Original Article

COMPARATIVE GROWTH FACTOR ANALYSIS: A-PRF AND S-PRF TO ENHANCE BONE REGENERATIVE CAPACITY OF STICKY BONE

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ABSTRACT

Platelet-rich fibrin (PRF) is an autologous biomaterial extensively used in regenerative dentistry because of its sustained release of growth factors. Among its various forms, standard PRF (S-PRF) and advanced PRF (A-PRF) differ in their centrifugation protocols, which may affect their biological properties and regenerative potential. This study aimed to compare the release of growth factors from sticky bone prepared using S-PRF and A-PRF. This in vitro experimental study was conducted using blood samples collected from healthy volunteers (n = 10). Sticky bone was prepared by mixing demineralized freeze-dried bone allograft (DFDBA) with either S-PRF or A-PRF. Growth factor analysis—including Angiopoietin-2, EGF, EPO, FGF, G-CSF, GM-CSF, PDGF-AA, PDGF-BB, SCF, TGF-α, VEGF, and HGF—was performed using the LEGENDplex multi-analyte flow cytometry assay. The A-PRF group demonstrated significantly higher levels of EPO, Angiopoietin-2, G-CSF, and HGF compared with the S-PRF group ($p < 0.05$), whereas FGF levels were similar between the two groups. Within the limitations of this study, A-PRF appears to be a more effective option for sticky bone preparation and may enhance bone regeneration outcomes.

Key words: Platelet-rich fibrin, Advanced PRF, Sticky bone, Growth factors, Bone regeneration, DFDBA.

Introduction

A natural remedy to enhance the healing of wounds is a derivative from our own blood, i.e., platelet-rich fibrin (PRF) [1]. Blood drawn from patients is put in sterile test tubes in a centrifugation machine. The resultant separation of various parts of blood allows a platelet-rich layer full of growth factors to be separated from red blood cell concentrate. This platelet-rich layer comes in a variety of consistencies depending on duration and speed of centrifugation. These platelet concentrates, like platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), have a major role in regenerative medicine [2, 3].

Platelet-rich fibrin(PRF) was first introduced by Choukroun in 2001 in France [4]. Choukroun's platelet-rich fibrin (PRF) is prepared from blood without the addition of anticoagulants. Based on the preparation protocol, two types of PRF are available. Standard platelet-rich fibrin (S-PRF) is prepared by centrifuging blood at 2700 rpm for 12 minutes. Advance PRF (A-PRF) was first introduced by Ghanaati *et al*. [4] in 2014, in which the revolution time and speed are altered. A-PRF utilizes the protocol of 1500 rpm for a time frame of 14 minutes [5, 6]. Decreasing the rpm, while increasing the centrifugation time in the A-PRF group, enhances the presence of platelets and neutrophilic granulocytes. Neutrophilic granulocytes contribute to the differentiation of monocytes into macrophages, so when A-PRF is implanted into the host socket, it influences the differentiation of host macrophages and already existing macrophages in the clot [7, 8].

Recent advancements in tissue engineering have led to the introduction of new methods to concentrate the platelet-derived growth factors [9]. New formulations of PRF have been developed with an aim to increase the growth factor concentration and provide superior regenerative potential compared to the conventional PRF [10-14].

Benign pathologies in oral and maxillofacial surgery require enucleation, which leads to the formation of the bony defect. Also, extraction sockets need adequate bone fill for dental implant placement at a later date [15, 16]. Both these bony defects, when not filled with appropriate material, may lead to the collapse of the soft tissue flap. It may hamper the esthetics in the anterior front region of the jaw [17, 18]. Previously, a bone graft alone was used to fill such bony defects. Now, sticky bone, which is a combination of PRF and bone graft, is found to be better due to its superior handling properties and abundant growth factors. This is found to aid faster and better bone regeneration [19].

Routinely, S-PRF is the choice of PRF used to prepare sticky bone [20-22]. It is proven in many studies that A-PRF has more bone regeneration potential than S-PRF. But, whether this change in centigution rate and time enhances the bone

regeneration potential of sticky bone prepared with different types of PRF needs to be assessed and proven yet. Here, we hypothesize that A-PRF, with its greater amount of growth factors, will be a better choice of PRF preparation to use in sticky bone and will lead to enhanced bone formation. Thus, the purpose of this study was to assess the bone regeneration efficacy of sticky bone prepared with S-PRF and A-PRF by growth factor analysis.

