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Comparative Evaluation Of Antimicrobial Efficacy Among Various Generations Of PRF From A Systemically Healthy Population - An In Vitro Study

Research Article

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Abstract

Aim: The aim of the study is to study the antibacterial efficacy of different generations of PRF.
Materials And Method: A volume of 5 mL of intravenous blood was obtained from the median cubital vein, PRF- centrifuged at 3300 rpm for 13 minutes , I-PRF - 700 rpm for 3 minutes, CGF- centrifuged using a one-step protocol: 30sec acceleration, 2min 2700 rpm, 4min 2400 rpm, 4min 2700 rpm, 3min 3000 rpm, 36sec deceleration and stop, A-PRF+ 1300 rpm for 8 minutes using a centrifuge. The PRF clot was removed from the tube with sterile tweezers and separated from the RBC base with scissors after centrifugation.
Results: The zone of inhibition was maximum for CGF followed by i-PRF, A-PRF and PRF. The zone of inhibition for PRF-7MM, I-PRF- 14MM, CGF-15MM and A-PRF- 9MM was seen.
Conclusions: Within the limitations of the study, all the PRF generation PRF, I-PRF, CGF, A-PRF showed antimicrobial efficacy against oral pathogens. CGF showed maximum inhibiton against the bacteria followed by I-PRF, A-PRF and PRF. This can be used to advantage for treating periodontal therapy and this might act as aid in tissue regeneration as well.

Introduction

Periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment and bone loss and is characterized by pocket formation and/or recession of the gingiva. It is recognized as the most frequently occurring form of periodontitis. It is prevalent in adults, but can occur at any age. The disease is usually associated with the presence of plaque and calculus. Progression of attachment loss usually occurs slowly, but periods of rapid progression can occur. Associated with a variable microbial pattern. The goal of soft and hard tissue regeneration is to replace disfigured periodontal tissues that have been lost due to periodontal diseases, as well as to reclaim previously lost alveolar bone and gingiva.One of the materials used for regenerative purpose is platelet concentrates. It is used as membrane and as regenerative material as such. Platelets are 2–3 m in diameter anucleate cytoplasmic fragments originating from bone marrow megakaryocytes. Many granules, few mitochondria, and two prominent membrane structures, the surface-connected canalicular system and the thick tubular system, are found in these cells. Granules are spherical or oval structures with diameters ranging from 200 to 500 nm that are each surrounded by a unit membrane.[1] Platelet-derived growth factor (PDGF), transforming growth factor (TGF-), and insulin-like growth factor (IGF-) form an intracellular storage pool of proteins essential for wound healing (IGF-I). After activation, the granules fuse with the platelet cell membrane. At least some secretory proteins are converted into biologically active forms. The active proteins are then secreted, allowing them to bind to the target cells' transmembrane receptors. Intracellular signal proteins are activated after they have been bound. This causes a gene sequence to be expressed, which regulates cellular proliferation, collagen synthesis, and osteoid formation, among other things.[2] Chemotactic and mitogenic properties of these GFs facilitate and modulate cellular functions involved in tissue healing, regeneration, and cell proliferation.[3] Platelet concentrates release growth factors (GFs) and other molecules that modulate the wound-healing response in both hard and soft tissues when triggered, which is why they're used. Furthermore, Platelet concentrates's' anti-inflammatory effects have resulted in a substantial reduction in postoperative pain and swelling.[4]

Platelet concentrates have been classified as first and second generation- first generation is platelet rich plasma (PRP) and second

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generation is plasma rich fibrin (PRF)[5]. Plasma rich fibrin has many paradigms under it like Injectable PRF, Leukocyte PRF, Advanced PRF and so on. The aim of the study is to study the antibacterial efficacy of each type of PRF.

Materials And Method

This study was conducted in Saveetha dental college and hospital. Blood samples were collected from systemically healthy patients from out patient department of periodontics after getting consent from the patients. Total of 5 subjects in each group.

Inclusion criteria

1. Systemically healthy patients.
2. Aged between 20-45 years
3. Non-smokers
4. Not on any medication for the past 3months.

Exclusion Criteria

1. Patients with medical history of any systemic illness or surgery.
2. Patients on any medication.
3. Smokers

Preparation of PRF

Plasma Rich Fibrin

A volume of 5 mL of intravenous blood was obtained from the median cubital vein, which lies within the cubital fossa anterior to the elbow in the plain bulb and centrifuged at 3300 rpm for 13 minutes using a centrifuge. The PRF clot was removed from the tube with sterile tweezers and separated from the RBC base with scissors after centrifugation. By squeezing out the fluids in the fibrin clot, PRF was obtained in the form of a membrane.

Injectable Plasma Rich Fibrin

A volume of 5 mL of intravenous blood was obtained in the plain bulb and centrifuged at 700 rpm for 3 minutes using a centrifuge. The PRF is in liquid form.

