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Antimicrobial Efficacy of I-PRF from Normal vs. Diabetic Donors in Combination with Triple and Modified Triple Antibiotic Pastes: An In-Vitro Evaluation against *Enterococcus faecalis*

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KEYWORDS

I-prf,TAP,m-TAP

ABSTRACT:

Introduction: Context: To assess the antibacterial potential of I-PRF alone, with TAP, and with modified TAP against *E. faecalis*, including its performance from diabetic donors under compromised healing conditions.

Objectives: To evaluate and compare the antimicrobial efficacy of injectable platelet-rich fibrin from healthy and diabetic individuals when combined with Triple Antibiotic Paste and Modified Triple Antibiotic Paste against *Enterococcus faecalis*.

Methods: Five systemically healthy individuals and five diabetic individuals (HbA1c >7.0) were selected, and 5 mL of peripheral blood was drawn from each to prepare injectable platelet-rich fibrin (I- prf) using standardized centrifugation. The antimicrobial activity of I- prf alone, I- prf + TAP (ciprofloxacin, metronidazole, minocycline), and I- prf + mTAP (ciprofloxacin, metronidazole, cefaclor) was tested against *E. faecalis* using agar diffusion method. Zones of inhibition were measured after 24 hours of anaerobic incubation at 37°C to compare efficacy between normal and diabetic groups.

Results: After 24 hours of incubation, clear zones of inhibition were observed around all platelet concentrates against *E. faecalis*. The mean inhibition zones in normal subjects were 3.56 mm (I-prf), 4.6 mm (I-prf+TAP), and 5.02 mm (I-prf+mTAP), while in diabetic subjects they were 2.84 mm, 3.84 mm, and 4.54 mm respectively, with normal groups showing significantly greater antimicrobial activity.

Conclusions: Within the study's limitations, I- prf demonstrated significant antibacterial activity against *E. faecalis*. Its combination with antibiotic pastes may enhance root canal disinfection, even in compromised systemic conditions.

Key Messages: I- prf boosts antibacterial effect against *E. faecalis*, even in compromised cases.

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1. Introduction

Tissue engineering in dentistry is rapidly advancing, yet finding an ideal biomaterial for optimal regeneration remains a challenge.[1,2] Platelet-rich concentrates like PRP and PRF release growth factors that promote cell division, differentiation, and healing. PRF is preferred over PRP for its better handling, affordability, and lack of anticoagulants.[3] In 2014, Injectable prf (I- prf) was developed using low-speed centrifugation, offering a flowable form with enhanced regenerative properties.[4] I-prf promotes mesenchymal stem cell proliferation, migration, osteogenic differentiation, exhibits anti-inflammatory, antimicrobial activity and wound healing associated with angiogenesis. Among the platelet concentrates, differential expression of VEGF-A was superior in I-prf.[5,6]

Regenerative endodontics, particularly for immature necrotic teeth, often involve disinfection with calcium hydroxide, TAP, or DAP.[7,8,9] However, these can weaken roots or harm stem cells.[10] Recent studies suggest incorporating antibiotic pastes into I- prf scaffolds to enhance disinfection while preserving stem cell viability.[11] Studies have shown that I-PRF can lead to good outcomes in endodontic treatments. However, in patients with uncontrolled diabetes and existing periradicular lesions, the chances of success may be lower.[12]

2. Objectives

This study compares the antibacterial effects of I- prf with TAP and modified TAP against *E. faecalis*, including I- prf from diabetic individuals, to evaluate its efficacy in compromised healing conditions.

3. Methods

This single-center, forward-looking clinical study was conducted with volunteers from the department. All participants were fully informed of the study protocol and provided written consent prior to enrolment. The study included five systemically healthy individuals with no history of medication use (Group A) and five diabetic participants with HbA1c levels >7.0 (Group B). Participants with blood disorders, those receiving anticoagulant or immunosuppressive therapy, or with a history of alcohol dependence were excluded.

Blood Collection and I-PRF Preparation: Venous blood was collected from the antecubital region under sterile conditions. From each participant, 5 mL of peripheral blood was drawn into plain plastic tubes (without anticoagulant) and centrifuged at 700 rpm for 3 minutes using the Intra-spin system (Intra-Lock, Boca-Raton, FL, USA) to obtain injectable platelet-rich fibrin (I-PRF).

Group A – Healthy Individuals

Samples from healthy participants were divided into three subgroups:

1. Subgroup A1 – Plain I-PRF: Fresh I-PRF applied directly onto agar plates inoculated with a standardized strain of *Enterococcus faecalis*.
2. Subgroup A2 – I-PRF + Triple Antibiotic Paste (TAP): Preparation of TAP: Ciprofloxacin (500 mg; *Arion Healthcare, Chandigarh, India*), Metronidazole (400 mg; *Abbott India Ltd., India*), and Minocycline (100 mg; *generic capsule available locally*) were crushed into fine powders using a sterile mortar and pestle. Equal weights were mixed (1:1:1 ratio), followed by the addition of sterile distilled water or vehicle (propylene glycol/macrogol) to obtain a homogenous paste.
3. Subgroup A3 – I-PRF + Modified TAP (mTAP): Preparation of mTAP: Prepared in the same manner as TAP, but Minocycline was replaced with Cefaclor (500 mg; *Health Biotech Ltd., India*). Equal weights of Ciprofloxacin (Arion Healthcare), Metronidazole (Abbott India), and Cefaclor (Health Biotech) were blended and combined with sterile vehicle to form a smooth, uniform paste.

