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# Antimicrobial activity of injectable platelet rich fibrin with antibiotics on periodontopathic bacteria

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**Abstract:**
Injectable platelet-rich fibrin (i-PRF) is used in periodontal regenerative procedures and may also serve as a carrier for locally delivered antimicrobial agents. Therefore, it is of interest to evaluate the antimicrobial activity of i-PRF combined with penicillin, metronidazole, or moxifloxacin against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Standardized agar well-diffusion assays were performed at 100%, 50%, and 25% concentrations, with i-PRF mixed with saline serving as the control. Antibiotic-containing i-PRF preparations produced zones of inhibition against both organisms, the reported group comparisons were statistically significant ($p < 0.01$). Thus, data support further standardized studies but do not establish clinical efficacy or the optimal antibiotic formulation.

**Keywords:** Injectable platelet-rich fibrin (i-PRF), periodontitis, anaerobic culture, platelet concentrates

**Background:**
Periodontitis is a chronic inflammatory disorder, associated with dysbiotic plaque biofilms, resulting in progressive destruction of the tooth-supporting apparatus and intrabony periodontal defects **[1].** Such defects present risk factors for further disease progression and their therapy improves tooth prognosis **[2].** Platelet Rich Fibrin is a second-generation platelet concentrate comprising of complex network of micro fibrins with entrapped platelets and leucocytes. The reason for their application is that the enriched platelet and leucocytes release intra cellular growth factors, bioactive molecules and bioactive peptides that enhance both hard and soft tissues healing process **[3].** In addition to this, the physiological architecture of the micro fibrines favors wound healing **[4].** Platelets are a vital component of the wound healing process. Upon activation, they release a range of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor (TGF-β) and insulin-like growth factor I (IGF-I) **[5].** Among the plethora of biologically active molecules present in blood platelet growth factors are of particular significance in the context of tissue healing and repair **[6].** PRF exhibits a consistent and uninterrupted release of growth factors over a 10-day interval **[7].** They exert a direct influence on the promotion of osteoblast and endothelial cell proliferation and differentiation, in addition to that of chondrocytes and various fibroblast sources **[8, 9].** i-PRF was developed as an advanced product of PRF by altering the centrifugation protocol by lowering the centrifugation speed and force to 700 rotations per minute (RPM) and 40 grams of force in the year 2014 **[10].** This results in segregation of the blood into 2 compartments, the top layer being the liquid platelet rich fibrin (Liquid PRF) and the bottom red blood cells. The top layer of liquid PRF can be aspirated in a syringe and stays in the injectable form for an average of 5 - 10 minutes **[11].** During this the liquid fibrinogen is slowly polymerising into a fibrin matrix after which it becomes a fibrin clot. Thus, the transient liquid form of i-PRF allows for the clinician to utilise it in injectable forms for various clinical applications like for both soft and hard tissue management in periodontal and oral surgical procedures. Similar to PRF, i-PRF brings about clinical benefits because of the fibrin matrix, their degradation and release of various cytokines including growth factors into the surgical wound area for regeneration and healing. Study by Jasmine *et al.* **[12]** evaluated the biofilm formation by Staphylococcus aureus pathogen reported that i-PRF showed a potent inhibition of biofilm formation by staphylococcus pathogen which was concentration dependent showing week to moderate effect at MIC concentrations and strong antibiofilm effect at MBC of i-PRF. Miron *et al.* have showed that i-PRF can be employed as a drug carrier when a pharmaceutical agent is introduced into i-PRF matrix before the onset of clot formation and discussed the possibility of combining various bioactive materials with i-PRF to produce an advanced local delivery system for small and large biomolecules **[13].** Therefore, it is of interest to evaluate the antimicrobial efficacy of injectable platelet rich fibrin incorporated with different antibiotics such as penicillin mu or ml, metronidazole 5mg or ml and moxifoxicin 150mg or ml on Aggregatibacter actinomycetemcomitans and Porphyromonas Gingivalis.

**Materials and Methods:**
**Study design:**
The study protocol was approved by the institutional ethical committee. In this in vitro study, a total of 56 samples of agar plates were included and were divided into 2 groups based on the microbial strain to be tested. Each group is further divided into 4sub groups based on the antibiotics which is added with i-PRF, one of group is kept as a control where in normal saline is added with i-PRF before centrifugation.

**Group A (Microbial strain - *Aggregatibacter actinomycetemcomitans*):**

**[1]** Sub Group 1- Injectable Platelet rich fibrin treated with penicillin 1mU/ml (**Figure 4**)
**[2]** Sub Group 2- -Injectable Platelet rich fibrin treated with metronidazole 5mg/ml (**Figure 5**)
**[3]** Sub Group 3-Injectable Platelet rich fibrin treated with moxifloxacin 150mg/ml (**Figure 6**)
**[4]** Sub Group 4-Injectable Platelet rich fibrin treated with normal saline 2ml (**Figure 7**)

**Group B (Microbial strain -*Porphyromonas Gingivalis*):**
**[1]** Sub Group 5- Injectable Platelet rich fibrin treated with penicillin 1mU/ml (**Figure 8**).
**[2]** Sub Group 6- -Injectable Platelet rich fibrin treated with metronidazole 5mg/ml (**Figure 9**).
**[3]** Sub Group 7-Injectable Platelet rich fibrin treated with moxifloxacin 150mg/ml (**Figure 10**).
**[4]** Sub Group 8 -Injectable Platelet rich fibrin treated with normal saline 2ml (**Figure 11**).

