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# Use of injectable platelet rich fibrin (IPRF) as LDD vehicle containing doxycycline in periodontal Therapy – In vitro study

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## ABSTRACT

**Purpose:** Local drug delivery systems (LLD) are preferred over systemic antibiotic therapy in indicated cases to avoid unnecessary large doses of drug, development of antibiotic resistance etc. The search for the more suitable novel vehicle for the local drug delivery that can render controlled release of drug at periodontally infected sites are widely researched. i-PRF being autologous and injectable could be a suitable vehicle for local delivery of drugs. This study aims to evaluate the possibility of using i-PRF as a controlled release drug vehicle in periodontal therapy.

**Methods:** i-PRF obtained from centrifugation of 10 ml of blood collected from volunteers are mixed with known concentration of doxycycline drug and allowed to become a gel. The drug loaded gel is dispensed in artificial saliva and allowed to degrade. At specific time intervals (1 hr, 3 hr, 5 hr, 7 hr, 3d, 5d, 7d, 9d, 14d) aliquot of the 200 microliter were collected from each sample and subjected for spectrophotometric analysis.

**Results:** The spectrophotometric results show that the drug was detected in all the samples obtained from the 1 hr to the 14th day. Final concentration in the eluted samples seem to be gradually reducing from the 1 hr to the 7 th hour and a steep downward pattern in the concentration was absorbed from the 3 rd day until 14th day.

**Conclusions:** The controlled drug release profile of the i-PRF shows its a potential and suitable vehicle for LDD system in periodontal therapy. Additionally, properties like the syringeability, in-situ gel formation, and autologous fibrin nature may facilitate its direct delivery into the periodontal pocket, getting moulded to the pocket shape with attachment to the soft and hard tissue thereby ensuring the retention of the drug loaded i-PRF in the confined pocket environment.

**Keywords:** *Platelet-Rich Fibrin; Drug Delivery Systems*

## INTRODUCTION

Periodontitis is an inflammatory disease of the supporting structures of the teeth which is considered as a multifactorial origin.[1] However, polymicrobial biofilm is required for the initiation of the disease resulting in an host microbial interaction leading to destruction of the host tissue including alveolar bone, cementum, and periodontal ligament.[2]

Conventional periodontal therapy targets at eliminating these polymicrobial biofilms by mechanical therapy,[3] however, there is evidence of residual microbes like P Gingivalis still present in the connective tissue of the periodontal pockets due to infiltration capacity of periodontal pathogens.[4]

Thus adjunct periodontal therapies like use of systemic antibiotics were later inducted to eliminate these residual periodontal pathogens after mechanical therapy and has shown improved clinical results.[5,6] Besides these clinical benefits, the possibility of occurrence of antibiotic resistance and other side effects from oral dosage have necessitated the use of local drug delivery systems in periodontal therapy in indicated cases.[7,8]

Local drug delivery in periodontal therapy has been in practice for the past 3 decades in localised pockets with clinical & microbiological results comparable to that of adjunctive systemic antibiotic therapy.[9], [10] Additionally it has benefits of low dose of drug use, that is sufficient enough to attain the required minimal inhibitory concentrations (MIC) in periodontal tissue, less systemic effects, absence of resistance formation etc.[8]

However the use of conventional local drug delivery (LDD) systems like fibres, chips, films and gels has some drawbacks like, synthetic in origin, time consumed for the placement (Fibers), local inflammatory reaction to the degraded products, irritation to the gingiva, chances of displacement/ dislodgement from the site, need to be removed after the therapy expensive and unclear data about transient antimicrobial resistance.[11–13]

Advancements in LDD systems have advocated the use of novel (In-situ gel forming formulations) vehicles like hydrogels, polysaccharides, polymers that are liquid from, which then form strong gels after application at the delivery site, more biocompatible with sustained release nature.[13][14] In this context the search for vehicles that are biomimetic in nature which can provide a sustained release of drugs will be of greater advantage and injectable Platelet Rich Fibrin could be an viable option.[15]

Platelet-rich fibrin is a second generation platelet concentrate that has been introduced by Choukroun J et al in 2001.[16,17] Since then it has been widely used in oral, dental and periodontal applications for its accelerated healing capacity.[18–22] Accumulating data supports its beneficial effects due to its 3 dimensional fibrin matrix that has platelets and leukocytes entrapped within it and an enormous amount of growth factors released from these cells.[23] The fibrin matrix mimics a scaffold loaded with growth factors which gradually degrades resulting in sustained release of the content into the wound area.[24,25] Injectable platelet rich fibrin (i-PRF) is a recent development in the PRF family which has the added advantage of being available as a liquid consistency period of 10 - 15 mins after centrifugation and gets polymerised slowly to form a fibrin clot.[26] It can be injected into tissue for therapeutic purposes and has reported extended benefits.[27,28]

