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Comparative Analysis of Platelet-derived Growth Factor Release from Novel Injectable Titanium-PRF and Other Platelet Concentrates: An In Vitro Study

Mounika Reddy[a], Pratibha Gopalkrishna[a]*, Shobha U Kamath[b], YS Phaneendramallimoggala[c], Vidya Sagar Sisinty[d], Santhosh Kumar[a]

[a]*Department of Periodontology, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal, India*

[b]*Department of Biochemistry, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India*

[c]*Department of Paediatrics, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India*

[d]*Department of Periodontology, Kamineni Institute of Dental Sciences, Narketpally, Nalgonda, Telangana, India*

*Corresponding author: pratibha.pk@manipal.edu

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ABSTRACT

Platelet concentrates, widely used as adjuncts to regenerative procedures, are available with varying specifications. The use of titanium tubes for low-speed centrifugation has previously been attempted for the preparation of titanium platelet-rich fibrin (T-PRF). Therefore, the present in vitro investigation aimed to prepare an injectable titanium platelet-rich fibrin (i-TPRF) and to compare growth factor release with that of known platelet concentrates. About 40 mL of blood was collected from six donors each for preparing platelet-rich fibrin (PRF), T-PRF, injectable platelet-rich fibrin (i-PRF) and i-TPRF. A volume of 5 mL of Dulbecco's Modified Eagle Medium (DMEM) was added to the platelet concentrates, and the platelet concentrates were incubated at 37 °C in a Shaker plate. An Enzyme-Linked Immunosorbent Assay was used to assess growth factor release on days 1 and 7. The results showed an increase in platelet-derived growth factor (PDGF) release from day 1 to day 7 in all the groups. At 24 hours, i-PRF showed the highest growth factor release, followed by T-PRF, i-TPRF and PRF. On day 7, T-PRF showed the highest PDGF levels, followed by i-TPRF, i-PRF, and PRF. The study demonstrated the potential of a new platelet concentrate preparation, i-T-PRF, with PDGF release comparable to that of known PRF preparations. In addition, platelet concentrates prepared in titanium tubes showed enhanced release of PDGF-AA.

Keywords: *Blood; Centrifugation; Enzyme-linked immunosorbent assay; Platelet-derived growth factor; Platelet-rich fibrin*

INTRODUCTION

Platelets are the second-most numerous blood cells (Borie *et al.*, 2015), with a lifespan of 7 to 10 days (Dohan *et al.*, 2006). Platelets play an important role in both haemostasis and wound healing. Fifteen per cent of the total platelet volume comprises alpha granules, which, when activated, release growth factors that promote healing and repair (Fernández-Delgado *et al.*, 2012). Developing biomimetic agents that modulate inflammation and enhance healing has been a challenge in clinical research. Growth factors are attracting interest within the tissue engineering triad, as their use may augment treatment with traditional bone grafts, which carry risks such as host rejection (Rojas *et al.*, 2019), inflammation, and the transmission of infection (Haugen *et al.*, 2019).

Platelet concentrates, currently used in periodontal surgery and dental implant procedures, are obtained from autologous blood and subjected to gradient centrifugation. This facilitates the delivery of platelets and their associated growth factors to the injury site at higher concentrations, thereby accelerating regenerative processes (Yamakawa & Hayashida, 2019). The addition of growth factors during the regenerative procedures accelerates the new bone formation and ridge preservation (Jamjoom & Cohen, 2015).

Bone contains growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGFß), fibroblast growth factor, insulin-like growth factor, and bone morphogenetic proteins (BMPs) (Sánchez-González *et al.*, 2012). BMPs can help undifferentiated mesenchymal cells develop into osteoblasts to induce new bone formation.

Tissue injury exposes intravascular platelets to subendothelial collagen, thereby activating thrombin generation. Platelets, when activated by thrombin, release growth factors, which form a haemostatic plug. Growth factors released by activated platelets diffuse into the surrounding tissues, attracting neutrophils and monocytes to the wound via chemotaxis. Monocytes differentiate into macrophages, which are involved in several processes essential for effective wound healing.

Historically, the first platelet concentrate, platelet–rich plasma (PRP), was introduced for clinical use in 1997 (Whitman *et al.*, 1997). The preparation protocol involved two centrifugation steps (Miron, Fujioka-Kobayashi, Bishara *et al.*, 2017) and required biochemical handling. PRP was also prone to antibody formation and coagulopathy, limiting its utility. Subsequently, several modifications were introduced, resulting in the evolution of platelet preparations over the years.

Platelet-rich fibrin (PRF) is a second-generation platelet concentrate consisting of platelets and growth factors in the fibrin membrane form (Choukroun *et al.*, 2006). This inexpensive and straightforward autologous platelet concentrate is prepared from the patient's blood with no anticoagulant or artificial biochemical modification (Preeja & Arun, 2014) and requires only a single centrifugation step (Ghanaati *et al.*, 2018). The blood sample is immediately centrifuged at 3,000 rpm (800 g) for 10 min.

