



Advanced Platelet- Rich Fibrin (A-PRF): A Gender Based Study on Cell Population

Harini Kumaran¹, Radhika Arjunker² and R. Abilasha^{3*}

¹Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

²Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 77, India.

³Department of Oral Pathology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 77, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors R. Arjunker and R. Abilasha designed the study and wrote the protocol. Author HK carried out the study, performed literature search and wrote the first draft of the manuscript. Authors R. Arjunker and R. Abilasha managed the analyses of the results and the discussion. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i2830864

Editor(s):

(1) Dr. R. Deveswaran, M. S. Ramaiah University of Applied Sciences, India.

Reviewers:

(1) Nataliya Ashukina, Sytenko Institute of Spine and Joint Pathology, Ukraine.

(2) Ajili Faïda, Université de Tunis El Manar, Tunisia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/59774>

Original Research Article

Received 08 July 2020

Accepted 12 September 2020

Published 09 November 2020

ABSTRACT

Platelet Rich Fibrin (PRF) is a natural fibrin-based biomaterial prepared from an anticoagulant-free blood harvest without any artificial biochemical modification that allows obtaining fibrin membranes enriched with platelets and growth factors. PRF is superior to other platelet concentrates like Platelet Rich Plasma (PRP) due to its ease and inexpensive method of preparation and also it does not need any addition of exogenous compounds like bovine thrombin and calcium chloride. A blood sample of 10 individuals with healthy periodontium and no systemic diseases was collected and centrifuged to get platelet rich fibrin. Platelet rich fibrin is studied histologically to compare the ability of tissue regeneration and wound healing capacity between males and females. Neutrophils and lymphocytes are increased in A-PRF compared to S-PRF. Neutrophils and lymphocytes are proportionately more in males than females in both A-PRF and S-PRF. Monocytes are similar in A-PRF & S-PRF. Cells are more concentrated in S-PRF. Cells are more widely distributed in A-PRF.

*Corresponding author: E-mail: abilasha@saveetha.com;

Fibrin is more dense in A-PRF compared to S-PRF. Hence A-PRF especially in males could have a greater potential of tissue regeneration and wound healing capacity. Although it is not conclusive due to limited sample size, further increase in sample size can throw more light on the clinical implications. The difference in regenerative potential of platelet concentrates can pave the way for specialised and focussed treatment strategies resulting in more favourable patient outcomes.

Keywords: *Advanced Platelet Rich Fbrin (A-PRF); lymphocytes; neutrophils; platelet rich fibrin; Standard Platelet Rich Fibrin (S-PRF).*

1. INTRODUCTION

In clinical research, the development of biomaterial that are used to regulate the inflammation and increase the speed of healing process is one of the great challenges [1]. Healing is a complex process, which is initiated by clot formation, followed by proliferative stage which comprises of epithelialization, angiogenesis, granulation tissue formation, collagen deposition and finally collagen maturation and contraction [2,3]. But understanding this entire process is still incomplete; however, it is known that platelets play a crucial role not only in hemostasis, but also in the wound healing process [4]. Materials like hydroxyapatite, freeze dried bone graft, tricalcium phosphate, bioactive glass etc. have been widely used and tested for their contribution in healing and regeneration of soft and hard tissues [5]. Recent research on growth factors has recognized that the best tissue regenerative stimulus is present amongst the autologous growth factors, which have clinically proven to induce regeneration and tissue healing [6]. It led to the introduction of Platelet rich plasma (PRP). PRP is the autogenous material that is obtained by mixing of bovine in platelet rich concentrate. It is known as the first generation of platelet concentrate [7]. Currently, the studies have been focussed on the use of an autogenous material called Platelet Rich Fibrin (second generation platelet concentrate) that provides an osteoconductive scaffold along with growth factors to stimulate patient's own cells towards a regenerative response. It was first described by Dr. Joseph Choukroun in France to promote wound healing in implants [5]. PRF is a natural fibrin-based biomaterial prepared from an anticoagulant-free blood harvest without any artificial biochemical modification that allows obtaining fibrin membranes enriched with platelets and growth factors. PRF is superior to other platelet concentrates like PRP due to its ease and inexpensive method of preparation and also it does not need any addition of exogenous

