Antimicrobial effect of platelet-rich plasma and platelet-rich fibrin

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ABSTRACT

Context: Platelet concentrates have been extensively used in a variety of medical fields to promote soft- and hard-tissue regeneration. The significance behind their use lies in the abundance of growth factors (GFs) in platelets α-granules that promote wound healing. Other than releasing a pool of GFs upon activation, platelets also have many features that indicate their role in the anti-infective host defense.

Aim: The aim of this study is to evaluate the antimicrobial activities of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) against periodontal disease-associated bacteria.

Subjects and Methods: Blood samples were obtained from ten adult male patients. PRP and PRF were procured using centrifugation. The antimicrobial activity of PRP and PRF was evaluated by microbial culturing using bacterial strains of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans.

Results: P. gingivalis and A. actinomycetemcomitans were inhibited by PRP but not by PRF.

Conclusions: PRP is a potentially useful substance in the fight against periodontal pathogens. This might represent a valuable property in adjunct to the enhancement of tissue regeneration.

Key words: Antimicrobial effect, blood platelets, platelet-rich fibrin, platelet-rich plasma

Periodontitis is initiated by oral biofilm formation if untreated progress to gingivitis further leading to periodontal disease. The link between periodontal disease and systemic diseases has been scientifically proven over the last two decades. The principle reason for this oral-systemic connection is dissemination of locally produced proinflammatory mediators such as C-reactive protein, interleukins-1 beta (IL-1β) and IL-6, and tumor necrosis factor alpha.[1] Although conventional periodontal therapy initially decreases the bacterial load at the infected loci, it may show increased counts of periodontal pathogens weeks after treatment. Prevention of such a bacterial contamination is, therefore, important.

In the past two decades, the use of autologous platelet concentrates (PCs) has gained great popularity in a variety of medical fields such as dentistry, oral surgery, orthopedics, dermatology, ophthalmology, and cosmetic and plastic surgery. The rationale for their use arises from the fact that platelets when activated release growth factors (GFs) and other molecules that modulate the wound-healing response in both hard and soft tissues. In addition, anti-inflammatory properties of PCs have resulted in marked reduction of postoperative pain and swelling.[2,3]

Platelet-rich plasma (PRP) is a form of PCs whose regenerative potential is due to the release of various GFs such as platelet-derived growth factor, transforming growth factor-β, and vascular endothelial growth factor.[4,5] Thus, PRP may improve wound healing by increasing the levels of GFs in the wound site after degranulation of the platelets.[6] Recently, in vitro studies have demonstrated that PRP exerts positive effects on gingival fibroblasts,[7] oral osteoblasts,[8] and periodontal ligament (PDL) fibroblasts.[9]
making it an ideal candidate to facilitate complete periodontal regeneration. However, the precise role of PCs in periodontal tissue regeneration needs to be clarified.\(^{10}\) An antimicrobial effect of PRP has also been reported against \textit{Staphylococcus aureus}, 	extit{Escherichia coli}, and \textit{Klebsiella pneumonia} indicating that PRP is potentially useful to fight against postoperative infections. However, the disadvantage of using PRP is that its properties can vary depending on the concentration of platelets, amount of leukocytes, the type of activator used, and time of placement of fibrin scaffold after clotting.\(^{11}\)

Platelet-rich fibrin (PRF), the second-generation PC, has been introduced by Choukroun \textit{et al.} in 2000.\(^{12}\) It contains platelets and GFs in the form of fibrin membranes prepared from the patient’s own blood free of any anticoagulant.\(^{13}\) PCs accelerate the wound healing after periodontal treatment. In addition, there is release of certain substances from platelets that promote tissue repair, angiogenesis, inflammation, and immune response. Platelets also contain biologically active proteins. The binding of these secreted proteins with a developing fibrin mesh or to the extracellular matrix can create chemotactic gradients aiding the recruitment of the stem cells, stimulating cell migration, differentiation, and promoting repair. PRF has a dense fibrin network with leukocytes, cytokines, structural glycoproteins, and also GFs.\(^{14}\) Leukocytes that are concentrated in PRF scaffold play an important role in GF release,\(^{15}\) immune regulation, anti-infectious activities,\(^{16}\) and matrix remodeling during wound healing. The slow polymerization mode of PRF and cicatricial capacity create a physiologic architecture favorable for wound healing.\(^{17}\)

It has been demonstrated that periodontal diseases are intimately related to the presence of some bacterial species such as \textit{Porphyromonas gingivalis}, \textit{Aggregatibacter actinomycetemcomitans}, and \textit{Fusobacterium nucleatum}. PRP and PRF are creating new avenues in the field of tissue repair and regeneration in periodontal treatment. However, there is no much evidence regarding its antibacterial potential.

Hence, the aim of this study is to evaluate antibacterial effects of PRF and PRP against two periodontal pathogens: \textit{P. gingivalis} and \textit{A. actinomycetemcomitans}.