PRF is collected in special glass or plastic tubes without anticoagulant additives such as citrate, allowing fibrin to form naturally after centrifugation, thereby producing an autologous fibrin product. According to Mourao *et al.* (2014), 9 mL to 10 mL of blood in a plastic test tube without preservatives, centrifuged at 3,300 rpm for 2 min to 3 min, produced an orange-coloured fluid containing injectable platelet-rich fibrin (i-PRF). Miron, Fujioka-Kobayashi, Hernandez *et al.* (2017) reduced the centrifugal force (g-force) and the duration of spin (700 rpm, i.e., 60 g for 3 min) to produce a liquid PRF preparation, termed i-PRF, in uncoated tubes. The volume of i-PRF in a 10 mL tube is usually only 1 mL to 1.5 mL. The recommended tubes

are additive-free white-top polythene terephthalate (PET) plastic vacutainers. PET plastic, considered hydrophobic, repels water and platelets, preventing platelet activation during centrifugation and delaying the start of clot formation by 15 min to 20 min.

It was hypothesised that titanium test tubes might be more effective at activating platelets than glass tubes used in the Choukroun method (Tunali *et al.*, 2013). Medical-grade IV titanium tubes are highly biocompatible with blood components, avoiding the negative effects of dry glass or glass-coated plastic tubes. Titanium nanoparticles have a procoagulant effect on red blood cells (RBCs) and increased thrombus formation (Bian *et al.*, 2021). It was suggested that platelets were activated by mechanical interaction with rough, haemocompatible materials, such as titanium (Gomez *et al.*, 2023). Titanium platelet-rich fibrin (T-PRF) was proposed to prevent contamination of the fibrin structure with silica particles from the glass tubes. This platelet concentrate also showed greater polymerised fibrin formation, slower resorption (Tunali *et al.*, 2015), and an abundance of growth factors (Ravi & Santhanakrishnan, 2020).

Kobayashi *et al.* (2016) observed that PRF releases high quantities of PDGF to recruit progenitor cells at the wound site. In his study, PDGF-AA was released in the highest amounts by all platelet concentrates, followed by TGF-beta1, PDGF-BB, PDGF-AB, VEGF, EGF, and IGF. Furthermore, PDGF-AA released from all platelet concentrates was 6 to 10-fold higher than PDGF-AB and PDGF-BB. Treatment with PDGF combined with IGF resulted in the formation of new bone and cementum (Lynch *et al.*, 1989). A review describes oriented periodontal ligament fibres observed in PDGF, indicating periodontal regeneration (Meghil *et al.*, 2023). PDGF-AA is reported to influence stem cells and enhance wound healing by promoting angiogenesis (Wu *et al.*, 2019). Another study noted that i-PRF, prepared at 700 rpm for 3 min (60 g), could release higher concentrations of growth factors like PDGF-AA, PDGF-AB, EGF, and IGF-1 at 10 days. It also induces higher fibroblast migration. Growth factors in PRF were believed to be encapsulated within a fibrin matrix, but liquid formulations did not have a fibrin matrix (Miron, Fujioka-Kobayashi, Hernandez *et al.*, 2017). Furthermore, lower centrifugation speeds seemed to allow the concentration of greater numbers of cells, such as leukocytes and growth factors, before the fibrin clot forms (Ghanaati *et al.*, 2014).

The present study aimed to formulate a novel injectable titanium PRF (i-TPRF) by combining the low centrifugation speed of i-PRF with the use of titanium test tubes, and to evaluate its potential for wound-healing through growth factor release. Hence, a primary objective of the present study was to evaluate the *in vitro* release profile of PDGF-AA from i-TPRF at multiple time intervals. A secondary objective was to compare PDGF-AA release from i-TPRF with PRF, i-PRF, and T-PRF.

MATERIAL AND METHODS

Subjects and Study Selection

Blood samples for this *in vitro* study were obtained from subjects visiting the Department of Periodontology. The procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The experimental protocol was scrutinised by the Institutional Review Committee and subsequently approved by the Institutional Ethics Committee (IEC: 45/2021). This was followed by registration in the national registry (CTRI/2021/06/034112). All subjects were informed about the study, and each subject provided written informed consent. We selected systemically and periodontally healthy volunteers aged 18–35 years for routine haematological evaluation. Subjects with medical conditions, bleeding, or platelet disorders were excluded from the study. Others excluded were those on anticoagulant or antiplatelet drugs and those who smoked.

