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REVIEW ARTICLE

Antimicrobial effect of platelet-rich fibrin: A systematic review of in vitro evidence-based studies

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1 | INTRODUCTION

Platelet-rich fibrin (PRF) is a blood byproduct that has been used for over 20 years in the medical field. PRF belongs to the second generation of platelet concentrates which differs from its processors as it does not contain anticoagulants or other additives. Blood centrifugation produces PRF after a 9–10 mL sample is drawn.[1] There are different methods to prepare PRF. However, the final product must include fibrin mesh-containing leukocytes and platelets. Together, these cells produce an essential release of growth factors and cytokines that participate in critical phases of the healing process, such as collagen matrix formation and neovascularization[1,2] (Figure 1).

Recently, PRF has been used as an adjuvant in treating wounds (e.g., diabetic foot) to form a protective barrier to increase local cellular activity, causing tissue recovery.[3] This action is due to PRF's tissue regeneration potential.[4] PRF's involvement in wound healing may go beyond acting as a protective barrier. Several studies have pointed to the benefits of PRF as an autologous biomaterial with not only regenerative potential but also an antimicrobial activity.[5–8] This phenomenon could be caused by the blood cells present in the fibrin matrix or the chemotaxis caused by cytokines released in the recipient tissue.

The release of pro- and anti-inflammatory cytokines and growth factors from the PRF scaffolds is essential for tissue recovery. Platelets play a vital role in this process due to their production of α-granules, which serve as a significant storage pool of proteins that are essential for wound healing. The resulting byproducts, such as interleukin-8 (IL-8),[9] IL-15,[10] and IL-18,[11] and the chemokine MIP1-α,[12] act in the chemotaxis of neutrophils and natural killer cells, increasing protection against microorganisms at the level of the intervened tissue. Some studies have highlighted the antimicrobial activity of blood byproducts through the induction of human β-defensin 2 (hBD-2)[13] and hBD-3.[14]

Although the issue of PRF's antimicrobial activity requires future translational evidence, some studies have demonstrated the robust tissue regeneration potential of blood byproducts.[15,16] Well-defined protocols are required to produce PRF.[1,17] In addition, studies will need to focus on PRF's antimicrobial activity in affected tissues and the efficacy of its scaffold against various microorganisms. Therefore, this SR aimed to analyze the antimicrobial potential of different types of PRF often used in regenerative treatments.

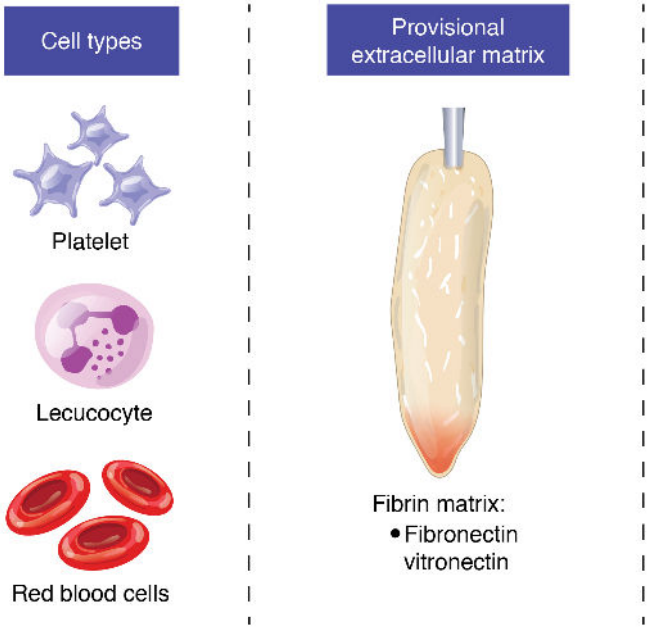

FIGURE 1 The composition of Leukocyte Platelet-Rich Fibrin (L-PRF) includes various cell types, a 3-D provisional extracellular matrix scaffold made of autologous fibrin enriched with fibronectin and vitronectin, and a collection of molecules such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and transforming growth factor-beta (TGF-ß). *Source*: Figure adapted from Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. Tissue Eng Part B Rev 2017;23(1):83–99.

2 | MATERIALS AND METHODS

2.1 | Protocol and registration

The protocol of this SR was performed following the recommendations of PRISMA-P[18] and registered in the INPLASY database under the number INPLASY202340016 (DOI 10.37766/inplasy2023.4.0016). The final version of this SR followed the PRISMA guidelines.[19] There were no deviations between the created protocol and the final version of this SR.

2.2 | Focused question

The main question was structured according to the PICOS strategy[20]: What is the antimicrobial effect of human PRF in in vitro studies?