Materials and Methods

This is an in-vivo-in vitro study conducted in the Regenerative Laboratory of our institute from period of Jan 2023 to June 2023. Permission was obtained from the Institutional Research Board [DYPDCH/IEC/123/111/19] before starting the study. The inclusion criteria were healthy volunteers between the ages of 18 and 30, who willingly consented to donate blood for the preparation of PRF. Valid written informed consent was obtained from 5 volunteers for each group.

10 ml of blood was withdrawn from each volunteer and centrifuged to obtain either S-PRF or A-PRF, as per grouping. A protocol of 1500 rotations per minute (rpm) for a time frame of 14 minutes for A-PRF and of 2700 rpm for 12 minutes for standard platelet-rich fibrin was used to prepare the two types of PRFs.

Both were mixed with 10 mg of allograft (demineralized freeze-dried bone allograft, DFDBA) obtained from Tata Memorial Hospital (TMH) Tissue Bank, to prepare sticky bone. Sticky bone prepared with both PRF types from a patient was subjected to growth factor analysis for various factors such as Angiopoietin-2, Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Platelet-derived growth factor AA (PDGF-AA), Platelet-derived growth factor (PDGF-BB), Stem cell factor (SCF), Transforming growth factor Alpha (TGF-α), Vascular epithelial Growth Factor (VEGF). The data obtained was analyzed.

Growth factor analysis

Sticky bone made with bone graft (DFBDA) with either S-PRF and A-PRF was used to analyze the growth factor.

The growth factor analysis assay was performed using LEGENDplex Multi-Analyte Flow Assay Kit. To 25 µL of each sample, 25 µL of mixed beads were added and incubated for 2 hours. After incubation, the samples were centrifuged at 250rpm for 5 minutes, and washing steps were followed. After performing the washing steps twice, 25 µL of detection antibodies was added to each sample and incubated for 1 hour, and 25 µL of SA-PE was added to each sample. The samples were then centrifuged for 30 mins and washing steps were repeated. The samples were tested using a flow cytometer, and the results were analyzed. All the tests were done in triplicate to avoid bias.

Results and Discussion

The growth factor level was assessed using the LEGENDplex Multi-Analyze Flow Assay Kit by flow cytometry. S-PRF sticky bone showed higher secretion of FGF (Fibroblast Growth Factor), and A-PRF sticky bone showed higher secretion of EPO (Erythropoietin), angiopoietin -2, G-CSF (Granulocyte Colony Stimulating Factor), and HGF (Hepatocyte Growth Factor) ($p<0.05$). Our findings demonstrate that A-PRF will be a better option to treat dental patients in daily practice than S-PRF. It will enhance the healing of oral tissues in density **(Figures 1a-1e)**.