Preparation of Platelet Concentrates

Initially, 20 mL of blood was withdrawn from an arm of a volunteer using a syringe to prepare two of the four types of platelet concentrates, PRF and T-PRF. For PRF preparation, a 10 mL blood sample was transferred to glass test tubes and immediately centrifuged at 2,700 rpm (approx. 900 g) for 12 min using PRF DUO Clinical centrifuge (DuoQuattro, France). Similarly, another 10 mL blood sample was transferred to a titanium test tube and centrifuged at 2,800 rpm for 12 min at room temperature using a REMI R-8C laboratory centrifuge (Remi Elektrotechnik Ltd., India) equipped with a 16-position rotor to prepare T-PRF (Bian *et al.*, 2021). Later, after drawing 20 mL of blood from the other arm, 10 mL was transferred to a glass test tube (without additives) for i-PRF preparation, followed by immediate centrifugation at 700 rpm (approx. 60 g) for 3 min using PRF DUO Clinical centrifuge (DuoQuattro) (Tunali *et al.*, 2013). Another 10 mL of sample was centrifuged using a REMI R-8C laboratory centrifuge (Remi Elektrotechnik Ltd., India) equipped with a 16-position rotor, at 1,000 rpm for 4 min at room temperature in a titanium test tube to prepare i-TPRF. The same procedure is followed for the other five volunteers. A total of six volunteer donors (Fig. 1) provided blood samples (24 samples) for the four groups.

Transfer of Platelet Concentrates to the Culture Medium

The prepared PRF concentrates (6 PRF and 6 T-PRF) and injectable PRF samples (6 i-PRF and 6 i-TPRF) were transferred into coded 5 mL Eppendorf tubes. About 5 mL of culture medium [Dulbecco's Modified Eagle Medium (DMEM), High Glucose – HIMEDIA, India] was added, and the vials were placed on a plate shaker (Delfia Plate Shake 1296–003, Wallac Oy, Finland) at 37°C. On day 1 (after 24 hours), 5 mL of DMEM culture medium was collected in an Eppendorf vial and stored at –80°C (Panasonic Ultra-Low Temperature Freezer, MDF-U55V, Japan). On day 7 (168 hours), 5 mL of the culture medium was collected into a fresh vial and stored at –80°C. On day 7, the culture medium samples in the respective vials were subjected to ELISA to quantify growth factors. Before analysing the samples, the vials were left at room temperature and then quantified.

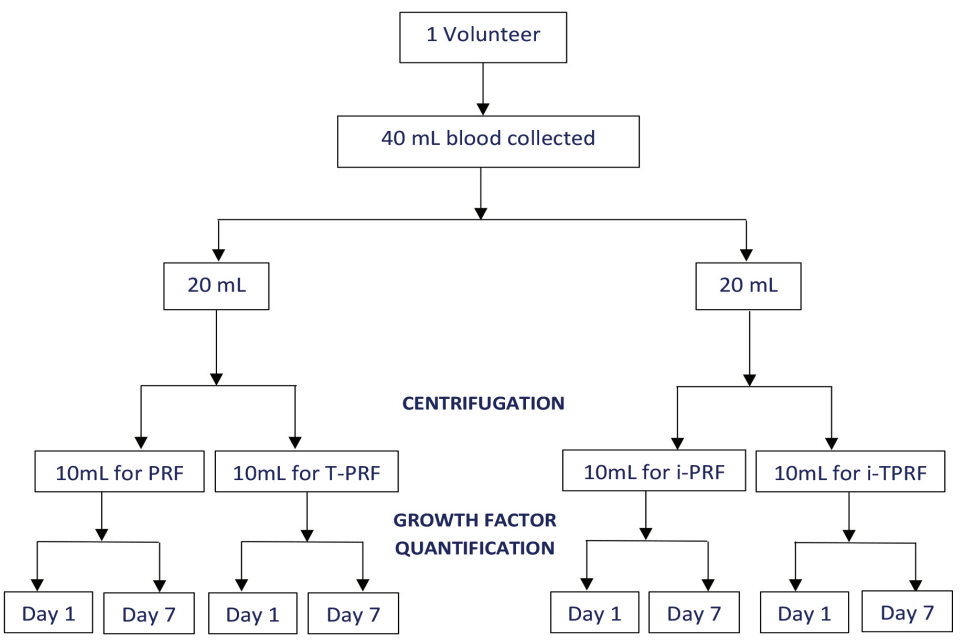

Fig. 1 Flow chart depicting the method of sample collection and distribution into groups.

Note: Total of 6 volunteers × 4 groups = 24 samples

Protein Quantification with ELISA

Protein quantification for PDGF-AA was carried out using ELISA (PDGF AA, make - ELK Biotechnology, Sensitivity: 0.065 ng/mL; Detection range: 0.16 ng/mL to 10 ng/mL, cat# ELK 1308, Lot# 17561362534, Wuhan City, China) according to the manufacturer's instructions. In the pre-coated ELISA plate wells, 100 µL of each standard working solution and sample were incubated for 80 min in the incubator (Selec TC544) at 37°C. A washing buffer was used to wash the wells. The wells were subsequently incubated with the antibody solution for 50 min and washed with a washing buffer. Later, 100 µL of the working solution was added to the wells using a micropipette, and the wells were incubated for 50 min at 37°C in an incubator (Selec TC544). The wells were subsequently washed with washing buffer, then 90 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) was added, and the wells were incubated with 50 µL of the stopping solution (provided in the kit) for 20 min at 37°C. A microplate reader (Infinite® 200 Pro, Tecan, Switzerland) was used to measure the absorbance at 450 nm.