2.3 | Eligibility criteria and study selection process

The eligibility criteria were determined using the PICOS strategy. The search and selection process were conducted by two independent authors (J.M.M. and V.M.). First, titles and abstracts were analyzed. Next, selected studies were read in full to verify whether they met all eligibility criteria. The search concordance between the two reviewers was evaluated by the Cohen's kappa (*k*) test. Disagreements between the reviewing authors were resolved through careful discussion.

Several eligibility criteria were established:

Population: PRF collections in healthy humans.

Intervention: Use of human PRF as an antimicrobial agent.

Comparison: Antibiotic efficacy between different types of PRF with other antibiotic agents and different microorganisms.

Outcome: Analyzes of microorganism growth, microorganism inhibition, and microorganism activity.

Study design: In vitro studies.

The exclusion criteria included animal studies, case series, case reports, and reviews. Studies that used PRF from animals or humans with chronic or hematological diseases were also excluded.

2.4 | Search strategy

The PubMed/MEDLINE, EMBASE, Web of Science, and Scopus databases were searched for relevant articles published prior to January 2023. There were no restrictions regarding the date of publication or language. Gray literature was searched using the Literature Report and OpenGrey databases. In addition, the study reference lists were evaluated (cross-referenced) to identify other potential studies for inclusion. Several search terms were used: ("platelet-rich fibrin" OR "leucocyte platelet-rich fibrin" OR "advanced platelet-rich fibrin" OR "injectable platelet-rich fibrin" OR "PRF") AND ("microbiome" OR "biofilm" OR "oral pathogen" OR "microbial" OR "antimicrobial" OR "microorganisms" OR "antibacterial" OR "antimicrobial" OR "infection") NOT ("review").

2.5 | Data extraction and management

Data were extracted by J.M.M. and systematically reviewed by V.M. When available, the data regarding authors, sample size, type of platelet concentrate, control group, types of microorganisms, type of culture, time of response, outcomes, results, and finding summaries were obtained. Missing information was investigated by contacting the authors via email. In the case of multiple publications of the same trial, data were extracted for general characteristics only. Specific data (i.e., outcomes and results of interest) were extracted from the most relevant publications.

2.6 | Risk of bias within studies

For the analysis of the risk of bias, the modified OHAT (Office of Health Assessment and Translation)[21] tool was used. The tool was developed to assess the risk of bias in all study designs, including in vitro studies. Nine domains were used to rank each study. Each domain was classified as "definitely low risk of bias," "probably low risk of bias," "probably high risk of bias," or "definitely high risk of bias." These ratings were coded as "++", "+", "−/NR" (not reported), and "−−", respectively. To estimate the risk of bias between studies, a percentage estimate of each four ratings was calculated.

2.7 | Summary of results

A quantitative analysis based on the meta-analysis could not be developed due to the heterogeneity observed in the design and methodologies adopted by the included studies. However, a qualitative analysis based on the synthesis of the results of the included studies was carried out.

3 | RESULTS

3.1 | Study selection

The initial search produced 306 titles from the MEDLINE/PubMed database, 454 from Embase, 702 from Web of Science, and 398 from Scopus. The first evaluation of titles and abstracts excluded 1842 articles that did not adhere to the eligibility criteria. After reading the full text of the remaining studies, two articles[22,23] did not meet the inclusion criteria and were excluded. The reasons for exclusion are reported in Figure 1. Therefore, 16 studies published between 2013 and 2021 were included in this SR. The coefficients of agreement (κ) between reviewers in relation to the search process were 0.97 (titles and abstracts) and 0.85 (inclusion of studies), an almost perfect agreement.[24] A flowchart demonstrating the selection process is shown in Figure 2.

3.2 | Characteristics of the included studies

3.2.1 | Design and methods

The characteristics and methods used by the included studies are shown in Table 1. The antimicrobial effects of PRF variations (PRF, I-PRF, agNP-PRF, and H-PRF) were compared to each other and with controls, such as phosphate-buffered saline, platelet-rich plasma (PRP), platelet-poor plasma (PPP), chlorhexidine, and antibiotics. One study[6] did not use a control group. Over 16 subgroups of bacteria from the oral, periodontal, and endodontic environments were analyzed. The most used culture medium was blood agar plates. The culture and response time ranged from 16[25] to 168 h.[26] The number of samples ranged from 5[26] to 64.[27]

3.3 | Interventions and comparisons

3.3.1 | Efficacy and comparison with control groups

With the exception of one article,[6] all other included studies reported a positive antibacterial action of PRF compared with the

