Original Article

DOI: 10.7860/JCDR/2026/81266.23507

Dentistry Section

Comparative Evaluation of Antimicrobial Efficacy of Leukocyte Platelet-rich Fibrin and Advanced Platelet-rich Fibrin against *Streptococcus Intermedius*: An In-vitro Study

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ABSTRACT

Introduction: Platelet-rich Fibrin (PRF) has emerged as a valuable biomaterial in oral and maxillofacial regenerative procedures. While its regenerative properties are well-documented, the antimicrobial potential of different PRF preparations remains incompletely understood, particularly against oral pathogens like *Streptococcus intermedius*.

Aim: To evaluate and compare the antimicrobial efficacy of Leukocyte Platelet-rich Fibrin (L-PRF) and Advanced Platelet-rich Fibrin (A-PRF) against *Streptococcus intermedius* in-vitro.

Materials and Methods: This in-vitro study design was conducted in the Department of Periodontics, Tagore Dental College and Hospital, Rathinamangalam, Tamil Nadu, India, from January 2025 to June 2025. A total of five participants were selected based on inclusion and exclusion criteria. A total of 5 blood samples (10 mL of blood samples were collected from each patient) were collected from five systemically healthy individuals diagnosed with chronic periodontitis. L-PRF and A-PRF were prepared using standardised centrifugation protocols (L-PRF: 3000 rpm/10 minutes; A-PRF: 1500 rpm/14 minutes). Antimicrobial activity against *S.intermedius* was assessed using the agar diffusion method. Zones Of Inhibition (ZOI) for L-PRF and A-PRF against *S.intermedius* were measured after 24-hour incubation at 37°C under microaerophilic conditions. Statistical analysis as applied on the data obtained, statistically significant.

Results: Vancomycin consistently demonstrated antimicrobial activity against *S. intermedius* with a mean inhibition zone of 18.76±1.32 mm. However, neither L-PRF nor A-PRF exhibited detectable ZOI against *S. intermedius* across all five samples, indicating absence of antimicrobial activity under the experimental conditions employed.

Conclusion: Within the limitations of the present in-vitro study, neither L-PRF nor A-PRF demonstrated antimicrobial activity against *S. intermedius* using the agar diffusion method. These findings suggest that the anticipated antimicrobial benefits of PRF may be species-specific and limited against certain oral pathogens. The clinical selection of PRF should therefore primarily consider its well-established regenerative properties rather than potential antimicrobial effects against *S. intermedius*.

Keywords: Chronic periodontitis, Growth factors, Zones of inhibition

INTRODUCTION

Oral and maxillofacial surgery has witnessed significant advancements in regenerative procedures over the past few decades. Among these innovations, platelet concentrates have emerged as valuable tools in tissue regeneration and wound healing processes. PRF, introduced by Choukroun J in 2001, represents a second-generation platelet concentrate characterised by a simplified preparation protocol that eliminates the need for biochemical blood manipulation through anticoagulants, bovine thrombin, or calcium chloride [1]. This autologous biomaterial contains a high concentration of platelets, leukocytes, growth factors, and cytokines embedded within a three-dimensional fibrin matrix that serves as a scaffold for cell migration and proliferation [2].

The clinical applications of PRF in dentistry are diverse and expanding. It has been utilised successfully in various procedures, including socket preservation following tooth extraction, sinus lift augmentation, periodontal regenerative surgery, and ridge augmentation [3]. The biological rationale behind PRF usage stems from its ability to release growth factors gradually, which stimulates angiogenesis, enhances cell proliferation, and promotes tissue regeneration [4]. Moreover, PRF has demonstrated favourable effects on soft-tissue healing and patient comfort by reducing postoperative pain and swelling [5].

Over time, modifications to the original PRF protocol have been introduced to enhance its biological properties and clinical outcomes [6]. A-PRF, developed by Choukroun J in 2014, utilises a lower centrifugation speed and longer centrifugation time compared to the conventional L-PRF protocol [7]. This modification aims to increase the presence of neutrophils and macrophages in the fibrin matrix while promoting a more sustained release of growth factors [8]. The structural and biological differences between L-PRF and A-PRF have been documented, with A-PRF exhibiting a more porous structure, higher leukocyte content, and extended growth factor release [9].

