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Original Article

Dentistry Section

Evaluation of Platelet Count, Anti-microbial Efficacy, and Fibrin Network Pattern for Advanced Platelet Rich Fibrin versus Titanium Platelet Rich Fibrin Prepared from Blood Samples of Young Adults: An Ex-vivo Study

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ABSTRACT

Introduction: Platelet concentrates have gained popularity in periodontal regenerative therapy owing to their autologous nature. Platelet Rich Fibrin (PRF) can be classified into various types based on the centrifugation protocol. Among these, Advanced PRF (A-PRF) and Titanium-prepared PRF (T-PRF) have gained recognition because of their regenerative potential.

Aim: To evaluate platelet count, antimicrobial efficacy, and fibrin network pattern for A-PRF and T-PRF in young adults.

Materials and Methods: This ex-vivo study was conducted at the department of Periodontology and Oral Implantology, Sree Sai Dental College and Research Institute, Andhra Pradesh, India, over a period of six months from January to June 2024 and it included a 15 systemically healthy subjects. From each subject, 10 mL of blood was collected, of which 5 mL was centrifuged to obtain A-PRF (1400 RPM, 15 minutes) and 5 mL for T-PRF (3000 RPM, 10 minutes in titanium tubes). The obtained PRF membranes were analysed for platelet count, antimicrobial activity, and changes in the fibrin network. Data were analysed using Statistical Package for the Social Sciences (SPSS) 20 with an independent t-test.

Results: A total of 15 patients (7 females and 8 males) were included in this study, with a mean age of 23.8±2.54 years. The T-PRF had greater antimicrobial efficacy than A-PRF, with statistical significance ($p<0.001$), whereas the platelet count and fibrin network pattern did not show any statistical significance ($p>0.05$).

Conclusion: According to finding of the present study result it was observed that T-PRF exhibited superior antimicrobial efficacy when compared to A-PRF.

Keywords: Centrifugation, Growth factor, Healthy subjects

INTRODUCTION

Wound healing is an important concept in regenerative surgical science. It occurs as a result of the interplay between various cells, such as epithelial cells, osteoblasts, and fibroblasts, as well as the signalling molecules released by platelets in the blood clot [1]. The alpha granules attached to the platelets release growth factors such as Transforming Growth Factor (TGF), Fibroblast Growth Factor (FGF), Epithelial Growth Factor (EGF), Insulin-Like Growth Factor (IGF), and Vascular Endothelial Growth Factor (VEGF), which are responsible for clot formation. Clinicians are continually searching for alternatives to provide a rapid and natural healing response. Platelet concentrates have been widely applied in various branches of dentistry for more than half a century to enhance biological processes. Fibrin glue has also been used as a surgical adjuvant. Platelet concentrates, which are topically injected, are bioactive surgical additives intended to accelerate wound healing; they are concentrated solutions of platelet growth factors [2].

Choukron J et al., introduced platelet concentrates in the late 1990s. PRF is the second generation of platelet concentrates, which has become popular since 2001 [3]. Choukroun's PRF is derived from blood and includes growth factors with strong tissue regeneration potential, along with a variety of blood cells, including platelets, B- and T-lymphocytes, monocytes, stem cells, and neutrophilic granulocytes [4]. The distribution of neutrophils has changed as a result of modifications to the centrifugation procedure. With advancements in techniques, numerous improvements have occurred in PRF using different Relative Centrifugal Forces (RCF), Rotations Per Minute (RPM), and centrifugation times. A third generation of PRF, known as A-PRF, was introduced by Ghanaati S et al., based on a preparation protocol that employs low speed centrifugation at 1500 RPM for 14 minutes. A-PRF has shown a sustained release of growth factors, making it favourable for regenerative therapy. Ghanaati S et al., stated that the decrease in centrifugation force combined with an increase in time has led to an improvement in the platelet count. A-PRF has also demonstrated a significant increase in the neutrophil and macrophage count in the distal part of the clot. Accordingly, a high microbial assault and inflammation may be mitigated by the presence of these cells [5].