compounds like bovine thrombin and calcium chloride. It is advantageous than autogenous graft also because an autograft requires a second surgical site and procedure [8]. Applications of PRF has been found in various branches of dentistry such as endodontics, oral and maxillofacial surgery, periodontics, tissue engineering. In the branch of endodontics, it is used in the treatment of open apex for regeneration of pulp-dentin complex, in combination with Mineral trioxide aggregate (MTA) to create root end barriers in apexification procedures to prevent extrusion of material, in regenerative pulpotomy and to fill in bony defect [5]. It also has a advantage of faster healing of cavitated periapical lesions when treated with PRF, graft stabilisation, wound sealing [9,10]. In the branch of oral and maxillofacial surgery, it is used as filling material in avulsion sockets, bone augmentation in sinus lifts for posterior maxilla augmentation for implants, bony defects etc, ridge preservation, guided bone regeneration [5]. In Periodontics, it is used as a treatment of intrabony defects [5,11–13], gingival recession, guided tissue regeneration, periapical lesions. In tissue engineering, it is used in vitro cultivation of human periosteal cells for bone [5]. Choukroun et al. reported that low speed centrifugation concept (LSCC) enhances the regeneration potential of fluid PRF-based matrices [14]. Richard et al. reported that females and old age produce larger PRF membranes due to the lower red blood cell count in their peripheral blood [15]. Hence a study was planned to know the efficacy of PRF between males and females and between two different centrifugation protocols standard platelet rich fibrin (S-PRF), (9 mL; 2700 rpm for 12 minutes) and (2) advanced platelet rich fibrin (A-PRF) 10 mL; 1500 rpm for 14 minutes) [16]. Previously we have worked on plenty of topics in Periodontology [17–29]. In this study, we assess the cells in platelet rich fibrin and compare the standard platelet rich fibrin (S-PRF) protocol and advanced platelet rich fibrin (A-PRF) protocol between males and females.

2. MATERIALS AND METHODS

2.1 Production of PRF

The classical technique for PRF preparation was invented by Dr. Joseph Choukroun in 2000. It is the current PRF technique authorized by the French Health Ministry in which PRF is prepared without using an anticoagulant during blood harvesting or bovine thrombin during gelling [8].

For preparation of PRF, blood sample without anticoagulant was collected from 10 individuals (5 males, 5 females) who were healthy volunteers with no periodontal problems or systemic disorders, in the age range between 18 to 22 years. For each individual, 2 tubes of peripheral blood were collected and immediately placed in a pre-programmed centrifuge (PC-O2, PROCESS for PRF, Nice, France). Centrifugation was performed according to the following two protocols: (1) Standard PRF, sterile glass coated plastic tube (9 mL; 2700 rpm for 12 minutes) and (2) Advanced PRF, sterile plain glass-based vacuum tubes (A-PRF10 tube) (10 mL; 1500 rpm for 14 minutes) [16].

After centrifugation, 3 layers are obtained in the test tube (Fig. 1). The topmost layer consisting of acellular PPP (platelet poor plasma), PRF clot in the middle and red blood cells (RBCs) at the bottom of the test tube. The middle layer of PRF clot is then removed with sterile tweezers (Fig. 2) and separated from the underlying RBC layer using scissors and then transferred on a sterile dish and subsequently fixed with 4% paraformaldehyde for 24hrs. It is supposed that the junction of PRF to the RBC layer is rich in growth factors and therefore this region is preserved [30].