**Blood collection and i-PRF preparation:**
Blood samples were collected from 56 healthy volunteers into 10ml vacutainer without anticoagulants (red vacutainer) by a venipuncture from anti-cubital fossa. The volunteers provided written informed consent before the study. They were systemically healthy, nonsmokers, with no symptoms of infection and took no antibiotics for at least 3months before experiments began. After collection of blood the tubes were immediately centrifuged at 700 rpm for 3 min at room temperature. The upper separated liquid portion i.e. i-PRF collected and analyzed immediately **(Figure 1).**

**Antibiotics incorporation into i-PRF:**
Prior to centrifugation of vacutainers, 2ml of antibiotics will be added to the fresh blood using a syringe. The following antibiotics and concentrations were tested: metronidazole 5mg or ml, penicillin 1 mU or ml and moxifloxacin 150 mg or ml. Saline (in similar volumes) was used as control based on CLSI guidelines. All i-PRF with antibiotics will be diluted for 100%, 50% and 25 % (**Figure 2**).

**Bacterial culturing:**
The standard bacterial strain consists of *P. gingivalis* (ATCC33277) and *Aggregatibacter actinomycetemcomitans* (ATCC 43718) were maintained on brucella agar base supplemented with 5mg or ml hemin, 1 mg or ml menadione and 5 percent sterile horse blood **(Figure 3).** Each agar plate will be labelled on the bottom of the plate indicating the group and sub group. A well will be created in the center of agar plate for the placement of i-PRF with antibiotics for proper diffusion. Blood agar plates inoculated with *P. gingivalis* (ATCC No. 33277) will be incubated in an anaerobic jar at 37°C for 3–4 days. Blood agar plates inoculated with *A. actinomycetemcomitans* (ATCC No. 43718) will be incubated in a 5–10% $CO_2$ jar at 37°C for 48 hours.

**Anti-biogram assay:**
It is used to determine the sensitivity or resistance of microorganisms to antimicrobial agents and helps in selecting appropriate antibiotic therapy. Pure bacterial isolates were cultured on suitable media and the inoculum was adjusted to match 0.5 McFarland turbidity standards. Stock concentrations of each test compound are prepared and kept ready. On a freshly prepared agar plate, standardized suspension of test organism is inoculated. The standardized inoculum was uniformly swabbed onto anaerobic agar plates using sterile cotton swabs. i-PRF with different antibiotics placed in each well. The plates were incubated at appropriate temperature conditions for 18–24 hours. After incubation, the zones of inhibition around each antibiotic well were measured in millimetres. The test compound diffuses into the medium in a radial fashion. If the diffused product is able to inhibit the test organism, then a zone of inhibition will be seen around the well. If there is no bacterial inhibition, then the organisms will grow up to the edge of the well. The antibacterial activity of the tested material will be determined by measuring the diameter of the CFU-free area. The diameter will be measured using a UNC probe.

**Statistical analysis:**
Inter-group comparison will be done using One-Way ANOVA or Kruskal-Wallis ANOVA. Intra-group comparison will be done using Repeated Measures ANOVA or Friedman's test, depending on the normality of the data. Categorical data will be compared by Chi-square test or a suitable test like Fischer's Exact Test or McNemar Test, based on the data obtained. For analysis, by keeping the alpha error at 5% and beta error at 20% with a power of 80%, a p-value less than 0.05 will be considered statistically significant

**Results:**
In this *in vitro* study two important periodontopathic bacteria such as *Aggregatibacter actinomycetemcomitans* (Group A) and *P. gingivalis* (Group B) were tested for its colony inhibition by platelet concentrate that is treated with different antibiotics such as i-PRF with penicillin (Group A, sub group 1, Group B sub group 5), i-PRF with metronidazole (Group A, sub group 2 and Group B sub group 6) , i-PRF with moxifloxacin (Group A, sub group 3 and group B, sub group 7) and i-PRF with saline (Group A, sub group 4 and Group B sub group 8). **Table 1** describes the zone of inhibition by i-PRF with different antibiotics on an agar plate having *A. actinomycetemcomitans* growth. i-PRF with antibiotics was diluted from 100%, 50%and 25% dilution before it is tested with *A. actinomycetemcomitans* for its antibacterial activity. In group A i-PRF with penicillin had a colony inhibition of 46mm, i-PRF with metronidazole had a colony inhibition up to 44.71mm and i-PRF with moxifloxacin had colony inhibition of 46mm and with saline there was no inhibition of the colonies, with all the subgroup 1, 2 and 3 showed statistically significant difference with a p value of less than 0.01. Among all the antibiotics. i-PRF with metronidazole showed less colony inhibition compared to penicillin and moxifloxacin on *A. actinomycetemcomitans*. Even after dilution of i-PRF at different