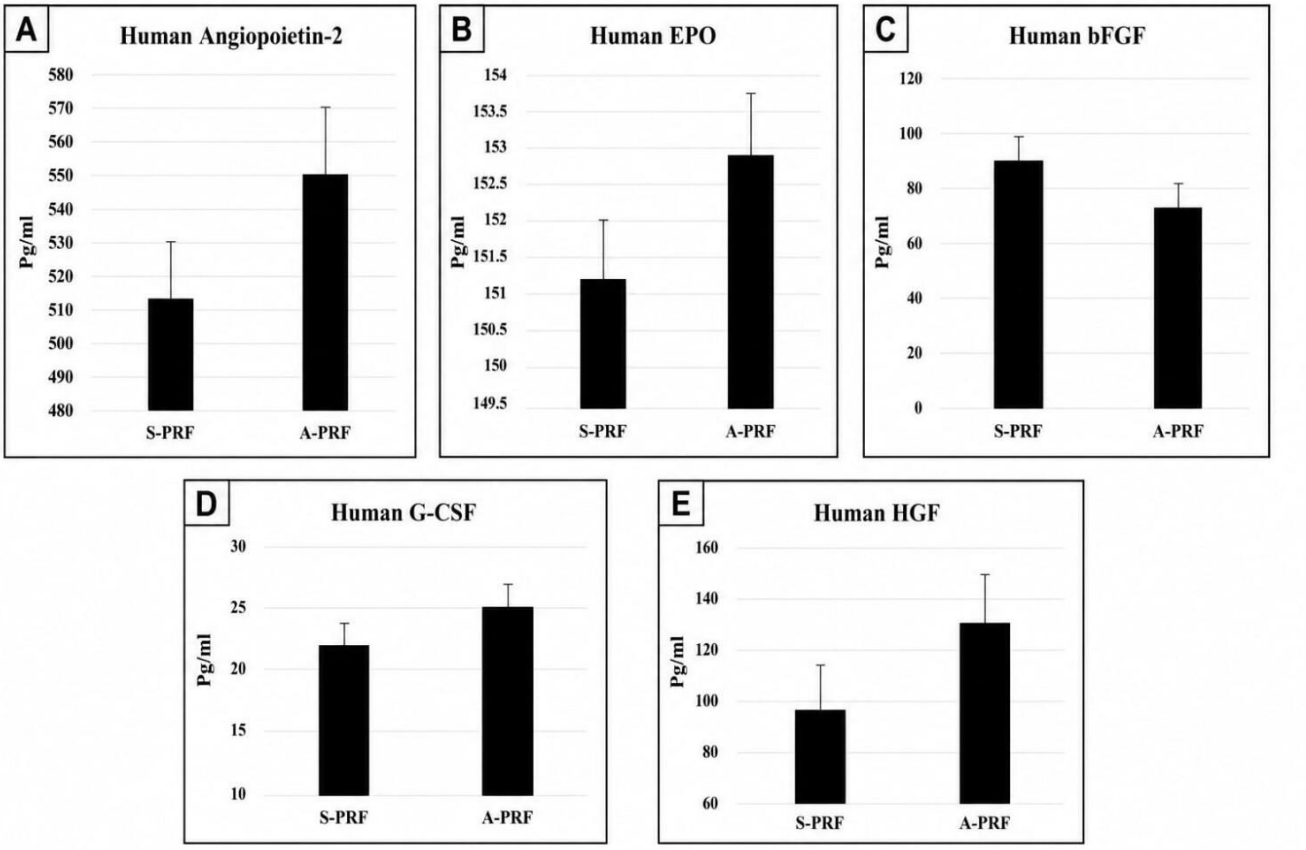

Figure 1.

PRF is a concentrate of growth factors that can be used in surgery to enhance both soft and hard tissue healing. This study was conducted to analyze whether the sticky bone prepared with A-PRF showed more growth factor than S-PRF or not.

A-PRF has several advantages over S-PRF. A-PRF uses a modified centrifugation protocol with lower g-force and longer centrifugation time. This results in less cellular damage and better preservation of growth factors. The lower centrifugation speed allows for more natural clot formation and better matrix consistency. The cellular composition of A-PRF contains a more diverse and intact cellular composition. It preserves more viable platelets, leukocytes, and stem cells. It also has better cellular migration and proliferation potential. A-PRF's improved biological properties, enhanced wound healing characteristics, and better tissue regeneration potential have been found in many researchers [23-27]. Studies have shown that A-PRF creates a more stable and sustained growth factor release, slower, more controlled growth factor delivery, which promotes longer-term tissue regeneration and provides a more conducive environment for bone healing [28, 29].

The results of this study clearly showed that A-PRF had an increased number of Human G-CSF, Human Angiopoietin-2, Human EPO, and Human HGF. Human bFGF was comparable in both types of sticky bone.

Studies show that Human G-CSF significantly increases the expression of BMP-2 in the fracture healing process [30, 31]. Human Angiopotin-2 has a direct effect on angiogenesis, which helps in bone regeneration [32, 33]. Human EPO is able to play a key role in the regeneration of newly resorbed bone by stimulating JAK-STAT signaling pathways in HSCs through Epo-R. This then triggers the production of BMPs, most notably BMP2 and BMP6 [34, 35].

HumanHGF aids upregulation of BMP-2 expression in osteocytes [36]. This aids in bone regeneration. Synergy between the bFGF and other factors affects the ability to promote angiogenesis and osteogenesis and provides a more robust and effective approach to bone regeneration [37, 38].

Thus, it is evident that increased growth factors by altering the PRF process the bone regeneration can significantly alter and enhance bone regeneration [13, 39]. The sticky bone with A-PRF will lead to faster and better bone regeneration process.

The comparison of growth factors in sticky bone with A-PRF and S-PRF showed that sticky bone with A-PRF had more growth factors. While both A-PRF and S-PRF are valuable in bone grafting, current evidence suggests that sticky bone with A-PRF offers superior biological properties, cellular preservation, and regenerative potential. Thus, we, the authors, clinicians, should consider

transitioning to A-PRF protocols for preparing sticky bone, potentially improving surgical outcomes and bone regeneration.

