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Dissertação apresentada à Universidade Católica Portuguesa para obtenção do grau de Mestre em Medicina Dentária

Por:
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Agradecimentos

Ao meu orientador, Prof Doutor Júlio Souza pela sua disponibilidade ao longo desse ano, pelos seus conhecimentos científicos e técnicos nesta área tão importante.

Ao nosso trinómio, Jorge e André sem os quais esse capítulo da minha vida não teria sido tão especial. Obrigado por esses momentos de alegria que permanecerão para sempre na minha memória. Que a nossa amizade dure para o resto das nossas vidas.

À minha mãe e ao meu pai, sem os quais esse apoio todo nunca teria existido. Por terem sempre acreditado em mim, tanto nos bons, como nos maus momentos. Sou o homem que eu sou hoje, graças a vocês.

Aos meus irmãos, André e Mathias que sempre estiveram presentes comigo apesar da distância. Estou realmente orgulhoso de ter-vos como irmãos e orgulhoso pelos homens que são.

À minha família, em particular, à minha avó, meus primos, meus tios pelas numerosas chamadas que me permitiram passar estes cinco anos com mais apoio.

ABSTRACT

Purpose: The purpose was to perform a scoping review on the antimicrobial effects of the injectable platelet rich fibrin.

Materials and Methods: A literature pursuit was conducted on PubMed and Scopus electronic database, within English studies published in the last 15 years. The following keywords were used: “antimicrobial” OR “antibiofilm” OR “antibacterial” AND “platelet rich fibrin” OR “PRF” OR “injectable platelet rich fibrin” OR “i-PRF” OR “injectable PRF” AND “bacteria” OR “Periodontal pathogen” OR “Aggregatibacter actinomycetemcomitans” OR “Prevotella intermedia” OR “Porphyromonas gingivalis”.

Results: The literature search identified a total of 720 articles although eleven studies were selected for this review. The findings from the selected studies showed inhibitory effect of the injectable platelet rich fibrin against biofilm-growing of around 40 and 50%. A peak of the inhibitory effect was reported after 12 h incubation in the medium containing *Aggregatibacter actinomycetemcomitans*. The studies validate the hypothesis on the i-PRF content of leukocytes on the inhibition of pathogenic bacteria. Furthermore, the association between an injectable PRF and traditional antimicrobials also revealed a high localized antimicrobial activity.

Conclusions: Injectable platelet rich fibrin reveals a significant inhibitory effect against a variety of oral microbial species, that become a noteworthy potential to decrease the risks of further infection in wound healing.

Keywords: i-PRF, PRF, antimicrobial, Injectable platelet rich fibrin, platelet rich fibrin.

RESUMO

Objetivo: o objetivo foi realizar uma revisão de escopo sobre o efeito antimicrobiano da fibrina rica em plaquetas injetável.

Materiais e métodos: Foi realizada uma pesquisa bibliográfica nas bases de dados eletrônicas da PubMed e Scopus, com artigos em inglês publicados nos últimos 15 anos. As palavras-chaves seguintes foram usadas: “antimicrobial” OR “antibiofilm” OR “antibacterial” AND “platelet rich fibrin” OR “PRF” OR “injectable platelet rich fibrin” OR “i-PRF” OR “injectable PRF” AND “bacteria” OR “Periodontal pathogen” OR “Aggregatibacter actinomycetemcomitans” OR “Prevotella intermedia” OR “Porphyromonas gingivalis”.

Resultados: A pesquisa bibliográfica identificou um total de 720 artigos, embora onze estudos foram selecionados para esta revisão. Os resultados dos estudos selecionados mostraram efeito inibitório da fibrina rica em plaquetas injetável contra o crescimento de biofilme de aproximadamente 40-50%. Um pico do efeito inibitório foi relatado após 12 horas de incubação em meio contendo *Aggregatibacter actinomycetemcomitans.* Os estudos validam a hipótese sobre inibição das bactérias pela presença dos leucócitos no i-PRF. Além disso, a associação entre um PRF injetável e antimicrobianos tradicionais também revelou uma elevada atividade antimicrobiana localizada.

Conclusões: A fibrina rica em plaquetas injetável possui um efeito inibitório significativo contra uma variedade de espécies microbianas orais, o que é clinicamente atrativo em contexto pós-operatório para minimizar possíveis infeções, sem ocultar o seu potencial regenerativo.

Palavras-chaves: i-PRF, PRF, antimicrobiano, fibrina rica em plaquetas injetável.

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1. Introduction

In the last years, several scientific research has been performed on the oral microbiome knowledge focusing on pathogen's interactions to understand the efficiency of antimicrobial agents for the prevention of biofilm-induced inflammatory reactions (1,2). Systemic antibiotic therapy is the most common treatment of biofilm-associated infections. However, such therapy can cause a bacterial resistance of pathogenic species considering the formation of biofilms. Antimicrobial resistance is a real issue across the world that increased in the last decades by the non-controlled use and prescription of antibiotics by health professionals (3). It should be highlighted that biofilms consist of a well-organized microbial community embedded in an extracellular polymeric matrix composed of polysaccharides, nucleic acids, proteins, minerals, and water (1,2). Bacteria organized in multispecies biofilm are more resistant to antibiotics when compared with other types of bacteria (3). Although inflammatory response depends on the precise nature of the initial stimulus and anatomic location, the immune response share common pathways as follows: (i) cell surface pattern receptors recognize detrimental stimuli (PAMPs); (ii) release of inflammatory markers (macrophages and production of inflammatory cytokines like IL-6, TNF-□); and (iii) inflammatory cells are recruited (monocytes, lymphocytes, T cells and B cells) (4,5) .

Nowadays, several bone substitutes are used to enhance bone healing including autogenous, xenogenous, allogenous, and synthetic or alloplastic graft materials (6–8). Although autogenous bone is often selected as the first choice, some disadvantages are reported such as deficient bone graft volume, morbidity, and surgical complications (8,9). Platelet-rich fibrin (PRF) an autologous biological product from patient blood that is applied to enhance healing effects in different applications like after tooth extraction (9). PRF consists of fibrin clotenriched with platelets, leukocytes, immune cytokines, and circulating stem cells (10). The first guidelines for PRF production were introduced by Choukroun et al in 2001, that requires 10 mL blood sample for centrifugation at 2,700 rpm for 12 minutes (10). Actually, there are several types of guidelines for PRF

preparations which result in different products such injectable (i-PRF), advanced-based (APRF), titanium-induced (T-PRF), or leukocyte-based (L-PRF).

An injectable PRF, namely i-PRF, can be produced by changing the spin centrifugation forces, and using non-glass centrifugation tubes resulting in a slowly cross-linking of fibrin (9–11). Thus, i-PRF is a low-viscosity or flowable PRF which can be handled with syringes (9,11,12). The flowable i-PRF is also rich in leukocytes, fibrinogen, platelets, fibrin, and growth factors as reported by previous studies (8–10). Leukocytes are part of the immune system that provide antimicrobial elements, like antimicrobial peptides or enzymes, like lactoferrin, defensins which thus allow a response from our organism against inflammatory mediators (11,13). The stimulated leukocytes degranulate and release their contents into the phagosomes, thus killing ingested microorganisms via oxidative and non-oxidative reactions (13–15). Platelets secondary granules positioned towards the leading control of neutrophil chemotaxis via distinct antimicrobial proteins and peptides (13,15,16). However, a few studies have reported the antimicrobial effects of PRF products.

1.1. Objective and hypothesis

The main aim was to perform on a scoping review on the antimicrobial effects of the injectable platelet rich fibrin. It was hypothesized that the injectable platelet rich fibrin reveals antimicrobial effects due to the presence of leukocytes.

2. State of art

2.1. Overview on platelet rich fibrin: composition, guidelines, and effects

Over the past few decades, changes and developments in autogenous bioactive materials have brought novel approaches in the field of dentistry. One of the innovations in tissue engineering has been the use of platelet concentrates, namely: platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) (17-20) is formed

by a three-dimensional matrix of polymerized fibrin, which serves as a fibers' network for a cell adhesion, proliferation, and differentiation (20,21). PRF is composed of leukocytes, fibrin, cytokines, platelet derived growth factor (PDGF), structural glycoproteins, vascular endothelial growth factor (VEGF) and more growths factors (22). Leukocytes concentrated in PRF samples play an important role in the release of growth factors, anti-infectious activities and immune regulation, which are precious characteristics/activities when using PRF (15). Kawase and Tanaka (23) proposed a classification on the platelet concentrates resulting into four types of PRF, as follow: the Choukroun's PRF, Sacco's PRF, other machine-made type, and home-made type. Thus, different protocols of PRF preparation are quite similar, except regarding the time and speed of centrifugation (Figure 1). Actually, the protocol proposed by Choukroun et al., is one of first ones in literature. I-PRF is produced using a low centrifugation speed at 800 rpm for 3 min (*Figure 2*). L-PRF is produced using a centrifugation of 2700 rpm for 12 min (10).

