2013; 24(4):1088-94.
45. Jankovic, S, Aleksic, Z, Klokkevold, P, Lekovic, V, Dimitrijevic, B, Kenney, EB, Camargo, P. Use of platelet-rich fibrin membrane following treatment of gingival recession: a randomized clinical trial. International Journal of Periodontics and Restorative Dentistry 2012; 32(2): e41-50.
46. Kulkarni, MR, Thomas, BS, Varghese, JM, Bhat, GS. Platelet-rich fibrin as an adjunct to palatal wound healing after harvesting a free gingival graft: A case series. Journal of Indian Society of Periodontology 2014; 18(3): 399-402.
47. Kumar, A, Yadav, A, Shetty, N. One-step apexification using platelet rich fibrin matrix and mineral trioxide aggregate apical barrier. Indian Journal of Dental Research 2014; 25(6):809-12.
48. Soydan, SS, Uckan, S Management of bisphosphonate-related osteonecrosis of the jaw with a platelet-rich fibrin membrane: technical report. Journal of Oral Maxillofacial Surgery 2014; 72(2): 322-6.
49. Montanari, M, Callea, M, Yavuz, I, Maglione, M. A new biological approach to guided bone and tissue regeneration. BMJ Case Reports 2013 9; 2013; pii: bcr2012008240. doi: 10.1136/bcr-2012-008240.
50. Peck, MT, Marnewick, J, Stephen, L. Alveolar ridge preservation using leukocyte and platelet-rich fibrin: a report of a case. Case Reports in Dentistry 2011; 2011; 345048. doi: 10.1155/2011/345048. Epub 2011 Jul 3.
51. Peck, MT, Marnewick, J, Stephen, LX, Singh, A, Patel, N, Majeed, A. The use of leukocyte- and platelet-rich fibrin (L-PRF) to facilitate implant placement in bone-deficient sites: a report of two cases. South African Dental Journal 2012 ; 67(2): 54-6, 58-9.