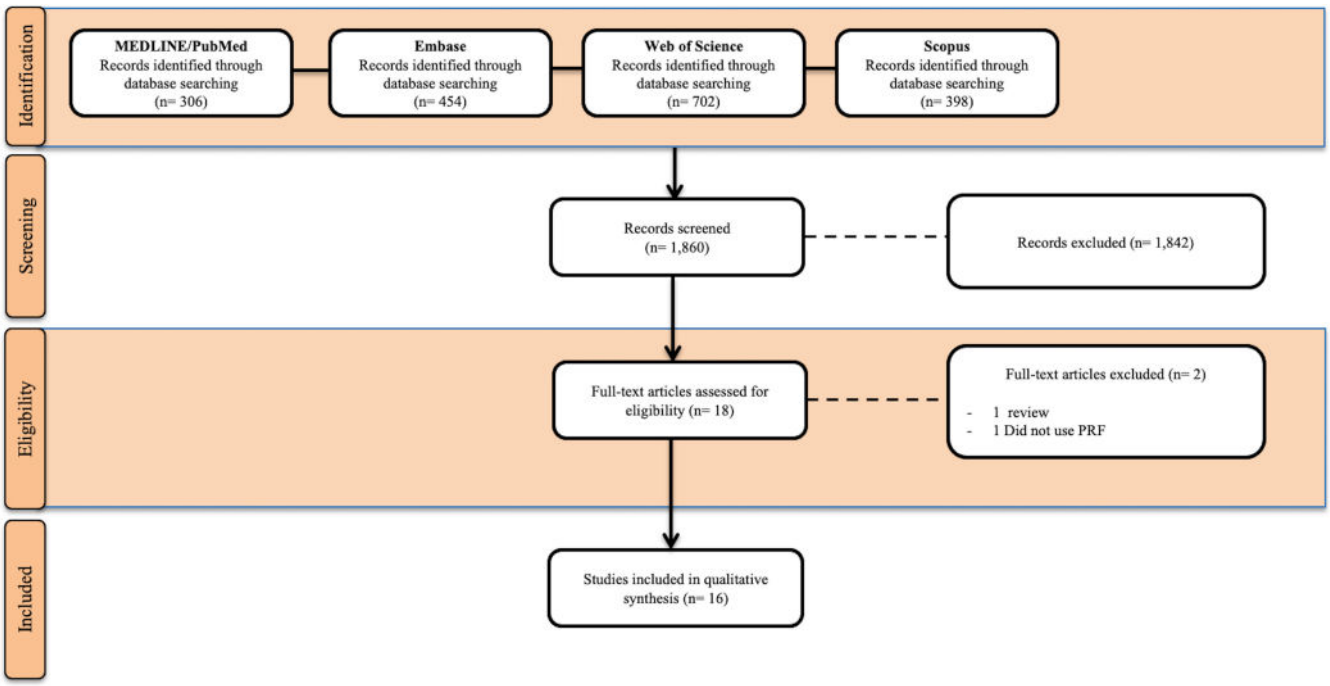

FIGURE 2 Flow diagram (PRISMA format) of the screening and selection process.

TABLE 1 Main characteristics of the included studies.

Author/Year	Samples	Platelet concentrates	Control Group	Micro-organisms tested
Wu et al., 2013	14	PRF	- Phosphate-buffered saline - PRP - PPP	*Escherichia. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*
Joshi et al., 2016	15	PRF	Sterile BHI broth containing tube served as a negative control. In addition, test tubes with BHI broth along with patient's plaque sample without PRF and with metronidazole (5 μg) were used independently as controls for each individual	Subgingival plaque of individuals with active periodontal disease
Badade et al., 2016	10	PRF	PRP	*Porphyromonas gingivalis* and *Aggregatibacter. actinomycetemcomitans*
Karde et al., 2017	10	I-PRF and PRF	- PRP - A volume of 2 mL blood collected in ethylenediaminetetraacetic acid anticoagulant containing vacutainer which was not subjected to any centrifugation	Human supragingival plaque
Kour et al., 2018	10	PRP, PRF, and I-PRF	- RPMI medium - PRP	*Porphyromonas gingivalis* and *Aggregatibacter. actinomycetemcomitans*
Haddadi et al., 2018	18	PRF and agNP-PRF	RPMI medium without any microorganism and 0.5 McFarland microorganism suspensions were considered as negative and positive controls	*Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Streptococcus mitis*
Khorshidi et al., 2018	10	PRF and agNP-PRF	Positive and negative controls in this study were the saliva cultured in RPMI and RPMI medium	*Streptococcus viridans* and *Klebsiella pneumonia*
Polak et al., 2019	5	PRF with the addition of antibiotics (5 mg/mL metronidazole; 150 mg/mL clindamycin; 1 mU/mL penicillin) and PRF free antibiotics	Collagen sponges saline	*Staphylococcus aureus* and *Fusobacterium nucleatum*
Castro et al., 2019	9	PRF and PRF exudate	CHX 0.12%	*Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*