Tunali M et al., introduced a new type of PRF using titanium tubes instead of glass or silica-coated tubes. The centrifugation protocol for T-PRF is 2800 RPM for 12 minutes. T-PRF has shown a tightly woven and thicker fibrin network, along with higher cellular entrapment, resulting in increased cellularity at the required site [6]. The antimicrobial efficacy of autologous platelet concentrates has made them the best choice for periodontal regenerative therapies [7]. The growth factors present are protected from being proteolysed, resulting in an extended time for the release of growth factors at the defect site [8]. T-PRF was developed based on the hypothesis aimed at eliminating the potential hazardous effects of silica-activated PRF in glass test tubes. Since titanium particles, as opposed to silica, are used to activate platelets, T-PRF exhibits distinct characteristics, including improved biocompatibility. The varying advantages associated with the recent centrifugation protocols, considering

RCF, RPM, and time, have led to alterations in platelet activation, fibrin network patterns, and antimicrobial properties [9].

The present study aimed to compare the fibrin network structure, platelet count, and antibacterial activity of A-PRF and T-PRF in young adults. This study is the first of its kind to compare A-PRF and T-PRF. According to the null hypothesis, there is no difference between A-PRF and T-PRF in relation to platelet count, antimicrobial efficacy, and fibrin network pattern.

MATERIALS AND METHODS

This ex-vivo study was conducted at the department of Periodontology and Oral Implantology, Sree Sai Dental College and Research Institute, Andhra Pradesh, India, over a period of six months from January to June 2024. The study was approved by the Institutional Ethics Committee under the reference number SSDCRI/IEC/2021-22/8/1. Informed consent was obtained from each individual prior to the study.

Inclusion and Exclusion criteria: The study included participants who were free of periodontal diseases, in good general health (assessed by a self reported questionnaire), and had no history of infection. Individuals who had received antibiotics within the six months preceding the study's start date, as well as alcohol dependent women who were nursing or pregnant, anyone with a history of immunosuppressive or anticoagulant medications that interfered with the body's natural coagulation process, and tobacco users in any form were also excluded.

Sample size calculation: A total of 15 healthy volunteers aged between 20 and 30 were included in this study. The sample size was calculated using G*Power version 3.1.9.2, with an alpha error of <5% ($p<0.05$) and 80% power.

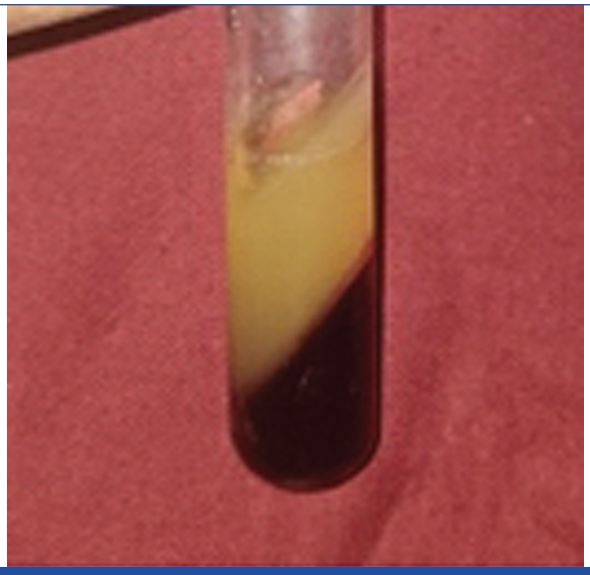
Study Procedure

Ten millilitres of blood were collected from each individual from the antecubital vein. From the 10 mL of blood collected, 5 mL was used to prepare T-PRF in titanium tubes with a centrifugation protocol of 3000 RPM for 10 minutes [Table/Fig-1] [10], while 5 mL was used to prepare A-PRF using a centrifugation protocol of 1500 RPM for 14 minutes [Table/fig-2] [5]. After obtaining the PRFs, the following parameters were assessed.

