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IN-VITRO ANALYSIS TO ANALYSE THE DISINTEGERATION PROPERTY OF PRF WHEN TREATED WITH VARIOUS CONCENTRATION OF TRANEXAMIC ACID

V. Radha * and Sheeja S. Varghese

Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technological Sciences, Saveetha University, Chennai - 600077, Tamil Nadu, India.

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Correspondence to Author:
V. Radha

Postgraduate Student, Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600077, Tamil Nadu, India.

E-mail: dentist.radha@gmail.com

ABSTRACT: Aim: To assess whether Tranexamic acid has any effect in inhibiting degradability of PRF membrane and also to compare the degradability of PRF at different concentrations of Tranexamic acid (200 mg, 150 mg and 50 mg). **Material and Methods:** The degradability of PRF membrane was compared between various concentrations of Tranexamic acid such as 200 mg, 150 mg, 50 mg against negative control of phosphate buffer solution (PBS). Group 1-PBS (negative control) n = 5, Group 2-PBS containing 200 mg of Tranexamic acid n = 5, Group 3-PBS containing 150 mg of Tranexamic acid n = 5, and Group 4-PBS containing 50 mg of Tranexamic acid n =5. PRF procured from each donor was weighed in a micro weighing machine and they were later dropped in each of the 4 groups of eppendorfs (5 in each group). After 1 week of storing in room temperature, the PRF pieces were retrieved and percentage of remaining PRF was calculated. ANOVA and post hoc tests were used to statistically compared this data between the groups. **Results:** There was reduction in the weights of the PRF in all the groups at 1 week when compare to baseline. The mean percentage of remaining weight of PRF in groups 1-4 was 35.59 ± 14.23, 85.23 ± 7.23, 87.27 ± 4.28, and 83.65 ± 6.22 respectively. **Conclusion:** Tranexamic acid at different concentration delayed the degradability of PRF efficiently.

INTRODUCTION: Periodontal disease is a chronic inflammatory/infectious disease, characterized by the loss of both tooth supporting soft and hard tissues. The goals of today's treatment of periodontitis are to reduce infection, resolve inflammation and create a clinical condition, which is compatible with periodontal health [1].

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The periodontal therapy mainly aims to be regeneration of the attachment structures of teeth, including new bone, periodontal ligament (PDL), and cementum, which have been destroyed by periodontal diseases or trauma.

Periodontal regeneration is said to be a process involving cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence [2]. Various regenerative therapies in practice are hard and soft tissue grafts, guided tissue regeneration, root biomodification, *etc*. There are evidences that the presence of growth factors and cytokines in platelets play key roles in inflammation and periodontal wound healing [3].

Platelet-rich fibrin (PRF) was first described by Choukroun *et al*., in 2000 [4]. PRF is a second-generation platelet concentrate containing platelets and growth factors in the form of fibrin membranes free of anticoagulants which is prepared from the patient's own blood. This PRF is a natural fibrin matrix, which concentrates most of the platelets and growth factors [5]. The fact that the compound PRF membrane contains not only the structural scaffold components fibrin, leukocytes, and platelets, but also a multitude of growth factors such as TGF-β1, VEGF, IGFs and PDGF-AB, as well as matricellular proteins such as thrombo-spondin-1, made us use PRF. PRF was first used by Choukroun *et al*., [4] and he also proposed a protocol for its preparation for use to improve bone healing in implant dentistry. Also, PRF shows a complex architecture as a healing matrix with unique mechanical properties, which makes it distinct from other platelet concentrates.

In a study, YC Chang *et al*., [6] showed that PRF upregulated ALP activity, increased extracellular signal related protein kinase phosphorylation and osteoprotegerin in periodontal ligament fibroblasts. And in a trial, Panda *et al*., [7] concluded that the adjunctive use of PRF with barrier membrane is more effective in the treatment of intrabony defects in chronic periodontitis as compared with barrier membrane alone. Lundquist R *et al*., [8] in their *in-vitro* study concluded that PRF provides sustained release and protection against proteolytic degradation of endogenous fibrogenic factors important for wound healing.