Various antimicrobial agents have been tested against control of periodontal infection during periodontal therapy.[29–33] But due to the polymicrobial nature of the periodontal infection the possibility of one antimicrobial agent that can target most of the pathogenic microorganisms in the plaque biofilm is preferred. Antimicrobials like amoxicilline,, metronidazole, ciprofloxacine etc have been widely advocated in the treatment of periodontal therapy however each of the mentioned drugs have their own limitations and disadvantages.

Doxycycline belongs to a broad spectrum of antimicrobial agents that are used for treating periodontal disease.[34], [35] doxycycline has the benefits of having anti collagenase effect in addition to the antimicrobial effect which could limit the collagen destruction seen in periodontal disease thus also having an additional host modulating effect.[36,37] Also it is the preferred drug of choice while treating periodontitis patients who are diabetic.[36]

Here we propose the possibility of i-PRF as a vehicle to locally deliver drugs into the

periodontal pockets for periodontal therapy. Thus, the aim of this study is to assess the use of a biomimetic substance (i-PRF) as a vehicle for delivery of doxycycline in periodontal therapy.

## MATERIALS AND METHODS

The study was designed as an initial in-vitro study and was approved by the institutional ethical committee. Dental graduate students with an age range of 20 - 22 years in the university hospital who were willing to participate were recruited into study as blood donors for i-PRF preparation. The inclusion criteria were subjects who are systemically healthy and willing to donate 10 ml of blood. Exclusion criteria were the presence of history of bleeding episodes, any illness in the past 6 months, under any medication in the past 6 months, blood donation in the past 3 months. Based on the above criteria 5 subjects volunteered and gave informed consent to be included in the study.

### *Preparation of the drug stock solution*

Analytical grade doxycycline was obtained from Sigma Aldrich ltd. The drug stock solution was made by mixing 10 mg of the doxycycline in 10 ml of deionized water vortexed for 3 minutes to make a final concentration of 1 mg/ ml. The stock solution was prepared fresh just before the commencement of the study and stored at 2-5 degree celsius and protected from light prior to use.

### *Collection of IPRF*

The i PRF was prepared according to the protocol developed by Miron & Choukron in 2017.[38,39] Briefly it involves collection of 10 ml intravenous blood from each volunteer using venipuncture under sterile conditions. The collected blood is transferred to a plain sterile test tube without any anticoagulant and immediately subjected to centrifugation at 70 g force, 700 rpm for 3 minutes. After centrifugation the blood separates into 2 parts, the bottom layer consisting of a red blood cell compartment and top layer as platelet rich fibrin plasma which is still in liquid consistency. The top platelet rich fibrin layer is aspirated in a 2 ml syringe and maintains in liquid consistency for about 10 - 15 minutes until it clots by slow polymerisation of fibrin structure.

### *Preparation of the drug loaded I-PRF*

Before the collection and centrifugation of the blood from volunteers, 200 microliter of the drug solution is dispensed at the bottom of the microtube vial microtube and kept ready. Once iPRF is obtained after centrifugation, 2 ml of it is added to the microtube vial containing the drug and vortexed for 10 seconds to obtain a homogenous mix of the drug and iPRF. This mixture is further allowed to become a gel as a result of the natural polymerisation process of the fibrin within the iPRF.

### *Pharmacokinetic evaluation*

The doxycycline loaded iPRF gel is divided into 3 equal parts and each one is placed in an microtube vial containing 1.5 ml of phosphate buffered saline (PBS), initiating solvent exchange. At specified time intervals of 1, 3, 5, 7 hours, 100 microliter of drug eluted SFA sample is collected and replaced with an equal amount of fresh PBS solution. The collected samples are then stored at -20 degree celsius temperature until further analysis. This is repeated at 3rd day, 5th, 7th, 9th and 14th day intervals (Figure 5).