Culture medium	Time of response	Evaluated outcome	Protocol of PRF	Summary of finding
Bacteria were maintained on nutrient agar slant (Oxoid) at 4°C	16–18 h	Inhibition zone	NR	PRF compared with commercial products, had remarkable antimicrobial activity against gram negative bacteria relevant to fistula colonization. The presence of platelets and leukocytes may play an important role in bacterial defense.
BHI broth and a disc of PRF. The test tube was then incubated at 37°C in an anaerobic chamber	48 h	Colorimetric analysis	3000 rpm for 10 min	The use of PRF will be benefit in reducing microbial load at periodontally infected sites
Blood agar plates	48–96 h	Inhibition zone	3000 rpm for 10 min	*P. gingivalis* and *A. actinomycetemcomitans* were inhibited by PRP but not by PRF
Blood agar plates	48 h	Disc diffusion method	I-PRF 700 rpm for 3–4 min PRF 3000 rpm for 10 min	I-PRF has maximum antimicrobial efficacy and higher platelet count in comparison to PRF and PRP, thereby indicating to have a better regenerative potential
Blood agar plates	24 h	Turbidity (absorbance) of medium (negative control)	I-PRF 700 rpm for 3 min PRF 3000 rpm for 10 min	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
Sabouraud dextrose agar and blood agar	24 h	The biofilm formation of each membrane was evaluated by crystal violet (0.1%) staining and optical density ELISA reader device	2700 rpm for 12 min	agNP-PRF as a biological material presented the more inhibition of biofilm formation of contaminating microorganisms and it can be used as anti-infectious material in the surgical sites.
Blood agar plates and thioglycolate broth	24 h	Turbidity (absorbance) of medium (negative control)	2700 rpm for 12 min	agNP-PRF improves the antibacterial activity of the PRF. It can play an important role in regenerative procedures.
Staphylococcus aureus (clinical isolate) was cultured in aerobic condition at 37°C for 24 h. *Fusobacterium nucleatum* was cultured in anaerobic condition at 37°C for 24 h	168 h	Antibiogram assay	2700 rpm for 12 min with a g-force of 735	PRF incorporated with antibiotics showed long-term antibacterial effect against *F. nucleatum* and *S. aureus*. This modified PRF preparation may be used to reduce the risk of post-operative infection in addition to the beneficial healing properties of PRF
Blood agar plates	72 h	Inhibition zone	NR	This study demonstrated the antibacterial effect of the PRF membrane against *P. intermedia*, *F. nucleatum*, and *A.actinomycetemcomitans*, but especially against *P. gingivalis*.

(Continues)

TABLE 1 (Continued)

Author/Year	Samples	Platelet concentrates	Control Group	Micro-organisms tested
Mamajiwala et al., 2020	60	PRF	Different ages groups 20–34 years, 35–49 years, 50–65 years	Subgingival biofilm from health patients
Rafiee et al., 2020	64 intact, caries-free single-rooted human mandibular first premolars	Triple antibiotics mixture, I-PRF containing triple antibiotics mixture, antibiotics free I-PRF	Biofilm untreated and bacteria-free untreated	*Actinomyces naeslundii* and *Enterococcus faecalis*
Schuldt et al., 2020	12	PRF	No control group	Subgingival plaque of individuals with healthy periodontium
Nagaraja et al., 2020	16	PRF and PRF matrix	Calcium hydroxide	The microbial samples from the root canal and *Candida albicans*
Jasmine et al., 2020	NR	I-PRF	*Staphylococcus. epidermis* (biofilm forming strain) and *Staphylococcus. epidermis* (biofilm negative strain)	The bacterial strains of *Staphylococcus aureus* and *Staphylococcus epidermis* were isolated from patients with oral and dental abscess
Feng et al., 2020	8	Horizontal-PRF and PRF	NR	*Staphylococcus aureus* and *Escherichia coli*
Melo-Ferraz et al., 2021	6	PRF	CHX 0.12%	*Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans*

Abbreviations: agNP-PRF, platelet–rich fibrin with silver nanoparticles; Anti-biogram assay; BHI, brain–heart infusion; CHX, chlorhexidine; I-PRF, injectable platelet-rich fibrin; MBC, minimal bactericidal concentration; MIC, minimal inhibitory concentration; NR, not reported; PPP, platelet-poor plasma; PRF, Platelet–rich fibrin; PRP, platelet-rich plasma; RPM, revolutions per minute; TBS, trypticase soy broth.