Sam *et al*., [9] have proven that PRF has a higher degradeability with less rigidity when compared to other commercially available barrier membranes. Also states that, this causes PRF to get resorbed faster and get collapsed thus causing failure in space maintenance in guided tissue regeneration. Yamashita *et al*., [10] studied the stability of PRF in rats, and concluded that histologically and immunohistochemically, the PRF became small and sparse at 21 days, almost disappearing at 28 days. Hence, although PRF can potentially stimulate tissue regeneration, its degradation time should be considered in its clinical applications. It is also obvious that PRF alone cannot work as a scaffold or a barrier membrane for maintaining the space of regeneration.

Tranexamic acid is known to be a synthetic derivative of the amino acid lysine, which exerts its anti fibrinolytic effect through the reversible blockade of lysine binding sites on plasminogen molecules [11]. Dunn CJ *et al*., [12] in his review stated that Tranexamic acid is a useful synthetic drug in a wide range of hemorrhagic conditions. It reduces postoperative blood loss and transfusion requirements. Also, Tranexamic acid reduces menstrual blood loss where it is a potent alternative to surgery in menorrhagia. He also mentioned that Tranexamic acid is more potent in terms of binding to plasminogen or plasmin than any other synthetic antifibrinolytic agent.

A study by Senghore *et al*., [13] showed that one intravenous pre-operative dose of Tranexamic acid is effective in preventing excessive post-operative bleeding in patients undergoing third molar extraction under a day case general anesthesia. Forbes *et al*., [14] in their double-blinded RCT stated that 1 g of Tranexamic acid for five days significantly reduced blood loss and transfusion requirements after dental extraction in patients with hemophilia and christmas disease. Also no side effects were seen in either group of patients. Screening tests showed no toxic action of Tranexamic acid on the liver, kidney, or heart. In the present study we hypothesized that Tranexamic acid may prolong the degradation of PRF.

Thus in this *in-vitro* study, we aimed to assess whether Tranexamic acid has any effect in inhibiting degradability of PRF membrane and also to compare the degradability of PRF at different concentrations of Tranexamic acid (200 mg, 150 mg and 50 mg).

MATERIALS AND METHODS: The study was conducted in the Dental Research Lab in Saveetha Institute of Medical and Technological Sciences with approval from the Institutional Review Board. In this study, the degradeability of PRF membrane was compared between various concentrations of Tranexamic acid such as 200 mg, 150 mg, 50 mg against negative control of phosphate buffer solution (PBS).

The different groups in this study was: Group 1-PBS (negative control) n = 5, Group 2-PBS containing 200 mg of Tranexamic acid n = 5,

Group 3- PBS containing 150 mg of Tranexamic acid n = 5, and Group 4- PBS containing 50 mg of Tranexamic acid n = 5. Commercially available Tranexamic acid ampoule (Tramy-Z 500 mg / 5 ml) was used. For preparation of various concentrations (200mg, 150 mg, 50 mg) of Tranexamic acid, the solution was measured with a micropipette and mixed in 1 ml, 1.5 ml and 2.5 ml of PBS solution in separate eppendorfs.

The selected blood donors for PRF procurement were systemically healthy student volunteers. 30 ml venous blood from 5 different donors was collected. The preparation of PRF was done based on the procedure described by Choukran *et al*., [4]. The blood collected was dispensed in 20 test tubes. It was run on a centrifuge at 2500 rates per min for 15 min in small batches after which it settles into the following layers: upper straw coloured cellular plasma, middle fraction containing the fibrin clot and the red lower fraction containing red blood cells. The upper straw coloured layer is then removed and middle fraction is collected, 2 mm below lower dividing line, which is the PRF.