In this way, PRF is slowly absorbed in the wound site and replaced by localized cells, vessels, and tissue. Endothelial cells, leukocytes, and platelets have an important role for the formation of a platelet plug when the hemostasis begins over the tissue healing process. A study on cell culture reported a stimuli of the migration of fibroblasts onto implants surfaces coated with i-PRF that can accelerate the osteogenic differentiation (24). Also, a growth named FGR-10 was detected suggesting the regulation of the cells' differentiation and proliferation. The presence of growth factors supports the optimal wound healing maintaining a tissue structure (25).

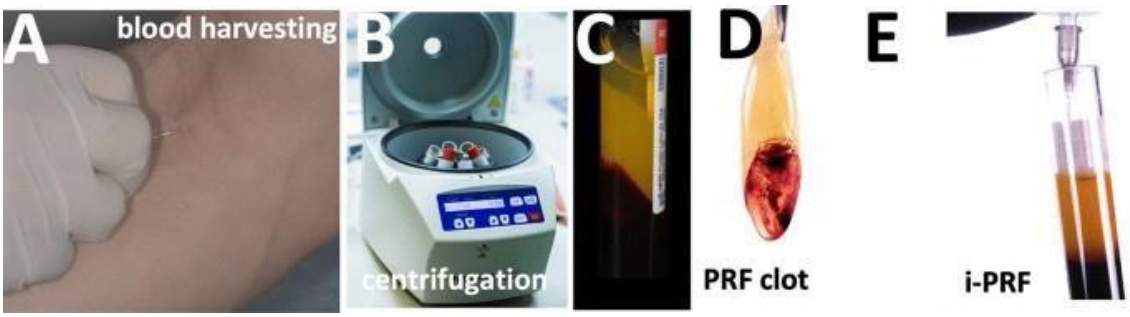

Figure 1. Processes of i-PRF preparation. (A) Blood harvesting from a patient followed by (B) centrifugation. (C) Three different phases of the centrifuged blood in tubes. (D) PRF clot. (E) I-PRF

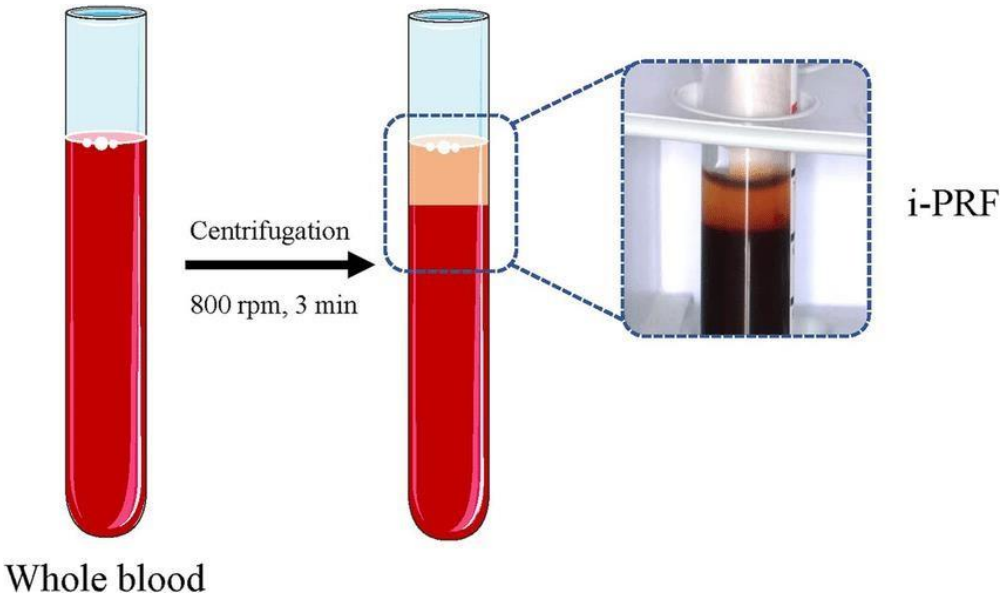

Figure 2. Sample of blood centrifuged during at 800 rpm for 3 min to produce and i-PRF.

An in vitro study evaluated the anti-inflammatory effect of PRF on wound healing (26). Cell culture assays revealed a lack of anti-inflammatory effect, since there is a reduction in the secretion of proinflammatory cytokines such as: the factor of vascular endothelial growth (VEGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), bone morphogenetic protein (BMP), and other molecules. Thus, the efficiency of wound healing was higher with an increase in the secretion of cytokines and different pro-angiogenic growth factors which seem to be involved in wound healing processes (26).

In fact, PRF promotes a faster and proper wound healing by improving neovascularization on the presence of high contents of growth factors when compared to PRP (27). Additionally, the fibrin matrix in PRF becomes a drug delivery system once it releases growth factors continually promoting the emergence of new vascular networks. On bone augmentation procedures like

sinus grafting and/or alveolar ridge preservation, PRF becomes the first choice on enhancing recession coverage and intrabony defect repair (28).

An in vivo study examined the use of i-PRF administration to treat root resorption in ten healthy patients. According to the findings, all individuals had their signs and symptoms resolved over the 12-month follow-up period. Additionally, the radiographic assessment revealed a significant decrease in the mean volume of periapical lesions and inner inflammatory root resorption (29). Another study reported that i-PRF in conjunction with dental implant placement promote faster wound healing, particularly in connective tissues. Since periimplantitis is caused by soft tissue breakdown, the potential for i-PRF to enhance collagen synthesis during the healing process could potentially strengthen host tissues' defenses against bacteria-induced infections while leukocytes can destroy pathogens in the surrounding tissues (30).

PRF can also increase dental implant stability (31) mainly when in combination with bioactive materials such as deproteinized bovine bone mineral (DBBM). The association also is beneficial to enhance the support of new bone formation and the cell activities by the release of growth factors. PRF and DBBM have a synergistic impact that improves the healing process of bone and soft tissue, shortens the healing period, and raises the overall success rate of grafting procedures.

2.2. Current challenges in antimicrobial therapy

Antimicrobial resistance is a terrible event from a clinical, social and economic point of view and it is a public health problem. One of the major issues is the improper use of antimicrobial agents, due to poor diagnosis by an increasingly high number of patients, highlighting the prescription uncertainty of and the pressure exerted on professionals in the field of medicine. In fact, countries with a low percentage of antibiotic prescriptions have a much lower rate of antimicrobial resistance than countries that often use antibacterial therapies (1). On the increase of the antimicrobial resistance, a constantly renewal of antimicrobial compounds is required (32). Inadequate prescription and consumption completely contrary to recommendations can results in high risks to

the worldwide population over pandemic periods. The number of deaths directly associated with resistant-to-infections antimicrobial therapies could reach 10 million lives per year by 2050 (33). In some regions across the world, the access to health care is particularly demanding, just like the access to effective antimicrobial therapies. So, global health and infection control are both linked by the fact that infections inhibit potential interventions planned for the patients. Thus, the economic factor cannot be excluded in our society once it weighs heavily on the affected populations (1).

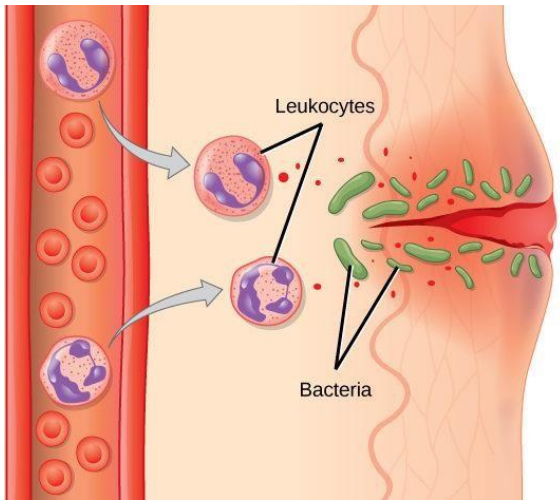

Figure 3. Pathogen recognition by leukocytes.