While the regenerative properties of these platelet concentrates have been extensively studied, their antimicrobial potential has garnered increasing attention. Several studies have demonstrated that PRF possesses antimicrobial activity against various oral pathogens, attributable to the presence of leukocytes, antimicrobial peptides, and specific growth factors [10]. The leukocytes in PRF, particularly neutrophils and monocytes, contribute to the defence mechanism through phagocytosis and the release of antimicrobial substances [11]. Additionally, platelets themselves contain antimicrobial peptides such as platelet factor 4, Regulated on Activation, Normal T cell Expressed and Secreted (RANTES), and thymosin βeta-4, which exhibit bactericidal properties [12].

Streptococcus intermedius a member of the *Streptococcus anginosus* group is a commensal organism of the oral cavity, gastrointestinal tract, and urogenital tract [13]. However, it is an opportunistic pathogen frequently associated with deep-seated purulent infections,

including dental abscesses, endocarditis, and brain abscesses [14]. In the oral environment, *S. intermedius* has been isolated from various pathological conditions, including periodontal disease, endodontic infections, and peri-implantitis [15]. Its virulence factors, including intermedilysin (a human-specific cytolysin) and hyaluronidase, contribute to tissue invasion and abscess formation [16].

Despite the growing body of literature on PRF's antimicrobial properties, comparative studies evaluating the antimicrobial efficacy of different PRF preparations against specific oral pathogens is limited. The variations in preparation protocols between L-PRF and A-PRF may influence their leukocyte content, growth factor composition, and consequently, their antimicrobial potential [17]. Understanding these differences could have significant clinical implications, especially in situations where infection control is critical for successful treatment outcomes.

Therefore, the aim of the present in-vitro study was to evaluate whether L-PRF and A-PRF have antimicrobial efficacy against *streptococcus intermedius* or not.

Null Hypothesis: There is no association between antimicrobial efficacy of L-PRF and A-PRF against *streptococcus intermedius*.

Alternate hypothesis: There is an association between antimicrobial efficacy of L-PRF and A-PRF against *streptococcus intermedius*.

MATERIALS AND METHODS

The present in-vitro study design was conducted in the Department of Periodontics, Tagore Dental College and Hospital, Rathinamangalam, Tamil Nadu, India, from January 2025 to June 2025. The study protocol was approved by the Institutional Ethics Committee of Tagore Dental College and Hospital. (IEC/TDCH/95/2024).

Sample size calculation: The pilot study was done prior with a sample size of two participants (n=2). The results demonstrated that both L-PRF and A-PRF showed absence of antimicrobial efficacy against *streptococcus intermedius*. The sample size was determined based on a pilot study and power analysis. Assuming an expected population proportion of one and a sample proportion of 0.884, with a power of 80% and a two-sided alpha error of 5%, a sample size of five participants was calculated as adequate for this study [18]. To account for potential attrition, with attrition rate of 37% the final sample size was maintained at five participants (n=5).

Inclusion criteria: Male and female patients aged 30-50 years, diagnosed with chronic generalised periodontitis according to the 2017 classification of periodontal diseases (Since Chronic generalised periodontitis has a particular strains of *streptococcus intermedius*) [19], systemically healthy individuals, no history of bleeding disorders, non-smokers, not on any medication that could alter platelet function {e.g., aspirin, Non steroidal Anti-Inflammatory Drugs (NSAIDs)} for at least two weeks prior to blood collection.