### *Spectrophotometry analysis*

The drug release kinetics of doxycycline from the iPRF were assessed over a period of 9 days. The collected samples were diluted and subjected to Ultra-Violet (UV) - visible spectrophotometric (Jasco V-730) analysis for the presence and quantification of doxycycline eluted from the PRF. An UV range of 190 - 1100 nm was used and the peaks for identification of doxycycline is noted at 268 nm.[40] Further the cumulative amount of doxycycline released is calculated based on a calibration curve of ciprofloxacin in deionized water to water (1:1).[41]

***Release kinetics***

Different release kinetics was used to analyse the mechanism of drug release. Release rate data were fitted into different release kinetics mechanisms like zero order, first order, Higuchi model, Korsemeyer- Peppas. Based on R2 value; the best fitted model was selected.

***Statistical expression***

All the drug concentrations obtained at each time interval were calculated as mean of all the 5 samples and expressed as mean ± SD.

## RESULTS

The spectrophotometric results show that the drug was detected in all the samples obtained from the 1 hr to the 14th day. The release pattern showed a burst release in the first day of observation after which there was gradual release followed by a short burst release between the 7th to 9th day. Further release till 14th day observation showed a sustained release pattern.

## DISCUSSION

Local drug delivery in periodontal therapy has its specific indications like presence of localised periodontal pockets after scaling root planing (SRP), recurring periodontal disease at localised sites, medically compromised patients where surgical periodontal therapy is contraindicated etc.[42] Although some of the commercially available local drug delivery systems have satisfied most of the the ideal requirement of a LDD such as, controlled/ sustained release of drugs, biocompatibility, MIC of drug at the pocket environment,[14] nevertheless there are few shortcomings that prevent the widespread use of these commercial products.[43] One among them is the poor adaptation of these delivery systems to the dynamic gingival environment. Inturn to make it compatible and adaptable to the local periodontal environment (mechanical properties) plasticizers and fillers are added however resulting in a risk of inflammatory reaction from the degraded byproducts.[44,45] To overcome this we assessed the use of an autologous biomaterial iPRF which has a naturally polymerized 3 dimensional fibrin matrix as a vehicle for drug delivery in periodontal therapy.

In this in vitro assessment the slow polymerization process of the iPRF allowed for the incorporation of the doxycycline drug in it with a working time of 5 - 10 minutes before it became a gel. The natural polymerization of the PRF liquid could result in either the drug becoming a part of the fibrin protein network or getting entrapped in the liquid phase between the fibrin network. The spectrophotometric evaluation showed that, throughout the 9 day study period, all the samples collected showed the presence of the drug (Table 1). Further the concentration of drug eluted from the samples at different time intervals shows that there was an initial burst release within the first 3 hours after which there was a gradual release extending till the study period of 9th day (Table 1). Since there were no similar studies earlier, direct comparison of our results was not possible. However, it is similar to the pattern of release of growth factors from the iPRF, where the growth factors entrapped within the fibrin network of PRF showed a gradual release pattern for a study period of 10 days.[24]

This pattern of drug release may be influenced by the fibrin architecture and the nature of interaction of the drug with the fibrin. The initial burst release of the drug could be due to the diffusion of a drug that is loosely attached in the fibrin matrix. The latter stage showing controlled release may be a result of the enzymatic degradation of the fibrin matrix thereby releasing the drug that has tightly attached to it. This shows that the PRF is being degraded gradually resulting in release of the drug entrapped within the fibrin architecture in a controlled pattern. This is further supported by a similar pharmacokinetic profile with drug vehicles that are similar to PRF architecture.[43]

The pharmacokinetic profile was studied for a period of 9 days based on the fact that the antibiotic course for most of the drugs in periodontal therapy preferably extends from 1 week to 2 weeks.[46,47] Further extended use of more than 10 days may not be justified and may

pose additional risk of antibiotic resistance development and cytotoxicity to the host cell.[13]

Another important observation is that the drug concentrations were well above the minimal inhibitory concentration (MIC) for most of the periodontal pathogens at all the time points of assessment.[48,49] This assures that PRF degradation although initially resulted in bust release, continued to show a sustained release of the drug to the local environment.

The in vitro release data were kinetically analyzed for establishing kinetics of drug release. Model fitting was done using SwissADME, a free software. Zero-Order, First-Order, Higuchi, Hixon-Crowell, Korsmeyer-Peppas, and Weibull modes were tested. The results indicate that all the iPRF follow a first order mechanism of drug release.