The use of i-PRF become an interesting approach for controlling potential bacteria-related infections. The presence of lymphocytes and stimuli of immune cells' migration (Figure 3) by PRF drastically reduce the risks of infections (13). The major antimicrobial pathways of platelet-derived compounds like i-PRF deal with the release of leukocytes which attack pathogenic bacteria species, the immune system modulation by the action of cytokines, enzyme-derived biofilm disruption (14). The storage and handling of i-PRF are important to maintaining its effectiveness and guarantee patient safety. After preparation, i-PRF needs to be used right away since its biological components like platelets and growth factors (10).

3. Materials and methods

3.1. Information sources and search strategy

A bibliographical analysis was carried out on PubMed (via National Library of Medicine) and Scopus regarding such database includes the major studies in the field of oral microbiology and dentistry. The present approach was conducted in accordance with the examination plan utilized in previous studies on integrative or systematic reviews (8-12-23). The following combination of pursuit items was used in this study: “antibiofilm” OR “antibacterial” AND “platelet rich fibrin” OR “PRF” OR “injectable platelet rich fibrin” OR “i-PRF” OR “injectable PRF” AND “bacteria” OR “Periodontal pathogen” OR “Aggregatibacter actinomycetemcomitans” OR “Prevotella intermedia” OR “Porphyromonas gingivalis”.

The inclusion criteria comprised studies published in the English language, up to May 2024, reporting the antimicrobial effects of the injectable platelet rich fibrin. The eligibility inclusion criteria used for the bibliographical examination also concerned in vitro studies; meta-analyses; randomized controlled trials; and prospective cohort studies. Also, a hand-search was carried out on the reference lists of all initial sources and eligible studies of this review for further relevant publications. The exclusion criteria were the following: systematic scoping reviews; reviews without PRF preparations; papers without abstract; case report with short follow-up period. Studies based on publication date were not restricted during the search process.

3.2. Study selection and data collection process

The scientific studies found by the bibliographic search route were evaluated into three stages. Studies were primarily scanned for relevance by title and then the abstracts were assessed. Two of the authors (JCMS, DDC) individually analyzed the titles and abstracts of supposedly relevant studies while a third author (BH) accomplished a final appraisal in occurrence of divergence.

The total of studies was gathered for each grouping of search items, and therefore, the duplicates were disregarded using Mendeley citation manager (Ed. Elsevier). The second step covered the careful assessment of the abstracts and non-excluded studies, according to the eligibility criteria in the abstract evaluation. A primary appraisal of the abstracts was performed to determine whether the previous studies match the purpose of the current review. Selected studies were independently read and evaluated regarding the aim of this review. At last, the eligible studies received a revision identification tag, linking first author and year of publication. The following aspects were recovered for this review: authors' names, publication year, journal, objective, study design, preparation of PRF, bacteria growth, analyses, and antibiofilm effects. PICO question was adjusted to the issue where "P" was related to the adult patients or type of PRF, while "I" referred to the methods of analyses, "C" to the different PRF preparation and (O) to the antimicrobial properties (Table 1). The PICO strategy was designed considering the following question: "Can the injectable PRF show antimicrobial effects linked to the leukocytes-rich content?" Data of the reports were harvested directly into a single data collection form to avoid multiple data record in the case of multiple reports in the same study (e.g., reports with different set-ups).

Table 1. PICO strategy.

P	I	C	O
Patient, population or problem	Intervention	Comparison	Outcome
Adult patients Types of PRF	Methods of analyses (CFU, MIC, SEM, optical microscopy, MBC, MTT, RT-PCR)	Comparison to other blood products (PRFP, PRF, blood clot)	Major antimicrobial pathways

4. Results

The literature search identified a total of 720 studies in PubMed and Scopus, as shown in Figure 1. After removal of non-related studies, eleven studies were carefully evaluated regarding the inclusion criteria. Of those

articles, one study was removed since it did not contain comprehensive data related to the purpose of the present review. Thus, 10 studies were considered in this review although 3 hand-held studies were added concerning complementary data for this scoping review.

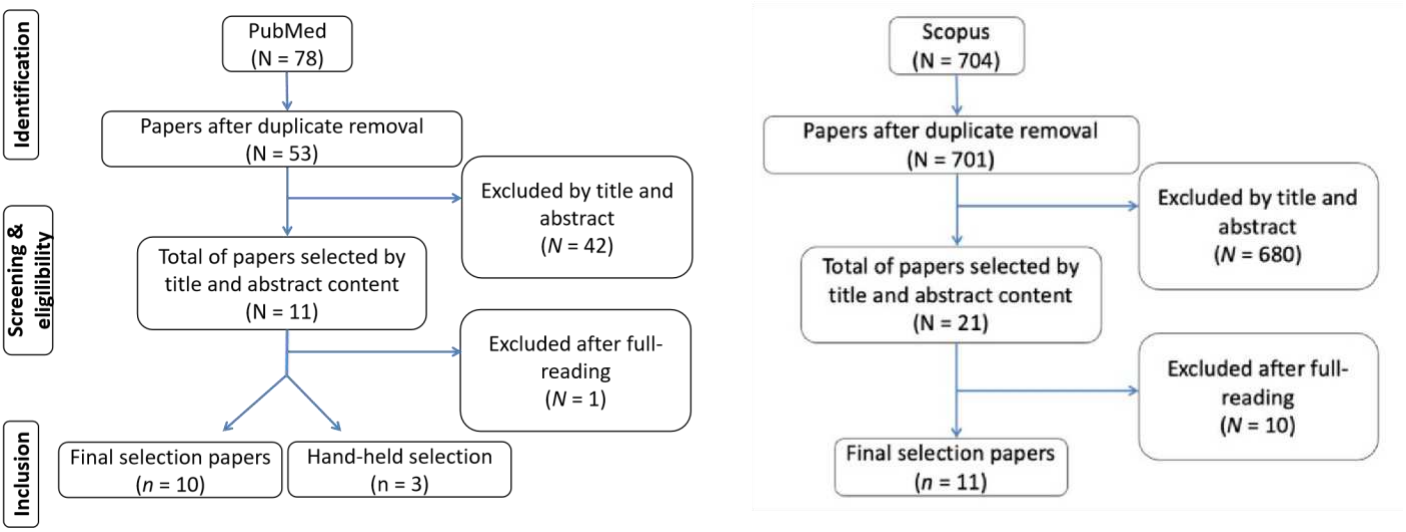

Figure 5. Flow diagram of the search strategy used in this study (PRISMA).

According to Badade *et al.*, they used 5 ml of intravenous blood collected and centrifuged using centrifuge (Manual Centrifugation Machine, e-Tek) at 3000 rpm for 10 min. For the PRP preparation, they used 5 mL of intravenous blood collected into the blood collection tube coated with 3.2% sodium citrate solution and centrifugation machine (Manual Centrifugation Machine, e-Tek) at 1000 rpm for 13 min. PRP against *P. gingivalis* created a zone of inhibition (in mm and diameter) ranged between 12 and 15 and against *Aa* ranged between 10 and 22. These results demonstrate that PRP significantly inhibited the growth of both pathogens, which was statistically significant ($P < 0.05$) compared to PRF, where no inhibitory activity was observed against these two pathogens (15). These results didn't validate our hypothesis concerning platelet rich fibrin's inhibition of pathogens.

A study realized the preparation of L-PRF, with four blood samples who were collected from each volunteer (n = 9) in a 9 mL glass-coated plastic tube and immediately centrifuged at 408 g for 12 minutes (Intraspin™, Intra-Lock, Boca

Raton, FL, USA). In a vitality qPCR analysis, in presence of *Aa* resulted in reductions in growth of 86%, 38%, and 24% with the respective ratios of 1:1, 1:2, and 1:4. Conversely, in the presence of *P. gingivalis*, growth increased by 86%, 72%, and 50% with the corresponding ratios of 1:1, 1:2, and 1:4, respectively. Another test has been realized where the mean area of inhibition for *P. gingivalis*, *P. intermedia*, *F. nucleatum*, and *A. actinomycetemcomitans* was 11.8 ± 5.0 mm², 2.7 ± 5.2 mm², 2.6 ± 3.0 mm², and 0.6 ± 1.7 mm², respectively. Significantly more inhibition was observed for *P. gingivalis* compared to the other pathogens (16).

In vivo study encompassing 60 volunteers, stratified into three groups: 20 healthy individuals, 20 diagnosed with gingivitis, and 20 with periodontitis, spanning an age range of 20 to 65 years. A total of 22 mL of blood was extracted from each participant, meticulously processed using the Dou Quattro Choukroun PRF system, innovatively designed by PROCESS for PRF®, headquartered in Nice, France. The percent reduction in i-PRF samples was found to be 49 ± 8%, 47 ± 10%, and 45 ± 10%, respectively, in the periodontitis, gingivitis, and healthy groups. Conversely, in A-PRF samples, the percent reduction was slightly lower, measuring at 42 ± 9%, 41 ± 9%, and 39 ± 9%, across the same groups. In summary, i-PRF exhibited superior antibacterial properties compared to A-PRF+. Specifically, the percentage reduction in bacterial count within the periodontitis group was notably higher for i-PRF than for A-PRF+ (32).

