INTRODUCTION

The initial objective of periodontal therapy involves the mechanical disruption of the biofilm concomitant with the correction of modifiable host factor, but a rebound of subgingival colonies to pre-treatment levels can occur just weeks after debridement. The risk of bacterial contamination and re-infection is one of the serious complications impairing wound healing and tissue regeneration. Even with the application of strict conventional disinfection protocols, bacteria can still infiltrate and colonize the underlying tissues of the wound.\(^1\) The increased risk of antibiotic resistance has led to newer research in an effort to search for alternatives to combat infection.

The use of autologous platelet concentrates (PCs) has gained popularity in a various fields. Being a completely natural, physiologic and relatively economic source of autologous product, PCs possess beneficial effects of eliminating concerns about immunologic reactions and disease transmission. The rationale for their use arises from the fact that platelets, when activated, release growth factors (GFS) and other molecules that modulate the wound healing response in both hard and soft tissues. In addition, anti-inflammatory properties of PCs have resulted in marked reduction of postoperative pain and swelling.\(^2,3\) The regenerative potential of PCs has been studied and used in variety of periodontal surgical procedures, but a relatively few amount of published studies regarding their antimicrobial potential are available. Dual effect i.e. antibacterial efficacy and healing promoting properties may be advantageous in promoting healing while preventing infection. Its antibacterial efficacy will certainly add whole new meaning as well as dimension to its use.

This study evaluated the antimicrobial effects of PRP, PRF and i-PRF against the putative periodontal pathogen \textit{F. nucleatum}. It plays a key role in subgingival biofilm formation by bridging the early colonizers and the late colonizers that make up the red complex initially described by Socransky that are strongly associated with active periodontal lesions.\(^4\)

METHODS

Ethical clearance from the institutional review board was obtained prior to the start of the experiment. The inclusion
criteria of the volunteer required that he/she be systemically healthy, a non-smoker, with no symptoms of active infection and has not taken any antibiotics for at least 6 months before the experiment for him/her to be included in this study.

Blood samples were obtained from one systemically healthy volunteer who signed an informed consent. Prior to the sample collection, the volunteer underwent a comprehensive blood screening test. *F. nucleatum* (ATCC no. 25586) was obtained from American Type Culture Collection through Fil-Anaserv Inc. Once prepared and delivered upon their respective TSA agar plates, a standardized inoculum of the culture was prepared in accordance to the McFarland Standard. The density of the suspension was measured by visual inspection of the turbidity.

**Preparation of platelet-rich plasma (PRP)**

A volume of 10ml of blood was collected in tubes with 1ml of sodium citrate solution (3.2%) as anticoagulant. The first centrifugation (soft spin) was at 1000 rpm for 10 minutes, allowing the separation of the blood sample in 3 distinct layers: acellular plasma (platelet-poor plasma or PPP) layer, a layer of maximum platelet concentration, and a layer of RBC’s. A sterile syringe was used to aspirate PPP, PRP and some RBCs (which are systemically attracted during operation). Then the material was transferred to another tube, which underwent a second spin.

The second spin (hard spin) proceeded at 2000 rpm for 20 minutes. The result of this second centrifugation had sediment the RBC at the bottom-most area, followed by a layer of PPP, and in-between a buffy layer of PRP. With a syringe, the major part of PPP is discarded. The PRP was drawn using a sterile pipette and then transferred in a sterile container. The PRP was then activated by 10% calcium chloride (0.02 ml per ml of PRP) before being utilized for the disc diffusion.

**Preparation of platelet-rich fibrin (PRF)**

A volume of 20ml of blood was collected in a sterile tube devoid of additives. Then it was immediately centrifuged at 3,000 rpm for 10 minutes. After the centrifugation cycle, the PRF clot was removed from the tube using sterile tweezers, and subsequently separated from the RBC base with scissors. This clot was placed on the surface of the PRF grid box and the lid was placed on top of it (the PRF clot) for 5-10 minutes. The PRF was then obtained in the form of a membrane.

**Preparation of injectable platelet-rich fibrin (i-PRF)**

A collected volume of 10ml of blood in a sterile tube without any additives was centrifuged at 700rpm for 3-4 minutes. After centrifugation, the bevel of the needle penetrated the wall obliquely, followed by aspiration of i-PRF and its subsequent placement in a sterile container.

**Figure 2: Preparation of PRF**

**Figure 3: Preparation of i-PRF**

**Tryptic soy agar plate preparation**

A volume of 20ml of TSA was present on each petri dish. Once calibrated, 10µl of standardized culture suspension was inoculated onto each TSA agar plate and streak plate method was performed. The surface of the agar plate was inoculated by streaking the swab three times over the entire agar surface; the plate was rotated approximately 60 degrees each time to ensure an even distribution of the inoculum.

