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Titanium platelet–rich fibrin (T-PRF) as doxycycline delivery system: An in-vitro study

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ABSTRACT

Background: Locally delivered anti-infective agents are one of the frequently used treatment strategies for the treatment of periodontal disease. Doxycycline (Doxy) is frequently preferred in periodontal therapy. Various carrier systems are available like Collagen sponges, mucoadhesive polymers. The use of PRF is a good strategy for drug delivery systems with its 3D matrix structure as a scaffold, as it can naturally degrade within a certain period of time without causing any allergic/inflammatory conditions in the body. Titanium platelet–rich fibrin (T-PRF) has a denser fibrin structure than PRF with long resorption time and may be a good candidate for long-term drug delivery and release.
Aim: To study and compare physical and antibacterial properties of T-PRF and Doxycycline loaded T-PRF.
Methodology: It is an in vitro study. The T-PRF and T-PRF loaded with Doxycycline as T-PRF/Doxy was prepared and their physical properties like length and consistency was evaluated. Antibacterial activities against *S. aureus* and *P. aeruginosa* will be investigated by measuring zone of inhibition after 24 hrs and 48 hrs.
Result: The antimicrobial activity of T-PRF against significantly increases after addition of doxycycline ($p<0.05$) without significantly altering physical property of T-PRF.
Conclusion: T-PRF loaded with doxycycline can be used as local drug delivery agent. However, very little literature is available in this field. And in vivo studies are required to determine the efficiency in periodontitis and peri-implantitis cases.

1. Introduction

Periodontitis, an inflammatory disease of supporting tissues of the teeth, is caused by a specific microorganism or group of specific microorganisms and results in the progressive destruction of periodontal tissues and pocket formation, recession, or both.[1] Various approaches applied for the treatment of periodontitis include mechanical therapy, periodontal surgery and use of local and systemic antibiotics. Traditional methods of treatment start with mechanical scaling and root planning and in cases where indicated, surgical treatments are required to eliminate the pocket, and if possible, to regenerate the lost tissue. This treatment alone may not be enough and guarantee treatment success.[2] All these treatment approaches aim at controlling the infection which is important for proper wound healing and subsequent regeneration of periodontal tissues, but there is always a risk of bacterial contamination with surgical procedures, and sometimes even after stringent disinfection, bacteria may still be able to survive and infiltrate into deeper tissues.The additional use of antibiotics in adjunct to periodontal treatment began in the late 1970s, and it can provide additional clinical benefits in some patient groups. Adjunctive antibiotics may be delivered systemically or locally. Locally delivered anti-

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infective agents are one of the frequently used treatment strategies for the treatment of periodontal diseases in recent years.[3] They have many advantages providing high drug concentration in the target region without systemic side effects and bacterial resistance.[4] Doxycycline (Doxy) is frequently preferred in periodontal therapy. Its concentration is 2-4 times higher than serum and it also possess anti-collagenase activity.[2,5] Various carrier systems are available like Collagen sponges, mucoadhesive polymers such as sodium carboxymethyl cellulose, sodium alginate; polyvinyl alcohol, polyvinylpyrrolidone, starch, and Carbopol were reported as an inhalation delivery of Doxycycline.[6] The success of these non-autogenous carrier systems is closely related to the immune response that occurs against these foreign materials placed in the body.

Recently, platelet concentrates are increasingly being used for the successful management of the manifestations of periodontal diseases. It improves wound healing by releasing a variety of growth factors and positively affects the gingival and periodontal fibroblasts and osteoblasts.[7] Platelets can contribute to host defence through several mechanisms of action. Three different types of granules are found in the platelets: α, δ and λ granules. Alpha granules in fact, contain various groups of proteins with antimicrobial properties.[8,9] The use of PRF is a good strategy for drug delivery systems with its 3D matrix structure as a scaffold, as it can naturally degrade within a certain period of time without causing any allergic/inflammatory conditions in the body. Titanium platelet–rich fibrin (T-PRF) has a denser fibrin structure than PRF with long resorption time and may be a good candidate for long-term drug delivery and release.[10]

The current study aimed to further improve PRF by providing it with anti-bacterial activity. The aim of the present study is to study & compare physical & antibacterial properties of T-PRF & Doxycycline loaded T-PRF.

2. Materials and Methods

The study was conducted in Department of Periodontology. The sample size (n) is derived by using the "comparing two means" formula. Total 12 patients were enrolled for the study. Written informed consent was taken from the patients.