The study comprised ten individuals, all free from systemic and periodontal conditions, aged 25 years and above. Each individual contributed 10 ml of blood, allocated as follows: 3 ml for PRP preparation, 3 ml for PRF preparation, and another 3 ml for i-PRF preparation. The remaining 1 ml was utilized to determine the platelet count. For i-PRF preparation, 3 ml of blood was collected into a tube and centrifuged at 700 rpm for 3 minutes. For PRF preparation, 3 ml of blood was collected into a glass blood-collecting tube and centrifuged at 3000 rpm for 10 minutes. For PRP preparation, 3 ml of blood was drawn into a blood-collecting tube containing 3.2% sodium citrate. Initial centrifugation was performed at 1000 rpm for 13 minutes. The resultant top layer of plasma was then extracted and subjected to a second centrifugation at 2000 rpm for 10 minutes. The width of the inhibition zones (in mm) against *P. gingivalis* indicated 15.2 mm for i-PRF, 8.8 mm for PRF, and 12.9 mm for PRP. For Aa, the inhibition zones measured 9.8 mm for

i-PRF, 10.0 mm for PRF, and 12.5 mm for PRP. l-PRF and PRP have demonstrated significant efficacy as potent tools in combating periodontal pathogens (34).

Blood samples were collected from ten patients diagnosed with chronic generalized marginal gingivitis. *In vitro*, 8 ml of blood was drawn from each patient, with 2 ml allocated for the preparation of i-PRF, PRF, and PRP, while the remaining 2 ml, treated with anticoagulant, served as a control. For i-PRF preparation, 2 ml of blood was collected in a non-coated vacutainer and centrifuged at 700 rpm for 3–4 minutes. For PRF preparation, 2 ml of blood was collected in a silicon-coated vacutainer and centrifuged at 3000 rpm for 10 minutes. Platelet quantification yielded the following results: 1,434,000 ± 75,233, 1,343,000 ± 81,486, 249,000 ± 61,319, and 291,000 ± 51,575 platelet count/mm3 for i-PRF, PRP, and PRF, respectively. Antimicrobial potential was assessed, revealing mean zones of inhibition (in cm) around i-PRF measuring 1.42 ± 0.25, around PRF measuring 1.3 ± 0.16, and around PRP measuring 1.02 ± 0.12. IPRF offers a more feasible and minimally invasive application, requiring no additives for preparation. With the highest platelet count among platelet concentrates, i-PRF also exhibits superior antimicrobial potency compared to other alternatives (35).

Sixty-four intact, caries-free single-rooted human mandibular first premolars, extracted for orthodontic purposes, were gathered for the study. For i-PRF production, blood samples were acquired from volunteer donors, aged between 20 and 35, with their informed consent. Subsequently, 9 mL tubes of whole blood without anticoagulant were promptly centrifuged at 60 g for 3 minutes. Following biofilm formation, both infected and uninfected samples underwent the initial sampling procedure. Each tooth was securely held with a sterile clamp, and the external surface was disinfected sequentially with 30% hydrogen peroxide, 2.5% NaOCl, and subsequently inactivated with 5% sodium thiosulfate. The inclusion of a triple antibiotic mixture in i-PRF samples led to a significant reduction in the number of live bacteria, reaching up to 91.62 ± 2.069%, more compared with antibiotic-free i-PRF scaffold, reaching up to 84.92 ± 3.107% (36).

Egle *et al.*, required blood samples from three healthy volunteers exhibiting vitamin D levels exceeding 30 ng/mL. These samples were collected in 13 mL iPRF+ tubes manufactured by PROCESS FOR PRF in Nice, France, and promptly subjected to centrifugation using a "PRF DUO Quattro" centrifuge. Centrifugation occurred at 700 rpm for 5 minutes (for women) or 6 minutes (for men). Following centrifugation, the upper layer of liquid PRF, measuring 1 mL, from one donor of each tube was pooled into a 50 mL tube for subsequent utilization. 0.5 mL of liquid PRF was utilized to generate one PRF sample. To create PRF samples with CLP (PRF_CLP), 0.5 mL of PRF was combined with pre-weighed 0.5 mg of CLP. The average MIC values for PRF_CLP samples from donors ranged between 52.1 and 62.5 µg/mL, demonstrating lower values compared to pure CLP samples, which ranged from 125 to 250 µg/mL. The antibacterial effect of CLP was affected by the addition of PRF, thus providing a reduction of MIC (37).

Pham and Tran in a *in vivo* study encompassing 60 participants, consisting of 20 healthy individuals, 20 diagnosed with gingivitis, and 20 with periodontitis, was conducted. Blood samples were collected from all the 60 participants. A-PRF preparation involved blood centrifugation at 1300 rpm for 8 minutes, whereas IPRF preparation required blood centrifugation at 700 rpm for 3 minutes. The zone of inhibition (in mm^2) for i-PRF was measured at 70.05 ± 7.34, 77.45 ± 9.70, and 83.33 ± 8.62, respectively, for the healthy, gingivitis, and periodontitis groups. In contrast, for A-PRF, it was recorded as 65.50 ± 9.48, 66.28 ± 10.40, and 76.57 ± 10.80, respectively, for the healthy, gingivitis, and periodontitis groups. In patients with gingivitis, the zone of inhibition was greater with i-PRF (83.3 mm^2) compared to A-PRF (76.5 mm^2). Similarly, in patients with gingivitis, the zone of inhibition was also higher with i-PRF (77.4 mm^2) compared to A-PRF (66.2 mm^2) (38).

This study comprised 60 patients, aged between 20 and 65 years. All participants volunteered for the study and met specific criteria: they were nonsmokers with no recent history of infection, antibiotic, or anti-inflammatory usage for at least three months prior to the study. Among these 60 patients, 20 were healthy individuals, 20 had gingivitis, and 20 had periodontitis. Each participant contributed 22 mL of blood for analysis. In the case of *P. gingivalis*, the

percent reduction observed in i-PRF samples was 0.05 ± 0.05, 0.05 ± 0.05, and 0.07 ± 0.08, respectively, for the periodontitis, gingivitis, and healthy groups. Conversely, in A-PRF samples, the percent reduction was lower, measuring at 0.03 ± 0.05, 0.03 ± 0.06, and 0.05 ± 0.09 for the same groups. I-PRF exhibited a higher percentage reduction in the number of bacteria within the periodontitis group compared to A-PRF+ (39).

5. Discussion

First of all, it has been showed that i-PRF exhibited betters antibacterial properties when compared to A-PRF, PRF or PRP by different inhibition ring tests where the circle of inhibition was greater in i-PRF samples. Relatively about the platelet count, one more time, i-PRF showed a significant positive difference with others platelet-derived compounds which strengthens the fact that healing tissue is possible with i-PRF.