RESULTS

Preparation of PRF, T-PRF, i-PRF, and i-TPRF

All four platelet concentrates were prepared according to the specifications. Among the four platelet concentrates, PRF and T-PRF were in the form of a fibrinous mesh, while the other two concentrates, i-PRF and i-TPRF, were in the liquid form (Fig. 2). Comparing the two injectable forms of platelet concentrates, i-TPRF was more viscous than i-PRF. Subsequently, PDGF–AA was quantified from the concentrates obtained on days 1 and 7.

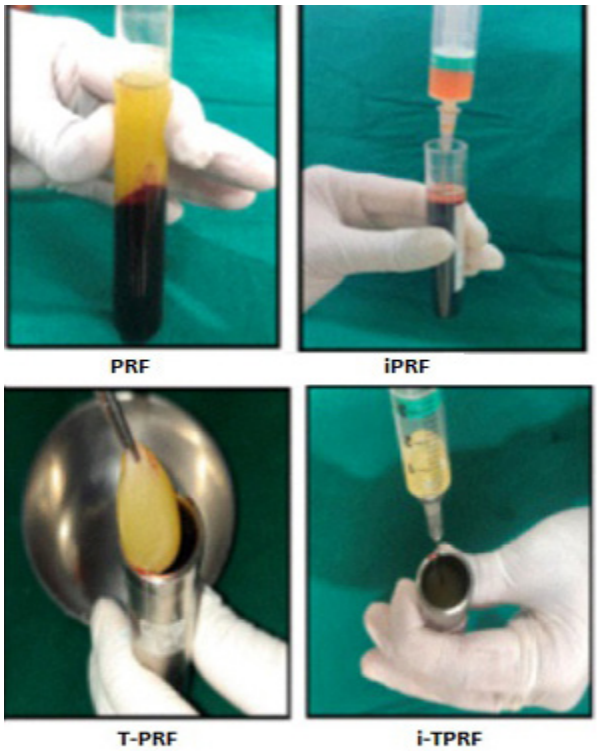

Fig. 2 Retrieval of platelet concentrates after centrifugation.

Quantity of PDGF from PRF, T-PRF, i-PRF and i-TPRF on day 1 (24 hours)

Injectable PRF showed the highest amount of growth factor release (median release of 0.3900 ng/mL) at 24 hr, ranging from a minimum of 0.240 ng/mL and a maximum of 0.699 ng/mL, followed by T-PRF. The average (median) quantity of T-PRF was 0.3175 ng/mL, with a minimum of 0.229 ng/mL and a maximum of 0.513 ng/mL. i-TPRF, averaging 0.2825 ng/mL with a minimum of 0.205 ng/mL and a maximum of 0.495 ng/mL, was next in growth factor release, followed lastly by PRF (median release of 0.2310ng/mL), with a minimum of 0.202 ng/mL and a maximum release of 0.58 1ng/mL. However, the differences were not statistically significant (Table 1). On day 1, i-PRF showed the greatest growth factor release (Fig. 3).

Table 1 Comparison of PDGF among the groups on day 1 using the Kruskal–Wallis test

Group	Min	Max	Median	IQR	F value	*p*-value
PRF	0.202	0.581	0.2310	0.224	0.38	0.15
T-PRF	0.229	0.513	0.3175	0.205		
i-PRF	0.240	0.699	0.3900	0.350		
i-TPRF	0.205	0.495	0.2825	0.192		

Note: Units – ng/mL; IQR – Interquartile range; $p < 0.05$ significant

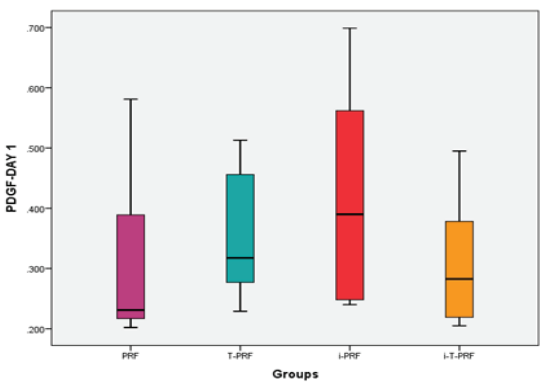

Fig. 3 Comparison of PDGF among the groups on day 1 using a box plot. The box plot shows the amount of growth factor release on day 1. The X-axis shows the types of platelet concentrates, and the Y-axis shows the amount of growth factor release. On day 1, i-PRF showed the highest amount of growth factor release.

