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RESEARCH ARTICLE

A NOVEL PROTOCOL FOR ENHANCING THE ANTIMICROBIAL EFFICACY OF PLATELET-RICH FIBRIN: AN IN VITRO STUDY

Sowmya Ila[1], Hema Duddukuri[2]*, Surya Prasanna Jammula[3], Mounika Gundapu[4], Gayathri Muralidaran[5], Ramanarayana Boyapati[6]

[1]Bachelor of Dental Surgery, Mamata Dental College, Khammam, Telangana, India.

[2]Assistant Professor, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India.

[3]Professor, Department of Periodontics, Panineeya Institute of Dental Sciences & Research Centre, Hyderabad, Telangana, India.

[4]Dental Assistant Surgeon, Government General Hospital, Warangal, Telangana, India.

[5]Associate Professor, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India.

[6]Professor, Department of Periodontics, Sibar Institute of Dental Sciences, Takkellapadu, Guntur, Andhra Pradesh, India.

Address for correspondence: Dr. Duddukuri Hema, Assistant Professor, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India. **Contact number:** (+91) 8008486222
E-mail address: hemaduddukuri@outlook.com

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Abstract

Background: Since its inception, platelet-rich fibrin (PRF) has undergone numerous modifications to obtain a biomaterial capable of promoting regeneration. Given its limited antimicrobial activity, research is now being carried out to enhance PRF's antimicrobial efficacy. In this regard, this study aimed to enhance the antimicrobial efficacy of PRF by immersing it in antibiotic solutions.

Materials and methods: PRF membranes procured from 10 blood samples were equally divided into 3 parts and allocated into Group I (PRF alone), Group II (PRF immersed in 5mg/ml Tetracycline hydrochloride solution), and Group III (PRF immersed in 12mg/ml Amoxicillin-Potassium clavulanate solution). All samples were tested for antimicrobial efficacy following 24, 48, and 96 hours incubation.

Results: Group I showed no antimicrobial activity. The mean values and standard deviations of zones of inhibition of the groups II and III were 19.5(2.06)mm and 20.8(2.52)mm respectively, following 24 hours of the incubation period. This had slightly declined to 19.1(2.18)mm (Group II) and 20.3(1.94)mm (Group III), after 48 hours. Bacterial growth was detected in all the groups after 96 hours. The antimicrobial activity differed significantly between groups I & II (P = 0.000) and I & III (P = 0.000) following 24, and 48 hours of incubation periods.

Conclusion: Antibiotic-immersed PRF exhibited improved antimicrobial efficacy sustained for 48 hours. This enhancement could potentially reduce the need for postoperative systemic antibiotics in regenerative procedures by creating a bacteria-free environment. As a result, this method may facilitate uneventful wound healing and desired regeneration while mitigating the adversities of systemic antibiotic therapy.

Introduction

Platelet-rich fibrin (PRF), a fibrin bio-scaffold entrapped with blood cells-primarily platelets and various biological mediators such as growth factors and cytokines, is being used extensively in the field of regenerative medicine.[1]

PRF has the potential to promote wound healing, angiogenesis, and also modulates host inflammatory and immune responses with the release of growth factors and cytokines.[2] However, the antimicrobial activity of PRF remains debatable over years. In an invitro study, Platelet-rich plasma (PRP) showed antimicrobial activity against P. gingivalis and A. actinomycetemcomitans but not PRF.[3] Karde et al.[4] found that among injectable platelet-rich fibrin (i-PRF), PRP and PRF, i-PRF had greater antimicrobial efficacy followed by PRF and PRP.

Various techniques have been developed and studied by researchers to amplify the antibacterial efficacy of PRF. Bahaa et al.[5] incorporated antibiotics in powder, ampoule, and solution forms into blood samples before centrifugation. In another study, antibiotics were given orally one hour before blood was drawn for PRF preparation.[6]

Simonpieri et al.[7] used a mixture of bone graft and PRF soaked in metronidazole solution for sinus augmentation. Rafiee et al.[8] compared the immersion and integration approaches to improve i-PRF's antimicrobial efficacy. More recently, a randomized split-mouth trial injected antibiotic solutions into the PRF clot and evaluated its effects on post-extraction socket healing. [9]

The type of technique employed for improving PRF's antimicrobial efficacy was shown to affect its structural properties as well as the duration of antimicrobial activity obtained. In this context, the present study aims to enhance the antimicrobial efficacy of PRF by immersing the PRF membrane in antibiotic solutions and evaluated its antimicrobial activity using the agar diffusion method.