PRF procured from each donor was pressed between sterile gauze pieces. They were cut into equal sizes of 7 × 3 mm. They were thoroughly dried in a blotting paper and excess water was removed to eliminate errors in weight measurement. The pieces were weighed in a micro-weighing machine (REMI 1MLH). They were later dropped in each of the 4 groups of eppendorfs (5 in each group). After 1 week of storing in room temperature, the PRF pieces were retrieved, dried in a blotting paper and weighed again. Percentage of remaining PRF [(weight at 1 week / weight at baseline) × 100] is calculated in each eppendorf. The mean value of all the percentage is calculated in each group. The data was analysed using SPSS software (Statistical Presentation System Software, 1999, SPSS Inc, New York, version 10.0). ANOVA and post hoc tests were used to statistically compare this data between the groups.

RESULTS: The mean weight of PRF at baseline in the groups 1(PBS - Control) Group 2 (200 mg of Tranexamic acid - 1 ml of PBS + 2 ml of Tranexamic acid), group 3 (150 mg of Tranexamic acid - 1.5 ml of PBS + 1.5 ml of Tranexamic acid), Group 4 (50 mg of Tranexamic acid - 2.5 ml of PBS + 0.5 ml of Tranexamic acid) were 14.6 ± 2.83, 14.3 ± 3.43, 17.34 ± 3.87, 15.44 ± 2.78. The mean weights of PRF at 1 week of groups 1-4 were 5.18 ± 0.85, 12.2 ± 3.04, 15.2 ± 3.9, and 12.98 ± 2.85 respectively. There was reduction in the weights of the PRF in all the groups at 1 week when compared to baseline. The mean percentage of remaining weight of PRF in groups 1-4 was 35.59 ± 14.23, 85.23 ± 7.23, 87.27 ± 4.28, and 83.65 ± 6.22 respectively.

The percentage of remaining weight of PRF at 1 week was maximum in Group 3 (150 mg of Tranexamic acid - 1.5 ml of PBS + 1.5 ml of Tranexamic acid), followed by Group 2 (200 mg of Tranexamic acid - 1 ml of PBS + 2 ml of Tranexamic acid), Group 4 (50 mg of Tranexamic acid - 2.5 ml of PBS + 0.5 ml of Tranexamic acid) and the least in Group 1 (PBS - Control).When the remaining weight of PRF in 4 groups were compared statistically using ANOVA, the difference was statistically significant with a P value of 0.000.

On Post hoc comparison, when each of the test groups (Group 2 - 4) was compared against the control group (Group -1) individually, the difference was statistically significant. When Group 2, 3 and 4 where compared with each other, there was no statistically significant difference. On analyzing the result, it was found that, all the three concentrations of Tranexamic acid were significantly better than the control group.

TABLE 1: MEAN WEIGHT OF PRF AT BASELINE AND 1 WEEK IN EACH GROUP

	Control group 1 (PBS)	Tranexamic acid		
		Group 2 (200 mg)	Group 3 (150 mg)	Group 4 (50 mg)
Mean weight at baseline	14.6 ± 2.83	14.3 ± 3.43	17.34 ± 3.87	15.44 ± 2.78
Mean weight at 1 week	5.18 ± 0.85	12.2 ± 3.04	15.2 ± 3.9	12.98 ± 2.85
% of remaining PRF (M ± SD)	35.59 ± 14.23	85.23 ± 7.23	87.27 ± 4.28	83.65 ± 6.22

TABLE 2: COMPARISON OF DIFFERENCE IN PERCENTAGE OF REMAINING PRF BETWEEN THE GROUPS BY ANOVA

	Control group 1 (PBS)	Tranexamic acid			P
		Group 2 (200 mg)	Group 2 (200 mg)	Group 2 (200 mg)	
% of remaining PRF (M ± SD)	35.59 ± 14.23	85.23 ± 7.23	87.27 ± 4.28	83.65 ± 6.22	0.000*

TABLE 3: COMPARISON OF DIFFERENCE IN PERCENTAGE OF REMAINING PRF BETWEEN THE GROUPS BY POST HOC

Variable I	Variable J	Mean Difference (I-J)	Std. Error	Sig.
Group 1	Group 2	-48.53	4.61	.000*
	Group 3	-50.57	4.61	.000*
	Group 4	-46.95	4.61	.000*
Group 2	Group 1	-48.53	4.61	.000*
	Group 3	-2.04	4.61	.970
	Group 4	1.58	4.61	.986
Group 3	Group 1	50.57	4.61	.000*
	Group 2	2.04	4.61	.970
	Group 4	3.62	4.61	.860
Group 4	Group 1	46.95	4.61	.000*
	Group 2	-1.58	4.61	.986
	Group 3	-3.62	4.61	.860