Inclusion criteria were: Healthy volunteers.

1. No systematic or blood diseases.
2. Non-smokers.
3. Did not take any kind of antibiotics during the last month.

The blood samples of 20 mL were drawn from the antecubital vein of the subject's arm. The blood was quickly transferred to titanium T-PRF tubes. The tubes were immediately centrifuged at 2700 rpm for 12 min. After centrifugation, T-PRF clots were removed from the tubes via sterile tweezers, separated from the red blood cells, and placed on sterile woven gauze. Compression was done to form membrane. For preparation of T-PRF loaded with doxycycline, 2 mL of Doxy solution at 3 mg/mL concentration was injected into the T-PRF tube immediately prior to centrifugation. Physical properties assessed by measuring the length (tested by compression) of the PRF clot. (Figure 1) The antibacterial activities of the prepared T-PRF, T-PRF/ Doxy were investigated by employing the disc-diffusion technique against P. aeruginosa ATCC 10,145 and S. aureus ATCC 6538. Bacterial stock culture was revived in 10 mL of nutrient broth for 24 h in a 35 °C incubator. Immediately, 5 mg of T-PRF, T-PRF/Doxy was transferred onto the nutrient agar. The plates were incubated at 35 °C in an incubator for 24 h. The next day, the inhibition zone diameters (IZD, mm) was measured.

Figure 1: a): 20ml of blood is withdrawn from antecubital vein; **b):** Titanium tubes were used for preparation of PRF; **c):** 2ml of Doxycycline solution was added to test group prior to centrifugation; **d):** The tubes were immediately centrifuged at 2700 rpm for 12 min; **e):** T-PRF clots were removed from the tubes via sterile tweezers; **f):** Compression was done to form membrane

2.1. Statistical analysis

The data was expressed in mean and standard deviation. Statistical Package for Social Sciences (SPSS 20.0) version was used for analysis. Unpaired t test was used to find the statistical significant between the groups. p value less than 0.05 was considered statistically significant at 95% confidence interval.

3. Results

Changes in physical properties were assessed by measuring the length of T-PRF membrane after compression. Result showed that addition of doxycycline solution did not showed significant difference in physical property of T-PRF membrane. (Table 1).

Table 1: Comparison of mean length between the group-I and group-II

Groups	Length (MEAN±SD)	p value
Group-I (T-PRF)	15.28±4.53	0.504
Group-II(T-PRF/DOXY)	14.13±3.71	

(p>0.05 no significant difference compared group-I with group-II)

3.1. *Antibacterial effect of T-PRF, T-PRF/Doxy*

The antibacterial activities of T-PRF, T-PRF/Doxy, and Collagen/Doxy were investigated against *P. aeruginosa and S. aureus.* Interestingly, T-PRF alone without the drug showed slightly antibacterial effect on P. aeruginosa and S. aureus, but the bacterial inhibition capacity of T-PRF was significantly enhanced upon drug loading into the matrix

Table 2: Comparison of mean zone of inhibition of *S. aureus* between the group-I and group-II

Groups	*S. aureus* Zone of inhibition (MEAN±SD)	p value
Group-I (T-PRF)	11.77±3.41	0.0001
Group-II (T-PRF/DOXY)	25.15±4.98*	

*p<0.05 significant difference compared group-I with group-II

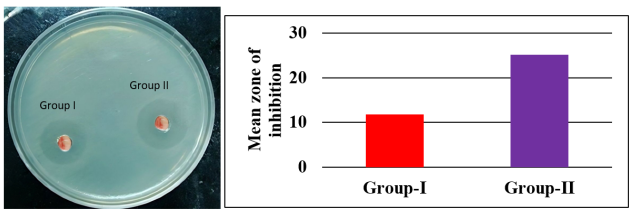

Figure 2: Comparison of mean zone of inhibition of *S.aureus* between the group-I and group-II

4. Discussion

Platelet concentrates are increasingly being used in periodontal surgeries, particularly because of their regenerative potential. Platelets play a vital role in wound healing. They release growth factors such as platelet-derived growth factor, transforming growth factor (TGF-), vascular endothelial growth factor, epidermal growth factor, and

Table 3: Comparison of mean zone of inhibition of *P. aeroginosa* between the group-I and group-II