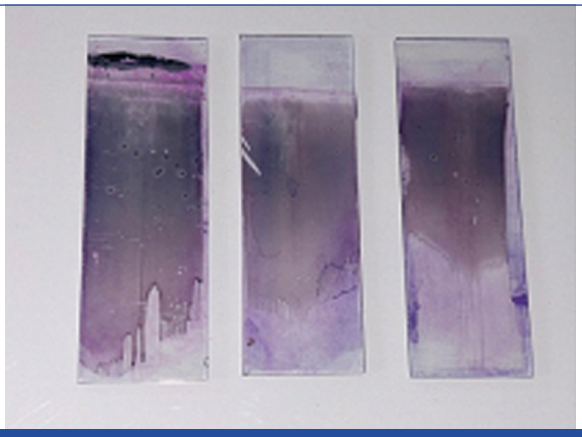
[Table/Fig-1]: T-PRF.

Parameters assessed: The peripheral smear method was used to estimate the platelet count, as suggested by Webb DI et al., [7]. A total of 0.1 mL of blood was used to estimate the platelet count of whole blood. The platelet count of PRF was assessed indirectly by calculating the platelet count of whole blood and the platelet count of the residual serum and then subtracting the platelet count of the residual serum from that of the whole blood [11].

Platelet count of PRF= Platelet count of whole blood- Platelet count of residual serum [Table/Fig-3].



[Table/Fig-2]: A-PRF.



[Table/Fig-3]: Leishman staining for assessing platelet count.

To evaluate antimicrobial effectiveness, supragingival plaque samples were obtained from subjects using Gracey curettes 1/2 and 7/8 (Gracey curette, Hu-Friedy Mfg. Co., LLC, Chicago, USA). An agar well diffusion test was performed in Petri dishes measuring 90 mm in diameter and containing 4 mm of blood agar. The plaque samples were immediately transferred to a glass test tube containing 5 mL of saline, followed by vortexing for five minutes to create a homogeneous suspension. This solution was then added to the blood agar using the lawn technique by evenly flooding the sample onto the agar plate. A sample with sufficient antimicrobial activity diffuses through the agar, forming a zone of inhibition [11]. The antibacterial activity was evaluated in compliance with the accepted guidelines for the evaluation of inhibition zones and the agar disk diffusion method, utilising a zone inhibition scale. [Table/Fig-4] [11].

[Table/Fig-4]: Zone of inhibition of T-PRF & A-PRF.

Isolated PRF clots were prepared onto slides using the cell block cytology method to examine the fibrin network pattern. The haemotoxylin and eosin-stained PRF was photographed under a light microscope, and the results were analysed using the Blood Elements Adhesion Index (BEAI) [11], which is defined as follows:

Score 0: Absence of fibrin network;

Score 1: Scarcely distributed fibrin network;

Score 2: Thin fibrin network pattern with poor interlacing;

Score 3: Dense fibrin network pattern with rich interlacing.

STATISTICAL ANALYSIS

The results were subjected to statistical analysis using SPSS version 20. An independent-samples t-test was employed to interpret the data. A p-value of less than 0.01 ($p<0.01$) was considered significant.

RESULTS

A total of 15 patients (7 females and 8 males) were included in this study, with a mean age of 23.8±2.54 years. The platelet count was assessed by an indirect method, calculating the platelet count of the residual serum and subtracting it from the platelet count of the whole blood. The mean platelet count for A-PRF was 0.125±0.09×10^6 / µL, while for T-PRF it was 0.15±0.16×10^6 /µL. However, there was no statistical significance (p=0.530) in platelet count between the two groups [Table/Fig-5].

