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Original Article

Dentistry Section

Pharmacokinetic Evaluation of Injectable Platelet-rich Fibrin Containing Metronidazole as a Local Drug Delivery Agent in Periodontal Therapy: An In-vitro Study

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ABSTRACT

Introduction: Periodontitis is a complex disease influenced by microorganisms, systemic factors and environmental factors. Traditional treatments like scaling often fail in moderate to severe cases due to limited access to deep pockets. Local Drug Delivery (LDD) systems, such as films and gels, offer higher drug concentrations at infection sites but are flushed out by saliva and Gingival Crevicular Fluid (GCF). Injectable Platelet-rich Fibrin (i-PRF) provides a slow, sustained release of metronidazole, enhancing the outcomes of periodontal therapy.

Aim: To evaluate the possibility of using i-PRF as a controlled release drug vehicle in periodontal therapy.

Materials and Methods: This study was an in-vitro analysis conducted at the Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India, in September 2024. A patient with a chief complaint of food lodgement and periodontitis was selected and blood was collected for further analysis. A drug stock solution was prepared by mixing 10 mg of metronidazole in 10 mL of deionised water, which was vortexed for three minutes to achieve a final concentration of 1 mg/mL. The i-PRF was prepared. The top PRF layer was aspirated into a 2 mL syringe and maintained in liquid consistency for about 10-15 minutes until it clotted through the slow polymerisation of the fibrin structure. Samples were collected at the 1st, 3rd, 5th and 7th hours and were repeated on the 3rd, 5th, 7th, 9th and 14th days, followed by spectrophotometric analysis.

Results: In the present study, the spectrophotometric analysis indicated the presence of the drug in all samples taken over the period from one hour to the 14th day. Initially, the concentration of the eluted samples showed a gradual decline between the 1st and 7th hours. A pronounced and rapid decrease in drug concentration was observed up to the 3rd day and drug elution continued steadily until the 14th day.

Conclusion: The i-PRF shows promise and can be effectively used as an LDD system in periodontal therapy. Its favourable characteristics, such as its syringeable form, which starts as a liquid and later transforms into a gel, make it an ideal candidate for precise application. Moreover, being composed of autologous fibrin, i-PRF can be directly delivered into the periodontal pocket, where it adapts to the shape of the pocket and adheres to both soft and hard tissues. This flexibility of i-PRF improves the retention of drug-loaded i-PRF in the restricted environment of the periodontal pocket, ensuring prolonged contact and efficacy of the treatment.

Keywords: Drug delivery systems, Drug elution, Periodontal pockets, Spectrophotometer

INTRODUCTION

Periodontitis is a complex disease affecting the periodontium, characterised by structural damage caused by specific microorganisms and influenced by factors such as tooth developmental issues, systemic conditions impacting oral tissues, socioeconomic status and environmental factors. Successful periodontal therapy depends on selecting the right antimicrobial agent and the appropriate drug delivery method. Traditional non surgical treatments, such as scaling and root planing, often fail to achieve disease remission. These methods can be time-consuming, challenging and sometimes ineffective, particularly for moderate to severe periodontitis, due to their inability to access deep periodontal pockets and the extent to which microorganisms infiltrate periodontal connective tissue [1]. These limitations underscore the need for innovative strategies that enhance drug delivery to specific sites of infection and inflammation.

Local Drug Delivery (LDD) systems are favored over systemic antibiotic therapy because by delivering drugs directly into the periodontal pocket, higher concentrations of the medication can be achieved locally compared to systemic administration. This can lead to more effective eradication of bacteria and reduction of inflammation [2]. Additionally, local delivery minimises systemic exposure, reducing the risk of adverse reactions and avoiding the unnecessary administration of large amounts of drugs, which can contribute to the development of antibiotic resistance. However, when using LDD agents alone, the continuous flow of gingival crevicular fluid (GCF) and saliva can clear the drug from the periodontal pocket [3]. This can result in the removal of the drug before it has had sufficient time to exert its therapeutic effect, limiting its duration of action.

Doxycycline-loaded Injectable Platelet-Rich Fibrin (i-PRF) has been studied for its slow release and sustained antibacterial effect in periodontal pockets [1]. Doxycycline can also help modulate the inflammatory response by reducing collagenase activity, thus improving tissue healing. Previous research has shown that doxycycline can be effectively delivered locally for extended periods, potentially reducing the need for systemic antibiotics [1].