levels there is inhibition of colonies seen with A. actinomycetem comitans. **Table 2** describes Group B with sub group 5, 6, 7 and 8 showing the zone of inhibition of i-PRF with different antibiotics on an agar plate having *P. gingivalis* growth. In group B with 100% dilution penicillin subgroup had a colony inhibition of 46mm, metronidazole had a colony inhibition up to 46mm and moxifloxacin had colony inhibition of 46mm and in sub group 8 where saline is used as control there was no inhibition of the colonies. All the subgroup 1, 2 and 3 showed statistically significant difference with a p value of less than 0.01. With respect to *P. gingivalis* colony inhibition is concerned all the antibiotics with i-PRF showed equal amount of inhibition. When the colony inhibition by i-PRF with different antibiotics was tested for the microorganism such *A. actinobacilluscomitans* (Group A) and *P. gingivalis* (Group B) at all dilution showed a statistically significant difference with p value of less than 0.01. All the antibiotics were equally effective on both the organisms in colony inhibition even after two dilutions. **Table 4** describes the intergroup pairwise comparison of group A and Group B, i-PRF with penicillin on *A. actinomycetemcomitans* and *P. gingivalis* showed non-significant difference with the p value of more than 0.05. This means to say that I- PRF with penicillin is equally effective in colony inhibition of both the organism **(Figure 12).** **Table 5** describes Intergroup pair wise comparison of sub group 2 and subgroup 6 i.e. i-PRF treated with metronidazole, when tested for colony inhibition of both the organism at all dilution showed statistically significant difference with p value less than 0.01. I-PRF with metronidazole is more effective against p. gingivalis compared to *A. actinomycetemcomitans*. **Table 6** describes Inter group pair wise comparison of sub group 3 and sub group 7 was made statistically i.e. i-PRF with moxifloxacin on both the organism was statistically non-significant with p value of more than 0.05. i-PRF when combined with moxifloxacin showed equal inhibition against both organisms. There was a statistically non-significant difference seen for the values between the sub-Group 4 (p>0.05) for all values in sub-Group 8. Inter group comparison of sub group 4 and sub group 8 i.e. when i-PRF treated with saline did not exhibit inhibition on both the organism, showing statistically non-significant results. The overall intergroup comparison is presented in **Table 3,** while the pairwise comparison of the saline controls is presented in **Table 7.**

**Table 1:** Showing Group A with different dilution at 100, 50 and 25% in all 1, 2, 3 and 4 sub group

| | | N | Mean | Std. Deviation | Median | Mean Rank | Chi square value | p value of Kruskal-Wallis Test |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 7 | 46 | 0 | 46 | 20 | 23.243 | 0.000** |
| | 2 | 7 | 44.71 | 1.38 | 45 | 14 | | |
| | 3 | 7 | 46 | 0 | 46 | 20 | | |
| | 4 | 7 | 0 | 0 | 0 | 4 | | |
| 0.5 | 1 | 7 | 46 | 0 | 46 | 21 | 22.502 | 0.000** |
| | 2 | 7 | 44.57 | 1.512 | 45 | 13.36 | | |
| | 3 | 7 | 45.86 | 0.378 | 46 | 19.64 | | |
| | 4 | 7 | 0 | 0 | 0 | 4 | | |
| 0.25 | 1 | 7 | 45 | 0 | 45 | 20.5 | 21.953 | 0.000** |
| | 2 | 7 | 43.71 | 1.254 | 43 | 14.21 | | |
| | 3 | 7 | 44.86 | 0.378 | 45 | 19.29 | | |
| | 4 | 7 | 0 | 0 | 0 | 4 | | |

*Kruskal-Wallis Test; **indicates statistically highly significant p<0.01*

**Table 2: Shows Well diffusion assay for P. gingivalis at different concentrations in various antibiotics**

| | | N | Mean | Std. Deviation | Median | Mean Rank | Chi square value | p value of Kruskal-Wallis Test |
|---|---|---|---|---|---|---|---|---|
| **1** | 5 | 7 | 46 | 0 | 46 | 18 | 27 | 0.000** |
| | 6 | 7 | 46 | 0 | 46 | 18 | | |
| | 7 | 7 | 46 | 0 | 46 | 18 | | |
| | 8 | 7 | 0 | 0 | 0 | 4 | | |
| **0.5** | 5 | 7 | 46 | 0 | 46 | 18 | 27 | 0.000** |
| | 6 | 7 | 46 | 0 | 46 | 18 | | |
| | 7 | 7 | 46 | 0 | 46 | 18 | | |
| | 8 | 7 | 0 | 0 | 0 | 4 | | |
| **0.25** | 5 | 7 | 45 | 0 | 45 | 18 | 27 | 0.000** |
| | 6 | 7 | 45 | 0 | 45 | 18 | | |
| | 7 | 7 | 45 | 0 | 45 | 18 | | |
| | 8 | 7 | 0 | 0 | 0 | 4 | | |