Group B – Diabetic Individuals

The same protocol was followed for diabetic participants, ensuring methodological consistency.

1. Subgroup B1 – Plain I-PRF: I-PRF obtained from diabetic individuals was placed directly onto *E. faecalis* inoculated agar plates.
2. Subgroup B2 – I-PRF + TAP: TAP (Ciprofloxacin, Metronidazole, Minocycline; 1:1:1 ratio) was prepared as described above

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and mixed with I-PRF in 1:1 or 2:1 ratio before placement on the agar plates.

3. Subgroup B3 – I-PRF + mTAP: mTAP was prepared by substituting Cefaclor for Minocycline (Ciprofloxacin, Metronidazole, Cefaclor; 1:1:1 ratio), then mixed with I-PRF in 1:1 or 2:1 ratio and applied to the agar plates.

Antimicrobial Testing: For both groups, agar plates inoculated with *E. faecalis* were divided and labelled according to subgroup. Plates were incubated at 37°C for 24 hours under anaerobic conditions. Following incubation, the zones of inhibition were measured in millimetres to evaluate and compare the antimicrobial efficacy of plain I-PRF, I-PRF + TAP, and I-PRF + mTAP between healthy and diabetic individuals.

4. Results

After 24 hours of incubation clear zones of inhibition were observed around the platelet concentrates. The mean widths for the zones of inhibition for I- prf on the agar plates inoculated with e-faecalis.

As described in Table 1, among normal subjects, mean zones of inhibition were 3.56 mm for I-prf, 4.6 mm for I-prf +TAP, and 5.02 mm for I-prf +mTAP. One-way ANOVA showed a highly significant difference between groups ($F = 67.792$, $p < 0.001$), and Tukey's post hoc test confirmed significant pairwise differences, with the I- prf +mTAP group exhibiting the highest antimicrobial activity.

As described in Table 2, in diabetic subjects, the mean zones of inhibition were 2.84 mm for I- prf, 3.84 mm for I- prf +TAP, and 4.54 mm for I- prf +mTAP. A statistically significant difference was observed among groups ($F = 61.173$, $p < 0.001$), and post hoc analysis revealed enhanced antimicrobial effects with TAP and mTAP, although overall inhibition was lower compared to the normal group.

As described in Table 3, a comparison between normal and diabetic groups showed that all normal groups had significantly higher zones of inhibition than their diabetic counterparts ($p < 0.001$ for Group I, $p = 0.002$ for Group II, and $p = 0.009$ for Group III), indicating a reduced antimicrobial response in diabetic conditions.

5. Discussion

Intracanal medicaments are widely used to evaluate the effectiveness of root canal disinfection before completing treatment. In complex regenerative cases, such as guided endodontic repair or teeth with open apices, past formulations like Frank's paste and Grossman's poly-antibiotic paste have been recommended. [13]

With rising antibiotic resistance, interest is shifting to alternatives like platelet concentrates, valued in dentistry for their antimicrobial, regenerative, and healing properties. Promising results with PRF highlight its potential as a versatile aid in dental therapy. [14] Two common types used are Platelet-Rich Fibrin (PRF) and its injectable form, I-PRF. While both work on similar principles, I-PRF stands out because it can be easily combined with other materials, including intracanal medicaments. [15] While research in this area is still evolving, several studies have begun to investigate the properties of platelet concentrates such as PRP, PRF, and I-PRF and its contribution in management of large periapical lesions. [16] Interestingly, they've shown promise even in diabetic patients, where healing is often more complicated. [17]

Enterococcus faecalis is a key focus in endodontic research due to its persistence in root canals and its survival strategies, including expression of multiple survival genes and alternative pathways like pyrimidine biosynthesis.[18] In the present in-vitro study, we tested the antimicrobial action of I-PRF alone and when mixed with two types of antibiotic pastes—Triple Antibiotic Paste (TAP) and Modified TAP (MTAP)—against *E. faecalis*, using the zone of inhibition technique. Since diabetic patients are more prone to infections and re-infections, largely due to issues like impaired collagen, reduced blood circulation, and weaker immune cell responses, [19] it's especially important to evaluate how effective these intracanal medicaments are in such individuals.

bacteria. [2]tudies, have shown that I-PRF can offer antibacterial effects that improve over time, mainly due to the gradual release of proteins that kill bacteria.[20] This is likely due to the immune functions of platelets, which help guide white blood cells to the infection site and activate processes like phagocytosis—where harmful