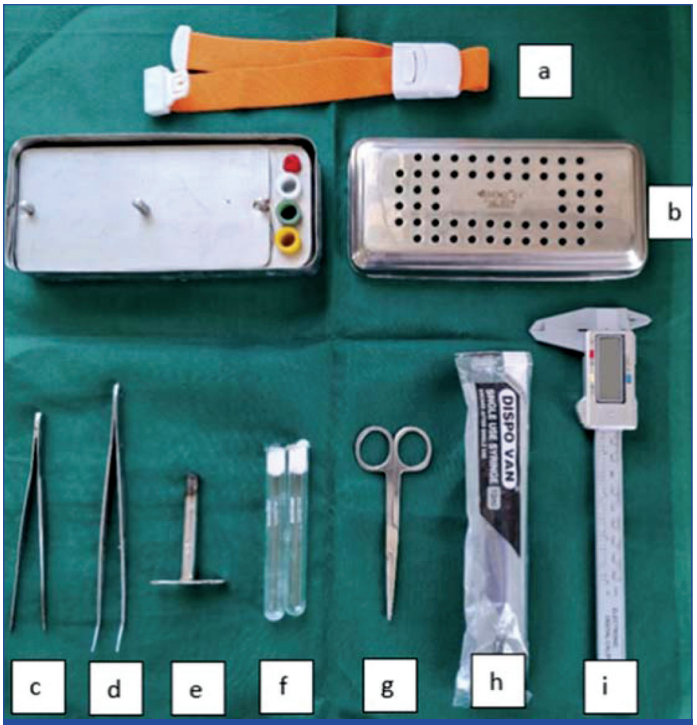
Exclusion criteria: Presence of systemic diseases that could affect periodontal health (e.g., diabetes mellitus, immunodeficiency disorders), haemoglobin levels <10 g/dL or platelet count <150,000/mm^3, history of antibiotic therapy within the past three months, pregnancy or lactation, unwillingness to participate in the study.

All participants received detailed information about the study objectives, procedures, potential risks, and benefits. Written informed consent was obtained from each participant before enrollment in the study.

Study Procedure

Materials: Materials used were Torniquet, PRF box, non toothed forceps, Tweezer, Gel punch, Test tubes, Scissors, 10 mL syringe, Digital calliper [Table/Fig-1].

Microbiological Methods (Bacterial strain and culture conditions): A standard strain of *streptococcus intermedius* were obtained from Microbial Type Culture Collection (MTCC) 15008 and



[Table/Fig-1]: Materials used in the study.

Gene Bank was used in this study. The strain was cultivated in Brain Heart Infusion Broth (BHIB) at 37°C for 24 hours in microaerophilic conditions (5% CO_2). The bacterial suspension was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using a densitometer [20].

Preparation of agar plates: Brain Heart Infusion Agar (BHIA) plates were prepared according to the manufacturer's instructions. Briefly, 52 g of BHIA powder was dissolved in one litre of distilled water, autoclaved at 121°C for 15 minutes, cooled to 45-50°C, and poured into sterile petri dishes (20 mL per plate). After solidification, the plates were stored at 4°C until use.