Materials and Methods

Study design and participants: An in vitro experimental study was carried out in a teaching dental hospital located in Hyderabad, India. Volunteers who were aged between 20-30 years, systemically healthy, who were not using any medication within the past 3 months, and who were willing to consent and participate in the study were included in the study. Smokers, pregnant and lactating women, and those with diminished autonomy were excluded. Ethical approval was obtained from the Institutional ethics review Board (PMVIDS&RC/IEC/PERIO/PR/0277-18).

Materials used: The antibiotics used for the study were Tetracycline hydrochloride (Resteclin 500mg, Abbott Healthcare Pvt. Ltd., India) and Amoxicillin-Potassium clavulanate (Clavam 1.2g, Alkem Laboratories LTD, India). A total of 30 samples of PRF were obtained from 10 eligible volunteers, who provided a written informed consent.

The PRF procured from each patient was equally spilt into three parts and used in all three groups, categorized based on the antibiotic solution added to PRF:

- **Group I:** PRF alone (n=10)
- **Group II:** PRF immersed in Tetracycline hydrochloride solution (n=10)
- **Group III:** PRF immersed in Amoxicillin-Potassium clavulanate solution (n=10)

Methodology:

Platelet-rich fibrin preparation: 5 ml of intravenous blood (antecubital site) was collected into a glass test tube and centrifuged for 10 min at 3000 rpm. After centrifugation, the PRF clot obtained was separated from the RBC base and squeezed to form a membrane.

Addition of antibiotics to PRF: The PRF membrane was equally split into three parts, two of which were immersed in the antibiotic solutions for 60 seconds before antibiotic sensitivity testing. Amoxicillin-Potassium clavulanate (Clavam 1.2g) and Tetracycline hydrochloride (Resteclin 500 mg) solutions were prepared by mixing the powder with 100 ml of distilled sterile water to obtain a concentration of 12mg/ml of Amoxicillin-Potassium clavulanate and 5mg/ml of Tetracycline hydrochloride solutions.[10]

Plaque collection: Using a sterile curette, a sample of supragingival plaque was taken from the patients, transferred to saline, vortexed for five minutes to create a homogeneous suspension, and then incubated. Later, 0.1 ml of this suspension was used to inoculate blood agar using the lawn streaking method for evaluation of the antimicrobial activity.

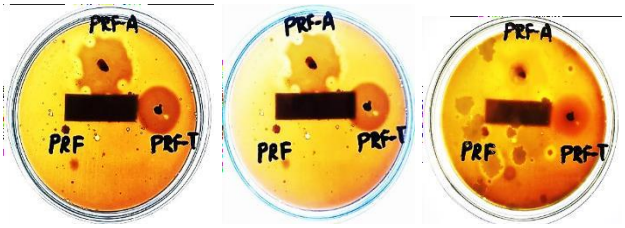
Evaluation of antimicrobial activity: The antimicrobial activity was assessed using the agar diffusion method. PRF membrane immersed in antibiotic solutions (group II & III) and PRF membrane alone (group I) were placed on the inoculated blood agar plate and incubated aerobically at 37°C. Later, the clear zones of inhibition produced on blood agar following incubation at 24, 48, and 96 hours intervals were measured. The same procedure was followed for all the samples.

Statistical analysis: The data obtained were analyzed using the SPSS V.22 (Statistical Package for Social Science, IBM, 2009). Descriptive statistics were used to estimate the mean and standard deviations of the measured zones of inhibition for each group, and one-way ANOVA test with post-hoc

Tukey HSD (Highly significant difference) test was employed to determine the statistical significance of the observed results.

Results

Throughout all of the tested time intervals, group I did not exhibit a zone of inhibition (0 mm). However, groups II & III displayed mean zones of inhibition of 19.5 (2.06) mm and 20.8 (2.52) mm respectively, following incubation for 24 hours (Fig. 1(a)). However, after 48 hours, the observed values of zones of inhibition had decreased slightly for groups II (19.1 (2.18) mm) and III (20.3 (1.94) mm) (Fig. 1(b)). Following a 96-hour incubation period, bacterial growth was detected in all the groups (Fig. 1(c)).



a)After 24hrs b)After 48hrs c)After 96hrs

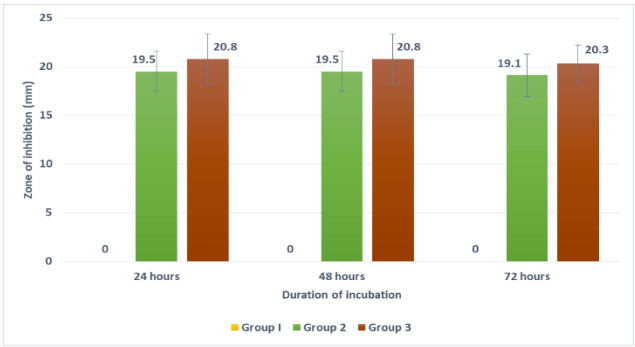
Figure-1. Zones of inhibitions produced by PRF (Group I) and PRF immersed in antibiotic solutions (Groups II & III) following 24, 48, and 96 hours incubation period

The mean values and standard deviations of the measured zones of inhibition of all the groups following incubation after 24 and 48 hours were represented in Table 1 & Graph 1.