Metronidazole is a potent antimicrobial agent useful for preventing periodontal infection due to its broad-spectrum activity, particularly against obligate anaerobes [4]. Metronidazole can be successfully administered in periodontal pockets in the form of LDD agents via various vehicles such as films, sponges, gels and fibres. The use of these agents can lead to the release of antibiotics over different durations of time [1]. Literature suggests that metronidazole, when

used in different forms of drug delivery systems, is found to be effective against periodontal disease when administered locally rather than systemically [5].

Research has been conducted on the local delivery of drugs at periodontally infected sites, offering controlled release of the medication. An autologous and injectable vehicle such as injectable Platelet-Rich Fibrin (i-PRF) could serve as a suitable novel vehicle for LDD [6]. Introduced Platelet-Rich Fibrin (PRF), which is a second-generation platelet concentrate. i-PRF is based on alterations in centrifugation protocols and G-forces [7]. i-PRF is prepared using low-speed centrifugation, transforming from a liquid to a gel form after approximately 10-15 minutes, preserving platelets, leukocytes and growth factors for slow and sustained release.

The aim of the present study was to evaluate the possibility of using i-PRF as a controlled release drug vehicle in periodontal therapy. The present study combined metronidazole with i-PRF, leveraging metronidazole's antimicrobial effect along with i-PRF's growth factors and regenerative properties. The anti-inflammatory and regenerative effects of i-PRF, combined with the antimicrobial action of metronidazole, might provide a synergistic effect, helping not only to control the infection but also to enhance the healing process in periodontal therapy.

MATERIALS AND METHODS

The present study was an in-vitro analysis conducted at the Department of Periodontology, Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India, in September 2024. Blood (10 mL) was collected from a patient with a chief complaint of food lodgement and further analysis was conducted. The study received approval from the Institutional Ethical Committee (EC/NEW/INST/2021/MH/0029).

Inclusion criteria: Dental graduate students aged 20 to 22 years reported to the Department of Periodontology with a chief complaint of food lodgement in the gums. Those who expressed willingness to donate 10 mL of blood were recruited as blood donors for the preparation of i-PRF and for the study.

Exclusion criteria: Exclusion criteria included a history of bleeding episodes, any illnesses such as diabetes, bleeding disorders, cardiovascular diseases and the use of medications such as antibiotic prophylaxis within the last six months. Additionally, individuals who had donated blood within the past three months were excluded from the study.

Study Procedure

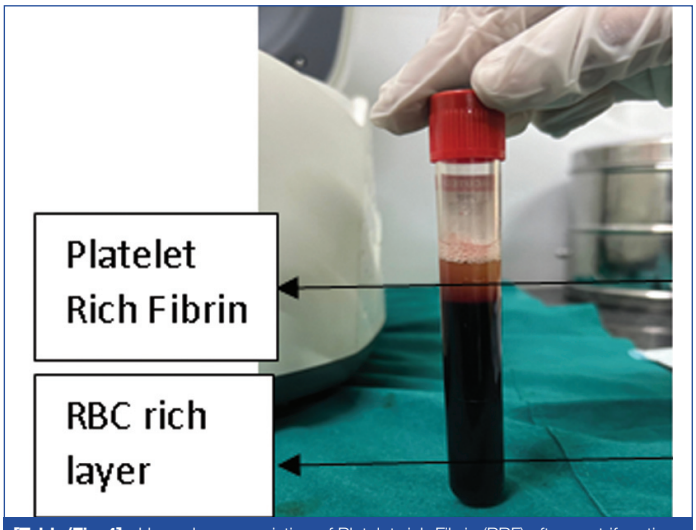
A drug stock solution was prepared by mixing 10 mg of metronidazole in 10 mL of deionised water and vortexing it for 3 minutes to achieve a final concentration of 1 mg/mL [1]. The i-PRF was prepared according to the protocol developed by Fujioka-Kobayashi M et al., in 2017 [7,8]. The top PRF layer was aspirated into a 2 mL syringe and maintained in liquid consistency for about 10-15 minutes until it clotted through the slow polymerisation of the fibrin structure. Samples were collected at the 1st hour, 3rd hour, 5th hour and 7th hour and were repeated on the 3rd, 5th, 7th, 9th and 14th days, followed by spectrophotometric analysis.

Preparation of the drug stock solution: The study used analytical-grade metronidazole obtained from Sigma-Aldrich Limited. A stock solution of metronidazole was prepared manually by dissolving 10 mg of the drug in 10 mL of demineralised water and vortexing for three minutes to achieve a final concentration of 1 mg/mL [Table/Fig-1] [9]. The solution was freshly prepared before the study began and kept at 2-5°C, away from light, until it was used.