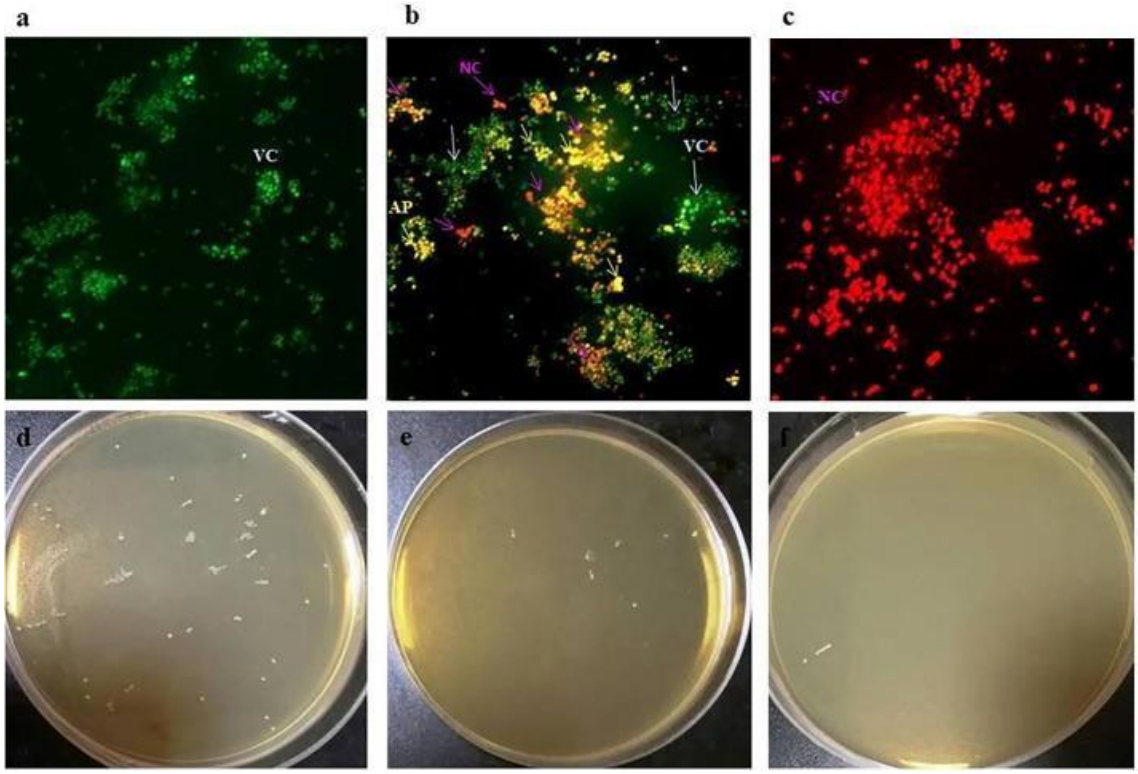

Figure 6. Fluorescence microscopic image of Live/Dead staining assay of i-PRF, (a) Untreated cells (100% viable cells), (b) Cells treated at MIC, (c) Cells treated

at MBC, S. aureus colony formation on MHA - (d) untreated control (e) i-PRF treated at 80 µl/ml, (f) i-PRF treated at 160 µl/ml.

Periodontitis is an inflammatory disease, which results in loss of bone and attachment. In this case, the objective of the periodontal therapy consists of regenerating the anatomy lost. Nowadays, platelet concentrates have gained in importance like Karde *et al.*(35) demonstrated through the quantification of platelets between i-PRF, PRF and PRP, which are the following: 1,434,000 ± 75,233, 1,343,000 ± 81,486, 249,000 ± 61,319, and 291,000 ± 51,575 (platelets count/mm^3), respectively for i-PRF, PRP, and PRF. The results showed a greater number of platelets with i-PRF compared to PRP and PRF, statistically significant which can be explained by the fact that the low centrifugation time and speed result in a greater number of platelets. Relatively to the PICO question, the “P” refers to patients with periodontitis, “I” to platelet count, “C” to i-PRF, PRF and PRP, and “O” antimicrobial potency.

In Kour *et al.* study (34), they compared the antibacterial effect against *P. gingivalis* and *A. actinomycetemcomitans* (*Aa*) of PRP, PRF and i-PRF. These two bacteria species have been associated to periodontal pathologies like periodontitis. For *P. gingivalis*, the width of zones of inhibition was larger for iPRF rather than PRF or PRP; explainable by the number of cells present in the i-PRF. On the other hand, in contact with *A. actinomycetemcomitans*, PRP showed a wider inhibition zone compared to i-PRF and PRF. The fact that *Aa* is resistant to platelet concentrates who are present in higher quantities in i-PRF. PRF seems to have a lower antimicrobial property when compared to PRP and i-PRF, because of the lower concentration of cells type, like leukocytes and platelets. Relatively to the PICO question, the “P” refers to adult patients, “I” to Wilcoxon signed-rank test, “C” to i-PRF, PRF and PRP, and “O” effect against periodontal pathogens.

Another PRF preparation that exists is the A-PRF+ which has a more porous structure, which allows more space for other cells such as leukocytes or immune cells and thus a higher release of growth factors. Pham (39) attempted, through this study, to compare the antimicrobial activity against *P. gingivalis* between A-PRF+ and i-PRF. By comparing the percent reduction of *P. gingivalis*

when in contact with A-PRF+ and i-PRF through a biofilm inhibitory assay, the percent reduction of *P. gingivalis* was higher with i-PRF rather than with A-PRF+, 49% and 39%, respectively for i-PRF and A-PRF+. In the mature biofilm inhibitory assay, the percent reduction was only around 3 to 7%, this percent reduction result from a greater resistance by part of the mature biofilm. Relatively to the PICO question, the “P” refers to adult patients, “I” to biofilm inhibitory assay, “C” to i-PRF and A-PRF+, and “O” antimicrobial activity.

Tran and Pham (32), investigated in their study the antimicrobial capacity of i-PRF and A-PRF+ in adult patients with or without periodontal disease against a pathogenic bacteria, *A. actinomycetemcomitans*. Three different groups were created, healthy, gingivitis and periodontitis groups. For each group, minimum inhibitory concentration (MIC) values were obtained. For the A-PRF+, MIC values were higher than MIC for i-PRF, in the three groups. With these results, we can say that i-PRF has a better inhibition effect compared to A-PRF+. An inhibition ring test has been performed o blood agar plates incubated at 37°C for 24h with *Aa*. This test demonstrated the greater antimicrobial capacity of i-PRF in relation to A-PRF+, due to its significant wider inhibition zone in the three patient groups. Relatively to the PICO question, the “P” refers to adult patients, “I” to MIC, “C” to i-PRF and A-PRF+, and “O” antimicrobial activity.

To compare the antimicrobial properties of some platelet concentrates, in this case, i-PRF and A-PRF+, against *Aggregatibacter actinomycetemcomitans* with different periodontal groups (healthy, gingivitis and periodontitis), Pham and Tran (45) through testing the biofilm susceptibility and the antibiofilm formation. The antibiofilm formation assay showed that i-PRF and A-PRF+ inhibited the adhesion of Aa. However, A-PRF+ had a lower anti-adhesion effect than i-PRF from the gingivitis and periodontitis groups. About the biofilm susceptibility assay, both i-PRF and A-PRF+ decreased the number of Aa adhered, but not as much as with the anti-adhesion test. This decrease can be explain by the capacity of iPRF and A-PRF+ to reduce the adherence of pathogen species and to promote their de-adhesion. Relatively to the PICO question, the “P” refers to adult patients, “I” to MIC, “C” to i-PRF and A-PRF+, and “O” antimicrobial activity.

Jasmine *et al*. (13) performed this study to evaluate the capacities of iPRF, which are the antibiofilm effect and antimicrobial effect. To analyze the antimicrobial and antibiofilm activity, they injected i-PRF in different oral pathogenic Staphylococcal isolates with different MIC and MBC. Against weak, moderate and strong biofilm forming staphylococcus isolates, i-PRF demonstrated a wide spectrum of effectiveness. All oral biofilm producers indicated a significant reduction in biofilm formation in the presence of i-PRF. I-
PRF's wide range of inhibitory and bactericidal activity can be attributed to its composition of immune cells, like leukocytes and other cells like it's the case of the fibrin or peptides. The wide spectrum of activity which is characteristic of iPRF seems related to lactoferrin, defensins who can interfere with the metabolic activities of bacterial cells, leading to apoptosis and necrotic phases. Relatively to the PICO question, the "P" refers to patients with dental or oral abscess, "I" to MIC and MBC, "C" to blood clot, and "O" antimicrobial activity.

Human lactoferrin (Lf) is a multifunctional glycoprotein having multiple biological functions like antiviral, antitumoral or antimicrobial. Thus, Pall *et al*. (40) decided to evaluate the possible cumulative antimicrobial and antibiofilm impact of i-PRF functionalized with Lf. Through a cell viability assay, they have demonstrated that the average cell viability was high after 24h in cultures treated with different concentrations of Lf. This average cell viability was higher after 48h contact. An impact of association between i-PRF and Lf dose-dependent is a reality. The antibiofilm formation assay revealed that depending on the concentration of Lf, a significant reduction of biofilm formation was observed, result that correlates the fact that a cumulative antibiofilm effect is possible. Relatively to the PICO question, the "P" refers to female patients, "I" to MIC and
MBC, "C" Lf, and "O" antimicrobial and antibiofilm activity.

The advancement of clinical practice involving i-PRF is a promising and evolving field in regenerative medicine and infection control. Its clinical applications are growing due to its ability to promote tissue regeneration, accelerate wound healing, and provide antimicrobial effects. However, in order to fully integrate i-PRF into standard clinical practice, several key areas need to be addressed and further developed (26).

Proper training and education of healthcare providers are crucial for the successful implementation of i-PRF in clinical practice. This involves not only the technical aspects of i-PRF preparation and application, but also a comprehensive understanding of its biological mechanisms and potential clinical benefits. By developing accredited training programs and continuous professional development courses, it would help ensure that health professionals would be skilled and conscious in using i-PRF effectively (20).