Groups	*P. aeruginosa* Zone of inhibition (MEAN±SD)	p value
Group-I (T-PRF)	8.95±1.90	0.0001
Group-II (T-PRF/DOXY)	20.74±4.61*	

(*p<0.05 significant difference compared group-I with group-II)

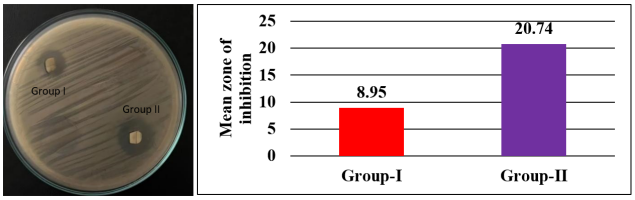

Figure 3: Comparison of mean zone of inhibition of P. *aeroginosa* between the group-I and group-II

insulin-like growth factor on activation.[11] Platelets provide matrix for connective tissue by secreting fibrin, fibronectin, and vitronectin, which also help in cell migration. In this way, platelets plays an important role in proliferation of cells, synthesis of collagen, and formation of osteoid. The fibrin matrix of PRF contains a large quantity of platelet and leukocyte cytokines and they are progressively released over time (7–11 days) as the fibrin network disintegrates.[11,12] This study showed that PRF alone carries a mild inhibitory activity for the growth of both S. aureus, and P.aeroginosa. Yang et al. in 2015 compared the antimicrobial activity of four plasma fractions, that is, PRP, platelet-poor plasma, platelet-depleted plasma, and PRF against *Pg, Aa*, and *Fusobacterium nucleatum* and found that PRP had the highest antibacterial activity.[13]

PRF use has been reported for many surgical applications such as treatment of bony defects, dental implant surgery, post-extraction healing and reducing rate of post-surgery complications.[14–16]

Castro et al (2019) demonstrated that L-PRF membrane has antibacterial effects against periodontopathogens including P. *intermedia, F. nucleatum*, and *A. actinomycetemcomitans*.

Various studies have been conducted to further enhance the antimicrobial properties of PRF using various antibiotics.[15] Cieslik-Bielecka, et al (2007) showed that IV administration of amoxicillin and clavulanic acid 30 min prior to preparation of PRF lacks strong antibacterial activity against *Enterococcus faecalis*.[17] Furthermore, Miron and Zhang, in an extensive review, discussed the possibility of combining various bioactive materials with liquid PRF to produce an advanced local delivery system for small and large biomolecules.[18]

Doxycycline is highly recommended drug used for treatment of periodontitis.

This study showed that incorporation of antibiotics in PRF preparation did not changed physical properties of membrane. These results were similar to study conducted by Polak et al & Ercan et al. Polak et al. showed that addition of 0.5ml of clindamycin/ metronidazole/ penicillin did not changes physical properties of PRF. Polak et al (2019) showed that addition of metronidazole, penicillin or clindamycin to PRF produced antimicrobial preparation with long-term anti-bacterial activity, up to 4 days of the experiments against *S.aureus* and *F. nucleatum*.[19] Similar results were obtained by this study.

Study showed that the zone of inhibition against *S. aureus* & *P.aeroginosa* increased significantly after addition of Doxycycline. This was in accordance to the study conducted by Ercan et al. Ercan et al.[20] compared collagen & T-PRF loaded with doxycycline and found that in comparison with collagen, approximately sevenfold more Doxy**,** 281 mg/g, was loaded into T-PRF. Their study showed that there was more drug release with T-PRF compared to collagen. This results shows that T-PRF can be used as doxycycline delivering agent.

However, there are certain limitations to this study which includes small sample size, inability to compare different concentration of doxycycline and use of doxycycline and T PRF against numerous periodontal pathogens.

In future, combination of other antibiotics with T-PRF should be tested against periodontal pathogens. As T-PRF is used for various surgical procedures in periodontics including infrabony defects, furcation, mucogingival surgery and for post-operative healing, addition of antibiotic such as doxycycline will further improve the clinical outcome of these treatment modalities. In vivo study with larger sample size and longer follow up should be conducted.

5. Conclusion

T-PRF showed promising results as doxycycline delivery agent. As T-PRF alone possess antimicrobial activity addition of antibiotic will further enhances its activity. Addition of doxycycline does not affect the physical property of T-PRF. These can be used as an effective treatment for periodontitis and peri-implantitis.

6. Source of Funding & Conflicts of Interest

Nil.

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