ORIGINAL RESEARCH PAPER

Volume-7 | Issue-12 | December-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARATIVE EVALUATION OF I-PRF & PRF ON ANTIMICROBIAL EFFICIENCY A- CLINICOMICROBIOLOGICAL STUDY

Periodontology

Dr. Ritu D. Solanki*	Part -2 PG Student, College Of Dental Sciences And Research Centre, Opp. Pleasure Club, Maipur, Bopal, Ahmedabd, CDSRC, Ahmedabad. *Corresponding Author
Dr. Anita. H. Panchal	MDS, HOD, College Of Dental Sciences And Research Centre, Opp. Pleasure Club, Maipur, Bopal, Ahmedabd
Dr. Rahul. N. Shah	MDS, Reader, College Of Dental Sciences And Research Centre, Opp. Pleasure Club, Maipur, Bopal, Ahmedabd
Dr. Satish Somoori	M.Sc, Microbiology, AP , College Of Dental Sciences And Research Centre, Opp. Pleasure Club, Maipur, Bopal, Ahmedabd
Dr. Khoobi. M. Shah	MDS, Senior Lecturer, College Of Dental Sciences And Research Centre, Opp. Pleasure Club, Maipur, Bopal, Ahmedabd
Dr. Ganesh. R. Nair	Part 3 PG Student, College Of Dental Sciences And Research Centre, Opp. Pleasure Club, Maipur, Bopal, Ahmedabd

ABSTRACT

Periodontal therapy aimed at regenerating lost periodontal structure. Growth factors of different platelet concentrates gained popularity in promoting soft and hard tissue regeneration. Efficacy of i-PRF is required to be explored. This was designed to evaluate the antimicrobial efficacy of i-PRF,PRF and blood. Blood sample obtained from 20 subjects and platelet concentrate was prepared using centrifugation protocol i.e, i-PRF and PRF. i-PRF considered as Group A, PRF considered as Group B and blood as Group C. Platelet count was done by manual counting method using Neubauer's chamber. Antimicrobial activity examined by blood agar using well diffusion method by measuring zone of inhibition and quantifying inhibitory effect of platelet concentrate. Statistical analysis done by one-way ANOVA, $P < 0.05$ considered statistically significant. Inhibition zone of bacteria around i-PRF($P \leq 0.05$) and PRF ($P \leq 0.05$) was statisticaly significant compared to blood. Antimicrobial efficacy of i-PRF is better compared to PRF and control.

KEYWORDS

periodontitis,platelets,i-PRf,antimicrobial efficacy

I. INTRODUCTION

- Periodontitis has been defined as "an immuno-inflammatory disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss".[1] Advance chronic periodontitis leads to severe periodontal destruction and ultimately tooth loss. Hence, the periodontal therapy is aimed at regenerating the lost periodontal structures.[2]

- There are various methods used for regenerating the lost periodontal structures. Such as 1) Bone grafts 2) Guided tissue regeneration 3) Stem cells 4) Tissue engineering 5) Platelet concentrates. Platelet concentrates for topical and infiltrative use are blood extracts obtained after various processing of a whole blood sample, mostly through centrifugation.[3]

- The objective of the processing is to separate the blood components in order to discard elements considered as not usable (mostly the red blood cells, heavy and easily separated) and concentrate the elements that may be used for therapeutic applications (fibrinogen/fibrin, platelets, growth factors, leukocytes and other forms of circulating cells, in solution in liquid plasma).[4] These preparations are used on a surgical or wounded site in order to stimulate, improve and accelerate healing.[5]

- Platelet concentrates have gained popularity in periodontal regenerative therapy because of their autologous nature. Platelets have presence of various growth factors in the α–granules of platelets which are released at local site on their activation.[6]

- Platelets exhibit hallmark structural and functional characteristics that are associated with their participation in host defense against infection. Platelets are capable of binding, aggregating, and internalizing microorganisms, which enhances the clearance of pathogens from the bloodstream. Platelets participate in antibody dependent cell cytotoxicity functions to kill protozoal pathogens. When stimulated by microorganisms or agonists generated in the setting of infection, platelets release an array of microbial peptide.[7]

- Recently introduced platelet concentrate by Joseph Choukron in 2014 is **injectable platelet-rich fibrin**.[8] because of their antimicrobial functions veroius platelet concentrates have been studied.

- Aim of the present study was to compare and evaluate the platelet count and antimicrobial efficacy of platelet concentrate i.e; i-PRF, PRF & Blood.