Quantity of PDGF from PRF, T-PRF, i-PRF and i-TPRF on day 7 (168 hrs)

On day 7, T-PRF showed the highest amount of growth factor released (median release of 2.762 ng/mL), ranging from a minimum of 0.242 ng/mL and a maximum of 7.164 ng/mL, followed by i-TPRF (median release of 2.573 ng/mL), ranging from a minimum release of 0.714 ng/mL and a maximum release of 2.852 ng/mL. This was followed by i-PRF (median release of 1.299 ng/mL), with a range from 0.327 ng/mL to 4.832 ng/mL. PRF (median release of 0.541 ng/mL) with a range from a minimum of 0.283 ng/mL and a maximum of 1.277 ng/mL was seen to have the least release (Table 2, Fig. 4).

Table 2 Comparison of PDGF among the groups on day 7 using the Kruskal-Wallis test

Group	Min	Max	Median	IQR	F value	*p*-value
PRF	0.283	1.277	0.541	0.708	5.18	0.15
T-PRF	0.242	7.164	2.762	5.215		
i-PRF	0.327	4.832	1.299	2.271		
i-TPRF	0.714	2.852	2.573	1.855		

Note: Units – ng/mL; IQR – Interquartile range; $p < 0.05$ significant

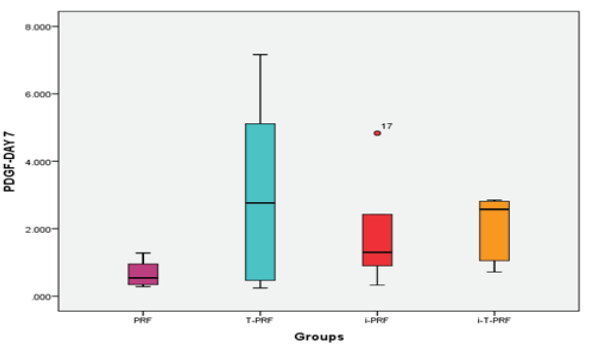

Fig. 4 Comparison of PDGF among the groups on day 7 using a box plot. The box plot shows the amount of growth factor release on day 7. The X-axis shows types of platelet concentrates, and the Y-axis shows the amount of growth factor release. On day 7, T-PRF showed the highest growth factor.

Comparison of PDGF-AA concentrations in the four platelet preparations at these time intervals

Comparison of PDGF within groups using the Wilcoxon Signed Rank test showed a statistically significant ($p < 0.05$) increase in the amount of growth factor release from day 1 to day 7 in all groups (Table 3). The inter-group comparison of PDGF using the Mann-Whitney U test between the groups at day 1 and day 7 showed no statistical significance between the groups ($p < 0.05$) (Table 4).

Table 3 Intra-group comparison of PDGF using the Wilcoxon Signed Rank test

Group	Time intervals	Min	Max	Median	IQR	*p*-value
PRF	Day 1	0.202	0.581	0.231	0.224	0.028*
	Day 7	0.283	1.277	0.541	0.708	
T-PRF	Day 1	0.229	0.513	0.317	0.205	0.046*
	Day 7	0.242	7.164	2.762	5.215	
i-PRF	Day 1	0.240	0.699	0.390	0.350	0.046*
	Day 7	0.327	4.832	1.299	2.271	
i-TPRF	Day 1	0.205	0.495	0.282	0.192	0.028*
	Day 7	0.714	2.852	2.573	1.855	

Note: $p < 0.05$; *significant

Table 4 Inter-group comparison of PDGF between the groups on day 1 and day 7 using the Mann-Whitney U test

Group	Day 1		Day 7	
	U value	*p*-value	U value	*p*-value
PRF vs. T-PRF	11.00	0.26	10.00	0.20
PRF vs. i-PRF	8.00	0.109	8.00	0.109
PRF vs. i-TPRF	16.00	0.74	3.00	0.016
T-PRF vs. i-PRF	14.00	0.52	15.00	0.63
T-PRF vs. i-TPRF	13.00	0.42	16.00	0.74
i-PRF vs. i-TPRF	12.00	0.33	14.00	0.52

Note: *p*-value set significant at 0.05/4 = 0.0125

DISCUSSION

The present study analysed the release profile of PDGF–AA from four platelet concentrates: PRF, T-PRF, i-PRF, and i-TPRF. With the growing use of platelet concentrates in surgical disciplines, it is essential to understand the types and quantities of growth factors released that contribute tohard and soft tissue healing.

Growth factors are proteins (Molecular weight 25,000 Daltons) stored in platelet alpha granules. Growth factor release profile corresponds to the proliferative and maturation phases of wound healing. Platelet concentrates play a vital role in releasing growth factors during tissue regeneration. Platelets and growth factors accumulate in a fibrin clot during PRF membrane preparation (Naik *et al.*, 2013).