*Kruskal-Wallis Test; **indicates statistically highly significant p<0.01*

**Table 3:** Showing inter group comparison of colony inhibition of group A and Group B by i-PRF with different antibiotics sub group 1-8

| | | N | Mean | Std. Deviation | Median | Mean Rank | Chi square value | p value of Kruskal-Wallis Test |
|---|---|---|---|---|---|---|---|---|
| **1** | 1 | 7 | 46 | 0 | 4.6 | 37.5 | 50.746 | 0.000** |
| | 2 | 7 | 44.71 | 1.38 | 0.45 | 25.5 | | |
| | 3 | 7 | 46 | 0 | 0.46 | 37.5 | | |
| | 4 | 7 | 0 | 0 | 0 | 7.5 | | |
| | 5 | 7 | 46 | 0 | 0.46 | 37.5 | | |
| | 6 | 7 | 46 | 0 | 0.46 | 37.5 | | |
| | 7 | 7 | 46 | 0 | 0.46 | 37.5 | | |
| | 8 | 7 | 0 | 0 | 0 | 7.5 | | |
| **0.5** | 1 | 7 | 46 | 0 | 4.6 | 38.5 | 49.788 | 0.000** |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | 7 | 44.57 | 1.512 | 0.45 | 23.36 | | | |
| | 3 | 7 | 45.86 | 0.378 | 0.46 | 35.64 | | | |
| | 4 | 7 | 0 | 0 | 0 | 7.5 | | | |
| | 5 | 7 | 46 | 0 | 0.46 | 38.5 | | | |
| | 6 | 7 | 46 | 0 | 0.46 | 38.5 | | | |
| | 7 | 7 | 46 | 0 | 0.46 | 38.5 | | | |
| | 8 | 7 | 0 | 0 | 0 | 7.5 | | | |
| **0.25** | 1 | 7 | 45 | 0 | 4.5 | 38 | 49.032 | 0.000** | |
| | 2 | 7 | 43.71 | 1.254 | 0.43 | 25.71 | | | |
| | 3 | 7 | 44.86 | 0.378 | 0.45 | 35.29 | | | |
| | 4 | 7 | 0 | 0 | 0 | 7.5 | | | |
| | 5 | 7 | 45 | 0 | 0.45 | 38 | | | |
| | 6 | 7 | 45 | 0 | 0.45 | 38 | | | |
| | 7 | 7 | 45 | 0 | 0.45 | 38 | | | |
| | 8 | 7 | 0 | 0 | 0 | 7.5 | | | |

*Kruskal-Wallis Test; **indicates statistically highly significant p<0.01*

**Table 4:** Inter group pair wise comparison of sub group 1vs sub group 5 using mann-whitney u test

| | Mann-Whitney U value | Z value | p value of Mann-Whitney U test |
|---|---|---|---|
| **1** | 24.5 | 0 | 1.000# |
| **0.5** | 24.5 | 0 | 1.000# |
| **0.25** | 24.5 | 0 | 1.000# |

*Mann-Whitney U test; #indicates statistically non-significant p>0.05.*

**Table 5:** Inter group Pair wise comparison of all sub-Group 2vs6 using Mann-Whitney U test

| | Mann-Whitney U value | Z value | p value of Mann-Whitney U test |
|---|---|---|---|
| **1** | 10.5 | -2.245 | 0.025* |
| **0.5** | 7 | -2.622 | 0.009** |
| **0.25** | 10.5 | -2.256 | 0.024* |

*Mann-Whitney U test; **indicates statistically highly significant p<0.01; *indicates statistically significant p<0.05.*

**Table 6**: Inter group Pair wise comparison of all sub Group 3vs7 using Mann-Whitney U test

| | Mann-Whitney U value | Z value | p value of Mann-Whitney U test |
|---|---|---|---|
| **1** | 24.5 | 0 | 1.000# |
| **0.5** | 21 | -1 | 0.317# |
| **0.25** | 21 | -1 | 0.317# |

*Mann-Whitney U test; #indicates statistically non-significant p>0.05.*

**Table 7:** Inter group Pair wise comparison of all sub-Group 4vs8 using Mann-Whitney U test

| | Mann-Whitney U value | Z value | p value of Mann-Whitney U test |
|---|---|---|---|
| **1** | 24.5 | 0 | 1.000# |
| **0.5** | 24.5 | 0 | 1.000# |
| **0.25** | 24.5 | 0 | 1.000# |