MATERIALS AND METHOD

- A randomized clinical trial was conducted in Department of Periodontology and Implantology, College Of Dental Science and Research Centre, Ahmedabad India. Total 20 Patient were included in study.

Inclusion criteria:

- Patients with Chronic generalized periodontitis.
- Systemically healthy patients.

Exclusion criteria:

- Patients having habit of tobacco chewing and smoking,
- Patients who undergone for periodontal therapy in last six months,
- Patients who had on antiplatelet therapy,
- Pregnant and lactating woman.

Sample collection

- A total 6 ml of blood was collected from all the subjects. 2ml blood was used for i-PRF preparation, 2ml blood was used for PRF preparation and rest used as a control.

Preparation of platelets concentrates

- A 2 ml of blood collected in noncoated vacutainer without any additives and it is centrifuged at 700-800 rpm for 3–4 min for i-PRF preparation, A volume of 2 ml of blood collected in silicon-coated vacutainer without any additives and centrifuged at 2400 rpm for 10 min for PRF preparation and blood is collected in EDTA containing vacutainer.

Plaque sample collection

- Plaque samples of subjects were collected using area specific gracey curette, The sample was transferred to 5 ml saline containing tube and vortexed for 5 min to obtain uniform solution. Later, 0.1 ml of this suspension was then used to plate blood agar using streak method.

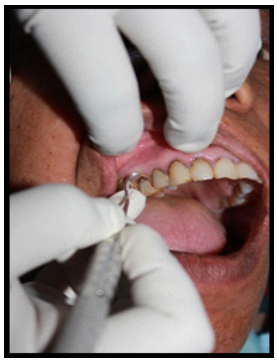

Figure 1.collection of plaque sample

Quantification of platelet

- 0.1 ml of all platelet concentrate were placed separately on glass slide. Using the corner of a clean slide, held at 45°, the drop of the sample was spread on that slide. Platelets were calculated by traditional estimation method. In this method, 10 fields of the smear were evaluated under oil immersion. The average was then multiplied by 15,000. This gave the platelet count/cu mm. PRF platelet count was assessed indirectly by subtracting the platelet count in the residual serum.

Agar plate preparation:

- In the inoculated blood agar plate with plaque solution, wells were prepaid. 0.1 ml of i-PRF, PRF, and control were placed in those wells. Inoculated blood agar plates were then incubated in an incubator aerobically at 37°C for 48 h.

Antimicrobial efficiency measurement:

- After 48 hrs antimicrobial efficiency was measured by measuring the mean zone of inhibition around each platelet concentrate wells.

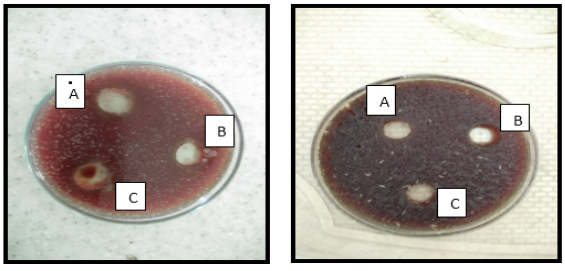

Figure 2 & 3 Zone of inhibition around each platelet concentrate A- i-PRF, B- PRF, C-Blood

Data analysis

- Statistical analysis For antimicrobial potential, the comparison between zones of inhibition obtained around all the samples was analyzed by one-way ANOVA.
- P <0.05 was considered statistically significant.

RESULTS

Table 1. Quantification of platelets Platelet count/mm3 obtained for i-PRF, PRP and control. The platelet count of i-PRF was statistically significant when compared to control ($P < 0.05$). It was also significant when compared to PRF ($P < 0.51$) and control ($P < 0.05$). This indicates that i-PRF has maximum number of platelets in comparison to other concentrates.

Table 1:- Mean platelet count of different platelet concentrate

Platelet concentrate	Platelet count(10^6)	Standard deviation(10^6)
i-PRF	1.40	1.91
PRF	1.25	1.48
Blood	1.37	0.67

P>0.05 is statistically significant

Table 2. Evaluation of antimicrobial potential The antimicrobial efficacy was demonstrated by appearance of a clear zone of inhibition around the samples. Mean zone of inhibition around i-PRF ($P < 0.05$) and PRF ($P < 0.05$) reached statistical significance. These results indicate that i-PRF has a significant inhibitory effect on growth of oral bacteria in comparison to other platelet concentrates.