Conclusion

Comparison of growth factor levels in sticky bone prepared with A-PRF and S-PRF demonstrated that A-PRF-containing sticky bone exhibited higher concentrations of growth factors [31, 32, 35-37]. Although both A-PRF and S-PRF are effective in bone grafting procedures, current evidence indicates that sticky bone prepared with A-PRF possesses superior biological properties, improved cellular preservation, and greater regenerative potential [23-26, 28]. Therefore, clinicians may consider adopting A-PRF protocols for sticky bone preparation to potentially achieve enhanced surgical outcomes and improved bone regeneration [40-43].

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Conflict of interest: None

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Ethics statement: This study involved human participants for blood sample collection and was conducted in accordance with the ethical standards of Dr. D. Y. Patil Vidyapeeth and the principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Ethics Committee of Dr. D. Y. Patil Vidyapeeth, Pune (Approval No. Ref. No. DYPV/EC/562/2020). Written informed consent was obtained from all participants prior to blood sample collection.

References

1. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, 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. doi:10.1016/j.tripleo.2005.07.008
2. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent. 2001;10(4):225-8.
3. Ahmed OH. GC-MS analysis and antioxidant effect of some Iraqi plants. J Nat Sci Biol Med. 2023;14(2):115-21. doi:10.4103/jnsbm.JNSBM_14_2_7
4. Choukroun J, Adda F, Schoeffler C, Vervelle A. An opportunity in perio-implantology: the PRF. Implantodontie. 2001;42:55-62.
5. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40(6):679-89. doi:10.1563/aaid-joi-D-14-00138
6. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig. 2016;20(9):2353-60. doi:10.1007/s00784-016-1719-1
7. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-rich fibrin and soft tissue wound healing: a systematic review. Tissue Eng Part B Rev. 2017;23(1):83-99. doi:10.1089/ten.TEB.2016.0233
8. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates. Trends Biotechnol. 2009;27(3):158-67.
9. Ahmed OH, Abbas AH, Yaseen YS. GC/MS analysis of crude extract of Fig leaves naturally grown in Iraq. J Adv Pharm Educ Res. 2023;13(3):96-101. doi:10.51847/TF4ouLslks
10. Miron RJ, Chai J, Zhang P, Li Y, Wang Y, Mourão CFAB, et al. A novel method for harvesting concentrated growth factors. Clin Implant Dent Relat Res. 2019;21(3):365-72.
11. Fujioka-Kobayashi M, Schaller B, Mourão CFAB, Zhang Y, Sculean A, Miron RJ. Biological characterization of PRF membranes. Int J Mol Sci. 2020;21(3):1025.
12. Kawase T. Platelet-rich plasma and its derivatives as promising bioactive materials. Jpn Dent Sci Rev. 2015;51(4):102-10.
13. Miron RJ, Zucchelli G, Pikos MA, Salama M, Lee S, Guillemette V, et al. Use of PRF in regenerative dentistry. J Clin Periodontol. 2017;44(3):336-46.
14. Thuy VTT, Hung DN, Oanh LTT, Tuyet VTA, Thu BT. Factors impact on business performance of enterprises: the case of Vietnam. J Organ Behav Res. 2023;8(2):27-39. doi:10.51847/2itmiM3CoE
15. Pham TT. Linking family supports and Vietnamese employee performance: the mediator role of work engagement. J Organ Behav Res. 2024;9(1):15-31. doi:10.51847/W3DMjBBfqq
16. Araujo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. J Clin Periodontol. 2005;32(2):212-8.
17. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes after tooth extraction. Int J Periodontics Restorative Dent. 2003;23(4):313-23.
18. Pakalapati A, Ranganadhareddy A, Kumar NNP. From formation to detection: understanding monoclonal antibody aggregation through analytical lenses. J Biochem Technol. 2024;15(2):27-32. doi:10.51847/MgvdN50FBW
19. Ahmed OH, Bdulqader AA, Ezghayer MA. Antibacterial activity of methanolic extract of leaves Indigofera suffruticosa naturally grown in Iraq. J Adv Pharm Educ Res. 2023;13(4):99-103. doi:10.51847/fRbzdpKkBV
20. Sohn DS, Moon JW, Moon KN, et al. The use of concentrated growth factors in sinus augmentation. Implant Dent. 2009;18(6):528-35.