*Mann-Whitney U test; #indicates statistically non-significant p>0.05.*

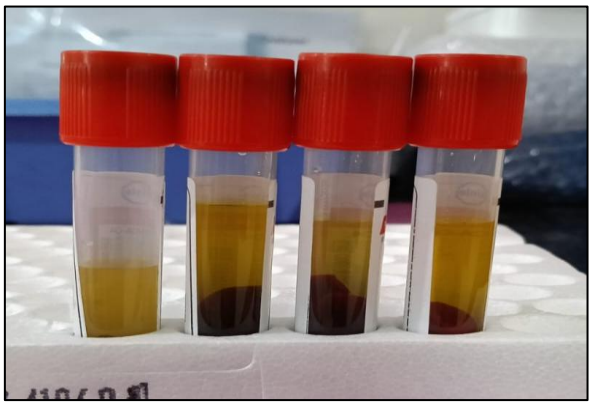


**Figure 1:** i-PRF obtained after centrifugation at 700rpm

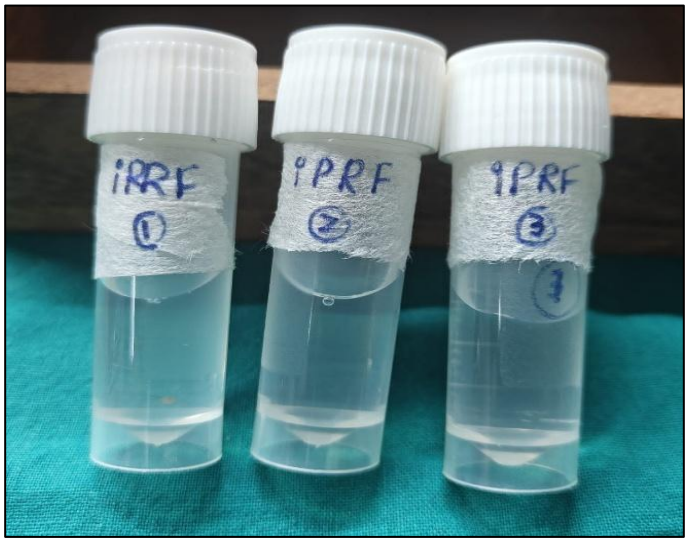



**Figure 2:** i-PRF with penicillin, i-PRF with metronidazole, i-PRF with moxifloxacin after dilution. (100%, 50%, 25%)

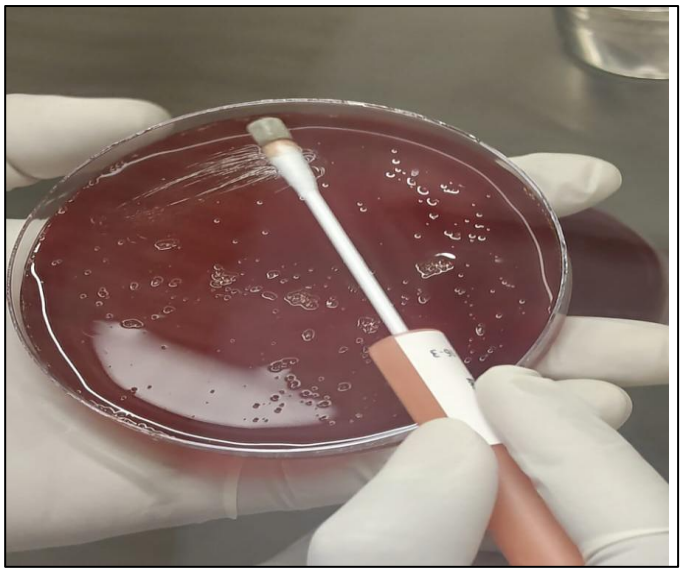


**Figure 3:** Bacterial inoculation on the agar plate

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**Figure 4:** Group A, subgroup 1 (*A. actinomycetemcomitans*, i-PRF with penicillin).

