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# Evaluating The Antibacterial Effects Of Three Platelet Concentrates On Periodontal Pathogens: An In-Vitro Study

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**ABSTRACT**
*Platelets have many features that indicate their role in the anti-infective host defense in addition to a pool of growth factors during activation. The antimicrobial activities of platelet-rich plasma (PRP) and related plasma preparations against periodontal disease–associated bacteria were evaluated in vitro in this study. Three distinct plasma fractions were extracted in the formulation used commonly in dentistry and were tested for their antibacterial properties against two periodontal pathogens: Porphyromonas gingivalis(Pg) and Aggregatibacter actinomycetemcomitans (Aa) with 0.12% chlorhexidine(CHX) as control. PRP and Leucocyte-Platelet-Rich Fibrin(L-PRF), Titanium- Platelet-Rich Fibrin (T-PRF) were obtained from 5ml (15 samples ) whole blood each of 5 healthy subjects. In vitro laboratory susceptibility was carried out using the modified agar diffusion method by measuring the diameters of inhibition zones on agar plates coated with selected microbial strains. Showed effective greater inhibition area around PRP and CHX than L-PRF and T-PRF, which was highly significant (p<0.0001) for both Pg and Aa. There was no significance when compared between L-PRF and T-PRF. PRP expressed antibacterial properties, which may be attributed to platelets possessing additional antimicrobial molecules. The application of PRP on periodontal surgical sites is advisable because of its regenerative potential and its antibacterial effects.*
***KEY WORDS:*** *Platelet rich plasma, Leukocyte platelet rich fibrin, Titanium platelet rich fibrin, in-vitro, Periodontal pathogen.*

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## INTRODUCTION

Periodontal Diseases are initiated by oral biofilm formation resulting into a polymicrobial infection in the periodontal pocket inflammatory bone destruction. The strategies to treat a periodontal disease is to mechanically debride (scaling and periodontal surgery) the dental biofilm and associated microbes at the infectious site[1-2] However, amazing challenge that have been observed with mechanical perinatal therapy is the the virus of any microbial contamination. This interference with the wound healing process and also tissue regeneration, ultimately doubting the success of the therapy. Hence, it is important to control any future infection formation. Platelet concentrate have shown its crucial role in regeneration therapy in almost all fields of dentistry[3]In the past few years, the use of platelet concentrates as an anti-inflammatory agent have been advocated by many author. However the use of platelet concentrate as an antimicrobial agents have not be explored much. The principal behind the antimicrobial action is true the generation of free radicals or metabolites that can bind and internalization of microorganisms[4]
Different types of PCs have been develop overtime, shows different characteristics. platelet rich plasma (PRP), leukocyte platelet rich fibrin (L-PRF), titanium platelet rich fibrin (T-PRF) contains growth factors (GFs) and biological active proteins that plays an important role antimicrobial action and the modelling of tissues in the area of healing. It is known now that the polymicrobial periodontal disease are specific to bacterial species. The effects of PCs on them still remains unclear. Hence with this prospect in mind, this in-vitro study was conducted to evaluate the antimicrobial effect of PRP, L-PRF and T-PRF against two common periodontal microbes.

## MATERIAL AND METHODS

### Subjects and Methods

Study was approved by the Institutional Ethical Committee of College of Dental Sciences, Davangere, India.Ten healthy subjects, age ranging from 25 to 45 years volunteered to participate in the study. A written and verbal informed consent was obtained before the start of the study. The participants who

were non-smokers, had no symptoms of any infection and took no antibiotics for more than 6 months were included in this study. Subjects who had undergone any periodontal therapy in less than 6 month or any treatment for infectious diseases, history of any systemic diseases or cancer, pregnant and lactating mothers were excluded from the study.

A total volume of 15 ml blood was drawn from each subject by intravenous puncher at antecubital fossa and 5 ml blood from each were used for L-PRF, T-PRF and PRP procurement. Antimicrobial culturing was done on blood agar plate using strains of *P. gingivalis* and *A. actinomycetemcomitans* separately for PRP, L-PRF, T-PRF and 0.12% Chlorhexidine (Positive Control).

**Procurement of Platelet Concentrates**

The blood was collected into their respective tubes for each type of PC preparation. For PRP, the blood was mixed with 3.2% sodium citrate solution coated tube and was inverted several times for proper mixing. For L-PRF the blood was directly collected into the silica coated tube that had no anticoagulant and for T-PRF, titanium tube was used instead of silica coated tubes for the preparation. The tubes were then centrifuged in centrifugation machine (ROTEK Centrifuge) at specific rpm and time.