The exploration of the synergistic effects of i-PRF in conjunction with existing therapies can enhance and optimize its clinical application. The utilization of i-PRF in conjunction with other treatments, such as antibiotics, stem cells, or biomaterials, has the potential to enhance patient outcomes. Research into these combined approaches could help develop more comprehensive treatment protocols and expand the therapeutic potential of i-PRF (20).

One of the limitations is the diversity of preparations of i-PRF preparations. The diversity of preparations can expose the difference of quality of different patient's blood samples, and so the clinical outcomes could not be so positive, and the patient may not be satisfied. Reducing the variability, the efficacity of iPRF therapies could be significantly enhanced, and thus to increasingly be accepted and have a more important weight in different areas of medicine.

The fact that there is a lack of standardization and no universal accepted protocol for i-PRF preparation can expose challenges in order to compare study results and in a second time, establish clinical guidelines permitting to simplify these processes. The initial investment when purchasing the equipment for i-PRF preparations can have a great impact on the dentist's final decision. When acquiring equipment of this type, we have to be sure that in the future, patients will be receptive to this universe that is PRF and thus have additional motivation to continue on this path.

Combining PRF with traditional antimicrobial compounds can be explored for enhance inhibition of microbial infections. Further research is needed to understand the synergistic effects and optimize the combination for specific microbiological contexts.

Also, assessing an antimicrobial compound against a multi-species biofilm involves evaluating its efficacy on inhibiting or eradicating different species in a

bacterial co-aggregation. Such microbiological assessment should account for the diverse microbial composition within the biofilm and the compound's ability to penetrate and disrupt the bacteria. The effects of the PRF and antimicrobial against a mono-species biofilm model can reveal different results when compared to a multi-species model. Rigorous testing under conditions resembling the natural environment is crucial for a comprehensive evaluation of the antimicrobial's potential against multi-species biofilms.

The release of antimicrobial agents from PRF can contribute to its effectiveness in controlling infections. The process involves the gradual release of antimicrobial compounds incorporated into the PRF matrix. The sustained release of antimicrobial compounds can provide an extended period of antimicrobial activity, promoting wound healing and minimizing the risk of infections. Research and optimization are necessary to understand the kinetics of release, ensuring an appropriate balance between the antimicrobial effect and tissue healing for specific applications in healthcare.

Assessing the effects of the proportion of leukocytes against different bacteria may involve the exposure of bacteria to different leukocyte-to-bacteria ratios and measuring growth inhibition or killing. The evaluation may include monitoring parameters like bacterial viability, leukocyte activation, phagocytosis efficiency and inflammatory response. By systematically varying leukocyte proportions and analyzing those parameters, studies can gather insights on the immune response against different bacterial strains to enhance host defense mechanisms.

6. Conclusions

- i-PRF has in its composition, leukocytes, fibrin, platelets and peptides that give this antimicrobial capacity. Regarding the proportion of leukocytes, it depends on the preparation chosen, but anyways, this proportion can range from 30 to 70% of the total cell.
- In literature, it has been shown that the zone of inhibition of i-PRF were wider compared with the other different PRF preparations. This conclusion was also verified for MIC, with a reduction of the concentration with i-PRF.

- It seems i-PRF might be superior to L-PRF because of the higher concentration of leukocytes and growth factors and the form of injectability of the i-PRF who is more precise.

References

1. Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017;10(4):369–78.
2. Alves CH, Russi KL, Rocha NC, Bastos F, Darrieux M, Parisotto TM, et al. Hostmicrobiome interactions regarding peri-implantitis and dental implant loss. J Transl Med. 2022 Sep 23;20(1):425.
3. Fioranelli M, Roccia MG, Flavin D, Cota L. Regulation of Inflammatory Reaction in Health and Disease. Int J Mol Sci. 2021 May 17;22(10).
4. Machado PRL, Araújo MIAS, Carvalho L, Carvalho EM. Mecanismos de resposta imune às infecções. An Bras Dermatol. 2004 Dec;79(6):647–62.
5. Belibasakis GN, Manoil D. Microbial Community-Driven Etiopathogenesis of PeriImplantitis. J Dent Res. 2021 Jan;100(1):21–8.
6. Apaza-Bedoya K, Tarce M, Benfatti CAM, Henriques B, Mathew MT, Teughels W, et al. Synergistic interactions between corrosion and wear at titanium-based dental implant connections: A scoping review. J Periodontal Res. 2017 Dec;52(6):946–54.
7. Galarraga-Vinueza ME, Passoni B, Benfatti CAM, Mesquita-Guimarães J, Henriques B, Magini RS, et al. Inhibition of multi-species oral biofilm by bromide doped bioactive glass. J Biomed Mater Res A. 2017 Jul;105(7):1994–2003.
8. Noronha Oliveira M, Varela HA, Caramês J, Silva F, Henriques B, Teughels W, et al. Synergistic Benefits on Combining Injectable Platelet-Rich Fibrin and Bone Graft Porous Particulate Materials. Biomedical Materials & Devices. 2023 Mar 8;1(1):426–42.
9. Sampaio N, Noronha Oliveira M, Carvalho O, Manzanares-Céspedes MC, Silva F, Henriques B, et al. Porous Zirconia Blocks Embedded with Platelet Rich Fibrin for Enhanced Bone Healing: Mechanical and Morphological Assessment. Biomedical Materials & Devices. 2023 Apr 24;
10. Pavlovic V, Ciric M, Jovanovic V, Trandafilovic M, Stojanovic P. Platelet-rich fibrin: Basics of biological actions and protocol modifications. Open Medicine. 2021 Mar 22;16(1):446–54.
11. Feng M, Wang Y, Zhang P, Zhao Q, Yu S, Shen K, et al. Antibacterial effects of platelet-rich fibrin produced by horizontal centrifugation. Int J Oral Sci. 2020 Dec 26;12(1):32.
12. Noronha Oliveira M, Varela HA, Caramês J, Silva F, Henriques B, Teughels W, et al. Synergistic Benefits on Combining Injectable Platelet-Rich Fibrin and Bone Graft Porous Particulate Materials. Biomedical Materials & Devices. 2022 Sep 8;
13. Jasmine S, A. T, Janarthanan K, Krishnamoorthy R, Alshatwi AA. Antimicrobial and antibiofilm potential of injectable platelet rich fibrin—a second-generation

platelet concentrate—against biofilm producing oral staphylococcus isolates. Saudi J Biol Sci. 2020 Jan;27(1):41–6.
14. Rodríguez Sánchez F, Verspecht T, Castro AB, Pauwels M, Andrés CR, Quirynen M, et al. Antimicrobial Mechanisms of Leucocyte- and Platelet Rich Fibrin Exudate Against Planktonic Porphyromonas gingivalis and Within Multi-Species Biofilm: A Pilot Study. Front Cell Infect Microbiol. 2021;11:722499.
15. Badade P, Mahale S, Panjwani A, Vaidya P, Warang A. Antimicrobial effect of platelet-rich plasma and platelet-rich fibrin. Indian Journal of Dental Research [Internet]. 2016 [cited 2023 Dec 4];27(3):300. Available from: https://journals.lww.com/ijdr/fulltext/2016/27030/antimicrobial_effect_of_platelet_rich_plasma_and.14.aspx
16. Castro AB, Herrero ER, Slomka V, Pinto N, Teughels W, Quirynen M. Antimicrobial capacity of Leucocyte-and Platelet Rich Fibrin against periodontal pathogens. Sci Rep. 2019 Jun 3;9(1):8188.
17. Borie E, Oliví DG, Orsi IA, Garlet K, Weber B, Beltrán V, et al. Platelet-rich fibrin application in dentistry: a literature review. Int J Clin Exp Med. 2015;8(5):7922–9.
18. Dhiman M, Kumar S, Duhan J, Sangwan P, Tewari S. Effect of Platelet-rich Fibrin on Healing of Apicomarginal Defects: A Randomized Controlled Trial. J Endod. 2015 Jul;41(7):985–91.
19. Naik B, Karunakar P, Jayadev M, Marshal VR. Role of Platelet rich fibrin in wound healing: A critical review. J Conserv Dent. 2013 Jul;16(4):284–93.
20. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Plateletrich fibrin (PRF): a second-generation platelet concentrate. Part II: plateletrelated biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Mar;101(3):e45-50.
21. Wu CL, Lee SS, Tsai CH, Lu KH, Zhao JH, Chang YC. Platelet-rich fibrin increases cell attachment, proliferation and collagen-related protein expression of human osteoblasts. Aust Dent J. 2012 Jun;57(2):207–12.
22. Chhabra P CPSK. Platelet rich fibrin – its structural design and composition. Int J Oral Health Dent. 2019;5(2):81–4.
23. Kawase T, Tanaka T. An updated proposal for terminology and classification of platelet-rich fibrin. Regen Ther. 2017 Dec;7:80–1.
24. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron RJ. Behavior of Gingival Fibroblasts on Titanium Implant Surfaces in Combination with either InjectablePRF or PRP. Int J Mol Sci. 2017 Feb 4;18(2).
25. Bahar ŞÇ, Karakan NC, Vurmaz A. The effects of injectable platelet-rich fibrin application on wound healing following gingivectomy and gingivoplasty operations: single-blind, randomized controlled, prospective clinical study. Clin Oral Investig. 2024 Jan 10;28(1):85.
26. Dohle E, El Bagdadi K, Sader R, Choukroun J, James Kirkpatrick C, Ghanaati S. Platelet-rich fibrin-based matrices to improve angiogenesis in an in vitro coculture model for bone tissue engineering. J Tissue Eng Regen Med. 2018 Mar;12(3):598–610.