Platelet count	Mean (/µL)	Std. Deviation	p-value
A-PRF	0.125333×10^6	0.0990575	0.530
T-PRF	0.1533×10^6	0.16286	

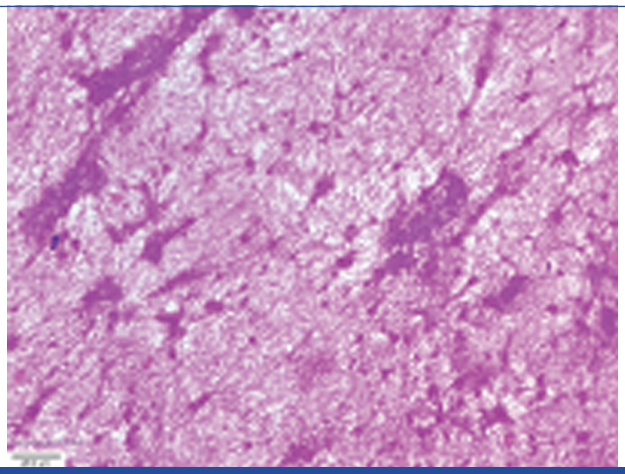
[Table/Fig-5]: Mean, Std.deviations and p-values of platelet count for A-PRF and T-PRF.

The widest zone of inhibition was observed in T-PRF, with a mean value of 13.3±1.6 mm, while the narrowest zone of inhibition was seen in A-PRF, with a mean value of 6.2±5.2 mm. There was a statistically significant difference when comparing both groups, with p<0.001, which was highly significant [Table/Fig-6].

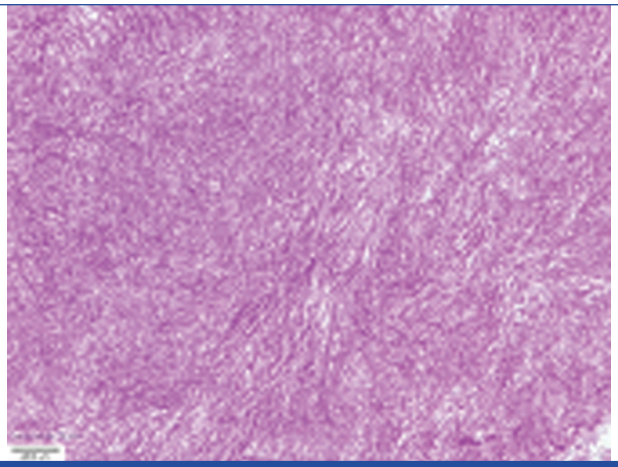
Antimicrobial efficacy	Mean (mm)	Std. Deviation	p-value
A-PRF	6.20	5.254	<0.001*
T-PRF	13.33	1.633	

[Table/Fig-6]: Mean, Std.deviations and p-values of Anti-microbial efficacy for A-PRF and T-PRF in mm.

The photographic analysis [Table/Fig-7,8] demonstrated a denser fibrin network pattern for T-PRF compared to A-PRF. The results indicated that the fibrin network pattern of A-PRF and T-PRF showed no statistically significant difference (p=0.082), with a mean score of 2.0±0.0 for A-PRF and 2.2±0.4 for T-PRF [Table/Fig-9].



[Table/Fig-7]: Fibrin network pattern of T-PRF under 40X magnification showing thick fibrin network pattern with rich interlacing.



[Table/Fig-8]: Fibrin network pattern of A-PRF under 40X magnification showing thin fibrin network pattern with poor interlacing.

Fibrin network pattern	Mean	Std. Deviation	p-value
A-PRF	2.00	0.000	0.082
T-PRF	2.20	0.414	

[Table/Fig-9]: Mean, Std.deviations and p-values of Fibrin network pattern for A-PRF and T-PRF.
Std.deviation: Standard deviation p-value < 0.000*, denotes high statistical significance A-PRF: Advanced platelet rich fibrin T-PRF: Titanium platelet rich fibrin

DISCUSSION

The present study aimed to evaluate and compare the platelet count, antimicrobial efficacy, and fibrin network pattern of A-PRF and T-PRF. Although the numerical values indicated an increase in platelet count for T-PRF compared to A-PRF, this difference was not statistically significant. The findings are consistent with previous studies, such as that of Tunali M et al., who reported that T-PRF enhances platelet activation more effectively than PRF prepared in silica tubes [6]. However, the lack of statistical significance in platelet count contrasts with the results of Ghanaati S et al., who found that decreasing centrifugation speed and increasing duration resulted in significantly higher platelet concentrations [5]. Differences in centrifugation protocols and sample sizes may explain this discrepancy.