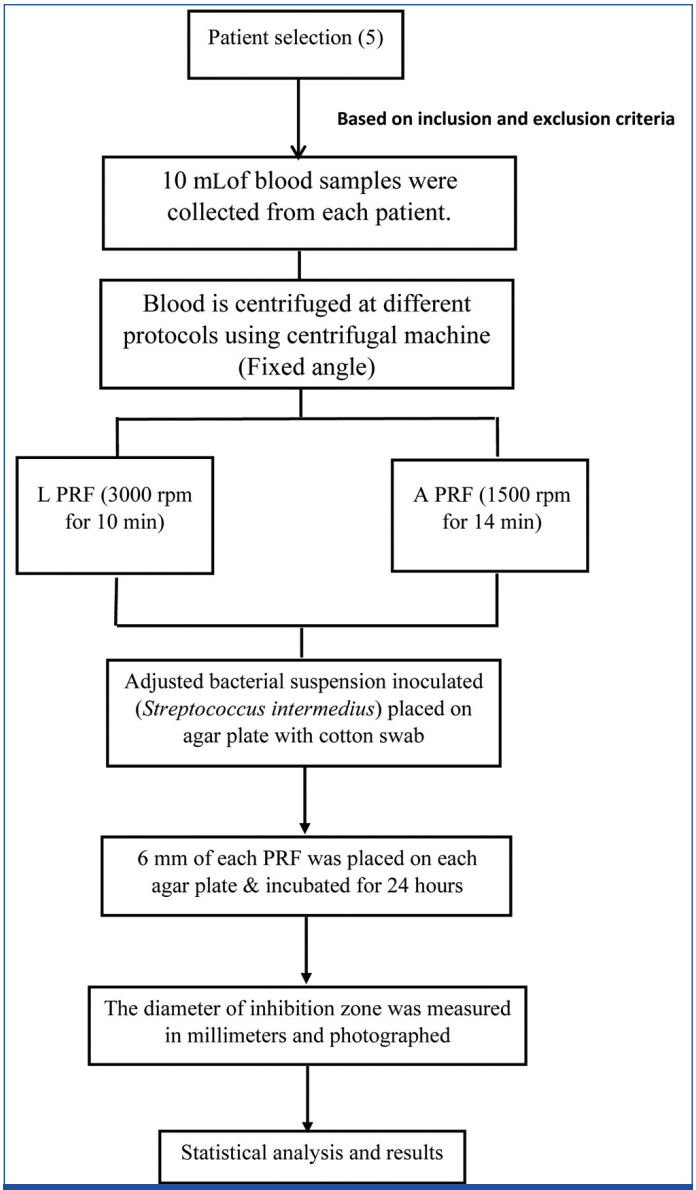
For the antimicrobial assay, the standardised bacterial suspension (0.1 mL) was lawn cultured on BHIA plates using sterile cotton swabs to ensure uniform growth. A total of five plates were prepared, one for each participant's PRF samples.

Preparation of Platelet-rich Fibrin (PRF) samples: Under aseptic conditions, 10 mL of venous blood was collected from each participant using a 10 mL disposable syringe. The blood was immediately transferred to two separate 10 mL vacutainer tubes without anticoagulant for the preparation of L-PRF and A-PRF [Table/Fig-2].

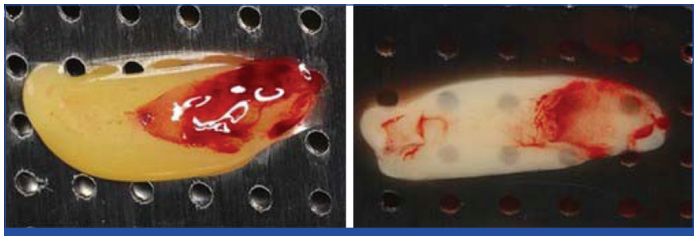
Leukocyte Platelet-rich Fibrin (L-PRF) preparation: The first vacutainer tube was immediately centrifuged at 3000 rpm for 10 minutes in the Remi R-8C centrifuge according to the protocol described by Pepelassi E and Deligianni M [21]. After centrifugation, the tube showed three distinct layers: a red blood cell layer at the bottom, a PRF clot in the middle, and acellular plasma at the top. The PRF clot was carefully removed from the tube using sterile non toothed forceps and separated from the red blood cell layer by gentle scraping [Table/Fig-3].

Advanced Platelet-rich Fibrin (A-PRF) preparation: The second vacutainer tube was centrifuged at 1500 rpm for 14 minutes following the protocol established by Ghanaati S et al., and Pepelassi E and Deligianni M [8,21]. After centrifugation, the PRF clot was retrieved from the tube using sterile forceps and separated from the red blood cell layer [Table/Fig-4].

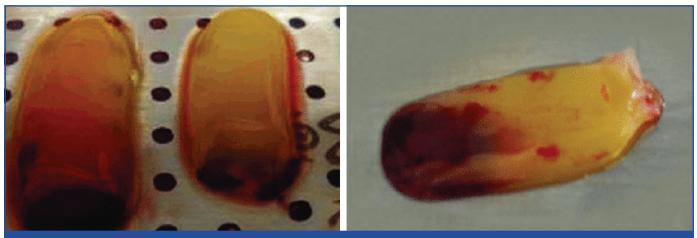
PRF membrane preparation: Both L-PRF and A-PRF clots were placed in the PRF Box (Process Ltd., Nice, France) and compressed under a constant pressure for five minutes to obtain standardised membranes of uniform thickness (approximately, 1 mm) [9]. From



[Table/Fig-2]: Methodology flowchart.