Fibroblasts, monocytes, vascular smooth muscle cells, macrophages, and endothelial cells also express PDGF-AA. PDGF-AA helps angiogenesis and wound healing by augmenting connective tissue matrix deposition at the leading edge of new granulation tissue. Kobayashi *et al.* (2016) observed greater amounts of PDGF-AA released than TGF-beta1, PDGF-BB, PDGF-AB, VEGF, EGF, and IGF in all the studied platelet concentrates. All platelet concentrations showed PDGF-AA release at 6–10-fold higher concentrations than PDGF-AB and PDGF-BB. Sites treated with the combination of PDGF and IGF showed significant histological new bone and cementum formation (Lynch *et al.*, 1989). A review by Meghil *et al.* (2023) hints at oriented periodontal ligament fibres, suggesting PDGF effects in periodontal regeneration. Moreover, PDGF-AA also recruits progenitor cells to the site of regeneration, like mesenchymal cells and adipose stem cells.

Our study has demonstrated the preparation of a novel injectable platelet concentrate (i-TPRF) that combines titanium tubes with low centrifugation speed and duration. As a further objective, we assessed PDGF-AA release from the injectable form of TPRF concentrate (i-TPRF) and compared it with that of other platelet concentrates.

Among the four platelet concentrates prepared in the present study, two platelet concentrates, i.e., PRF and T-PRF, were in the form of fibrin. The fibrin formed in both concentrates appeared clinically similar. Tunali *et al.* (2014) observed a strong fibrin network histologically in T-PRF specimens. Being denser, T-PRF is also pliable enough to handle in clinical situations. Although the other two platelet concentrates, i-PRF and i-TPRF, were in liquid form, the fluid obtained in the titanium tubes was relatively thick and viscous, with a yellow tinge.

In the present study, on day 1, injectable PRF showed the highest growth factor release. With regard to the amount of PDGF-AA on day 7, it was significantly higher than on day 1 in all the PRF groups. T-PRF showed the highest growth factor release on day 7, followed by i-TPRF. The observations suggest a spurt in growth factor release for T-PRF and i-TPRF, though the difference is not statistically significant. Therefore, the platelet concentrates prepared in the titanium tubes, T-PRF and i-TPRF, demonstrated relatively better growth factor release, which was comparable. Similarly, i-PRF showed a greater increase in growth factor release on day 7 than PRF. Hence, the fluid/injectable forms of platelet concentrates had higher growth factor levels on day 7 than PRF, possibly due to the lower centrifugation speed.

Platelet concentrates' growth factor release kinetics may vary depending on donor characteristics, production methods, and platelet count enrichment. It has been reported that leukocytes in the platelet concentrate prolong growth factor release for up to 7 days (Mariani & Pulsatelli, 2020). These PRF leukocytes help remove necrotic debris from the defect location, preventing infection. Moreover, the growth factor promotes angiogenesis between 7 and 21 days of wound healing, particularly in bone tissue repair (Hankenson *et al.*, 2011).

Previous studies suggest different durations of growth factor release. The A-PRF release from 11,048 pg/mL on day 1 increased to 9,262 pg/mL on day 10, which was notably higher than the PRP and PRF levels at 6,176 pg/mL and 9,262 pg/mL, respectively (Kobayashi *et al.*, 2016). The i-PRF released a higher amount of PDGF on day 3 than PRP (Miron, Fujioka-Kobayashi, Hernandez *et al.*, 2017). Growth-factor release from PRF and platelet-rich fibrin matrix (PRFM) membranes has also been observed for up to 23 days. The PRFM group released a higher amount of PDGF (173.24 pg/mL) than the PRF group (164.3 pg/mL) after 15 days (Chatterjee & Debnath, 2019).

Similarly, T-PRF rapidly released higher amounts of PDGF-AA at early time points (15 mins to 1 day) than L-PRF and A-PRF. A-PRF seemed to have a more sustained release (Ravi & Santhanakrishnan, 2020). Titanium is reported to influence soft tissue healing through cytokines and epithelial cell adhesion (Kasnak *et al.*, 2019). Studies suggest that T-PRF can also enhance osteoblast proliferation for up to 72 hours (Baygin *et al.*, 2024).

The release of other angiogenic factors has also been noted previously. A significant release of PDGF-AB, TGF-β1, and VEGF from L-PRF membranes over 7 days has been observed (Ehrenfest *et al.*, 2009). In another study, the higher release of PDGF-BB, transforming growth factor (TGF-β1), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) was observed at relatively low centrifugal forces (RCF) (Wend *et al.*, 2017). An increase in the release of VEGF, TGF-β1, and EGF from three platelet concentrates (PRF, A-PRF, A-PRF+) from 24 hours to 7 days has been reported, followed by a decline from day 7 to day 10 (El Bagdadi *et al.*, 2019).

Although the study is limited by its small sample size, it provides preliminary observations on platelet growth factor release. A longer assessment period may be needed to observe the decline in growth factor release. An additional step is required to prepare injectable PRF formulations. The shortcoming of i-TPRF is the difficulty in viewing and withdrawing the liquid that forms above the RBC layer due to the titanium test tube's lack of transparency.