DISCUSSION: This study aimed to assess whether Tranexamic acid has any effect in inhibiting degradation of PRF membrane and also to compare the degradability of PRF at different concentrations (200 mg, 150 mg and 50 mg) of Tranexamic acid. The percentage of remaining weight of PRF at 1 week was maximum in Group 3 followed by Group 2, Group 4 and the least in Group 1 (PBS - Control). When each of the test groups (Group 2 - 4) were compared against the control group (Group -1) individually, the difference was statistically significant. When Group 2, 3 and 4 where compared with each other, there was no statistically significant difference.

Number of studies [15, 16, 17, 18, 19] has confirmed the clinical and radiographic efficiency of PRF in periodontal defects. When PRF used as a membrane in coronally advanced flap, it showed an increase in width of keratinized gingiva [20, 21]. As it is sagacious to use PRF membrane for GTR applications as well, analysis of the mechanical properties of PRF membrane for GTR procedures is required before using clinically. Thus in this study, we hypothesized that addition of Tranexamic may have an inhibitory activity on PRF degradation thus prolonging its function as a barrier membrane and providing space maintenance for a longer time. Tranexamic acid has also been used widely studied and found efficient for various purpose, to reduce fibrinolysis during knee and hip athroplasty, as a mouth to control post operative bleeding in oral maxillofacial surgery, to reduce menstrual blood loss during pregnancy to reduce the risk of thrombo-embolic complications. It is affirmative that PRF degrades both *in-vitro* and *in-vivo*, as Yamashita *et al*., [10] has shown that PRF completely disappeared in 28 days in Wistar rat models *in-vivo*. Also, Sam *et al*., [9] compared the degradation rate of PRF *in-vitro* with two other commercially available collagen membranes to obtain a gross evaluation of its degradation profile. The membrane degradation test results showed that PRF membrane was comparable to other membranes in terms of maintaining its physical property up to 6 days. After 1-week, PRF membrane was found to have degraded to about 36% of initial weight, whereas fish collagen to about 8%, and bovine collagen to about 3%.

In our study, when treated with Tranexamic acid for 1 week, PRF was found to have degraded to about 10-20% only. This contributes to the mechanism of action of Tranexamic acid in fibrinolysis. Tranexamic acid almost completely blocks the interaction of plasminogen and the heavy chain of plasmin with the lysine residues of fibrin monomer, primarily through its binding to the high affinity lysine binding site of plasminogen [12]. Saturation of this site with Tranexamic acid prevents binding of plasminogen to the surface of fibrin, the process of which retards fibrinolysis.

Thus, the reduced fibrinolytic activity by Tranexamic acid is brought about by the inhibition of both t-PA activity (tissue plasminogen activator) and plasmin. There is no clinical evidence to differentiate the effect of Tranexamic acid or PRF *in-vitro* and *in-vivo*. This study being *in-vitro*, the property of the PRF and the effect of Tranexamic acid on it may change *in-vivo* condition. Also, in this study, we did not test whether Tranexamic acid has any influence in the PRF property of releasing growth factor.

Further studies are needed to check the effect of Tranexamic acid on the growth factor releasing property of PRF before establishing, thereby positively improving the clinical efficiency of PRF in periodontal regeneration.

CONCLUSION: Tranexamic acid is effective in inhibiting the degradation of PRF membrane. There was no significant difference between various concentrations of Tranexamic acid to positively inhibit the degradation of PRF. Different concentrations of Tranexamic acid (200 mg, 150 mg and 50 mg) had equal inhibition effect on degradation of PRF.

