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Original Research

Antibacterial and antifungal efficacy of platelet-rich fibrin (PRF) and platelet-rich fibrin matrix against root canal microflora

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ABSTRACT:

Background: The present study was conducted to assess antibacterial and antifungal efficacy of platelet-rich fibrin (PRF) and platelet-rich fibrin matrix against root canal microflora. **Materials & Methods:** The present study was conducted on 36 subjects of both genders. Blood samples were obtained from all subjects, PRF (group I) and PRFM (group II) were processed as per the protocol. The susceptibility test against microbiota in the root canal and Candida albicans was assessed through minimum inhibition zone by agar diffusion technique. **Results:** The mean antibacterial scores in group I was 4.16 and in group II was 1.20. The difference was significant (P< 0.05). The mean antifungal scores in group I was 1.72 and in group II was 0.46. The difference was significant (P< 0.05). **Conclusion:** Authors found that the antibacterial efficacy of PRF may prove beneficial as compared to PRF matrix.

Key words: Antifungal scores, Antibacterial scores, Platelet-rich fibrin.

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INTRODUCTION

Endodontic regenerative procedures are biologically based procedures which includes regeneration of pulp-like tissue, more idealistically the dentin-pulp complex, affected coronal dentin following a carious exposure or trauma; and regeneration of pathological root resorption in cervical, middle, or apical areas. Since two decades, an increased understanding of the physiological therapeutic role of platelets in wound healing has led to the use of platelet concentrates (PCs) as remedial tools for regenerative protocols.[1]

Regenerative endodontic treatment (RET) is based on the concept of tissue engineering, which requires the eradication of pathogens, the preservation of stem cells, and the presence of scaffolds and signal molecules.[2] To create a favourable microenvironment for stem cells to migrate, proliferate and differentiate, an ideal scaffold should facilitate spatial orientation and signal molecule release by cells. In most cases of tooth revascularization/revitalization, an endodontic explorer or file is introduced into the root canal and passes through the apical foramen to provoke bleeding from the periapical tissue into the canal to form a blood clot (BC) below the cemental enamel junction (CEJ).[3]

Platelet-rich fibrin (PRF) is the second generation of PCs. It was introduced by Choukroun et al. in 2000. These PCs contains biologically active protein that accelerates the wound healing and in addition, promotes angiogenesis, tissue repair, however, causes moderate inflammation, and an immune response[4]. The binding of these proteins with a developing fibrin mesh or to the extracellular matrix can create chemotactic gradients aiding the recruitment of stem cells, thereby, stimulating cell migration, differentiation, and this promotes repair and regeneration. There is a novel concept in PCs called PRF matrix (PRFM) which is processed using higher gravitational force without the use of bovine thrombin.[5] PRFM lacks leukocytes and is made of a dense fibrin network. The density of the fibrin matrix makes it suitable for regenerative endodontic treatment modalities.The present study was conducted to assess antibacterial and antifungal efficacy of platelet-rich fibrin (PRF) and platelet-rich fibrin matrix against root canal microflora.

MATERIALS & METHODS

The present study was conducted in the department of Endodontics. It comprised of 36 subjects of both genders. Ethical committee of the institute approved the study. All were informed and their consent was taken. Systemically healthy, non-smokers with no symptoms of infection or on antibiotics at least 3 months prior to experiments were selected.

PRF preparation was adopted from the protocol by Dohan et al. A total volume of 5 ml of blood was collected in vacutainer and centrifuged at 400 g for 15 min. After centrifugation, the PRF clot was removed from the tube using sterile tweezers, and is separated from the RBC base using scissors. PRF was then immediately transferred to the culture dish.

A total volume of 5 ml of intravenous blood was collected into the blood collection tube coated with 3.2% sodium citrate solution used as an anticoagulant. Each tube was vortexed several times to confirm proper mixing of the blood and anticoagulant. The first centrifugation done at 1100 g for 6 min. The result was the separation of the whole blood into its three basic components: red blood cells, platelet concentration, and platelet poor plasma. The platelet concentration and platelet-poor plasma transferred into a vacutainer containing calcium chloride under sterile conditions. Second centrifugation was operated at 4500 g for 25 min. A translucent, yellow-white platelet-fibrin matrix, with an inner diameter of 33 mm which is equal to the bottle (33 mm) formed at the bottom was retrieved. This fibrin matrix so obtained is termed PRFM and was immediately transferred to the culture dish.