Table 2:- Mean zone of inhibition around each platelet concentrate

Platelet concentrate	ZOI(cm)	Standard deviation(cm)
i-PRF	2.19	0.47
PRF	1.32	0.30
Blood	0.23	0.10

P>0.05 is statistically significant

DISCUSSION:

- During the past 20 years, autologous platelet-rich preparations have achieved great popularity in various fields of medicine. Regenerative potential of platelet concentrates has been extensively explored since their introduction in regenerative medicine in the oral and maxillofacial surgery field during the late 1990s.

- PRF and i-PRF have gained a lot of popularity as hard and soft tissues regenerative material. Its regeneration potential is thought to stem from the fact that platelets store growth factors.[7]

- Apart from their excellent regenerative properties, studies have reported an antimicrobial effect of platelet concentrates against bacteria such as Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia.

- The mechanism of the antibacterial effect of platelet- derived preparations is not yet fully understood. It is hypothesized that platelets demonstrate antimicrobial activity by the following ways: 1. They generate oxygen metabolites, including superoxide, hydrogen peroxide and hydroxyl free radicals. 2. They bind, aggregate and internalize microorganisms, which enhances the clearance of pathogens from the bloodstream. 3. Platelets release an array of potent antimicrobial peptides.

- In the present study, it was observed that i-PRF has the maximum number of platelet count. This can be attributed to the low centrifugation speed and also, i-PRF has shown the maximum zone of inhibition around the oral microflora compared to the PRF and blood.

- **Yeaman, (1997)**[8] suggested that direct interaction of platelets with microorganisms, participation in antibody-dependent cell cytotoxicity and engulfment by entrapped white blood cells within PRF could result in direct bacterial killing.

- **Drago L et al. (2013)**[10] used platelet concentrate in the form of P-PRP, they found that P-PRP inhibited the growth of E. fecalis, C.albicans, S.agalactiae and S.oralis but not of P.aeruginosa. i-PRF has added advantage of being used in injectable form. It contain larger proportion of leukocytes and blood plasma protein due to lower centrifugation speed and time.[9]

CONCLUSION

- Autologous i-PRF & PRF is a simple and inexpensive technique used for the regeneration of lost periodontal tissues. i-PRF being an autologous preparation, is free from any side effects which routinely encountered with other antimicrobial agents.
- The present clinical study of i-PRF demonstrated a better antimicrobial efficacy as compared to PRF. This could be used to reduce microbial load at periodontally infected sites. i-PRF can also be used with the bone graft. However, this is being an in-vitro study, its results should further be evaluated and validated through more in-vivo studies.

REFRENCES:

1. Flemmig TF: Periodontitis. Ann Periodontol 4:32–38, 1999.
2. Prerna Ashok Karde, et al; Comparative evaluation of platelet count and antimicrobial efficacy of injectable platelet-rich fibrin with other platelet concentrates: An in vitro study: Journal of Indian Society of Periodontology 2017;21(2), 97-101.
3. Bielecki T, Dohan Ehrenfest DM. Platelet-rich plasma (PRP) and Platelet-Rich Fibrin (PRF): surgical adjuvants, preparations for in situ regenerative medicine and tools for tissue engineering. Curr Pharm Biotechnol. 2012;13:21-30.
4. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet

concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L- PRF). Trends Biotechnol. 2009;27:58-67.
5. Cieslik-Bielecka A, Choukroun J, Odin G, Dohan Ehrenfest DM. L-PRP/L-PRF in esthetic plastic surgery, regenerative medicine of the skin and chronic wounds. Curr Pharm Biotechnol. 2012;13:66-77.
6. Michael R. Yeaman : The Role of Platelets in Antimicrobial Host Defense: Clinical Infectious Diseases 1997;25:951–70.
7. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fi brin (PRF): A second-generation platelet concentrate. Part III: Leucocyte activation: A new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:e51-5.
8. Michael R. Yeaman : The Role of Platelets in Antimicrobial Host Defense: Clinical Infectious Diseases 1997;25:951–70
9. Choukroun J. Advanced PRF & i-PRF: Platelet concentrates or blood concentrates? J Periodontal Med Clin Pract 2014;1:3.
10. Drago L, et al. : Plasma Components and Platelet Activation Are Essential for the Antimicrobial Properties of Autologous Platelet –Rich Plasma: An In Vitro Study : PLoS One, 2014; 9(9), 1-5