27. Miron RJ, Gruber R, Farshidfar N, Sculean A, Zhang Y. Ten years of injectable platelet-rich fibrin. Periodontol 2000. 2024 Feb;94(1):92–113.
28. Miron RJ, Moraschini V, Del Fabbro M, Piattelli A, Fujioka-Kobayashi M, Zhang Y, et al. Use of platelet-rich fibrin for the treatment of gingival recessions: a systematic review and meta-analysis. Clin Oral Investig. 2020 Aug;24(8):2543–57.
29. Nageh M, Ibrahim LA, AbuNaeem FM, Salam E. Management of internal inflammatory root resorption using injectable platelet-rich fibrin revascularization technique: a clinical study with cone-beam computed tomography evaluation. Clin Oral Investig. 2022 Feb;26(2):1505–16.
30. Wang X, Zhang Y, Choukroun J, Ghanaati S, Miron R. Behavior of Gingival Fibroblasts on Titanium Implant Surfaces in Combination with either InjectablePRF or PRP. Int J Mol Sci. 2017 Feb 4;18(2):331.
31. Guan S, Xiao T, Bai J, Ning C, Zhang X, Yang L, et al. Clinical application of platelet-rich fibrin to enhance dental implant stability: A systematic review and meta-analysis. Heliyon. 2023 Feb;9(2):e13196.
32. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MTS, Chinedu Ikem J, Victor Chigozie U, et al. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal. 2022 Sep;36(9):e24655.
33. Phuong Tran TT, Vu Pham TA. Effect of advanced and injectable platelet-rich fibrins against Aggregatibacter actinomycetemcomitans in subjects with or without periodontal diseases. J Dent Sci. 2023 Apr;18(2):491–6.
34. Kour P, Pudakalkatti P, Vas A, Das S, Padmanabhan S. Comparative evaluation of antimicrobial efficacy of platelet-rich plasma, platelet-rich fibrin, and injectable platelet-rich fibrin on the standard strains of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. Contemp Clin Dent. 2018;9(6):325.
35. Karde P, Sethi K, Mahale S, Khedkar S, Patil A, Joshi C. Comparative evaluation of platelet count and antimicrobial efficacy of injectable platelet-rich fibrin with other platelet concentrates: An in vitro study. J Indian Soc Periodontol. 2017;21(2):97.
36. Rafiee A, Memarpour M, Najibi Y, Khalvati B, Kianpour S, Morowvat MH. Antimicrobial Efficacy of a Novel Antibiotic-Eluting Injectable Platelet-Rich Fibrin Scaffold against a Dual-Species Biofilm in an Infected Immature Root Canal Model. Biomed Res Int. 2020 Dec 7;2020:1–8.
37. Egle K, Skadins I, Grava A, Micko L, Dubniks V, Salma I, et al. Injectable PlateletRich Fibrin as a Drug Carrier Increases the Antibacterial Susceptibility of Antibiotic—Clindamycin Phosphate. Int J Mol Sci. 2022 Jul 3;23(13):7407.
38. Pham TAV, Tran TTP. Antimicrobial effect against Aggregatibacter actinomycetemcomitans of advanced and injectable platelet-rich fibrin from patients with periodontal diseases versus periodontally healthy subjects. J Oral Biol Craniofac Res. 2023 Mar;13(2):332–6.
39. Pham TAV. Comparison of Antimicrobial Activity against Porphyromonas gingivalis between Advanced Platelet-Rich Fibrin and Injectable Platelet-Rich Fibrin. Int J Biomater. 2023 Mar 27;2023:1–7.

40. Pall E, Roman A, Olah D, Beteg FI, Cenariu M, Spînu M. Enhanced Bioactive Potential of Functionalized Injectable Platelet-Rich Plasma. Molecules. 2023;28(4).

Annexes

Table 1.

Authors (Year)	Purpose	Study design	PRF preparation	Analyses	Outcomes
Jasmine *et al.* (2019) India, Saudi Arabia	To evaluate the in vitro antimicrobial and antibiofilm effects of IPRF against oral pathogenic biofilm producing staphylococcus bacteria isolated from patient with dental and oral abscess	In vivo involving: Blood samples from healthy human donors Bacteria cultures from patients with oral and dental abscess Physicochemical analysis	i-PRF: blood sample centrifuged at 1000 rpm for 5 min	MIC: inhibition of the growth at the concentration of 80 □l/ml MBC: no growth at the concentration of 240 □l/ml Live/Dead bacterial assay: untreated control (100% viable cells), I-PRF at MIC (>50% dead cells) and at MBC (100% dead cells) Biofilm inhibitory assay: at MIC, more than 50% reduction of biofilm production and at MBC, 100% of biofilm production	In conclusion, the i-PRF can be easily prepared during surgery and possesses bactericidal and antibiofilm activity. IPRF could prevent postoperative infections after a surgery.
Pham (2023) Vietnam	To provide in vitro evidence of the antimicrobial properties of new generations of PRF, including I-PRF and	In vivo involving 60 volunteers (20 healthy, 20 with gingivitis and 20 with periodontitis) aged 20-65 years. 22 mL of blood were collected for each	A-PRF+: 10 ml of blood was centrifuged at 1 300 rpm for 8 min. I-PRF: blood centrifugation at	Biofilm Inhibitory Assay: percent reduction in I-PRF sample was 49 ± 8%, 47 ± 10%, and 45 ± 10%, respectively in	In conclusion, I-PRF had better antibacterial properties than A-PRF+. The percentage reduction in the number of bacteria in the periodontitis group

	A-PRF+ that were obtained from patients			periodontitis, gingivitis, and healthy groups; and in	

	with different periodontal conditions, *against P. gingivalis*	patient (placed in the Dou Quattro Choukroun PRF system; designed by PROCESS for PRF ®, Nice, France)) In vitro: Inoculum of *P. gingivalis*, which was cultured from subgingival plaque of patients with periodontitis at 37°C for 7 days	700 rpm for 3 min (60 x g)	A-PRF the percent reduction was lower, 42 ± 9%, 41 ± 9% and 39 ± 9%. Mature Biofilm Impact Assay: percent reduction of *P. gingivalis* in I-PRF was 0.05 ± 0.05, 0.05 ± 0.05, and 0.07 ± 0.08, respectively in periodontitis, gingivitis and healthy groups; and in APRF samples, the percent reduction was lower, 0.03 ± 0.05, 0.03 ± 0.06, and 0.05 ± 0.09 Time-Kill Kinetics Assay: after exposing to A-PRF and I-PRF, a higher percent reduction was present in I-PRF samples	was higher for I-PRF than A-PRF+

Pham and Tran (2023) Vietnam	To compare the antimicrobial effects of these PRF materials against the periodontal pathogenic bacterium *Aggregatibacter actinomycetemcomitans* (*Aa*) in patients with different periodontal conditions	In vivo involving 60 volunteers [20 with healthy systemic and periodontal conditions, 20 with gingivitis (severe gingival inflammation) and 20 with periodontitis stage III or IV)] In vitro: Use of *Aggregatibacter actinomycetemcomitans*	Blood samples were centrifuged at 1300 rpm for 8 min to prepare A-PRF+; Blood samples were centrifuged at 700 rpm for 3 min to generate I-PRF	Antibiofilm formation assay: better antiadhesion effect from I-PRF rather than A-PRF (in periodontitis group, 26% and 35%, respectively, IPRF and A-PRF) Biofilm susceptibility assay: this time, the reduction of number of	I-PRF has proven to be a powerful product in the fight against periodontal pathogens.

		isolated from subgingival plaque samples of patients with periodontitis and with 5% agar at 37°C for 5-7 days		attached Aa was not like the results in the antiadhesion test, so both APRF and I-PRF had a very similar effect (5% and 4%, respectively, I-PRF and APRF) Time-kill assays: no significant differences among the groups for the A-PRF and I-PRF samples. Both of them showed bactericidal effects.	