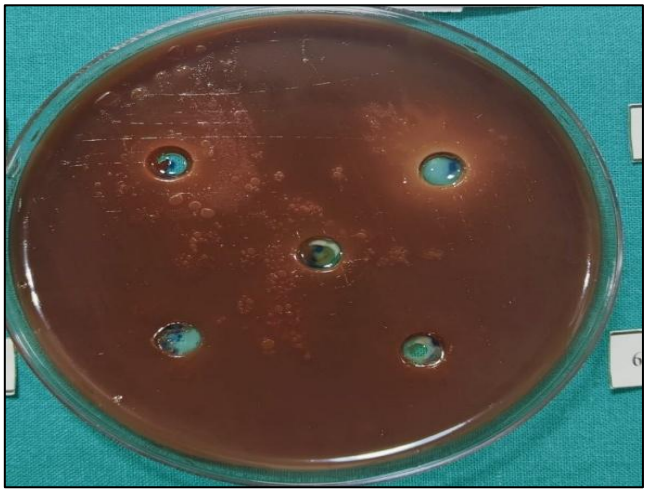


**Figure 7:** Group A sub group 4 (*A. actinomycetemcomitans*, I- PRF with saline)




**Figure 5:** Group A, subgroup 2 (*A. actinomycetemcomitans*, i-PRF with metronidazole)

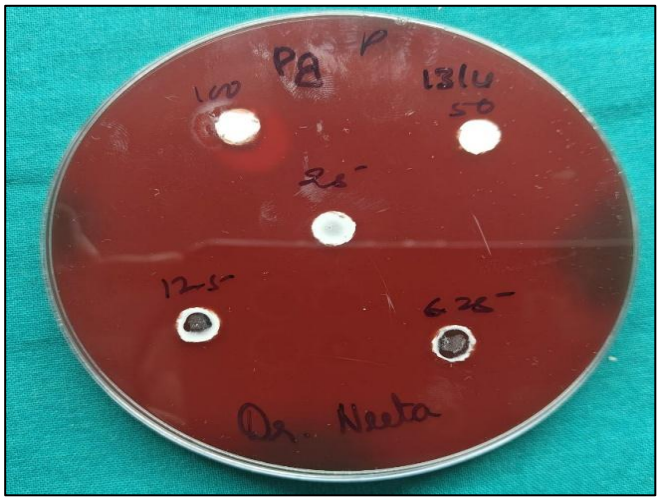



**Figure 8:** Group B subgroup 5 (*P. gingivalis*, i-PRF with penicillin**)**

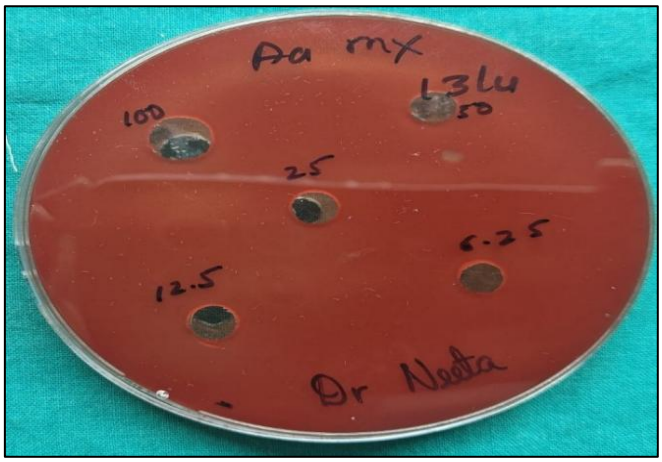



**Figure 6:** Group A subgroup 3 (*A. actinomycetemcomitans*, I- PRF with moxifloxacin)

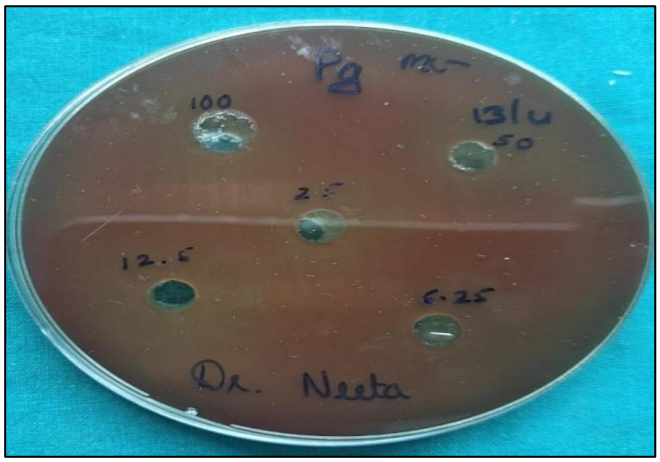



**Figure 9:** Group B subgroup 6 (*P. gingivalis*, i-PRF with metronidazole)

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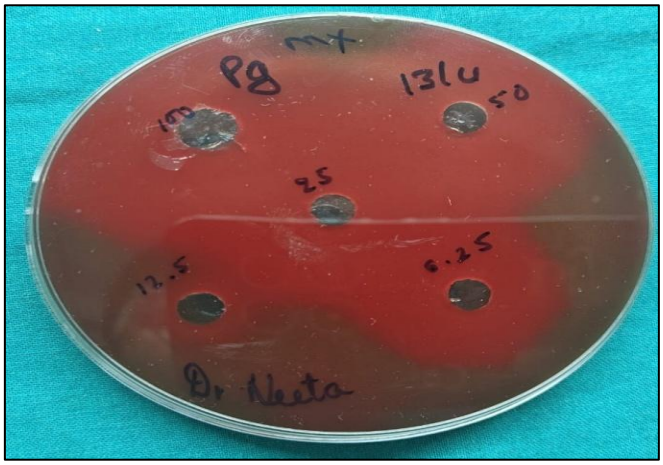