2.2 Microscopic Analysis of the Platelet Rich Fibrin (A-PRF & S-PRF)

For microscopic analysis, the samples were chemically fixed and processed in an alcohol series and xylene. Subsequently, paraffin embedding was performed, and 10 sections were cut with a semi-automated microtome and affixed on albumin coated glass slides. Before staining, samples underwent a deparaffinization and rehydration process by sequential immersion in xylene followed by descending concentrations of ethanol. Samples were histologically stained with standard protocols for hematoxylin and eosin (H&E). Microscopic analysis was done using Binocular research microscope (Lieca, 10X and 40 X magnification). The slides were subjected to

robust screening to check for the increase or decrease in the various cells.



Fig. 1. Photograph showing test tube with PRF



Fig. 2. Photograph showing PRF separated from plasma

3. RESULTS AND DISCUSSION

Histological analysis was done based on three cell types such as neutrophils, lymphocytes and monocytes as mentioned. The following results were obtained. Neutrophils and lymphocytes are proportionately more in A-PRF than S-PRF. Neutrophils and lymphocytes are proportionately more in males than females in both A-PRF and S-PRF. Monocytes are similar in A-PRF & S-PRF. Cells are more concentrated in S-PRF. Cells are more distributed in A-PRF. Fibrin is dense in A-PRF and loose in S-PRF (Figs. 3,4,5 and 6).

In this study, it is found that Neutrophils and lymphocytes are proportionately more in males than females in both A-PRF and S-PRF.

Neutrophil contribution to tissue repair is that neutrophils convert monocytes into macrophages which initiates release of multiple cytokines and growth factors. The neutrophils become apoptotic and are cleared by macrophages. It is also characterised by the release of the tissue-repairing cytokines transforming growth factor- β (TGF β) and interleukin-10 (IL-10) [31,32].

Robertson in his study reported that drugs that promote neutrophil apoptosis have a therapeutic potential to accelerate tissue repair [33]. Hence the higher the neutrophils the higher was the regenerative capacity of tissues. We can find that males have more neutrophils than females which suggest that S-PRF and A-PRF from males may have the more regenerative potential than females.

In this study, we found that neutrophils and lymphocytes are more in A-PRF than S-PRF. Choukroun, S. Ghanaati, in their study demonstrated that reducing the relative centrifugation force (RCF) from a high range to low range with in PRF based matrices lead to a significant increase of leucocyte and platelet number. It also resulted in the increase of growth factor concentration such as vascular endothelial growth factor (VEGF) and transforming growth factor- β (TGF- β 1). They concluded that the low speed centrifugation concept (LSCC) enhances the regeneration potential of fluid PRF-based matrices. Consequently, the reduction of relative centrifugation force by application of low speed centrifugation concept opens up new avenues for advanced PRF-matrices, in which the cell-cell communication between platelets and leukocytes and that of these cells within the recipient tissue might result in improved wound healing and enhanced tissue regeneration [14].

Bagdadi in his study, concluded that A-PRF+, prepared with a reduced relative centrifugation force, displayed significantly higher vascular endothelial growth factor (VEGF) concentration over the study period of 10 days than A-PRF and PRF. Epidermal growth factor (EGF) and transforming growth factor- β (TGF- β 1) were comparable in A-PRF and A-PRF+, which were significantly higher than PRF [34]. Wend in his study found that total growth factor release of Platelet derived growth factor (PDGF-BB), transforming growth factor- β (TGF- β) and epidermal growth factor (EGF) in low and medium relative centrifugation force (RCF) were both significantly higher than those in the high RCF group. Vascular endothelial growth factor

(VEGF) and MMP-9 were significantly higher in the low RCF group compared to high RCF. These findings support the LSCC (low speed centrifugation concept), which confirms that improved PRF-based matrices may be generated through RCF reduction [35]. In our study, neutrophils and lymphocytes are more in A-PRF than S-PRF. Hence it is clear that A-PRF which had a reduced centrifugation speed have improved PRF matrices which in turn have a greater potential of tissue regeneration.