The microbial samples from the root canal of mandibular first molar was collected under strict asepsis. The surface of the tooth was disinfected with 30% hydrogen peroxide for 1 min followed by 3% sodium hypochlorite and neutralization was performed using 5% sodium thiosulfate. Access was prepared and the root canal was irrigated using sterile saline. Samples were taken from each canal with K files (MANI) and paper points by inserting it into the root canal for 1 minute. The collected sample was immediately transported in a test tube containing peptone water, to microbial culture laboratory. It was incubated for 24 hours.

Samples were cultured on blood agar culture plate. A heated sterile platinum wire loop was used to streak the sample on the blood agar plate. The agar plate was then incubated for 2–3 days. If any macroscopic changes (cream-colored, raised colonies) seen indicated the growth of microorganisms.

For fungal culturing, the samples were streaked on the Sabouraud's dextrose agar plate using a sterile platinum loop followed by which the culture media was subsequently kept at 37°C for 2 days. Any macroscopic changes seen indicating the growth of fungi, was further subjected to gram staining and germ tube test to detect the presence or absence of Candida albicans.

Two groups PRF (group I) and PRFM (group II) were prepared. The susceptibility test of root canal microflora and C. albicans to PRF and PRFM was tested by agar diffusion method. Results thus obtained were subjected to statistical analysis using Mann Whitney test. P value less than 0.05 was considered significant.

RESULTS

Table I Mean distribution of the antibacterial scores

Groups	Mean score	P value
Group I	4.16	0.01
Group II	1.20	

Table I, graph I shows that mean antibacterial scores in group I was 4.16 and in group II was 1.20. The difference was significant (P< 0.05).

Graph I Mean distribution of the antibacterial scores

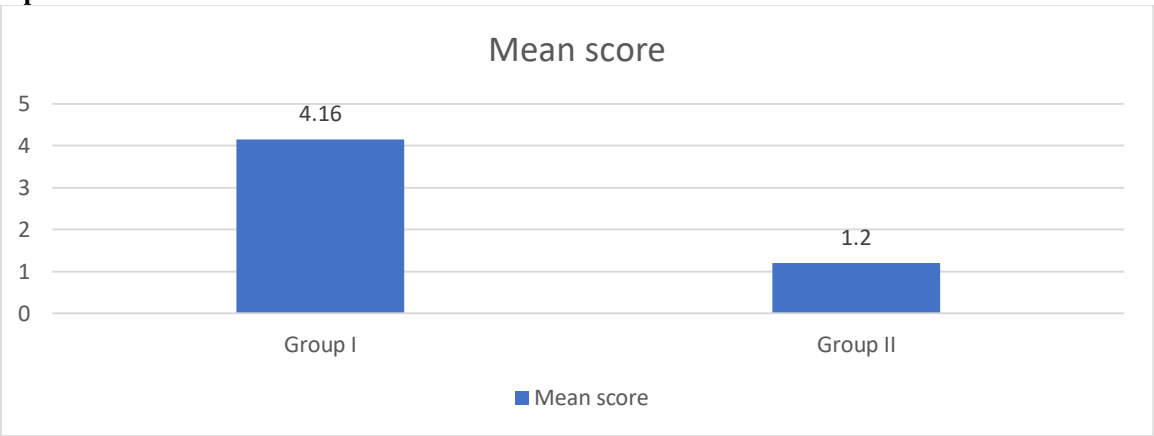
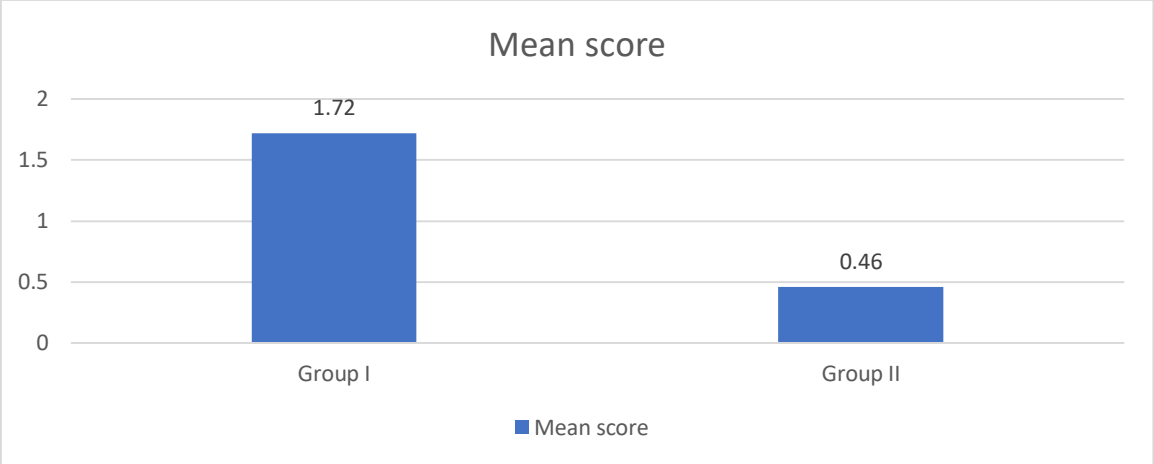