**SUBJECTS AND METHODS**

This study protocol was approved by the Institutional Ethical Committee. All the patients provided written informed consent before beginning the study. Blood samples were obtained from ten adult male patients, age ranging from 25 to 45 years. They were systemically healthy, nonsmokers, with no symptoms of infection and took no antibiotics for at least 3 months before experiments began.

A volume of 10 ml of blood was collected from each patient: 5 ml was used for PRF and 5 ml for PRP procurement. The ten samples were randomly divided into two groups: 5 samples in \textit{P. gingivalis} group and 5 samples in \textit{A. actinomycetemcomitans} group. Antimicrobial culturing was done on agar plates.

**Platelet-rich fibrin procurement**

A volume of 5 ml of intravenous blood (antecubital site) was collected in the plain bulb [Figure 1a] and centrifuged using centrifuge (Manual Centrifugation Machine, e-Tek) at 3000 rpm for 10 min [Figure 1b]. After centrifugation, the PRF clot was removed from the tube using sterile tweezers [Figure 1c], separated from the RBC base using scissors. PRF was obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.

**Platelet-rich plasma procurement**

A volume of 5 ml of intravenous blood (antecubital site) was collected into the blood collection tube coated with 3.2% sodium citrate solution used as an anticoagulant [Figure 2a]. Each tube was inverted several times to ensure proper mixing of the blood and anticoagulant.

After proper mixing, the tubes were placed in centrifugation machine (Manual Centrifugation Machine, e-Tek). The first centrifugation was done at 1000 rpm for 13 min. The result was the separation of the whole blood into its three basic components: Red blood cells, platelet concentration, and platelet-poor plasma [Figure 2b]. Because of differing densities, the layer of red blood cells was formed at the lowest level; platelet concentration comprised the middle layer and platelet-poor plasma the upper layer.

The platelet-poor plasma was drawn from the sodium citrate bulb with the help of Pasteur pipette and was discarded. The bulb was reinserted into the centrifuge machine at 2000 rpm for 10 min. The result after centrifugation was sedimented RBC at the bottom and PRP (sometimes referred to as “buffy coat”) at the top [Figure 2c].

![Figure 1: Platelet-rich fibrin procurement (a) venous blood collected in plain bulb; (b) platelet-rich fibrin obtained after centrifugation of blood at 3000 rpm for 13 min; (c) platelet-rich fibrin clot was removed from the tube using sterile tweezers](image-url)
The PRP was withdrawn using Pasteur pipette and placed in a sterile container. This PRP was activated by 10% calcium chloride and left undisturbed for 30–40 min so as to ensure proper gel formation [Figure 2d].

**Microbial culturing**

Agar plates were inoculated with bacterial strains: *P. gingivalis* ATCC No. 33277 and *A. actinomycetemcomitans* ATCC No. 43718. Each agar plate was then labeled and divided into two halves with the help of a marker on the bottom of the plate for PRP and PRF, respectively. A well was created for placing PRP for proper diffusion, whereas PRF was placed on the surface of the agar plate.

**Anaerobic culturing of Porphyromonas gingivalis in anaerobic jar**

Principle – in this system, anaerobiosis is achieved by the formation of the hydrogen and carbon dioxide gas mixture through the following reaction:

1. Citric acid + sodium bicarbonate → sodium citrate + carbon dioxide
2. Sodium borohydride + water → sodium metaborate + hydrogen.

The liberated hydrogen combines with oxygen in the presence of palladium catalyst to form water. At the same time, a trace of carbon dioxide released from the first reaction stimulates the growth of anaerobic bacteria.

Blood agar plates inoculated with *P. gingivalis* ATCC No. 33277 were incubated in anaerobic jar at 37°C for 3–4 days.

**Anaerobic culturing of Aggregatibacter actinomycetemcomitans in CO₂ jar**

Blood agar plates inoculated with *A. actinomycetemcomitans* ATCC No. 43718 were incubated in 5–10% CO₂ jar at 37°C for 48 h.

**Statistical analysis**

Analysis was performed using Mann–Whitney test as the data were non-normally distributed to compare PRP and PRF for *P. gingivalis* and *A. actinomycetemcomitans*. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

Zone of inhibition produced by PRP against *P. gingivalis* ranged between 12 mm and 15 mm (mean 13 mm) in diameter and against *A. actinomycetemcomitans* ranged between 10 mm and 22 mm (mean 13.8 mm) in diameter, whereas PRF showed no zone of inhibition against *P. gingivalis* and *A. actinomycetemcomitans* [Table 1]. This shows that PRP dramatically inhibited the growth of both *P. gingivalis* and *A. actinomycetemcomitans*, which was statistically significant \( P < 0.05 \) as compared to PRF where no activity was seen against these pathogens [Figure 3].

**DISCUSSION**

The regenerative potential of PCs has been studied extensively over the last 20 years. However, in the available literature, only a few reports can be found about their antimicrobial effects.