[Table/Fig-3]: Preparation of L-PRF.



[Table/Fig-4]: Preparation of A-PRF.

each membrane, three discs of 6 mm diameter were obtained using a sterile gel punch.

Antimicrobial assay (Agar diffusion test): The antimicrobial efficacy of L-PRF and A-PRF against *S. intermedius* was evaluated using the agar diffusion method [22]. For each participant, three wells of 6 mm diameter were created in the BHIA plate using a sterile gel punch. The wells were positioned equidistant from each other, approximately 30 mm apart.

In each plate, the following were placed:

1. L-PRF membrane disc (6 mm diameter) in the first well;
2. A-PRF membrane disc (6 mm diameter) in the second well;
3. Vancomycin disc (30 µg) in the third well as a positive control.

The plates were incubated at 37°C for 24 hours in microaerophilic conditions (5% CO_2). After the incubation period, the ZOI were measured in millimeters using a digital calliper. The diameter of the inhibition zone was recorded as the mean of two perpendicular measurements for each sample.

Antimicrobial activity assessment: The antimicrobial effect of L-PRF and A-PRF against *streptococcus intermedius* was evaluated using the agar diffusion method. All experiments were performed with samples from five different participants, and each experiment included vancomycin as a positive control.

STATISTICAL ANALYSIS

The data were statistically analysed using R Studio version 2024.12.1+563. Descriptive statistics including mean, standard deviation, and range were calculated for the inhibition zones produced by L-PRF, A-PRF, and vancomycin. The normality of data distribution was assessed using the Shapiro-Wilk test.

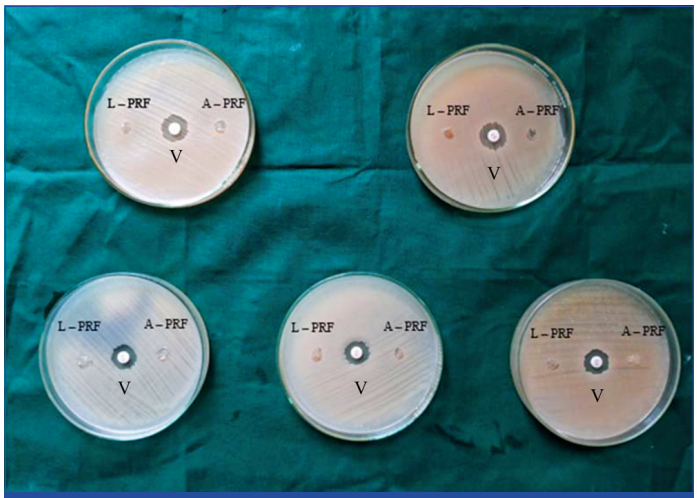
RESULTS

As all the data values were zero, statistical analysis was not feasible. Accordingly, only the descriptive data were presented and the comparative analysis could not be performed.

Zones of Inhibition (ZOI)

After 24 hours of incubation at 37°C under microaerophilic conditions, clearly defined ZOI were observed around all vancomycin discs (positive control) with a mean diameter of 18.76±1.32 mm (range: 17.2-20.5 mm), confirming the validity of the antimicrobial assay and the susceptibility of the *S. intermedius* strain to the control antibiotic.

However, no ZOI were observed around either the L-PRF or A-PRF membrane discs from any of the five participants, indicating absence of detectable antimicrobial activity against *S. intermedius* under the experimental conditions employed in this study [Table/Fig-5].



[Table/Fig-5]: Representative agar plate showing Zones Of Inhibition (ZOI). Note the clear inhibition zone around the Vancomycin disc (V) and the absence of inhibition zones around the L-PRF and A-PRF discs.

The results are summarised in [Table/Fig-6], which presents the mean diameters of inhibition zones for L-PRF, A-PRF, and vancomycin across all five participants.