control groups. Badade et al.[6] investigated the action of PRF on two types of bacteria (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) commonly associated with periodontal disease. There was no mean inhibition rate for either bacteria after 48 h of observation. However, the control group (PRP) demonstrated a positive action. Nagaraja et al.[28] demonstrated the antibacterial activity of PRF against bacteria isolated from the root canal. However, the membrane did not demonstrate antifungal efficacy against *Candida albicans*. Similarly, another study[29] did not observe a positive effect of PRF against *C. albicans*.

3.3.2 | Comparison between different types of PRF

Two studies[30,31] compared the antimicrobial effect of PRF and I-PRF in inhibiting periodontium-related bacteria (including the red

Culture medium	Time of response	Evaluated outcome	Protocol of PRF	Summary of finding
Blood agar plates	48 h	Agar well-diffusion method	PRF 1400, 2800, and 3500 rpm for 8 min while other 3 membranes were obtained after 14 min of centrifugation with a g-force of 228–1425	This study demonstrated that platelet count, antimicrobial efficacy, and fibrin network, get affected by centrifugation speed (rpm) and time duration in different age groups.
BHI +1% sucrose	168 h	Real-time PCR analysis	I-PRF 60 g for 3 min at 4 grades	The highest antibacterial activity against *A. naeslundii* belonged to (I-PRF containing triple antibiotic mixture. However, the other two groups had similar antibacterial property against *E. faecalis*.
The samples were placed in 2 mL of sterile phosphate-buffered saline	48 h	Decontamination by scanning electron microscopy	2700 rpm for 2 min 2400 rpm for 4 min; 2700 rpm for 4 min and 3000 rpm for 3 min with a g-force of 400	Application of PRF significantly reduced bacterial counts on contaminated SLA titanium surface, most likely through anti-microbial action by platelets.
Moistfree blood agar culture plate (bacterial). Sabouraud's dextrose agar plate (fungal)	48 h	Inhibition zone	3000 rpm for 10 min	PRF matrix had no demonstrable antibacterial or antifungal efficacy. PRF demonstrated antibacterial activity against root canal isolates but had no antifungal efficacy.
TSB	24 h	MIC and MBC	I-PRF 1000 rpm for 5 min at 37 grades	I-PRF exhibited bactericidal activity against both non biofilm and biofilm producing bacteria. I-PRF could be potential antimicrobial peptide used to combat postoperative infections caused by biofilm producing *staphylococcus*
Blood agar plates	24 h	Inhibition zone and colony-forming unit measurements	PRF was obtained with g-force of 700 for 12 min H-PRF was collected by a horizontal centrifuge at a g-force of 700 for 8 min	This study demonstrated that the PRF prepared by horizontal centrifugation exhibited significantly better antibacterial activities against both *S. aureus* and *E. coli* than traditional PRF.
Blood agar plates	24 h	Inhibition zone and molecular biomarkers	2700 rpm for 12 min	The PRF products are efficient in activating platelets of whole blood, and in inhibiting microbial growth of aerobic and facultative anaerobic microorganisms that frequently contribute to the failure of dental treatments

complex periodontal pathogens described by Socransky et al.[32]). Although PRF demonstrated a positive effect, the two studies concluded I-PRF demonstrated a significantly greater effect.

Other studies[29,33] compared the antimicrobial effect of PRF with agNP-PRF. In the study by Haddadi et al.,[29] the PRF membrane was not able to inhibit *C. albicans* after 24 h. However, when agNP-PRF was used, a significant decrease in biofilm was observed. The effectiveness of agNP-PRF was proportional to the concentration of agNPs (the more concentrated, the greater the antimicrobial effect). In the study by Khorsshidi et al.,[33] after 12 h of incubation, greater bacterial growth (e.g., *Streptococcus virudans and Klebsiella pneumonia*) was observed on PRF membranes compared to agNP-PRF membranes. Thus, the authors concluded that the modification of PRF with agNPs can prevent the growth of microorganisms in surgical wounds.