The thickness and viscosity of the obtained fibrin, and the effects of varying centrifugation speeds and times with titanium tubes, can be investigated in the future. Including i-TPRF in clinical trials will substantiate its clinical utility as a biomaterial. Furthermore, comparisons among the various PDGF types can be made.

CONCLUSION

The study, for the first time, developed a novel injectable variant of T-PRF, i-TPRF, using low centrifugation speed and time, and demonstrated relatively good growth factor release over the tested time intervals, comparable to existing platelet concentrate protocols. The growth factor concentration increased gradually from day 1 to day 7 across all platelet preparations. Overall, enhanced PDGF-AA release was observed when centrifuged in titanium tubes compared to routine glass tubes. However, only PDGF-AA release was assessed in the present study. Other growth factors may behave differently. The conditions present during periodontal surgery can indicate whether a fibrin clot or an injectable platelet concentrate is desired at the wound site. A viscid consistency facilitates manipulation during periodontal surgery. A fluid/injectable formulation can improve bone graft cohesion at the defect site and prevent the dissipation of osseous material due to bleeding.

REFERENCES

Baygin M, Cakiris A, Yabaci Tak A, Abaci N, Ekmekci SS, Gurkan Koseoglu B (2024). In vitro comparison of the effects of titanium-prepared platelet-rich fibrin and leukocyte platelet-rich fibrin on osteoblast behavior and their gene expression. *BMC Oral Health*, **24**(1): 1552. https://doi.org/10.1186/s12903-024-05223-4

Bian Y, Chung HY, Bae ON, Lim KM, Chung JH, Pi J (2021). Titanium dioxide nanoparticles enhance thrombosis through triggering the phosphatidylserine exposure and procoagulant activation of red blood cells. *Part Fibre Toxicol*, **18**(1): 28. https://doi.org/10.1186/s12989-021-00422-1

Borie E, Oliví DG, Orsi IA, Garlet K, Weber B, Beltrán V *et al.* (2015). Platelet-rich fibrin application in dentistry: A literature review. *Int J Clin Exp Med*, **8**(5): 7922.

Chatterjee A, Debnath K (2019). Comparative evaluation of growth factors from platelet concentrates: An in vitro study. *J Indian Soc Periodontol*, **23**(4): 322–328. https://doi.org/10.4103/jisp.jisp_678_18

Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL *et al.* (2006). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, **101**(3): e56–e60. https://doi.org/10.1016/j.tripleo.2005.07.011

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J *et al.* (2006). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, **101**(3): e37–e44. https://doi.org/10.1016/j.tripleo.2005.07.008

Ehrenfest DM, Rasmusson L, Albrektsson T (2009). Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*, **27**(3): 158–167. https://doi.org/10.1016/j.tibtech.2008.11.009

El Bagdadi K, Kubesch A, Yu X, Al-Maawi S, Orlowska A, Dias A *et al.* (2019). 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*, **45**(3): 467–479. https://doi.org/10.1007/s00068-017-0785-7

Fernández-Delgado N, Hernández-Ramírez P, Forrellat-Barrios M (2012). Platelet functional spectrum: From hemostasis to regenerative medicine. *Revista Cubana de Hematología, Inmunología y Hemoterapia*, **28**(3): 200–216.

Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J *et al.* (2014). Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol*, **40**: 679–689. https://doi.org/10.1563/aaid-joi-D-14-00138

Ghanaati S, Herrera-Vizcaino C, Al-Maawi S, Lorenz J, Miron RJ, Nelson K *et al.* (2018). Fifteen years of platelet rich fibrin in dentistry and oromaxillofacial surgery: How high is the level of scientific evidence? *J Oral Implantol*, **44**(6): 471–492. https://doi.org/10.1563/aaid-joi-D-17-00179

Gomez TW, Samuel A, Shankar R, Gopakumar R, Chandran D (2023). The effects of titanium on blood derived biomaterials pertaining to regeneration: A comparative study. *J Osseointegr*, **15**(1): 24–31. https://doi.org/10.23805/JO.2023.15.01.02

Hankenson KD, Dishowitz M, Gray C, Schenker M (2011). Angiogenesis in bone regeneration. *Injury*, **42**(6): 556–561. https://doi.org/10.1016/j.injury.2011.03.035

Haugen HJ, Lyngstadaas SP, Rossi F, Perale G (2019). Bone grafts: Which is the ideal biomaterial? *J Clin Periodontol*, **46**: 92–102. https://doi.org/10.1111/jcpe.13058

Jamjoom A, Cohen RE (2015). Grafts for ridge preservation. *J Funct Biomater*, **6**(3): 833–848.