Table 1. Mean values and standard deviations (SD) for the measured zones of inhibitions following incubation after 24 and 48 hours (n=10)

Groups	After 24 hours (mm)	After 48 hours (mm)
Group I	0	0
Group II	19.5 ± 2.06	19.1 ± 2.18
Group III	20.8 ± 2.52	20.3 ± 1.94

Graph 1. Graphical representation of mean and standard deviations for the measured zones of inhibitions following incubation after 24 and 48 hours



Intragroup analysis of zones of inhibition assessed after 24 and 48 hours for groups II (P = 0.67) and III (P = 0.62) revealed no statistically significant difference (Table 2).

Table 2. Intragroup comparison of zones of inhibition measured at 24 and 48 hours of incubation

Groups	Source of Variation	SS	F	P-value
Group II	Between Groups	1.06	0.12	0.88
	Within Groups	119.9		
	Total	120.9		
Group III	Between Groups	1.66	0.15	0.86
	Within Groups	149.3		
	Total	150.9		

Note: Test employed: one-way ANOVA; P < .05 is set as significant and P < .001 as highly significant.

After 24 and 48 hours, an intergroup comparison showed a highly statistically significant difference between groups I and II (P = 0.000) & groups I and III (P = 0.000). However, no significant difference was observed between groups II & III after 24 (P = 0.28) and 48 hours (P = 0.26). (Tables 3 and 4).

Table 3. Intergroup comparison of zones of inhibition

Duration of incubation	Source of Variation	SS	F	P-value
After 24 hours	Between Groups	2715.2	381.43	0.0000*
	Within Groups	96.1		
	Total	2811.3		
After 48 hours	Between Groups	2715.2	381.43	0.0000*
	Within Groups	96.1		
	Total	2811.3		
After 72 hours	Between Groups	2594.4	454.87	0.0000*
	Within Groups	77		
	Total	2671.4		

Note: Test employed: one-way ANOVA; P < .05 is set as significant and P < .001 as highly significant. * indicates P <0.001.

Table 4. Post Hoc Tukey HSD test for pairwise comparison of the groups

Duration of incubation	*Pairwise Comparisons*	$HSD_{.05} = 2.09$ $HSD_{.01} = 2.68$	$Q_{.05} = 3.50$ $Q_{.01} = 4.49$
After 24 hours	G_1:G_2	19.50	$Q = 32.69$ ($p = .00000$) *
	G_1:G_3	20.80	$Q = 34.86$ ($p = .00000$) *
	G_2:G_3	1.30	$Q = 2.18$ ($p = .28835$)
		$HSD_{.05} = 2.09$ $HSD_{.01} = 2.68$	$Q_{.05} = 3.50$ $Q_{.01} = 4.49$
After 48 hours	G_1:G_2	19.50	$Q = 32.69$ ($p = .00000$) *
	G_1:G_3	20.80	$Q = 34.86$ ($p = .00000$) *
	G_2:G_3	1.30	$Q = 2.18$ ($p = .28835$)
		$HSD_{.05} = 1.87$ $HSD_{.01} = 2.40$	$Q_{.05} = 3.50$ $Q_{.01} = 4.49$
After 72 hours	G_1:G_2	19.10	$Q = 35.77$ ($p = .00000$) *
	G_1:G_3	20.30	$Q = 38.01$ ($p = .00000$) *
	G_2:G_3	1.20	$Q = 2.18$ ($p = .26756$)

Note:Test employed: Post Hoc Tukey HSD (honestly significant difference); * indicates $P < 0.001$. G_1: Group I, G_2: Group II, G_3: Group III.

Discussion

Research assessing the antimicrobial efficacy of PRF has disclosed that it has less potential than other platelet concentrates such as PRP[3,7] and i-PRF.[4] This was speculated to be due to PRF's lower platelet and leukocyte concentrations compared to other platelet concentrates.[11] Mamajiwala et al.[12] observed that lower centrifugation time and speed increased the antimicrobial efficacy with increased platelets in the obtained PRF. Ensuring a bacteria-free environment is vital for an uneventful wound healing. With the controlled release of growth factors over time PRF aids in wound healing and regeneration.[13] Nevertheless, its weak antimicrobial activity may not be enough to prevent the emergence of post-operative infections. Enhancing PRF's antimicrobial efficacy is currently the topic of interest as it can control the infection and concurrently overcome the downsides of systemic antibiotic therapy while facilitating regeneration.