Collection of i-PRF [10]: A 10 mL sample of intravenous blood was collected from the median cubital vein of the volunteer through venipuncture, performed under sterile conditions [Table/Fig-2]. The collected blood was transferred to a plain sterile test tube without anticoagulants and immediately centrifuged in a Duo Quattro Advanced PRF Centrifuge at a force of 70 g (700 rpm) for three minutes [Table/Fig-3] [7]. After centrifugation, the blood separated into two layers: the bottom layer containing red blood cells and the top layer comprising liquid PRF plasma [Table/Fig-4]. The top layer was then aspirated into a 2 mL syringe [Table/Fig-5].

[Table/Fig-1]: Drug stock solution [9].

[Table/Fig-2]: Collection of blood for making i-PRF.
[Table/Fig-3]: Duo Quattro advanced PRF centrifuge [7]. (Images from left to right)



[Table/Fig-4]: Upper layer consisting of Platelet-rich Fibrin (PRF) after centrifugation at 700 rpm for 3 min.

Preparation of drug-loaded i-PRF: Before collecting and centrifuging the blood, 200 microliters of the drug solution were dispensed into the bottom of a microtube vial. Following centrifugation, 2 mL of i-PRF was added to the drug-containing microtube and vortexed for

[Table/Fig-5]: i-PRF was added to the drug-containing microtube.

10 seconds to ensure even distribution of the drug within the i-PRF [Table/Fig-5]. The mixture was then allowed to gel as a result of the natural fibrin polymerisation process in the i-PRF [Table/Fig-6].

[Table/Fig-6]: Conversion of liquid into gel form after 10-15 min.

Pharmacokinetic evaluation by spectrophotometric analysis: The in-vitro release data were kinetically analysed to establish the kinetics of drug release. Model fitting was performed using Swiss ADME, a free software tool. Zero-Order, First-Order, Higuchi, Hixon-Crowell, Korsmeyer-Peppas and Weibull models were tested. The results indicated that all the i-PRF samples followed a first-order mechanism of drug release.

The drug-loaded i-PRF gel was divided into three parts to avoid any potential sample deterioration [Table/Fig-7]. A vial containing 1 mL of Phosphate Buffered Saline (PBS) was prepared, into which the metronidazole-loaded i-PRF was added to initiate solvent exchange [Table/Fig-8].

At specified time points (1st hour, 3rd hour, 5th hour and 7th hour), 100 microliters of the drug-released PBS sample was withdrawn from the container and 100 microliters of PBS solution was added back into it. The withdrawn samples were preserved at -20°C until all the samples were collected. This procedure was repeated on the 3rd day, 5th day, 7th day, 9th day and 14th day, followed by spectrophotometric analysis to check the drug elution from the i-PRF preparation.

[Table/Fig-7]: Metronidazole enriched i-PRF gel divided into three equal parts.

[Table/Fig-8]: One part of metronidazole enriched i-PRF dispensed in 1 mL PBS solution.

The drug release kinetics of metronidazole from the i-PRF were evaluated in nine samples [Table/Fig-9], obtained at 1 hour, 3 hours, 5 hours, 7 hours, 3 days, 5 days, 7 days, 9 days and 14 days. The collected samples were diluted with PBS solution and analysed using a Ultraviolet (UV)-visible spectrophotometer [Table/Fig-10]

[Table/Fig-9]: Samples collected at specific time intervals 1, 3, 5 and 7 hours on 1st, 3rd, 5th, 7th, 9th, 14th day to analyse drug elution.

[Table/Fig-10]: UV Spectrophotometer.
(Thermo Fisher Scientific GENESYS 10S uv)

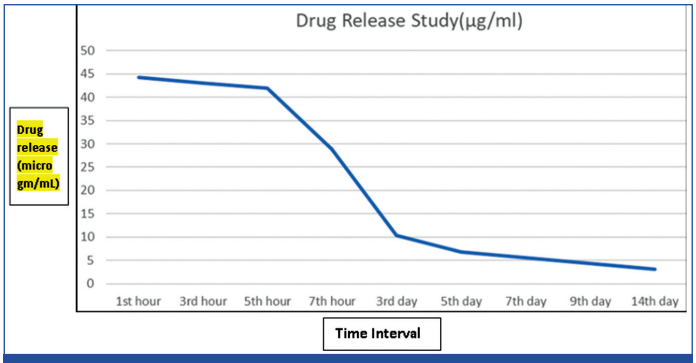
to detect and quantify the metronidazole eluted from the i-PRF. The analysis was conducted over a UV range of 190 to 1100 nm, with peaks indicating the presence of metronidazole observed at 278 nm [10]. Additionally, the total amount of metronidazole released was calculated using a standard curve of metronidazole in a 1:1 mixture of deionised water and distilled water [11].