One study[7] investigated the antimicrobial effects of H-PRF and PRF against *Staphylococcus aureus* and *Escherichia coli* after 24 h of

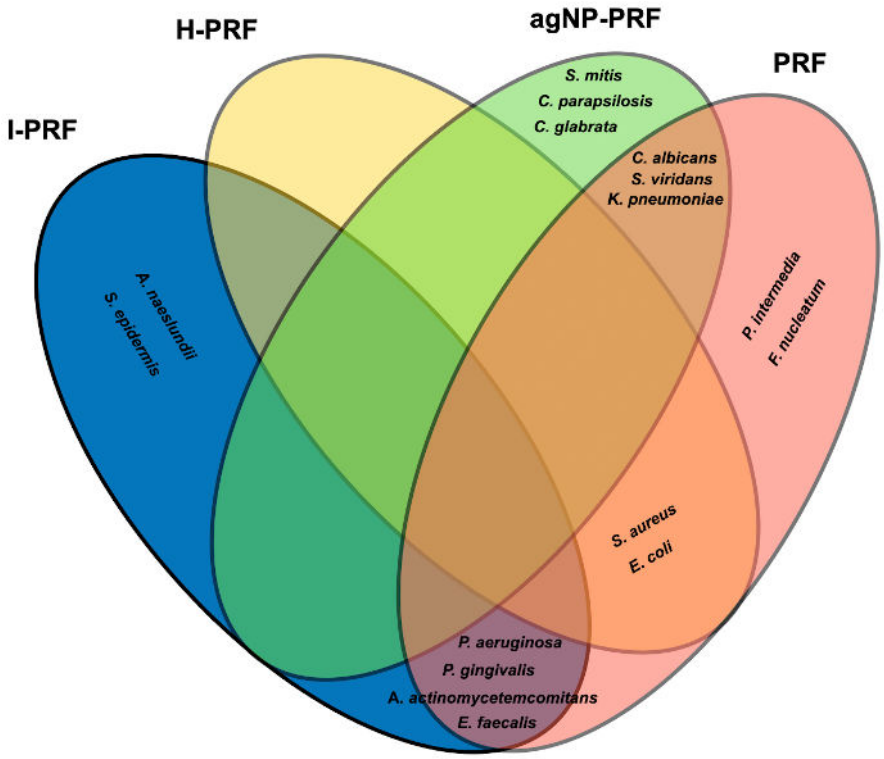

FIGURE 3 Venn diagram showing microbial sensitivity evidence in relation to the type of PRF used.

incubation. H-PRF was prepared by horizontal centrifugation, and PRF was produced using a fixed-angle centrifuge. The zone of inhibition in the H-PRF group was significantly ($p < 0.05$) larger than the PRF group for both bacteria. Figure 3 demonstrates the microbial sensitivity evidence in relation to the type of PRF used.

3.4 | Risk of bias within studies

Six studies[5,7,29,30,34,35] were rated at medium risk of bias. All the other studies had a low risk of bias. Items 2 (was allocation to study groups adequately concealed?), 4 (were research personnel blinded to the study group during the study?), and 7 (can we be confident in the outcome assessment?) were the topics least scored by the studies. The risk-of-bias analysis is detailed in Table 2.

4 | DISCUSSION

Antimicrobial activity is desirable for any biomaterial during tissue regeneration. Despite the majority of studies analyzing the regenerative potential of PRF, the literature remains scarce regarding its antimicrobial effectiveness. This SR aimed to analyze the antimicrobial potential of different types of PRF often used in regenerative treatments.

Some antimicrobial mechanisms of PRF have been described in the literature, such as thrombin-stimulated platelets that release proteins with antimicrobial activity against fungi and bacteria[36] and platelets that generate reactive oxygen species, bind and internalize microorganisms, and participate in antibody-dependent cellular cytotoxicity.[37] In addition, platelets can recognize, isolate, and neutralize possible antigens and signal leukocyte recruitment.[38]

Most of the included studies demonstrated the antimicrobial efficacy of different types of analyzed PRF. However, one study[6] did not report inhibition of two types of bacteria (*P. gingivalis* and *A. actinomycetemcomitans*) after 48 h of observation. Special attention has been given to these bacteria due to their strong association with periodontal and peri-implant diseases[32,39] and rheumatoid arthritis.[40,41] Conversely, two other studies,[5,31] concluded that PRF was effective in inhibiting these two bacteria after a maximum 72 h of observation. In a direct comparison between PRF and I-PRF, although both platelets were effective against these bacteria, the effect of I-PRF was significantly greater.[31]

In all comparative studies, I-PRF demonstrated a greater performance compared to standard PRF. One hypothesis explaining this greater performance is that a higher concentration of cells and cytokines are present in I-PRF due to the slower and longer centrifugation spin associated with its production.[42] Furthermore, another advantage of I-PRF is that it is presented in liquid form (injectable), which allows for its incorporation into other biomaterials providing new strategies to load antimicrobial effects within any biomaterial.