Now the question is, how to augment the antimicrobial activity of the PRF without compromising its regenerative potential? The present study attempted to enhance the antimicrobial efficacy by immersing the PRF membrane in antibiotic solutions for 60 seconds. The study found no antimicrobial activity for PRF alone (Group I), which was in accordance with the findings of Badade et al.[3] However, other in vitro studies detected minimal antimicrobial activity for the PRF membrane.[4,14] This might be due to the differences in the size of the PRF membrane as well as the bacterial strains used for antibiotic sensitivity testing which could influence the zones of inhibition detected. In the present study, the PRF membrane obtained from a patient was split into three equal parts and allocated into three groups to prevent sampling bias.

PRF immersed with antibiotic solutions (Groups II & III) demonstrated significant antimicrobial activity after 24 hours. This was observed to have declined non-significantly upon 48 hours of incubation. The enhanced antimicrobial activity was lost by the end of 96 hours with the detection of bacterial growth. On the contrary, when antibiotics were systemically administered to the patient before drawing the blood sample, the antimicrobial efficacy lasted for 24 hours.[6]

However, the incorporation of antibiotics into the blood sample prior to centrifugation resulted in different drug release profiles i.e. 3 days, 4 days, and 10 days as demonstrated by Siawasch et al.,[15] Polak et al.,[14] and Bahaa et al.[5] respectively.

Rafiee et al.[8] compared the drug release profile of i-PRF loaded with a triple antibiotic mixture using: immersion (antibiotic added to the i-PRF sample and shook at 150 rpm for one hour) and integration (antibiotic added to blood sample before centrifugation). Their findings revealed that the immersion approach produced an initial burst of drug release in the first 24 hours that lasted for up to 14 days, whereas the integration method produced an initial burst of drug release in the first 24 hours followed by a gradual diminution with no further release beyond 7 days.

The following are some of the possible limitations of the existing protocols proposed to enhance the antimicrobial activity of PRF: use of systemic antibiotics may have adverse effects and has been shown to have antimicrobial activity preserved for a shorter duration,[6] injecting antibiotic solutions into the PRF clot may affect its physical characteristics making it difficult to use as a membrane.[9]

The physical properties, fibrin formation, and growth factor release from PRF were shown to be influenced by the form, concentration, and volume of the antibiotic solutions incorporated into the blood sample before centrifugation.[13,16] Furthermore, there is a possibility of the destruction of the drugs that are incorporated into the blood sample during the centrifugation.[8] Although the addition of antibiotics to the i-PRF and centrifugation for one hour resulted in extended drug release for 14 days, platelet concentrates were transferred to the surgical area as early as possible due to the release of growth factors. The effect of centrifugation of fabricated PRF on platelet activation and growth factor release needs to be investigated. Immersion of the PRF membrane in

antibiotic solutions, on the other hand, may not modify its physical properties while increasing antibacterial activity comparable to that achieved by adding antibiotics prior to centrifugation. However, the effect of immersing PRF in antibiotic solutions on the release of growth factors has to be investigated further.

The clinical applications of PRF mixed with antibiotics were previously demonstrated with favorable therapeutic outcomes.[7,9,16,17] However, the type of antibiotics, concentrations (dosage), and the form of the drug (powder, solution, and ampoule) that are effective and safe for inclusion in PRF must be studied further. Interestingly, Egle et al[18] observed that clindamycin phosphate was hydrolyzed to clindamycin, a more active form with greater antibacterial potential when mixed with i-PRF. Therefore, it is also crucial to assess the effect of PRF on the drugs incorporated. All the existing methods must be compared to develop a standardized and effective protocol for enhancing the PRF's antimicrobial efficacy. These techniques must be evaluated in terms of safety, feasibility, clinical applicability, duration of antimicrobial activity attained, and the structural and biological changes produced in the PRF.

Conclusion

Within the study limitations, the findings indicate that immersion of the PRF membrane in antibiotic solutions can enhance its antimicrobial efficacy for at least 48 hours without affecting its physical characteristics. These findings should be substantiated further by conducting well-designed experiments with a larger sample to assess the possible biological alterations in PRF caused due to immersion in antibiotic solutions. The study points out the necessity for developing an effective and standardized protocol to enhance the antimicrobial efficacy of PRF without impeding its regenerative potential.

Clinical relevance of the study: This in vitro study demonstrated that immersing PRF membrane in antibiotic solutions improved its antimicrobial activity, implying its potential to be used as a bio carrier of antibiotics. In clinical scenarios, this enables both tissue regeneration along with an uneventful wound healing by preventing anticipated post-operative infections.

Declaration

Conflict of interest and financial disclosures

The authors declare no conflict of interest and there was no external source of funding.

Ethical approval & consent to participate Obtained

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