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REFERENCES:

1. Lang NP and Tonetti MS: Periodontal risk assessment (PRA) for patients in supportive periodontal therapy (SPT). Oral Health Prev Dent 2003; 1: 7-16.
2. Giannobile WV. The potential role of growth and differentiation factors in periodontal regeneration. J Periodontol 1996; 67: 545-53.
3. Giannobile WV: Periodontal tissue engineering by growth factors Bone. 1996; 19 (S-1): 23S-37S.
4. Choukroun J, Adda F, Schoeffer C and Vervelle A: PRF: an opportunity in perio-implantology. Implantodontie 2000; 42: 55-62.
5. Dohan DM, Choukroun J and Diss A: Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: platelet related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006; 101: E45e50.
6. Chang YC and Zhao JH: Effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects. Australian Dental Journal 2011; 56(4): 365-71.
7. Panda S, Sankari M, Satpathy A, Jayakumar D, Mozzati M and Mortellaro C: Adjunctive effect of autologus platelet-rich fibrin to barrier membrane in the treatment of periodontal intrabony defects. J Craniofac Surg 2016; 27(3): 691-6.
8. Lundquist R, Dziegiel MH and Ågren MS: Bioactivity and stability of endogenous fibrogenic factors in platelet-rich fibrin. Wound Repair and Regenera 2008; 16(3): 356-63.
9. Sam G, Vadakkekuttical RJ and Amol NV: *In-vitro* evaluation of mechanical properties of platelet-rich fibrin membrane and scanning electron microscopic examination of its surface characteristics. J Indian Soc Periodontol 2015; 19(1): 32.
10. Yamashita Y, Chen K, Kuroda S and Kasugai S: Stability of Platelet-rich Fibrin in-vivo: Histological study in rats. J Oral Tissue Eng 2016; 14(2): 83-90.
11. Svanberg L, Åstedt B and Nilsson IM: Abruptio placentae-treatment with the fibrinolytic inhibitor Tranexamic acid. Acta obstetricia et gynecologica Scandinavica 1980; 59(2): 127-30.
12. Dunn CJ and Goa KL: Tranexamic acid. Drugs 1999; 57(6): 1005-32.
13. Senghore N and Harris M: Oral Surgery: The effect of tranexamic acid (cyclokapron) on blood loss after third molar extraction under a day case general anesthetic. British Dental Journal 1999; 186(12): 634.
14. Forbes CD, Barr RD, Reid G, Thomson C, Prentice CR, Mc Nicol GP and Douglas AS: Tranexamic acid in control of hemorrhage after dental extraction in haemophilia and Christmas disease. Br Med J 1972; 2(5809): 311-3.
15. Lekovic V, Milinkovic I, Aleksic Z, Jankovic S, Stankovic P and Kenney EB: Platelet-rich fibrin and bovine porous bone mineral *vs* platelet-rich fibrin in the treatment of intrabony periodontal defects. J Periodontal Res 2012; 47(4): 409-17.
16. Sharma A and Pradeep AR: Treatment of 3-wall intrabony defects in patients with chronic periodontitis with autologous platelet-rich fibrin: a randomized controlled clinical trial. J Periodontol 201; 82(12): 1705-12.
17. Thorat M, Pradeep AR and Pallavi B: Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: a controlled clinical trial. J Clin Periodontol 2011; 38(10): 925-32.
18. Rosamma Joseph V, Raghunath A and Sharma N: Clinical effectiveness of autologous platelet rich fibrin in the management of infrabony periodontal defects. Singapore Dent J 2012; 33(1): 5-12.
19. Sharma A and Pradeep AR: Autologous platelet-rich fibrin in the treatment of mandibular degree II furcation defects: a randomized clinical trial. J Periodontol 2011; 82(10): 1396-403.
20. Aroca S, Keglevich T, Barbieri B, Gera I and Etienne D: Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. J Periodontol 2009; 80(2): 244-52.
21. Padma R, Shilpa A, Kumar PA, Nagasri M, Kumar C and Sreedhar A: A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's class-I and II recession defects. J Indian Soc Periodontol 2013; 17(5): 631-6.

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