Kasnak G, Fteita D, Jaatinen O, Kononen E, Tunali M, Gürsoy M *et al.* (2019). Regulatory effects of PRF and titanium surfaces on cellular adhesion, spread, and cytokine expressions of gingival keratinocytes. *Histochem Cell Biol*, **152**: 63–73. https://doi.org/10.1007/s00418-019-01774-8

Kobayashi E, Fluckiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B *et al.* (2016). Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clin Oral Investig*, **20**(9): 2353–2360. https://doi.org/10.1007/s00418-019-01774-8

Lynch SE, Williams RC, Polson AM, Howell TH, Reddy MS, Zappa UE *et al.* (1989). A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. *J Clin Periodontol*, **16**(8): 545–548. https://doi.org/10.1111/j.1600-051x.1989.tb02334.x

Mariani E, Pulsatelli L (2020). Platelet concentrates in musculoskeletal medicine. *Int J Mol Sci*, **21**(4): 1328. https://doi.org/10.3390/ijms21041328

Meghil MM, Mandil O, Nevins M, Saleh MHA, Wang HL (2023). Histologic evidence of oral and periodontal regeneration using recombinant human platelet-derived growth factor. *Medicina*, **59**(4): 676. https://doi.org/10.3390/medicina59040676

Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J (2017). Platelet-rich fibrin and soft tissue wound healing: A systematic review. *Tissue Eng Part B Rev*, **23**(1): 83–99. https://doi.org/10.1089/ten.TEB.2016.0233

Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, Ghanaati S *et al.* (2017). Injectable platelet-rich fibrin (i-PRF): Opportunities in regenerative dentistry? *Clin Oral Investig*, **21**(8): 2619–2627. https://doi.org/10.1007/s00784-017-2063-9

Mourao CF, Valiense H, Melo ER, Mourao NB, Maia MD (2014). Obtention of injectable platelet-rich fibrin (i-PRF) and its polymerization with bone graft: Technical note. *Rev Col Bras Cir*, **42**(6): 421–423. https://doi.org/10.1590/0100-69912015006013

Naik B, Karunakar P, Jayadev M, Marshal VR (2013). Role of platelet-rich fibrin in wound healing: A critical review. *J Conserv Dent*, **16**(4): 284–293. https://doi.org/10.4103/0972-0707.114344

Preeja C, Arun S (2014). Platelet-rich fibrin: Its role in periodontal regeneration. *Saudi J Dent Res*, **5**(2): 117–122.

Ravi S, Santhanakrishnan M (2020). Mechanical, chemical, structural analysis and comparative release of PDGF-AA from L-PRF, A-PRF and T-PRF - An in vitro study. *Biomater Res*, **24**: 16. https://doi.org/10.1186/s40824-020-00193-4

Rojas MA, Marini L, Pilloni A, Sahrmann P (2019). Early wound healing outcomes after regenerative periodontal surgery with enamel matrix derivatives or guided tissue regeneration: A systematic review. *BMC Oral Health*, **19**(1): 76. https://doi.org/10.1186/s12903-019-0766-9

Sánchez-González DJ, Méndez-Bolaina E, Trejo-Bahena NI (2012). Platelet-rich plasma peptides: Key for regeneration. *Int J Pept*, **2012**: 532519. https://doi.org/10.1155/2012/532519

Tunali M, Özdemir H, Küçükodacı Z, Akman S, Fıratli E (2013). In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): A new platelet concentrate. *Br J Oral Maxillofac Surg*, **51**(5): 438–443. https://doi.org/10.1016/j.bjoms.2012.08.003

Tunali M, Ozdemir H, Kucukodaci Z, Akman S, Yaprak E, Toker H *et al*. (2014). A novel platelet concentrate: Titanium-prepared platelet-rich fibrin. *Biomed Res Int*, **2014**: 209548. https://doi.org/10.1155/2014/209548

Tunali M, Ozdemir H, Kucukodaci Z, Ezirganli S, Baris E, Akman S *et al*. (2015). A novel platelet concentrate for guided bone regeneration: Titanium prepared platelet-rich fibrin (T-PRF). *Gulhane Med J*, **57**: 102–106.

Wend S, Kubesch A, Orlowska A, Al-Maawi S, Zender N, Dias A *et al*. (2017). Reduction of the relative centrifugal force influences cell number and growth factor release within injectable PRF-based matrices. *J Mater Sci Mater Med*, **28**(12): 188. https://doi.org/10.1007/s10856-017-5992-6

Whitman DH, Berry RL, Green DM (1997). Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg*, **55**(11): 1294–1299. https://doi.org/10.1016/s0278-2391(97)90187-7

Wu LW, Chen WL, Huang SM, Chan JY (2019). Platelet-derived growth factor-AA is a substantial factor in the ability of adipose-derived stem cells and endothelial progenitor cells to enhance wound healing. *FASEB J*, **33**(2): 2388–2395. https://doi.org/10.1096/fj.201800658R

Yamakawa S, Hayashida K (2019). Advances in surgical applications of growth factors for wound healing. *Burns Trauma*, **7**: 10. https://doi.org/10.1186/s41038-019-0148-1