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microbes are engulfed and destroyed.[21] These immune benefits may also come from proteins and peptides stored in platelet granules, along with components from the body's complement system. The antibacterial efficacy of PRF may prove beneficial when used in the revascularization procedure of immature necrotic teeth.[22]

The triple antibiotic paste (TAP: metronidazole, ciprofloxacin, minocycline) is effective against intracanal bacteria but may weaken dentin with prolonged use. A modified form (MTAP), substituting minocycline with cefaclor, is prepared by mixing equal parts of the three antibiotics in distilled water (1 mg/mL).[23]

This study aimed to test whether mixing I-PRF with TAP or MTAP would improve their antimicrobial effects. Since I-PRF already has some antibacterial properties due to the proteins it carries, combining it with antibiotics could create a stronger effect and possibly reduce the risk of bacterial resistance. [24] As hypothesized, the mixtures of I-PRF with TAP and MTAP showed significantly better antimicrobial performance. Although we still don't fully understand the mechanism, it might be related to I-PRF's ability to promote blood vessel growth (angiogenesis), which could help deliver antibiotics more effectively to the target area. [20]

Recent studies highlight TAP's use in scaffolds and I-PRF mixtures for disrupting biofilms of *A. naeslundii* and *E. faecalis*, a key step in successful root canal therapy. Low-dose, localized delivery through scaffolds appears effective with minimal stem cell toxicity, and emerging approaches include bioresorbable scaffolds, antibiotic-loaded fibers, and I-PRF for endodontic regeneration. [14]

MTAP with I-PRF achieved the greatest antibacterial effect against *E. faecalis*, likely due to the combined actions of ciprofloxacin (DNA disruption in gram-positive and gram-negative bacteria), metronidazole (DNA damage in anaerobes), and cefaclor (cell wall inhibition), producing a synergistic effect further influenced by bactericidal–bacteriostatic interactions. [8]

People with diabetes face roughly twice the risk of bacterial infections due to impaired innate and adaptive immunity in hyperglycemic conditions. In this study, I-PRF from diabetic donors showed slightly reduced antibacterial activity with TAP or MTAP compared to non-diabetic I-PRF, likely reflecting lower platelet counts. Nonetheless, diabetic I-PRF remained beneficial by providing metabolic support to healing cells, forming a fibrin barrier against bacterial invasion, and releasing growth factors that promote tissue repair.

Conclusion

This is the first comparative study which states that I-PRF from diabetic donors had slightly reduced antibacterial activity with TAP and MTAP against *E. faecalis* compared to non-diabetic I-PRF yet still demonstrated encouraging regenerative potential. Larger, long-term studies are needed to confirm these benefits and define its role in endodontic therapy, particularly for patients with systemic conditions like diabetes.

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Table 1: Comparison of zone of inhibition in Group A (Normal Patients Blood)

Normal Subjects	Mean	SD	One-way Anova F test	P value
Group AI (I- prf)	3.56	0.23	F =67.792	p<0.001**
Group AII (I- prf +TAP)	4.6	0.25		
Group AIII (I- prf +MTAP)	5.02	0.083		
Tukey's post hoc test to find pairwise comparison				
Group	Comparison Group		Mean Difference	P value
Group AI (I- prf) vs	Group AII (I- prf +TAP)		1.04	p<0.001**
	Group AIII (I- prf +MTAP)		1.46	p<0.001**
Group AII (I- prf +TAP)	Group AIII (I- prf +MTAP)		0.42	p =0.018*

p>0.05 – no significant difference *p<0.05 – significant **p<0.001 – highly significant

Table 2: Comparison of zone of inhibition in Group B (Diabetic)

Diabetic	Mean	SD	One-way Anova F test	P value
Group BI (I- prf)	2.84	0.11	F = 61.173	P<0.001**
Group BII (I- prf +TAP)	3.84	0.27		
Group BIII (I- prf +MBAP)	4.54	0.3		
Tukey's post hoc test to find pairwise comparison				
Group	Comparison Group		Mean Difference	P value
Group BI (I- prf) vs	Group BII (I- prf +TAP)		1.0	p<0.001**
	Group BIII (I- prf +MTAP)		1.7	p<0.001**
Group BII (I- prf +TAP)	Group BIII (I- prf +MTAP)		0.7	p=0.002*

p>0.05 – no significant difference *p<0.05 – significant **p<0.001 – highly significant

Table 3: Comparison between Group A(Normal) subjects and Group B (Diabetic) subjects in relation to zone of inhibitions (in mm)

	Group A (Normal)	Group B (Diabetic)	Unpaired t test	P value, Significance
Group I (I- prf)	3.56 (0.23)	2.84 (0.11)	t = 6.267	p < 0.001**
Group II (I- prf +TAP)	4.6 (0.25)	3.84 (0.27)	t =4.575	P =0.002*
Group III (I- prf +MTAP)	5.02 (0.083)	4.54 (0.3)	t = 3.394	p =0.009*

p>0.05 – no significant difference *p<0.05 – significant **p<0.001 – highly significant

Graph 1. Comparison between Group A (Normal) subjects and Group B (Diabetic) subjects in relation to zone of inhibitions (in mm)