Karde *et al.* (2017)	To evaluate the antimicrobial property, and platelet count of IPRF in comparison to other platelet concentrates, i.e., PRF, platelet-rich plasma (PRP), and control (whole blood)	In vivo: Blood samples were obtained from ten chronic generalized marginal gingivitis patients Invitro: A volume of 8 ml of blood was collected from each patient: 2 ml was used for preparation of I-PRF, PRF, and PRP, remaining 2 ml with anticoagulant served as a control.	I-PRF: A volume of 2 ml of blood collected in noncoated vacutainer without any additives and centrifuged at 700 rpm for 3–4 min PRF: A volume of 2 ml of blood collected in silico ncoated vacutainer without any additives and centrifuged at 3000 rpm for 10 min	Quantification of platelets: 1,434,000 ± 75,233, 1,343,000 ± 81,486, 249,000 ± 61,319, and 291,000 ± 51,575 (platelets count/mm^3), respectively for I-PRF, PRP, and PRF. Antimicrobial potential: the mean zone of inhibition (in cm) around I-PRF was 1.42 ± 0.25, for PRF was 1.3 ± 0.16 and for PRP was 1.02 ± 0.12	With I-PRF, its application can be more feasible and minimally invasive without using any additives for its preparation. I-PRF had the highest number of platelets. Its antimicrobial potency was also highest when compared to other platelet concentrates
Kour *et al.* (2018) India	I-PRF is being compared with the other two platelet concentrates, that is, PRP and PRF, for its antibacterial effect against two periodontal pathogens: Pg and Aa using well diffusion method and comparing the zones of inhibition	In vitro: Agar plates inoculated with bacterial strains: *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, the plates were then incubated at a temperature of 37°C for 24h in anaerobic environment	I-PRF: 3 ml of blood was taken into a blood-collecting tube without any additives and centrifuged at 700 rpm for 3 min PRF: 3 ml of blood was taken into a glass bloodcollecting tube without any additives and centrifuged at 3000 rpm for 10 min	Wilcoxon signed-rank test: the width of zones of inhibition (in mm) of *P. gingivalis* for I-PRF was 15.2 mm, for PRF was 8.8 mm and for PRP was 12.9 mm. For *Aa*, I-PRF was 9.8 mm, for PRF was 10.0 mm and for PRP was 12.5 mm	I-PRF and PRP have proved to be powerful weapons in the battle against periodontal pathogens.

Tran and Pham (2023)	To compare the antimicrobial capacity of A-PRF+ and i-PRF obtained from subjects with or without periodontal diseases against the pathogenic bacteria *Aa*	In vivo involving 60 humans (20 healthy, 20 with gingivitis and 20 with periodontitis) Blood samples from 60 humans In vitro: Bacteria cultures from the subgingival plaque of patients with periodontitis at 37°C for 72h.	A-PRF) Blood centrifugation at 1300 rpm for 8 min I-PRF) Blood centrifugation at 700 rpm for 3 min	MIC: for I-PRF, was 0.35 ± 0.09, 0.28 ± 0.13 and 0.26 ± 0.09, respectively healthy, gingivitis and periodontitis groups. For A-PRF, was 0.38 ± 0.15, 0.37 ± 0.07 and 0.35 ± 0.09, respectively healthy, gingivitis and periodontitis groups. Inhibition ring test: zone of inhibition (in mm^2) was for I-PRF, 70.05 ± 7.34, 77.45 ± 9.70 and 83.33 ± 8.62, respectively healthy, gingivitis and periodontitis groups. For A-PRF, was 65.50 ± 9.48, 66.28 ± 10.40 and 76.57 ± 10.80,	In conclusion, I-PRF exhibited better antibacterial activity than A-PRF+. In patients with gingivitis, the zone of inhibition was higher with I-PRF (83,3 mm^2) than APRF (76,5 mm^2). In patients with gingivitis, the zone of inhibition was higher too with I-PRF (77,4 mm^2) than A-PRF (66,2 mm^2).

				respectively healthy, gingivitis and periodontitis groups.	

Castro *et al.* (2019) Belgium, Chile	To evaluate the antibacterial properties of the L-PRF membrane and L-PRF exudate against the main periopathogens cultured on agar plates and in planktonic solution	In vivo involving nine participants, nonsmokers, systemically healthy, without a history of periodontal disease In vitro: BHI agar plates (Difco, Sparks, MD, USA) were seeded with an overnight culture 2 h before the application of an L-PRF membrane and incubated in anaerobic or aerobic conditions during 72h	Four blood samples were collected from each volunteer (n = 9) in a 9 mL glass-coated plastic tube and immediately centrifuged at 408 g for 12 minutes (Intraspin™, IntraLock, Boca Raton, FL, USA)	Vitality qPCR: in presence of *Aa*, a reduction in the growth of 86%, 38% and 24% with the ratios 1:1, 1:2 and 1:4, respectively. In presence of *P. gingivalis*, an increase in the growth of 86%, 72% and 50% with the ratios 1:1, 1:2 and 1:4, respectively was observed L-PRF exudate dilution test: bacteria-free area (in mm^2) for L-PRF exudate was 17 ± 2.6 against *P. gingivalis*, 0 for the other species.	This study demonstrated the antibacterial effect of the L-PRF membrane against *P. intermedia*, *F. nucleatum*, and *A. actinomycetemcomitans*, but especially against *P. gingivalis*
Moraschini *et al*. (2023) Brazil, USA, Switzerland	To evaluate the antimicrobial potential of different types of plateletrich fibrin used in regenerative treatments	In vitro: sixteen studies evaluated.	Blood centrifugation at 1000 rpm for 5 min at 37°C	MTT: the number of live bacteria has been drastically reduced with IPRF sample containing triple antibiotic mixture up to 91.62 ± 2.069%	In conclusion, it has been show that PRF has a antimicrobial activity, greater against bacteria rather than fungal.
Pall *et al*. (2023) Romania	The aim was to assess the various cumulative effects on i-PRF, which	In vitro: five different bacterial reference strains incubated with 6 mm diameter of	i-PRF: samples centrifugated at 700 rpm for three minutes.	Cell viability: in cultures treated with supplemented 2% i-PRF, the average cell viability was 96.33% ±	Indication that when iPRF is mixed with the highest concentration of human lactoferrin, an

	are antibacterial and antibiofilm effects	gentamicin at 37°C for 24h		0.85, for 7% i-PRF the average was 100.73% ± 0.33 and with 10% i-PRF the average was 97.21% ± 1.56.	increase of antimicrobial potential is observed.
Sánchez *et al*. (2021) Belgium, Spain	The aim was to describe the processes of antimicrobial consequences of L-PRF exudate against *P. gingivalis*	In vivo: L-PRF samples were obtained from the blood of a healthy adult In vitro: agar plate anaerobically incubated at 37°C with different bacterial stains	L-PRF: 9 ml blood sample centrifuged at 408 rpm for 12 min (IntraSpin, Intra-Lock, Boca Raton, FL, USA)	Zone of inhibition (mm^2): 61.2 ± 6.7 for undiluted LPRF and 59.9 ± 5.5 for LPRF exudate exposed to trypsin. A larger inhibition area was observed for undiluted L-PRF than for L-PRF exudate exposed to trypsin.	L-PRF seems to release peptides that may be responsible for the antimicrobial properties against *P. gingivalis*
Sindhusha and Ramamurthy (2023) India	To compare antimicrobial properties of L-PRF and i-PRF against oral pathogens	In vivo: 30 healthy adults (above 25 years old) were selected, and 10 ml of blood was taken from each one. In vitro: agar plates were prepared, containing bacteria stains (*Pg* and *Aa*) for 24h at 37°C	i-PRF: 5 ml of blood centrifuged at 700 rpm for 3 min L-PRF: 5 ml of centrifuged at 3000 rpm for 10min	Zone of inhibition (mm): 2.19 for i-PRF and 1.32 for L-PRF. Greater inhibition zone for i-PRF.	i-PRF demonstrated a better antimicrobial effect as compared to L-PRF against *Pg*.

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Data da prova pública: 20 de setembro de 2024