Table II Mean distribution of the antifungal scores

Groups	Mean score	P value
Group I	1.72	0.01
Group II	0.46	

Table II, graph II shows that mean antifungal scores in group I was 1.72 and in group II was 0.46. The difference was significant (P< 0.05).

Graph II Mean distribution of the antifungal scores



DISCUSSION

Platelet-rich plasma (PRP) is a first-generation platelet concentrate. Studies have demonstrated the reliability of PRP in improving periapical healing, apical closure and dentinal wall thickening. Platelet-rich fibrin (PRF), a second-generation platelet concentrate, has many advantages over PRP. Firstly, the preparation of PRF does not require the addition of exogenous agents, such as thrombin. Secondly, PRF forms an organized fibrin network in which platelets and leukocytes are trapped. These entrapped cells serve as a reservoir of various growth factors for long-term release. Important circulating immune cells and various cytokines in PRF clots also act against infection. In addition, the mechanical properties of PRF might facilitate the condensation of overlying MTA. Thus, it is rational to expect PRF to be an optimal bioscaffold for tooth revascularization/ revitalization.[6]

Pulp revascularization procedures are being researched on to manage immature teeth for a decade. However, cases with pulpal and periapical infection usually do not respond favorably to revascularization procedures due to inability to completely disinfect the canal.[7] Microorganisms that leads to endodontic infections are mainly of low virulence. Their pathogenesis and endurance are influenced by the release of lipopolysaccharide, toxins, and the synthesis of enzymes. Pulpal and periapical lesions are associated with a mixed microbiota, consisting of aerobic, anaerobic, Gram-positive, and negative microorganisms.[8] The present study was conducted to assess antibacterial and antifungal efficacy of platelet-rich fibrin (PRF) and platelet-rich fibrin matrix against root canal microflora.

In present study we found that mean antibacterial scores in group I was 4.16 and in group II was 1.20. Nagaraj et al[9] assessed the antibacterial and antifungal property of platelet-rich fibrin (PRF) and PRF matrix (PRFM) in blood samples obtained from 16 participants, PRF showed an effective antibacterial property, however, did not perform well against C. albicans strains. PRFM did not show any antibacterial or antifungal properties. The antibacterial efficacy of PRF may prove beneficial when used in the revascularization procedure of immature necrotic teeth.

In present study we found that mean antifungal scores in group I was 1.72 and in group II was 0.46. Sen et al[10] suggested that Candida is considered as dentinophilic microorganism. H Chen et al[11] compared the performance of platelet-rich fibrin (PRF) with BC in inducing root development and periapical lesion healing after tooth revascularization. Five patients receiving RET using PRF as a scaffold were matched 1:1 to a previous cohort of 5 patients who underwent tooth revascularization by provoking periapical bleeding. Clinical signs and symptoms were examined at follow-ups. Periapical lesion healing and root development were monitored radiographically. The resolution of clinical signs and symptoms as well as periapical radiolucency was observed in all patients (100%). Root elongation, dentinal wall thickening and apex closure were found in most cases (80% in both groups). There was no significant difference between the groups in terms of clinical sign resolution, root development and periapical healing.

Bielecki et al[12] made an extensive study on the impact of leukocyte in PCs and their role in wound healing and immune response. Elements of neutrophils such as polymorphonuclear neutrophilic granulocytes granule proteins, heparin-binding protein, cathepsin G, cathelicidin, Calprotectin, defensins, phospholipase A2, and eosinophils are effective immune mediators. The shortcoming of the study is small sample size.

CONCLUSION

Within the limitation of the study, it was concluded that antibacterial efficacy of PRF may prove beneficial as compared to PRF matrix.

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