**Figure 4: Tryptic soy agar plate preparation**

**Disk Diffusion Test**

The evaluation of the antimicrobial activity of PRP, PRF and i-PRF against was carried out using the disk diffusion method protocol with metronidazole antibiotic disc as the positive control. Each agar plate had four different samples and was done in 20 plates. The agar plate contained 6mm punched out Whatman #1 filter paper for PRP and i-PRF, the PRF was placed...
directly on the surface of the agar plate, and for metronidazole antibiotic impregnated disc was used. Using a micropipette, a fixed volume of 10 µl of PRP and i-PRF was loaded on disc one by one, taking precautions that the tip was in slight contact with the disc. After it has been absorbed, they were placed on the agar plate with its corresponding label. Since the study involves anaerobic microorganism, the agar plates were then incubated at 37 ºC for 48 hours using BD Gaspak.

The antimicrobial effects were determined by measuring the dimensions of the growth inhibition zone found around each disk after 48 hours of incubation. A ruler is placed across the zone of inhibition (ZOI) at the widest diameter while inverting the agar plate. The result is expressed in millimeters (mm) diameter and then subjected to statistical analysis.

Data analysis was done using the mean, standard deviation, median, minimum and maximum (values) was used to describe the observations of the experiment. The Shapiro Wilks test was used to assess the normality of the data. Analysis of variance was used to compare PRP, PRF, i-PRF. Independent t-test was used to compare metronidazole with PRP, PRF and i-PRF separately. The level of significance was established as p<0.05 for all tests and were carried out with the use of the SPSS software (version 25.0).

RESULTS

The bacteriostatic effect was assessed by measuring the zones of inhibition (in millimeters). A total of 80 samples (20 for each group) were evaluated and subjected to statistical analysis. The mean widths for the zones of inhibition for PRP, PRF, and I-PRF on the agar plates inoculated with Fusobacterium are listed in Table 1 along with the standard deviations.

Table 1: Descriptive statistics of Metronidazole, PRP, i-PRF and PRF

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>20</td>
<td>17.950</td>
<td>1.317</td>
</tr>
<tr>
<td>PRP</td>
<td>20</td>
<td>12.500</td>
<td>0.827</td>
</tr>
<tr>
<td>i-PRF</td>
<td>20</td>
<td>11.450</td>
<td>1.050</td>
</tr>
<tr>
<td>PRF</td>
<td>20</td>
<td>6.500</td>
<td>0.889</td>
</tr>
</tbody>
</table>

The resulting p-value was 0.000, indicating significant differences (Table 2) in the antimicrobial activities (as measured by their respective zones of inhibition) of platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and injectable platelet-rich fibrin (i-PRF).

Table 2: Comparison for different platelet concentrates

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP</td>
<td>12.500</td>
<td>0.827</td>
<td>0.000</td>
</tr>
<tr>
<td>i-PRF</td>
<td>11.450</td>
<td>1.050</td>
<td></td>
</tr>
<tr>
<td>PRF</td>
<td>6.500</td>
<td>0.889</td>
<td></td>
</tr>
</tbody>
</table>

The comparison of antimicrobial efficacy (represented by the zones of inhibition) between metronidazole and the platelet concentrates (PCs) are presented in Table 3. Consistent p-values of 0.000 indicate that the ZOI of metronidazole was statistically higher than that of the three platelet concentrates.

Table 3: Independent t-test between different platelet concentrates and metronidazole

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental Mean ± S.D.</th>
<th>Metronidazole Mean ± S.D.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP</td>
<td>12.500 ± 0.827</td>
<td>17.950 ± 1.317</td>
<td>0.000</td>
</tr>
<tr>
<td>i-PRF</td>
<td>11.450 ± 0.827</td>
<td>17.950 ± 1.317</td>
<td>0.000</td>
</tr>
<tr>
<td>PRF</td>
<td>6.500 ± 0.827</td>
<td>17.950 ± 1.317</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The resulting data was compared with the standard table of inferences as suggested by Guevara et al.7 Table 4 presents the interpretability of each mean observation against the cut-off values of each range. These results signify that antimicrobial effect of PRP and i-PRF is partially active against F.nucleatum, while PRF is inactive.