Regarding the antifungal activity of PRF, two studies[28,29] reported that PRF did not have an inhibitory effect on *C. albicans*. However, Mello-Ferraz et al.[43] reported that the PRF membrane was able to inhibit *C. albicans*, producing a better result than the control group (chlorhexidine 0.12%). The use of agNP-PRF was also found to inhibit *C. albicans*.[29] Oral candidiasis is seen in 8% of pediatric patients and 46% of patients with hematological disorders.[44] In

TABLE 2 Risk of bias tool from the National Toxicology Program's Office of Health Assessment and Translation (OHAT).

	Karde et al., 2017	Badade et al., 2016	Castro et al., 2019	Feng et al, 2020	Haddadi et al., 2018
1. Was administered dose or exposure level adequately randomized?	−−	++	−−	−−	−−
2. Was allocation to study groups adequately concealed?	−−	−−	−−	−−	−−
3. Were experimental conditions identical across study groups?	−−	++	++	++	++
4. Were research personnel blinded to the study group during the study?	−−	−−	−−	−−	−−
5. Were outcome data complete without attrition or exclusion from analysis?	++	++	++	++	++
6. Can we be confident in the exposure characterization?	+	+	+	+	+
7. Can we be confident in the outcome assessment (including blinding of assessors)?	−−	−−	−−	−−	−−
8. Were all measured outcomes reported?	++	++	++	++	++
9. Were there no other potential threats to internal validity	+	+	+	+	+
ROB estimation	Medium	Low	Medium	Medium	Medium

Joshi et al., 2016	Jasmine et al., 2020	Khorshidi et al., 2018	Mamajiwala et al., 2019	Nagaraja et al., 2019	Polak et al., 2019	Kour et al., 2019	Rafiee et al., 2020	Schuldt et al., 2020	Melo-Ferraz et al., 2021	Wu et al., 2013
−−	−−	++	++	++	−−	−−	−−	−−	−	−−
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++	++	++	++	++	++	++	++	++	++	++
+	+	+	+	+	+	+	+	+	+	+
Medium	Medium	Low	Low	Low	Low	Low	Low	Low	Low	Low

Abbreviations: ROB, risk of bias; ++, definitely low risk of bias; +, probably low risk of bias; −, probably high risk of bias; −−, definitely high risk of bias. Red: Definitely high risk of bias; Light green: Probably low risk of bias; Dark green: Definitely low risk of bias; Yellow: low risk of bias.

addition, *C. albicans* is the most common fungal species associated with root canals.[28] Controlling this fungus is essential, especially in wounds of immunocompromised patients.

Different studies demonstrated the antimicrobial potential of agNPs.[45,46] AgNP may play an anti-inflammatory role by inhibiting some matrix metalloproteinases, which may, in turn, enhance wound healing.[45] According to two of the included studies, the association of agNP with PRF results in increased antimicrobial activity when compared with PRF alone.[29,33]

Some recent evidence demonstrates that the preparation of PRF using horizontal centrifugation (H-PRF) increases the number of immune cells present.[47,48] One included study demonstrated that H-PRF exhibited significantly better antibacterial activities against *S. aureus* and *E. coli* when compared with standard PRF[7] produced via fixed-angle centrifugation. Furthermore, histological results demonstrate that cells (platelets and leukocytes) in standard PRF membranes are found unevenly along the back distal surface, while in H-PRF, they are more uniformly distributed along the entire membrane.[48] This characteristic may be particularly interesting for a more homogeneous release of growth factors and cytokines throughout all wound regions.

This SR presents some strengths, such as its broad and unrestricted search that included data from nonpublished outcome results, minimizing the risk of selective reporting bias. Furthermore, this SR was performed in line with a previous protocol registered at INPLASY and in accordance with the recommendations of Cochrane and PRISMA.

However, some limitations can be highlighted. The antimicrobial activity of PRF has been little explored in the literature, and the available evidence primarily involves in vitro studies. Therefore, most of the included studies demonstrated a moderate potential risk of bias. Because of this, these results should be interpreted with caution. The significant divergence in the methodology (e.g., method of preparation, type of tubes [plastic, glass, and glass coated], and method of application [liquid, membrane, and gel]) used by the included studies could result in reporting bias and deserves greater attention in future studies. The preparation of PRF and its standardization can have a direct influence on the final composition