Macroscopic Observations

Visual examination of the agar plates revealed that both L-PRF and A-PRF discs maintained their structural integrity throughout the 24-

Sample type	Mean zone of inhibition (mm)±SD	Range (mm)
L-PRF	0.00±0.00	0.00
A-PRF	0.00±0.00	0.00
Vancomycin	18.76±1.32	17.2-20.5

[Table/Fig-6]: Mean diameters of inhibition zones (mm) against *Streptococcus intermedius*.

hour incubation period. The bacterial lawn around the PRF discs appeared uniform and consistent with the rest of the plate, showing no visible reduction in bacterial growth in the immediate vicinity of the membrane discs.

Since no inhibition zones were detected for either L-PRF or A-PRF samples against *S. intermedius*, no statistical comparison could be performed between these two groups. The consistency of results across all five participants (0 mm inhibition for both PRF types) indicated complete reproducibility of the finding that neither L-PRF nor A-PRF exhibited antimicrobial activity against *S. intermedius* in the agar diffusion model under the conditions employed in the present study.

Statistical analysis was therefore limited to descriptive statistics for the vancomycin control group, which showed normal distribution according to the Shapiro-Wilk test (p=0.893).

DISCUSSION

The present in-vitro study aimed to evaluate and compare the antimicrobial efficacy of two different PRF preparations L-PRF and A-PRF against *streptococcus intermedius*, a clinically significant oral pathogen frequently implicated in deep-seated infections. Contrary to the authors' hypothesis, the results demonstrated that neither L-PRF nor A-PRF exhibited detectable antimicrobial activity against *S. intermedius* in the agar diffusion model employed in the present study.

These findings contrast with several previous studies that have reported antimicrobial properties of platelet concentrates against various oral pathogens. Badade PS et al., found that both PRF and PRP demonstrated significant antimicrobial activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, with PRF exhibiting superior effects compared to PRP [23]. Similarly, Kour P et al., reported antimicrobial efficacy of various platelet concentrates, including PRF, against periodontal pathogens [24]. A recent study by Castro AB et al., demonstrated that L-PRF exudates exhibited antimicrobial effects against *Porphyromonas gingivalis* and *Fusobacterium nucleatum* [25].

The absence of antimicrobial activity against *S. intermedius* in the present study could be attributed to several factors. Firstly, the susceptibility to antimicrobial components in PRF may vary among different bacterial species. *S. intermedius* possesses various virulence factors, including the production of intermedilysin and hyaluronidase, which may contribute to its resistance against antimicrobial peptides present in PRF [26]. Khalil W et al., proposed that the antimicrobial effect of platelet concentrates is species-specific, with certain bacteria being more susceptible than others [27].

Secondly, the methodology employed for antimicrobial assessment may influence the observed results. The agar diffusion test, while widely used for antimicrobial susceptibility testing, relies on the diffusion of active components through the agar medium [28]. Drago L et al., suggested that different methodological approaches, such as direct contact tests or broth dilution methods, might better elucidate the antimicrobial potential of platelet concentrates [29]. It is possible that the antimicrobial factors present in PRF, such as defensins, may have limited diffusion capacity in the agar medium due to their size or binding to the fibrin matrix [30].

Thirdly, the concentration and viability of leukocytes in the PRF preparations could significantly influence their antimicrobial properties. While both L-PRF and A-PRF contain leukocytes, their concentration, distribution, and functional capacity may vary based on the centrifugation protocol [31]. Ghanaati S et al., demonstrated that A-PRF contains a higher number of neutrophils compared to L-PRF, but the functional capacity of these cells in the fibrin matrix remains unclear [8]. The absence of antimicrobial activity in both PRF preparations suggests that either the leukocyte concentration was insufficient to exert antimicrobial effects against *S. intermedius*, or the leukocytes were not functionally active in the experimental conditions employed [32].

Additionally, the temporal dynamics of antimicrobial factor release from PRF must be considered. PRF membranes are known to release growth factors and cytokines gradually over time. Kobayashi E et al., reported that A-PRF exhibited a more sustained release of growth factors compared to L-PRF [33]. It is plausible that the 24-hour incubation period in the present study may not have been sufficient for the release of adequate concentrations of antimicrobial components to inhibit *S. intermedius* growth. Extended incubation periods or collection of PRF exudates over time for antimicrobial testing might provide different results.