RESULTS

In the present study, the spectrophotometric analysis indicated the presence of the drug in all samples collected over a period from 1 hour to 14 days [Table/Fig-11]. Initially, the concentration in the eluted samples showed a gradual decline between the 1st and 7th hours. A pronounced and rapid decrease in drug concentration was observed up to the 3rd day. Drug elution continued steadily up to the 14th day [Table/Fig-12].

Time interval	Mean drug concentration recovered (microg/mL)
1 hour	32.42±3.25
3 hour	31.24±2.72
5 hour	30.56±2.47
7 hour	19.31±1.83
3 day	7.01±0.88
5 day	4.75±0.71
7 day	3.60±0.31
9 day	1.94±0.10
14 day	1.01±0.02

[Table/Fig-11]: Spectrophotometric analysis.



[Table/Fig-12]: Drug elution.

DISCUSSION

Local Drug Delivery (LDD) in periodontal therapy involves the targeted administration of medications directly into the periodontal pocket to treat periodontal disease [12]. This approach aims to enhance treatment efficacy by delivering higher concentrations of therapeutic agents precisely where they are needed while minimising systemic side effects [13]. Commonly used drugs include antibiotics such as tetracycline and metronidazole, anti-inflammatory agents like corticosteroids and biologics that promote tissue regeneration. Delivery systems vary and can include gel-based formulations, microspheres, nanoparticles and resorbable membranes, each designed to release medication over time and improve targeting [14].

Kowshihan P et al., conducted a study in which chlorhexidine was used alongside i-PRF formulations to investigate the effects of both, finding that the controlled release profile of chlorhexidine from i-PRF shows its potential as a suitable vehicle for the LDD system in periodontal therapy [9].

Ram AJ et al., conducted a study in which they examined doxycycline-loaded i-PRF for its slow release and sustained antibacterial effect in periodontal pockets. They found that doxycycline can help modulate the inflammatory response by reducing collagenase activity, thus improving tissue healing. They demonstrated that doxycycline can be effectively delivered locally for extended periods, potentially reducing the need for systemic antibiotics [10].

The benefits of LDD are significant; it allows for increased concentrations of the drug at the infected site and reduces potential side effects by limiting systemic exposure. Additionally, it can enhance patient convenience by requiring fewer visits or procedures compared to traditional systemic treatments. LDD is particularly useful for patients with localised pockets that do not respond adequately to conventional scaling and root planing, serving as an effective adjunct to mechanical treatments [15]. However, its effectiveness may be limited by the depth of penetration within the pocket and patient adherence to oral hygiene practices. Overall, LDD represents a valuable tool in periodontal therapy, contributing to improved outcomes for patients suffering from periodontal disease.

Despite all the advantages of LDD, there are several limitations. For instance, when using LDD agents alone, the continuous flow of Gingival Crevicular Fluid (GCF) and saliva can clear the drug from the periodontal pocket. This can lead to the removal of the drug before it has had sufficient time to exert its therapeutic effect, thereby limiting its duration of action [16]. To address this, we evaluated the potential of i-PRF, which features a naturally formed 3-D fibrin structure, as a medium for drug delivery in periodontal treatment [17].

The inherent properties of i-PRF, such as its autologous nature, chair-side preparation in a short duration of time, initial syringeability and subsequent conversion into a gel-like consistency, help mould the i-PRF to the shape of the pocket while retaining and eluting the drug for up to 14 days [18,19].

The spectrophotometric analysis showed the presence of the drug in all samples at different time intervals. The synergistic effect of metronidazole and i-PRF provides a viable vehicle for LDD. Further in vivo studies with assessment of clinical parameters are warranted.

Limitation(s)

The study includes only one subject for the preparation of i-PRF, which significantly limits the generalisability of the results. A larger sample size is necessary to draw more reliable conclusions regarding the effectiveness of the metronidazole-loaded i-PRF in different individuals and various clinical conditions. Additionally, the study does not address the long-term release and effectiveness of the drug. The potential for extended antimicrobial effects or the stability of the drug delivery system over time (e.g., after 3-6 months) remains untested.

CONCLUSION(S)

The i-PRF demonstrates promising and effective potential as a delivery system for LDD in periodontal therapy. Its favourable

characteristics, such as its syringeable form, which starts as a liquid and later transforms into a gel, make it an ideal candidate for precise application. Moreover, being composed of autologous fibrin, i-PRF can be directly delivered into the periodontal pocket, where it adapts to the shape of the pocket and adheres to both soft and hard tissues. This adaptability improves the retention of drug-loaded i-PRF in the periodontal pocket's restricted environment, ensuring prolonged contact and efficacy of the treatment.

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