**Figure 10:** Group B subgroup 7 (*P. gingivalis*, i-PRF with moxifloxacin)

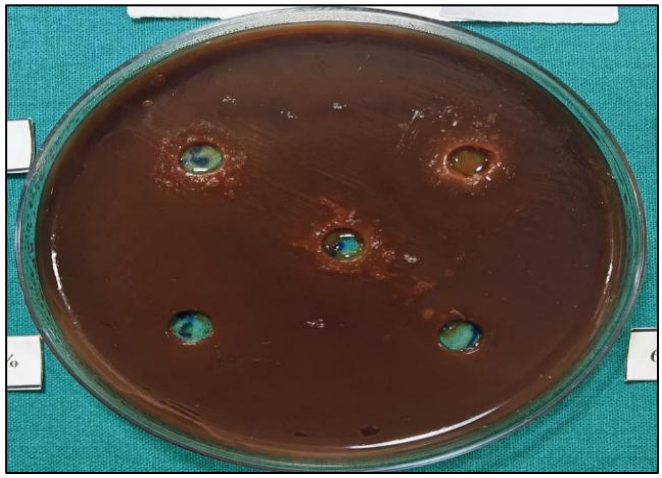


**Figure 11:** Group B sub group 8 (*P. gingivalis*, i-PRF with saline)

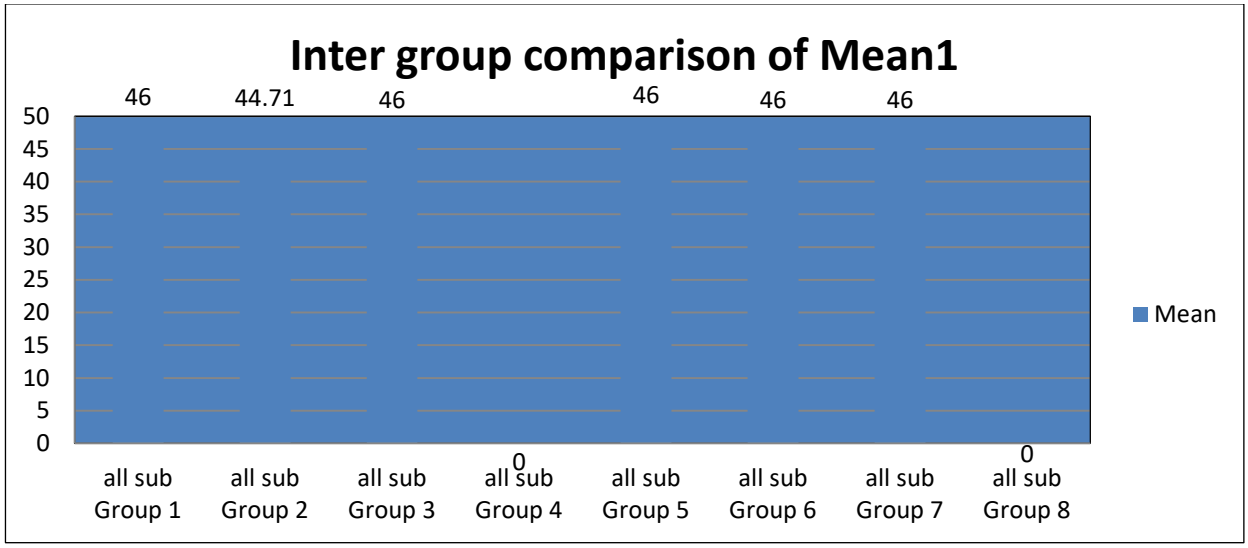



**Figure 12:** Describes both the groups and sub group comparison

**Discussion:**

Platelet concentrates are used in medical practice because of its regenerative potential in various periodontal surgeries, oral and maxillofacial surgeries **[14].** The growth factors which are released from platelets such as platelet derived growth factor, transforming growth factor (TGF-beta), vascular endothelial growth factor, epidermal growth factor and insulin like growth factor, provide matrix for connective tissue by secreting fibrin, fibronectin and vitronectin, which help in cell migration. Platelet helps in wound healing by proliferation of cells, matrix synthesis for connective tissue formation and collagen synthesis **[15].** They are a good option to be placed in fresh extraction sockets and also in in cases where bone grafting is required in cases of intrabony defects **[16],** periodontitis caused because of concrescence **[17]** and also GBR. Platelet concentrates in the form of autologous fibrin glue can be used in place of sutures after periodontal flap surgeries **[18].** In this study i-PRF is assed for its antimicrobial properties together with other antibiotics, which were added during its preparation. With Aggregatibacter actinomycetem comitans, i-PRF with penicillin showed a colony inhibition of 46mm, with metronidazole the colonies were inhibited with a mean of 44mm and moxifloxacin together showed colony inhibition in all dilution, with a mean of 46mm. In Group B, P. gingivalis with penicillin showed a mean of 46mm colony inhibition. With metronidazole and moxifloxacin the colonies inhibition was up to 46mm. when both the groups were compared to control that is i-PRF with saline was tested for both organisms showed no inhibition. Thus, it can be stated that i-PRF when combined with different antibiotics the efficacy of antimicrobial property can be greatly enhanced at all dilution. This is in accordance with many in vitro studies where they evaluated i-PRF with antibiotics, Chou *et al.* **[19]** evaluated i-PRF scaffold with a triple antibiotic mixture, ciprofloxicillin, metronidazole and minocycline against Actinomyces naeslundii and Enterococcus faecalis biofilms in an infected root canal model, showed highest antibacterial activity reducing live