For PRP[5]first centrifugation was done at 1000 rpm for 13 min resulted into formation of three separate layers. The upper layer (Platelet Poor Plasma) was removed using micropipette and discarded. The tube was reinserted into the centrifuge machine at 2000 rpm for 10 min resulting into formation ofRBC sediment at bottom layer and PRP at top layer. The PRP was withdrawn using Pasteur pipette and was placed in a sterile container. PRP was activated by adding 10% calcium chloride and was left undisturbed for 30–40 min.For L-PRF and T-PRF, tubes were centrifuged at 3000 rpm/10 minutes and 2800 rpm/12 minutes respectively for each preparation. After centrifugation, with the use of sterile tweezers, the PRF was removed and separated from the RBC layer.

**Microbial culturing**

Blood agar plates were inoculated with bacterial strains: *P. gingivalis* ATCC No. 33277 and *A. actinomycetemcomitans* ATCC No. 43718. Each agar plate was then labeled and divided into four sections arbitrary forChlorhexidine, PRP, L-PRF and T-PRF, respectively.

**Incubation**

The anaerobes, *A. actinomycetemcomitans* plates were incubate in the $CO_2$ jar and was keep in the incubator at 37 ºC and for *P. gingivalis*, the plates were incubate in the anaerobic jar at 37 ºC incubation. Observations were made on 2nd day and 7th day and diameter of the colonies free area across the well were recorded in millimeters. (Figure 1 and 2)

**Statistical Analysis**

Analysis was performed using Turkey Post-Hoc test as the data were non-normally distributed to compare PRP, L-PRF, T-PRF and Positive Control (PC) for *P. Gingivalis* and *A. Actinomycetemcomitans*. The level of significance was considered to beat p value< 0.05.

**RESULTS**

Analysis showed zone of inhibition around PRP of mean range of 11.8±0.83 mm at 2nd day and 18.2±0.83 mm at 7th day for *P. Gingivalis* and a mean range of 15±0.70mm at 2nd day and 21.2±1.30mm at 7th day for *A.actinomycetemcomitans*. Chlorhexidine CHX (Positive Control) has shown zone of inhibition of mean range of 11.2±0.83 mm at 2nd day and 14.2±0.83 mm at 7th day on *P.Gingivalis*and mean range of 20.4±1.14mm at 2nd day and 22.4±0.54mm at 7th day for *A.actinomycetemcomitans*. No zone of inhibition was seen with L-PRF and T-PRF with both microbial cultures.(Table 1).

When PRP was compared to L-PRF, T-PRF and CHX, the resultant was highly significant ($p < 0.0001$). Similar result was seen with CHX when compared to PRP, L-PRF and T-PRF ($p < 0.0001$). No significance was seen when L-PRF was compared to T-PRF ($p = 1.000$). This shows that PRP and CHX dramatically inhibited the growth of both *P. Gingivalis* and *A. actinomycetemcomitans* compared with each other and to PRF's. (TABLES 2)

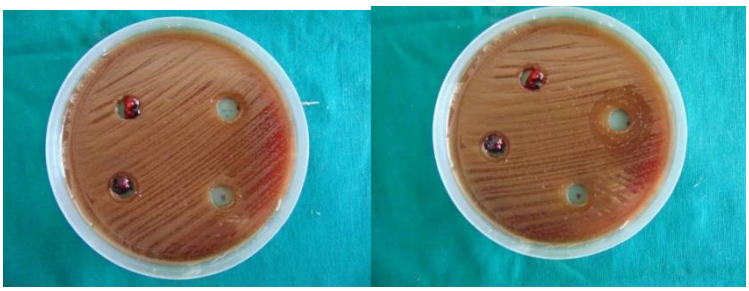

Figure 1: Antimicrobial effect of Platelet Concentrates and CHX on *P. gingivalis*.

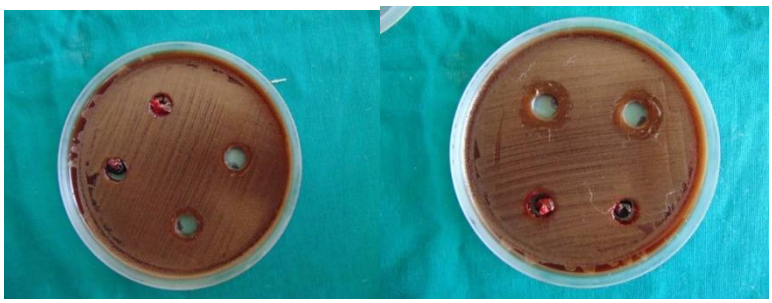

Figure 2: Antimicrobial effect of Platelet Concentrates and CHX on *A.actinomycetemcomitans*

Table 1: Comparative data on Platelet Concentrates for two perio-pathogens at 2nd and 7th day.