In a recent study,[35] the clinical, microbiological and immunological effects of local drug delivery (LDD)of doxycyline or photodynamic therapy (PDT), adjunctive to scaling and root planing in persistent or recurrent periodontal pockets in patients enrolled in supportive periodontal therapy (SPT) after one year was evaluated. A total of 105 patients enrolled in SPT with persistent/recurrent pockets were randomly treated with SI +PDT or SI + LDD or SI (control). The number of treated sites with bleeding on probing (n BOP+), probing pocket depths (PPD), clinical attachment level (CAL), full-mouth plaque and bleeding scores (gingival bleeding index, %bleeding on probing-BOP) was evaluated at baseline and after 12 months. Additionally, eight periodontopathogens and the immunomarkers IL-1β (interleukin)and MMP-8 (matrix metalloprotease) were quantitatively determined using real-time PCR and ELISA, respectively. All three treatments resulted in statistically significant clinical improvements ($p < 0.05$) without statistically significant intergroup differences ($p > 0.05$), which were maintained up to 12 months. The presence of BOP negatively affected the PPD and CAL. Moreover, statistically significantly fewer bleeding sites at 12 months were observed in the test groups ($p = 0.049$). Several periodontopathogens were reduced after 12 months. It was concluded that in periodontal patients enrolled in SPT, treatment of persistent/recurrent pockets with scaling root planing alone or combined with either PDT or LDD may lead to comparable clinical, microbiological and immunological improvements, which are maintained up to 12 months.

In another randomised controlled clinical trial[35,37] the antimicrobial effectiveness of locally administered 2% lemongrass gel and 10% doxycycline hyclate gel as an adjunct to scaling and root planing (SRP) in treating chronic periodontitis was evaluated. This is a double-blind parallel arm randomised controlled study in which 40 subjects were randomly divided into Group A and B for 2% lemongrass gel and 10% doxycycline hyclate gel, respectively. The clinical assessments of Gingival Index (GI), Plaque Index (PI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) together with microbial colony counts for Porphyromonas gingivalis, Actinomyces naeslundii, and Prevotella intermedia were done at baseline, 1st month, and 3rd month follow-ups. The results showed there was a significant reduction in the mean scores of GI, PPD, and CAL clinical indices from baseline to the 1st and 3rd month follow-ups in both the 2% lemongrass gel and 10% doxycycline gel groups ($p < 0.05$). Similarly, there was significant reduction in mean CFU scores for all periodontal pathogens from baseline to 1st and 3rd month follow-ups in both the 2% lemongrass gel and 10% doxycycline gel groups ($p < 0.05$). It was concluded that the local delivery of 2% lemongrass gel as an adjunct to scaling and root planing is effective and comparable to 10% doxycycline gel in the treatment of chronic periodontitis.

Other physical properties like the syringeability and the viscosity of the iPRF can facilitate its delivery and retention into the confined pocket environment. Apart from the controlled drug release pattern, the biomimetic nature of iPRF when used as a vehicle could alleviate the possible inflammatory reaction from otherwise synthetic drug vehicles, minimises dislodgement of LDD system from the periodontal pocket, and possibility of drug resistance thus altogether

making it an ideal vehicle for LDD in periodontal therapy. [50]

The observations of our study can strongly suggest the clinical use of iPRF as a tool for delivery of drug to the periodontal environment with many advantages as 1) the ease of handling, like it can be injected directly into the periodontal tissues and pocket 2) the polymerisation of iPRF to a moderately hardened consistency after being injected can facilitate to achieve a 3 dimensional shape of the periodontal pocket 3) additionally the possible adherence of iPRF to the host tissue like the root surface and gingival tissue ensures localisation of the PRF loaded drug inside the periodontal environment facilitating a sustained release of the drug.

The above results and the crucial features of iPRF may compel to conclude that iPRF as a potential and suitable vehicle for LDD system in periodontal therapy. However, further research on pharmacokinetics of other common drugs used in periodontal therapy, their nature of interaction with iPRF and influence on drug kinetics are underway to support and substantiate the current evidence.

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**TABLE 1:** Concentration of Doxycycline eluted from the i-PRF gel over time.

| Time | Concentration of Doxycycline eluted (microgram/ml) |
|---|---|
| 1 hr | 57.41± 7.34 |
| 3 hr | 45.53 ± 6.23 |
| 5 hr | 41.85 ± 4.76 |
| 7 hr | 32.57 ± 6.72 |
| 3 rd d | 12.82 ± 1.43 |

| | |
|---|---|
| 5th d | 11.29 ± 2.68 |
| 7th d | 9.40 ± 2.83 |
| 9th d | 7.54 ± 2.65 |
| 14th d | 7.04 ± 1.87 |