The structural characteristics of the PRF membranes could also influence their antimicrobial potential. The compression of PRF clots to form membranes, as performed in the present study, might alter the architecture of the fibrin network and affect the release kinetics of embedded components [34]. Dohan Ehrenfest DM et al., emphasised that the three-dimensional structure of PRF is crucial for its biological properties [35]. Future studies might compare the antimicrobial efficacy of compressed PRF membranes versus uncompressed PRF clots to elucidate the impact of structural modifications.

Another important consideration is the potential synergistic effect of PRF with other antimicrobial agents. While PRF alone did not demonstrate antimicrobial activity against *S. intermedius* in the present study, it might enhance the efficacy of conventional antibiotics through synergistic interactions. Jhansi G and Rao K reported that PRF in combination with antibiotics exhibited enhanced antimicrobial effects against periodontal pathogens compared to either component alone [36]. Future research could explore such combinatorial approaches for managing *S. intermedius* infections in clinical settings.

The clinical implications of the present study findings warrant careful consideration. While PRF is widely used in various regenerative procedures in oral and maxillofacial surgery [37], its selection should not be primarily based on anticipated antimicrobial benefits against *S. intermedius*. Instead, the well-documented regenerative properties of PRF, including enhanced wound healing, tissue regeneration, and reduced postoperative complications, should remain the principal considerations for its clinical application [38]. In situations where *S. intermedius* infection is a concern, conventional antimicrobial strategies should be employed alongside PRF to ensure comprehensive management.

Despite the negative results, where the null hypothesis is accepted, the present study contributes valuable insights to the understanding of PRF's biological properties. The species-specific nature of PRF's antimicrobial activity emphasises the importance of targeted microbiological investigations when evaluating biomaterials for specific clinical applications. Furthermore, the present study findings highlight the need for standardised methodologies in assessing the antimicrobial properties of platelet concentrates to enable meaningful comparisons across studies.

Future research directions should include alternative methodological approaches such as direct contact tests, time-kill assays, or biofilm models to comprehensively evaluate PRF's antimicrobial properties. Additionally, molecular investigations into

the interactions between PRF components and specific bacterial virulence factors could provide mechanistic insights into the observed results. Studies comparing the antimicrobial efficacy of various platelet concentrates against a broader spectrum of oral pathogens, especially in gram-negative bacteria, would also contribute significantly to the field.

Limitation(s)

Firstly, the agar diffusion method, while standardised and reproducible, may not fully represent the complex interactions between PRF and bacteria in the in-vivo environment. Secondly, the study focused on a single bacterial species and may not reflect PRF's antimicrobial potential against polymicrobial infections commonly encountered in clinical settings. Thirdly, when evaluating the antimicrobial activity at a single time point (24 hours), which may not capture the dynamic release of antimicrobial components from PRF over extended periods.

CONCLUSION(S)

In conclusion, within the limitations of the present in-vitro study, neither L-PRF nor A-PRF demonstrated antimicrobial activity against *Streptococcus intermedius* in the agar diffusion model. These findings suggest that the anticipated antimicrobial benefits of PRF may be limited against certain bacterial species, highlighting the importance of species-specific considerations in the clinical application of platelet concentrates. While PRF remains a valuable tool in regenerative procedures due to its well-established effects on tissue healing and regeneration, its selection should not be primarily based on expected antimicrobial properties against *S. intermedius*. Future research employing diverse methodological approaches and investigating the potential synergistic effects of PRF with conventional antimicrobials could provide further insights into optimising the therapeutic benefits of platelet concentrates in clinical practice.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 09, 2025
- Manual Googling: Feb 09, 2026
- iThenticate Software: Feb 11, 2026 (1%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jun 24, 2025**
Date of Peer Review: **Oct 29, 2025**
Date of Acceptance: **Feb 13, 2026**
Date of Publishing: **Jun 01, 2026**