| **2nd DAY** | ***P. gingivalis*** | | | | ***A. actinomycetemcomitans*** | | | |
|---|---|---|---|---|---|---|---|---|
| Sample no. | PRP (mm) | LPRF (mm) | TPRF (mm) | PC (mm) | PRP (mm) | LPRF (mm) | TPRF (mm) | PC (mm) |
| 1 | 12 | 0 | 0 | 15 | 11 | 0 | 0 | 14 |
| 2 | 12 | 0 | 0 | 15 | 12 | 0 | 0 | 15 |
| 3 | 13 | 0 | 0 | 14 | 10 | 0 | 0 | 13 |
| 4 | 11 | 0 | 0 | 15 | 11 | 0 | 0 | 15 |
| 5 | 12 | 0 | 0 | 16 | 12 | 0 | 0 | 14 |
| Mean: | 11.8(0.83) | 0 | 0 | 15(0.70) | 11.2(0.837) | 0 | 0 | 14.2(0.837) |
| **7th DAY** | | | | | | | | |
| 1 | 18 | 0 | 0 | 20 | 22 | 0 | 0 | 22 |
| 2 | 19 | 0 | 0 | 23 | 20 | 0 | 0 | 22 |
| 3 | 17 | 0 | 0 | 21 | 19 | 0 | 0 | 23 |
| 4 | 19 | 0 | 0 | 20 | 20 | 0 | 0 | 23 |
| 5 | 18 | 0 | 0 | 22 | 21 | 0 | 0 | 22 |
| Mean : | 18.2(0.83) | 0 | 0 | 21.2(1.30) | 20.4(1.14) | 0 | 0 | 22.4(0.54) |

p value <0.05 = * (significant), p value <0.001 = ** ( highly significant)

Table 2 : Inter-group comparison between Platelet Concentrates at for two perio-pathogens.

| | ***P. gingivalis*** | | ***A. actinomycetemcomitans*** | |
|---|---|---|---|---|
| At day 2 (Post hoc test ) | PRP vs LPRF | **0.0001**** | PRP vs LPRF | **0.0001**** |
| | PRP vs TPRF | **0.0001**** | PRP vs TPRF | **0.0001**** |
| | PRP vs PC | **0.0001**** | PRP vs PC | **0.001**** |
| | LPRF vs TPRF | **1** | LPRF vs TPRF | **1** |
| | LPRF vs PC | **0.0001**** | LPRF vs PC | **0.0001**** |
| | TPRF vs PC | **0.0001**** | TPRF vs PC | **0.0001**** |
| | | | | |
| At day 7 (Post hoc test ) | PRP vs LPRF | **0.0001**** | PRP vs LPRF | **0.0001**** |
| | PRP vs TPRF | **0.0001**** | PRP vs TPRF | **0.0001**** |
| | PRP vs PC | **0.0001**** | PRP vs PC | **0.0001**** |
| | LPRF vs TPRF | **1** | LPRF vs TPRF | **1** |
| | LPRF vs PC | **0.0001**** | LPRF vs PC | **0.0001**** |
| | TPRF vs PC | **0.0001**** | TPRF vs PC | **0.0001**** |

p value <0.05 = * (significant), p value <0.001 = ** ( highly significant)

## DISCUSSION

The primary goal of any periodontal therapy is regeneration and PCs have been used since few decades with this respect. However, the potential use of PCs as antimicrobial agents against periodontal pathogens is less study in the literature[6].PCs has a complex mixture of platelets, plasma and white blood cells that are responsible for its antimicrobial activity. It contain oval shaped alpha granules of diameter 200 to 500 nm that forms the pool of vital proteins required for wound healing[7].The antimicrobial action of platelets can be explained by the fact that the molecules of Alpha granules can cause internalization of bacteria.6 the present evidence suggest free radical species[8] and antibody - dependent cell killing of protozoans [9]can play an important role in the regeneration and wound healing of periodontal structures. With increase in the investigative findings, more antimicrobial factors were found in PCs like platelet antimicrobial proteins, complement - binding proteins and antigen - specific immune response via myeloperoxidase [10-12].

The present study have shown the capability of PRP to inhibit *P. gingivalis* and *A. actinomycetemcomitans* at second and seventh day, whereas the other PCs were unable to enable it the perio-pathogen under the in vitro conditions. The reason behind the significance of the increase antimicrobial activity of PRP and not seen with any other PRF can be due to the fact that there is a slow and progressive release of platelet-leukocyte aggregates from the thick interwoven fibre matrix over the time period of more than a week[13-14].Compare to L-PRF, T-PRF has even has both take fibrin matrix as, thus the release of factors from this tight mesh is ought to be more delayed[15]On contrary the PRP has an unorganized matrix with scattered platelet- leukocyte aggregates , large concentration of of weighted and leukocytes[16]
Therefore the release of antimicrobial agents from PRP is early within a week compared to other concentrates. Hence within the limitations of this study PRP is considered as an active PCs showing equivalent action compared to the be active control either chlorhexidine then the other concentrates- L-PRF and T-PRF.

## CONCLUSION

This study is the first to evaluate effect three different kinds of platelet concentrates on periodontal pathogens over the time interval of 2-7 days. PRP has shown a potential used in routine practice as an antimicrobial agent that have the ability to inhibit the pathogens in 7 days. Future investigation are required to evaluate the platelet rich fibrins potential using a more physiological in vitro condition.

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