Table 4: Observed values in comparison with cut-off values

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>ZOI range</th>
<th>Cut-off value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>17.950</td>
<td>14-19mm</td>
<td>14</td>
<td>Active</td>
</tr>
<tr>
<td>PRP</td>
<td>12.500</td>
<td>10-13mm</td>
<td>10</td>
<td>Partially active</td>
</tr>
<tr>
<td>i-PRF</td>
<td>11.450</td>
<td>10-13mm</td>
<td>10</td>
<td>Partially active</td>
</tr>
<tr>
<td>PRF</td>
<td>6.500</td>
<td>&lt;10mm</td>
<td>10</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

DISCUSSION

This study is an evaluation and comparative analysis between the different platelet concentrates and metronidazole against...
**Fusobacterium nucleatum.** All groups of metronidazole (positive control), PRP, i-PRF and PRF (the 3 experimental groups) consisted of 20 samples each. The results of this study clearly demonstrated that PRP and i-PRF are capable of inhibiting *F. nucleatum* growth at 48 hours, whereas PRF showed very minimal inhibition in just some samples (5 out of 20).

Despite being an autologous preparation, PRP requires the addition of calcium chloride for its activation. Yang et al. investigated the antimicrobial activity of PRP and other plasma preparations against periodontal pathogens. The authors found that all plasma preparations can inhibit bacterial growth, with PRP showing the superior activity. Similar results from studies by Badade et al. and Reham et al. also showed the bacteriostatic effects of platelet rich plasma against the selected oral and periodontal pathogens. PRP contains a large number of platelets, as well as a high concentration of leukocytes. In the role of immune defense against bacterial infection, these concentrated leukocytes within platelets enable PRP to play a vital role. In this study, the activity of PRP against *F. nucleatum* was significantly higher than i-PRF and PRF. These results corroborate other studies regarding the possible benefits that might be derived from PRP as an adjunct in periodontal therapy.

The i-PRF is a new alternative to the platelet aggregate in different areas of dentistry. Injectable PRF is used to make sticky bone graft, via induction of complete clotting of the bone graft in an effort to enhance the blood supply and maximize the delayed release of growth factors contained within. Having just being recently introduced, very few studies have actually explored i-PRF. Prerna et al. observed that i-PRF exhibited zones of inhibition around oral microorganisms, as was the case in this experiment as well (against *F. nucleatum*). Miron et al. demonstrated that in general, PRP had higher, early release of growth factors, whereas i-PRF showed significantly higher levels of long-term release of the same factors. Taking advantage of slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factors can be observed when compared to PRF.

In this study, the majority of the ZOI results from the PRF samples did not show any inhibition against *F. nucleatum*. One plausible reason might be due to the fact that PRF is a matrix of autologous fibrin, in which a large quantity of platelets and leucocyte cytokines are embedded intrinsically during centrifugation. This fibrin consistency has been suggested to be responsible for the progressive release of the platelet products over time (7 – 11 days), as the fibrin disintegrates hence the “delay” or outright absence of antibacterial activity within a relatively-short observation period. These findings are consistent with previous studies that evaluated the antimicrobial activity of PRF on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, which also failed to show any zone of inhibition.

These three autologous biomaterials differ with regards to the viability of the contained cells, their manner of activation (natural or chemical), difference in centrifugation speed and duration, the density of the resulting fibrin network, specific interactions between the cellular and extracellular components, and the release of a variety of proteins. These differences may significantly impact their respective anti-inflammatory and antimicrobial properties. Furthermore, the mechanisms and dynamics of the individual antimicrobial components contained within these biomaterials are poorly understood.

In the present study, platelet concentrates were able to demonstrate bacteriostatic activity against *F. nucleatum*. Though metronidazole showed significantly higher antimicrobial potency than the platelet concentrates as expected, PC’s, being an autologous preparation, are free from any side effects which are routinely encountered with other antimicrobial agents.

**CONCLUSION**

Based on the findings, it was concluded that, two of the three experimental groups (i.e. platelet-rich plasma & injectable platelet-rich fibrin) demonstrated bacteriostatic activity against *Fusobacterium nucleatum*, with both groups showing delineable zones of inhibition. Majority of the *F. nucleatum* samples with platelet-rich fibrin (PRF) displayed either contact inhibition or very minimal inhibition (bordering to none) around the discs. At its physical state, the potential antimicrobial property of PRF appears to be negligible *in vitro*. The antibacterial activity of both PRP and i-PRF could be categorized as “partially-active”. Based solely on the resulting zones of inhibition, none of the platelet concentrates’ bacteriostatic action against *F. nucleatum* was comparable to metronidazole’s.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST:** None

**FINANCIAL DISCLOSURE:** None

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**REFERENCES:**