bacterial counts by up to 92%. The antibiotics showed a burst release within 24hrs followed by sustained maintenance of the three antibiotics up to 14 days. Jia *et al.* **[20]** investigated the use of i-PRF incorporating vancomycin hydrochloride (VANKA) into two carrier system such as liposomes and microparticles, suspended in PRF before clot formation. The finding revelled that VANKA in PRF without a carrier system did not attain controlled antibiotic loading. Several techniques of platelet concentrates are available either in liquid form or clot form. In PRP technique the patient's own blood is collected with anticoagulant and immediately undergoes two centrifugation processes, later the platelet concentrates are activated with thrombin and/or calcium chloride **[7].** In PRF preparation the donor blood is collected without anticoagulant and undergoes single centrifugation to form fibrin structure. The fundamental concept of preparing autologous platelet concentrate (APC), including platelet, fibrin and potentially leukocytes in addition, possess inherent antimicrobial properties, together with regenerative capability, makes them suitable for clinical use **[10].** Moreover, using patient's own blood eliminates immune responses and also APCs offer a promising tool for promoting the local delivery of antibiotics, overcoming the limitations of conventional antibiotic therapy **[21, 22]**. There are three methods of platelet concentrate drug loading has been described in the literature, addition of antibiotics to PRP before coagulation, in PRF it is added before centrifugation and injection of antibiotics into PRF membrane after centrifugation **[23].** In a study by Badade *et al.* **[24]** wherein they evaluated the antimicrobial effect of PRP and PRF was done without the addition of antibiotics to platelet concentrate against the periodontopathic bacteria such as *A. actinomycetemcomitans* and *P. gingivalis* by culturing. Colony inhibition showed that PRP inhibited *P. gingivalis* in 3-4 days of incubation and *A. actinomycetemcomitans* at 48hrs of incubation but not PRF, the probable reason could be that PRF is a matrix of autologous fibrin, in which a large quantity of platelets and leukocytes, cytokines are embedded intrinsically during centrifugation leading to their progressive release over time (7-11days) as the network of fibrin disintegrates **[25, 26].** It is also suggested that the components of PRP and PRF are the same but the addition of calcium chloride in PRP may be the reason for the contribution of antibacterial activity. In platelet concentrates the antimicrobial activity is poorly understood, as it is a complex mixture of platelets, white blood cells and plasma. Existing evidence suggest that platelets generate oxygen metabolites such as superoxide, hydrogen peroxides and hydroxyl free radicals, **[27, 28]** they have the capability of binding and internalizing microorganisms enabling their clearance and participating in antibody-dependent cell cytotoxicity to kill protozoal pathogen and also, they have antimicrobial proteins and peptides such as platelet. Polyhydroxyalkanoate granules and complement binding proteins. Release of myeloperoxidase, activation of antioxidant responsive element and antigen specific immune response has also been suggested **[29, 30].** Activated platelets also release various growth factors which play important roles in improving the healing of ulcers and secreting platelet microbicidal proteins (PMPs). PMPs contain platelet factor 4, regulated upon activation of normal T cell expressed and secreted protein, connective tissue activating peptide 3, platelet basic protein, thymosin beta-4, fibrinopeptide A and fibrinopeptide B. On contacting bacterial membrane, changes membrane permeability, entering the cell and inhibiting the synthesis of big molecules **[29].** In this study when I-PRF was diluted to 50%, 25% of its concentration still there was colony inhibition with similar values to without dilution. Penicillin, moxifloxcilin with I-PRF had similar colony inhibition for both the organism, metronidazole was more effective against *P. gingivalis* compared to *A. A. comitans*, since the organism is anaerobic, metronidazole was effective. Since the control group, I-PRF with saline did not show inhibition in our study so the antimicrobial property of antibiotics was more efficient in colony inhibition. This is contradictory to the study done by Kour *et al.,* **[31]** where antimicrobial efficacy of Platelet-rich Plasma, Platelet-rich Fibrin and Injectable Platelet-rich Fibrin on the Standard Strains of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* was done, For Pg, I-PRF had the widest zone of inhibition which was significantly wider as compared to PRF. PRP had significantly wider zone of inhibition against PRF. For Aa, PRP had wider zone of inhibition which was significantly wider as compared to that of PRF and I-PRF. All the three platelet concentrates PRP, PRF and I-PRF have antibacterial activity, but PRP and I-PRF are more active as compared to PRF. Based on the available literature till now it can be stated that I-PRF since available in liquid form makes it easier to use, with abundant growth factors together can be used as a carrier for antibiotics for local application in periodontal regeneration.

**Conclusion:**

I-PRF with antibiotics inhibited main periodontal pathogens, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* in all concentrations. Hence, using antibiotics together with PRF offers advantages of both regeneration of tissue as well as continuous antibiotic release at the wound site.

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