Miron et al. in his study reported that the size of the membranes produced from females was 17% larger than those produced in males. As the role of centrifugation is to separate blood layers transitionally over time, these differences were thought to be observed due to females generally containing lower hematocrits levels within their peripheral blood compared to males [15].

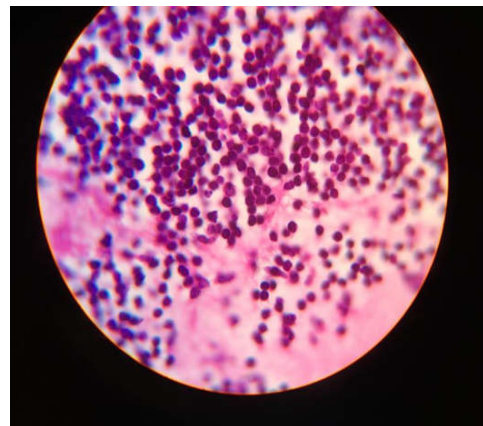


Fig. 3. Photomicrograph of A-PRF in males showing more number of neutrophils

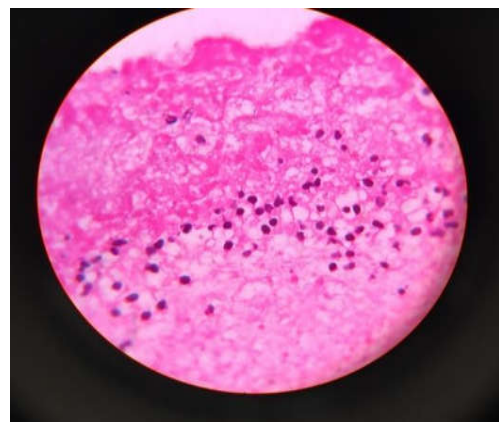


Fig. 4. Photomicrograph of A-PRF in females showing less number of neutrophils

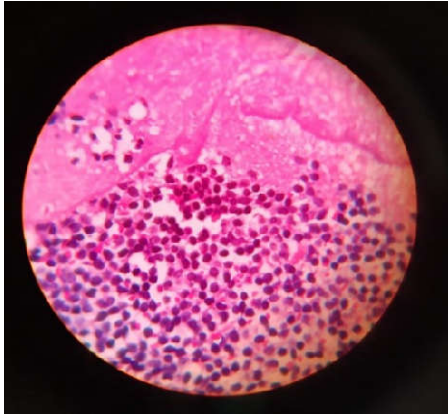


Fig. 5. Photomicrograph of S-PRF in males showing more number of neutrophils

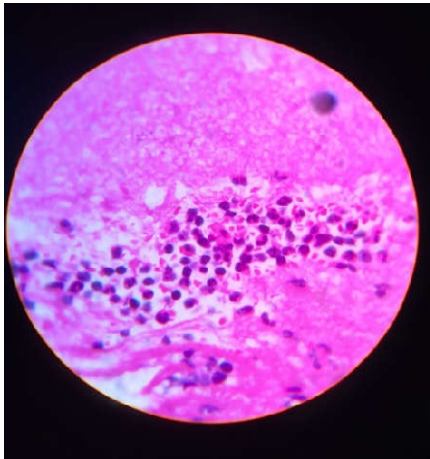


Fig. 6. Photomicrograph of S-PRF in females showing less number of neutrophils

4. CONCLUSION

There is an increase in neutrophils in A-PRF and S-PRF of males compared to females. This indirectly implies a greater potential for tissue regeneration and wound healing capacity in the A-PRF from males. As this study is limited in sample size, definitive conclusions cannot be made. This can be overcome by more detailed studies with histomorphometrical analysis of the various cells with larger sample sizes. The difference in regenerative potential of platelet concentrates can pave the way for specialised and focussed treatment strategies resulting in more favorable patient outcomes.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