Concentrated Growth Factor

A volume of 5 mL of intravenous blood was obtained in the plain bulb and centrifuged using a one-step protocol: 30sec acceleration, 2min 2700 rpm, 4min 2400 rpm, 4min 2700 rpm, 3min 3000 rpm, 36sec deceleration and stop. The CGF clot was removed from the tube with sterile tweezers and separated from the RBC base with scissors after centrifugation. By squeezing out the fluids in the fibrin clot, CGF was obtained in the form of a membrane. Advanced Plasma Rich Fibrin:

A volume of 5 mL of intravenous blood was obtained in the plain bulb and centrifuged at 1300 rpm for 8 minutes using a centrifuge. The PRF clot was removed from the tube with sterile tweezers and separated from the RBC base with scissors after centrifugation. By squeezing out the fluids in the fibrin clot, PRF was obtained in the form of a membrane.

Microbial assay:

Nutrient agar plate were cultured with streptococcus mutans strain. A total of four wells were created and labelled as PRF, i-PRF, A-PRF and CGF. All the type was PRF was placed in their respective wells and was incubated for 24 hours. Antimicrobial efficacy was evaluated by the zone of inhibition.

Result

The zone of inhibition was maximum for CGF followed by i-PRF, A-PRF and PRF. The zone of inhibition for PRF-7MM, I-PRF- 14MM, CGF-15MM and A-PRF- 9MM was seen.

Figure 1. Platelet rich fibrin.

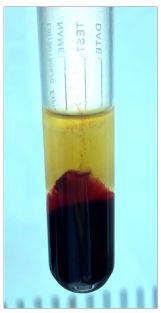

Figure 2. Injectable platelet rich fibrin.

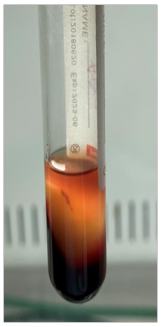

Figure 3. Concentrated growth factor.

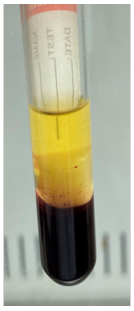

Figure 4. Advanced platelet rich fibrin.

Figure 5. Shows zone of inhibition for various generations of PRF.

Table 1. Zone of inhibition for various generations of PRF.

GENERATION OF PRF	ZONE OF INHIBITION (MM)
PRF	7
I-PRF	14
CGF	15
A-PRF	9

Discussion

Over the last 20 years, the regenerative capacity of computers has been thoroughly researched. However, there are only a few studies about their antimicrobial effects in the literature. In the present study the zone of inhibition was maximum for CGF followed by i-PRF, A-PRF and PRF. The zone of inhibition for PRF-7MM, I-PRF- 14MM, CGF-15MM and A-PRF- 9MM was seen.

Natural components of platelet-rich fibrin (PRF) include (A) cell types: platelets, leukocytes, and red blood cells; (B) a provisional extracellular matrix 3-D scaffold made of autologous fibrin, including fibronectin and vitronectin; (C) a large number of bioactive molecules, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), transforming growth factor-beta (TGF-ß), and bone morphogenetic protein-2; (BMP2) [6, 7] A study concluded that P. gingivalis and A. actinomycetemcomitans were inhibited by PRP but not by PRF. [8] Study done in 2018, included that in the case of Pg, I-PRF had the largest inhibition region, which was substantially larger than PRF. In addition, PRP had a slightly larger inhibition zone against PRF. In the case of Aa, PRP had a slightly larger zone of inhibition than PRF and I-PRF.[9] In 2015, a study compared the antimicrobial activity of four plasma fractions, that is, PRP, platelet-poor plasma, platelet-depleted plasma, and PRF against Pg, Aa, and Fusobacterium nucleatum and found that PRP had the highest antibacterial activity.[10] In 2016, Joshi et al. published a study in which PRF showed a strong zone of inhibition against a subgingival plaque sample, and calorimetric analysis verified that PRF caused the least amount of turbidity.[10] A study by Kardeet al. in 2017 where the antibacterial activity of PRP, PRF, and I-PRF was tested on the supragingival plaque and it was observed that I-PRF showed a maximum zone of inhibition followed by PRP and then PRF.[11]

Since platelets are present along with white blood cells and plas-

ma, the exact component of the platelet concentrates responsible for the antimicrobial activity has yet to be determined. The antibacterial effect of platelet concentrates has been attributed to a variety of mechanisms, including the development of oxygen metabolites such as superoxide, hydrogen peroxide, and hydroxyl-free radicals, the binding, aggregation, and internalization of microorganisms, and the release of antimicrobial peptides. [10] As suggested by Yeaman, direct bacterial killing could result from platelets' direct association with microorganisms, involvement in antibody-dependent cell cytotoxicity, and engulfment by entrapped white blood cells inside PRF. The leukocytes that are present in much greater concentrations along with the platelets in these platelet concentrates as compared to the whole blood are already known for their antibacterial activity.

Many anti-bacterials are used along phase I periodontal therapy to outgrow the periodontopathic pathogens, but due to bacterial resistance, alternative methods have been studied to bring about the antimicrobial activity against these bacteria. One of the methods is using PRF for its antimicrobial activity along with the release of growth factor for regenerative purposes. Future scope of the present study is to work on methods to enhance the anti-microbial property of various generations of PRF.

Conclusion

Within the limitations of the study, all the PRF generation PRF, I-PRF, CGF, A-PRF showed antimicrobial efficacy against oral pathogens. CGF showed maximum inhibiton against the bacteria followed by I-PRF, A-PRF and PRF. This can be used to advantage for treating periodontal therapy and this might act as aid in tissue regeneration as well.

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