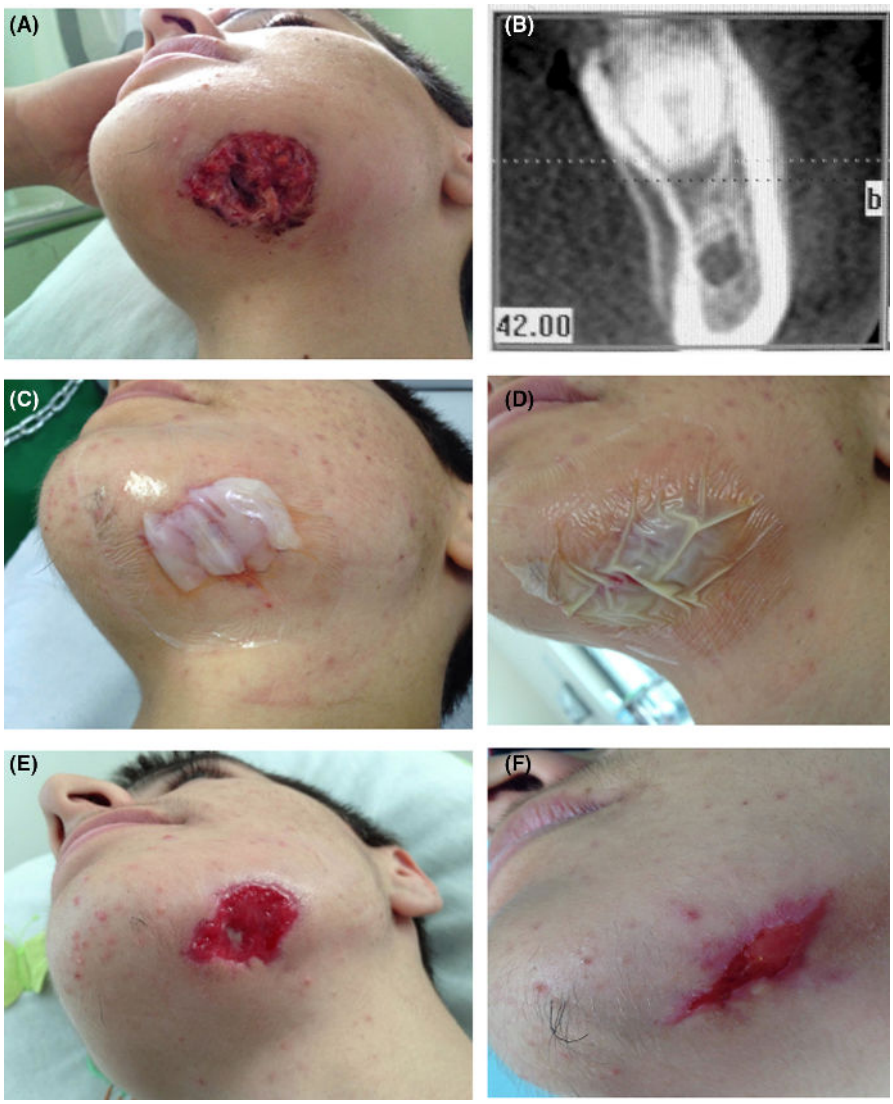

FIGURE 4 (A) Extra-oral lesion from endodontic dental abscess origin and (B) tomographic examination showing endodontic lesion. Clinical tests were also realized and confirmed the necrosis of the pulp; (C) the lesion was cleaned and covered with 4L-PRF membranes. The membranes were protected using an adhesive bandage. (D) Clinical aspect after 5 days post-treatment; (E) After 10 days the bandage was removed the previous lesion became epithelized and the border was covered with clinically healthy tissue; the lower lesion was observed that the lesion decreased; (F) 45 days after PRF treatment it was observed a clinical improvement of the initial extra-oral lesion. Courtesy of the Professor Ronaldo Iurovisk.

specifically of leukocytes within PRF and therefore present large variability in the final outcomes. Conducting future clinical studies on the antimicrobial effect of PRF is essential. In addition, methodological standardization for PRF preparation protocols and analysis techniques should be prioritized.

Based on the current state of evidence, the use of PRF should be considered in oral and reconstructive surgery. The increased expression of growth factors and immunological components (e.g., macrophages) in wounds, can act (isolated or associated with other biomaterials) by accelerating and inducing tissue neoformation. In addition, antimicrobial activity should be considered prophylactically in oral surgeries or as an adjunct in the treatment of chronic infectious wounds (Figure 4).

5 | CONCLUDING REMARKS

- The data observed suggest that the analyzed PRF variations have a significant antimicrobial effect.
- The data suggest a more robust bacterial effect compared with a fungal effect.
- The data indicate that first-generation PRF evolutions (I-PRF H-PRF, and agNP-PRF) improve antimicrobial activity.
- The evaluation of the microbiome in clinical studies could be beneficial for a better comprehensive analysis of the action of the PRF and complete the challenge presented in Figure 2.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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