The authors would like to thank Saveetha Dental College and Hospitals for providing a platform to conduct the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. 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.
2. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med.* 1999;341(10):738–46.
3. Agrawal M, Agrawal V. Platelet rich fibrin and its applications in dentistry-A review article. *National Journal of Medical and Dental Research.* 2014;2(3):51.
4. Gaßling VLW, Açil Y, Springer IN, Hubert N, Wiltfang J. Platelet-rich plasma and platelet-rich fibrin in human cell culture. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 2009;108(1):48–55.
5. Kanakamedala A, Ari G, Sudhakar U, Vijayalakshmi R, Ramakrishnan T, Emmadi P. Treatment of a furcation defect with a combination of platelet-rich fibrin (PRF) and bone graft-a case report. *Endodontic Practice Today [Internet].* 2009;3(2). Available: <http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=17532809&AN=43480961&h=AR6oV7XxFu%2BuKb6y38P9dApH8XjAJpldz84R1ugjrxw8LEsWtcpQuiQD92dQp31onkN5m%2F3TehpV0ejH44cF Sg%3D%3D&crl=c>
6. Nityasri AS, Pradeep KY. Role of CGF (Concentrated Growth Factor) in periodontal regeneration. *J Dent Health Oral Disord Ther [Internet];* 2018.

- Available:https://www.researchgate.net/profile/Pradeep_Kumar_Yadalam/publication/328097077_Role_of_CGF_Concentrated_Growth_Factor_in_periodontal_regeneration/links/5bd40e9c4585150b2b8a282c/Role-of-CGF-Concentrated-Growth-Factor-in-periodontal-regeneration.pdf
7. Prakash S, Thakur A. Platelet concentrates: Past, present and future. *J Maxillofac Oral Surg.* 2011;10(1):45–9.
 8. Preeja C, Arun S. Platelet-rich fibrin: Its role in periodontal regeneration. *The Saudi Journal for Dental Research.* 2014;5(2): 117–22.
 9. Vijayalakshmi B, Pradeep S. Role of platelet rich fibrin in dentistry. *Research Journal of Pharmacy and Technology.* 2016;9(11):2037–40.
 10. Hiremath H, Gada N, Kini Y, Kulkarni S, Yakub SS, Metgud S. Single-step apical barrier placement in immature teeth using mineral trioxide aggregate and management of periapical inflammatory lesion using platelet-rich plasma and hydroxyapatite. *J Endod.* 2008;34(8): 1020–4.
 11. Sharma A, Pradeep AR. Treatment of 3-wall intrabony defects in patients with chronic periodontitis with autologous platelet-rich fibrin: A randomized controlled clinical trial. *J Periodontol [Internet];* 2011. Available:<https://aap.onlinelibrary.wiley.com/doi/abs/10.1902/jop.2011.110075>
 12. Thorat MK, Pradeep AR. Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: A controlled clinical trial. *Journal of Clinical [Internet];* 2011. Available:<https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-051X.2011.01760.x>
 13. Panda S, Ramamoorthi S, Jayakumar ND, Sankari M, Varghese SS. Platelet rich fibrin and alloplast in the treatment of intrabony defect. *J Pharm Bioallied Sci.* 2014;6(2): 127–31.
 14. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: The first introduction to the low speed centrifugation concept. *Eur J Trauma Emerg Surg.* 2018;44(1):87–95.
 15. Miron RJ, Dham A, Dham U, Zhang Y, Pikos MA, Sculean A. The effect of age, gender, and time between blood draw and start of centrifugation on the size outcomes of platelet-rich fibrin (PRF) membranes. *Clin Oral Investig.* 2019;23(5):2179–85.
 16. Ghanaati S, Booms P, Orłowska 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.
 17. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. *J Oral Pathol Med.* 2019; 48(2):115–21.
 18. Gajendran PL, Parthasarathy H, Tadepalli A. Comparative evaluation of cathepsin K levels in gingival crevicular fluid among smoking and nonsmoking patients with chronic periodontitis. *Indian J Dent Res.* 2018;29(5):588–93.
 19. Kaarthikeyan G, Jayakumar ND, Sivakumar D. Comparative evaluation of bone formation between PRF and blood clot alone as the sole sinus-filling material in maxillary sinus augmentation with the implant as a tent pole: A randomized split-mouth study. *J Long Term Eff Med Implants.* 2019;29(2):105–11.
 20. Arjunkumar R. Nanomaterials for the management of periodontal diseases. In: Chaughule RS, editor. *Dental applications of nanotechnology.* Cham: Springer International Publishing. 2018;203–15.
 21. Ravi S, Malaiappan S, Varghese S, Jayakumar ND, Prakasam G. Additive effect of plasma rich in growth factors with guided tissue regeneration in treatment of intrabony defects in patients with chronic periodontitis: A split-mouth randomized controlled clinical trial. *J Periodontol.* 2017; 88(9):839–45.
 22. Kavarthapu A, Malaiappan S. Comparative evaluation of demineralized bone matrix and type II collagen membrane versus eggshell powder as a graft material and membrane in rat model. *Indian J Dent Res.* 2019;30(6):877–80.
 23. Murthykumar K, Arjunkumar R, Jayaseelan VP. Association of vitamin D receptor gene polymorphism (rs10735810) and chronic periodontitis. *J Investig Clin Dent.* 2019; 10(4):e12440.
 24. Ramesh A, Vellayappan R, Ravi S, Gurumoorthy K. Esthetic lip repositioning: A cosmetic approach for correction of gummy smile - A case series. *J Indian Soc Periodontol.* 2019;23(3):290–4.

25. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol.* 2018;89(10):1241–8.
26. Kavarthapu A, Thamaraiselvan M. Assessing the variation in course and position of inferior alveolar nerve among south Indian population: A cone beam computed tomographic study. *Indian J Dent Res.* 2018;29(4):405–9.
27. Ramesh A, Ravi S, Kaarthikeyan G. Comprehensive rehabilitation using dental implants in generalized aggressive periodontitis. *J Indian Soc Periodontol.* 2017;21(2):160–3.
28. Jain M, Nazar N. Comparative evaluation of the efficacy of intraligamentary and supraperiosteal injections in the extraction of maxillary teeth: A randomized controlled clinical trial. *J Contemp Dent Pract.* 2018;19(9):1117–21.
29. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol.* 2019;90(12):1441–8.
30. Malathi DK, Malathi K. Periodontal regeneration of an intrabony osseous defect with combination of platelet rich fibrin and bovine derived demineralized bone matrix: A case report [Internet]. *IOSR Journal of Dental and Medical Sciences.* 2013;4:20–6. Available:<http://dx.doi.org/10.9790/0853-0422026>
31. Wang J. Neutrophils in tissue injury and repair. *Cell Tissue Res.* 2018;371(3):531–9.
32. Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. *Nat Rev Immunol.* 2010;10(6):427–39.
33. Robertson AL, Holmes GR, Bojarczuk AN, Burgon J, Loynes CA, Chimen M, et al. A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism. *Sci Transl Med.* 2014;6(225):225ra29–225ra29.
34. El Bagdadi K, Kubesch A, Yu X, Al-Maawi S, Orłowska A, Dias A, et al. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: A proof of concept of LSCC (low speed centrifugation concept). *Eur J Trauma Emerg Surg.* 2019;45(3):467–79.
35. Wend S, Kubesch A, Orłowska A, Al-Maawi S, Zender N, Dias A, et al. Reduction of the relative centrifugal force influences cell number and growth factor release within injectable PRF-based matrices. *J Mater Sci Mater Med.* 2017;28(12):188.

© 2020 Kumaran et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/59774>