Ravi S et al., conducted a study on the mechanical, chemical, structural characteristics, and comparative release of Platelet-Derived Growth Factor (PDGF) – AA from Leukocyte-Platelet Rich Fibrin (L-PRF), A-PRF and T-PRF and found that A-PRF had a sustained release of growth factors compared to T-PRF [12].

The antibacterial effectiveness of PRF was assessed by the formation of inhibition zones surrounding the samples. Reducing the centrifugation speed and duration resulted in a significantly higher antibacterial potential due to the enhanced presence of neutrophilic granulocytes [5]. In the present study, however, T-PRF exhibited better antimicrobial efficacy, likely due to the increased platelet count when compared to A-PRF. A study conducted by Kour P et al., concluded that there was a significant amount of antimicrobial efficacy among different platelet concentrates [13]. The presence of leukocytes and platelets in PRF is primarily responsible for its antibacterial properties [14]. This fact was corroborated in the current investigation, which established a clear association between platelet concentrates and the antibacterial zone of inhibition. Nevertheless, the specific mechanism by which platelets impart their antibacterial properties remains unknown. Additionally, the presence of leukocytes may have contributed to the antimicrobial action of PRF membranes. Due to their phagocytic activity, white blood cells have an antibacterial effect and contain a high concentration of antimicrobial compounds such as myeloperoxidase, defensins, cathelicidins, and lysozyme [15]. However, one limitation of this study is that tests for leukocytes were not conducted.

The antibacterial action of T-PRF is of significant importance in clinical practice, as it may be used in surgical periodontal therapy as an adjuvant, thereby reducing bacterial count and assisting in healing and regeneration. Furthermore, it minimises the risk of postoperative infections. Another study conducted by Hoaglin DR and Lines GK demonstrated that using PRF to fill the extraction sockets of third molars reduced osteomyelitis infections by tenfold [16].

In 2014, Ghanaati S et al., developed a novel concept known as A-PRF (1500 rpm for 14 minutes) [5]. Their histochemical research revealed that A-PRF exhibited a looser fibrin network with higher interfibrin space compared to regular PRF, which had a denser fibrin clot and minimal interfibrin space. Miron RJ et al., recently proposed that reducing the speed and duration of centrifugation results in a smaller membrane size [17]. As previously demonstrated, lowering the centrifugation speed increases the quantity of cells and growth factors, thereby enhancing the regenerative capacity of PRF matrices [5].

Studies conducted by Tunali M et al., and Chatterjee A et al., showed a highly organised dense fibrin network pattern in T-PRF. In contrast, A-PRF displayed a looser fibrin network pattern with gaps in between [6,10]. In the present study, photographic analysis revealed that T-PRF had a denser fibrin network pattern when compared to A-PRF; however, this difference was not statistically significant. Further studies will be needed with a larger sample size, as well as across different age groups and stages of periodontitis.

Limitation(s)

Further studies need to be conducted with a larger sample size to enhance the statistical reliability of the findings. Additionally, the present study did not assess variations across different age groups and genders, which could provide a more comprehensive understanding of PRF's effects. Another important aspect that requires investigation is the leukocyte count, as it plays a crucial role in PRF's antimicrobial efficacy and regenerative potential. Future research should focus on these factors to establish a more detailed and clinically relevant analysis of PRF's therapeutic benefits.

CONCLUSION(S)

Within the limitations of the present study, T-PRF exhibited superior antimicrobial efficacy when compared to A-PRF. T-PRF displayed an equivalent platelet count and a similar fibrin network pattern when compared to A-PRF. Further studies need to be conducted with a larger sample size and across different age groups to ascertain the benefits of using T-PRF and A-PRF and to better understand the potential of the latest generation of platelet concentrates.

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