At present, the components responsible for the antimicrobial activity of PCs remain poorly understood because these materials are a complex mixture of platelets, white blood cells, and plasma. The impact of the plasma and cellular components has not been studied in detail yet. Existing evidence suggests that platelets may play multiple roles in antimicrobial host defense: They generate oxygen

![Figure 2: Platelet-rich plasma procurement (a) venous blood collected in sodium citrate bulb; (b) platelet-poor plasma obtained after centrifugation at 1000 rpm for 12 min which was discarded; (c) platelet-rich plasma obtained after centrifugation at 2000 rpm for 10 min; (d) platelet-rich plasma activated using 10% CaCl₂](image)

![Figure 3: Zone of inhibition seen around platelet-rich plasma and not around platelet-rich fibrin](image)

| Sample | Porphyromonas gingivalis | Aggregatibacter actinomycetemcomitans |
|--------|--------------------------|---------------------------------------|
| PRP (mm) | PRF | PRP (mm) | PRF |
| 1 | 13 | 0 | 1 | 22 |
| 2 | 15 | 0 | 2 | 12 |
| 3 | 13 | 0 | 3 | 13 |
| 4 | 12 | 0 | 4 | 10 |
| 5 | 12 | 0 | 5 | 12 |
| Mean | 13* | 0 | Mean | 13.8* |

*Statistically significant \( P < 0.05 \). PRP=Platelet-rich plasma, PRF=Platelet-rich fibrin
metabolites, including superoxide, hydrogen peroxide, and hydroxyl free radicals.\(^{18,19}\) Moreover, they are capable of binding, aggregating, and internalizing microorganisms, which enhances the clearance of pathogens from the bloodstream; they participate in antibody-dependent cell cytotoxicity functions to kill protozoan pathogens, and finally, platelets release an array of potent antimicrobial peptides.\(^{20,21}\)

Several antimicrobial factors have been proposed, including platelet antimicrobial proteins and peptides of the innate immune defense, or platelet \(\alpha\)-granules components, such as complement and complement-binding proteins.\(^{21-24}\) Yeaman in 1997 suggested that the antimicrobial activity of PCs could be due to direct interaction of platelets with microorganisms and participation in antibody-dependent cell cytotoxicity and white blood cells in direct bacterial killing. Release of myeloperoxidase, activation of the antioxidant responsive element, and antigen-specific immune response have also been suggested.\(^{19}\)

PRP contains a large number of platelets as well as a high concentration of leukocytes, which has been reported to be two to four times greater in PRP than in the whole blood. Among these leukocytes, neutrophils are known for their host defense actions against bacteria and fungi through the actions of myeloperoxidase, which presents in neutrophilic granulocytes; lymphocytes produce immunocompetent cells and one of their representative functions is found in immunologic defense; monocytes (precursors of macrophages) produce cytokines and chemotactic factors that participate in inflammation. Therefore, the concentrated leukocytes in PRP may enable PRP to play an important role in the immune defense against bacterial infection.

Activated platelets could release various GFs that play an important role in improving the healing of ulcers and secreting platelet microbicidal proteins (PMPs).\(^{19}\) PMPs contain an array of materials which have antibacterial activity, including platelet factor 4, regulated upon activation of normal T-cell expressed and secreted protein, connective tissue-activating peptide 3, platelet basic protein, thymosin beta-4, fibrinopeptide A, and fibrinopeptide B. PMPs could play a role through the following mechanisms: Contacting the bacterial membrane, changing the membrane permeability, entering the cell, and inhibiting the synthesis of big molecules.\(^{21}\)

Our data clearly demonstrated that PRP was capable of inhibiting \(P.\) gingivalis at 3–4 days of incubation and \(A.\) actinomycetemcomitans at 48 h of incubation. However, PRF was not able to inhibit these bacteria. This could be since PRF is a matrix of autologous fibrin, in which a large quantity of platelet and leukocyte cytokines are embedded intrinsically during centrifugation leading to their progressive release over time (7–11 days) as the network of fibrin disintegrates.\(^{25,26}\) The components in PRF and PRP are more or less the same, except the extra-added calcium chloride in PRP for activation of platelets, which may be the reason for the contribution of the antibacterial activity.

## CONCLUSION

For decades, periodontists have sought ways to repair the damage that occurs during periodontitis. This has included the use of a range of surgical procedures, the use of a variety of grafting materials (autologous bone and bone marrow, allograft, xenografts, and various manmade bone substitutes) and GFs, and the use of barrier membranes.\(^{27}\)

PRP has proved to be a powerful weapon in the battle against periodontal pathogens. This might be a valuable adjunctive to the enhancement of tissue regeneration. Choukroun’s technique is a simple and inexpensive technique for the successful regeneration of periodontal tissues. The main advantage is that PRF and PRP preparation utilizes the patient’s own blood reducing or eliminating disease transmission through blood. Further, long-term in vitro studies should be carried out to evaluate the mechanical properties and to assess the degradation profile of PRF. In addition, more studies and clinical trials are needed to investigate potential applications of PRF and PRP in the field of periodontal regeneration and tissue engineering.

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Conflicts of interest

There are no conflicts of interest.

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