21. Sohn DS, Heo JU, Kwak DH, Kim DE, Kim JM, Moon JW, et al. Bone regeneration using PRF in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(3):e1-6.
22. Sergun VP, Evgenia B, Burkova VN, Poznyakovsky VM, Danko NN, Tokhiriyon B. Treatment of menstruation disorders at puberty: a plant-based dietary supplement efficacy and safety. J Biochem Technol. 2023;14(3):13-17. doi:10.51847/r9vJrE0mHw
23. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. J Periodontol. 2017;88(1):112-21. doi:10.1902/jop.2016.160443
24. Ghanaati S, Herrera-Vizcaino C, Al-Maawi S, Lorenz J, Miron RJ, Nelson K, et al. Fifteen years of PRF. Eur Cell Mater. 2018;35:1-15.
25. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, et al. Comparative study of PRF and PRP. Int J Implant Dent. 2016;2(1):10.
26. Miron RJ, Pinto NR, Quirynen M, Ghanaati S. Standardization of PRF. Periodontol 2000. 2019;79(1):121-34.
27. Patricia A, Hailemeskel B. Turmeric, black pepper, and lemon hot infusion for joint and musculoskeletal pain: a case report. World J Environ Biosci. 2024;13(1):36-8. doi:10.51847/XeYTN4wNsa
28. He L, Lin Y, Hu X, Zhang Y, Wu H. Sustained release of growth factors in PRF. Clin Oral Investig. 2009;13(4):389-95.
29. Padma KR, Don KR, Anjum MR, Sindhu GS, Sankari M. Application of green energy technology for environmental sustainability. World J Environ Biosci. 2023;12(4):1-7. doi:10.51847/bAMKAPPZGe
30. Bona C, Camacho-Alonso F, Vaca A, Llorente-Alonso M. Oral biofilm control in patients using orthodontic aligners: evidence from a systematic review. Asian J Periodontics Orthod. 2025;5:33-42. doi:10.51847/siIhUaqfip
31. Takahashi Y, Yamamoto M, Tabata Y. G-CSF enhances bone regeneration. J Biomater Sci Polym Ed. 2005;16(3):327-40.
32. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science. 1997;277(5322):55-60. doi:10.1126/science.277.5322.55
33. Prada AM, Cicalău GIP, Ciavoi G. Resin infiltration for white-spot lesion management after orthodontic treatment. Asian J Periodontics Orthod. 2024;4:19-23. doi:10.51847/ZTuGEanCSV
34. Sakhnenkova TI, Abdul-Kadyrova LR, Akhilgova ZA, Brovikova AA, Markov OO, Saribekyan AA, et al. Morphological and biochemical analysis of 3D scaffold based on biocompatible polymer for tissue engineering. J Adv Pharm Educ Res. 2023;13(3):29-33. doi:10.51847/v8o0GbXJdN
35. Holstein JH, Orth M, Scheuer C, Tami A, Becker SC, Garcia P, et al. EPO and bone regeneration. Blood. 2007;109(3):1229-37.
36. Nakamura T, Mizuno S. HGF and bone regeneration. Biochem Biophys Res Commun. 2010;395(3):317-22.
37. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 2005;16(2):159-78. doi:10.1016/j.cytogfr.2005.01.004
38. Saadati N, Masihi S, Abouali N, Akiash N, Jafari RM, Tahmasebi Y. Evaluation of the cardiac function using GLS in speckle tracking echocardiography in preeclampsia and IUGR patients. J Adv Pharm Educ Res. 2024;14(4):1-6. doi:10.51847/QvxatTLHKC
39. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest DM. The relevance of PRF. Curr Pharm Biotechnol. 2012;13(7):1237-43.
40. Sharma A, Pradeep AR. Autologous PRF in periodontal defects. J Periodontol. 2011;82(10):1396-403.
41. Wu CL, Lee SS, Tsai CH, et al. Platelet-rich fibrin in regenerative medicine. J Dent Sci. 2012;7(2):93-9.
42. Miron RJ, Fujioka-Kobayashi M, Bishara M, et al. Platelet concentrates in tissue regeneration. J Biomed Mater Res A. 2017;105(11):3009-20.
43. Ehrenfest DM, Pinto NR, Pereda A, et al. The impact of PRF on implant dentistry. J Oral Implantol. 2018;44(3):220-9.