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Evaluation of Tensile strength and Degradation of Platelet Rich Fibrin after treating with Tranexamic Acid: An In-Vitro Study

[1]Dr. Triveni Kale, Professor, Department of Periodontology and Oral Implantology, MGV's KBH Dental College and Hospital, Nashik, Maharashtra.

[2]Dr. Tejashvi Seth, Postgraduate student, Department of Periodontology and Oral Implantology, MGV's KBH Dental College and Hospital, Nashik, Maharashtra.

[3]Dr. Shruti Lehndey, Postgraduate student, Department of Periodontology and Oral Implantology, MGV's KBH Dental College and Hospital, Nashik, Maharashtra.

[4]Dr. Shruti Lehndey, Postgraduate student, Department of Periodontology and Oral Implantology, MGV's KBH Dental College and Hospital, Nashik, Maharashtra.

Corresponding Author: Dr. Tejashvi Seth, Postgraduate student, Department of Periodontology and Oral Implantology, MGV's KBH Dental College and Hospital, Nashik, Maharashtra.

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Abstract

Background: PRF is a 2nd generation platelet concentrate, derived from the patient's own blood, consisting of a multitude of growth factors, with adjunctive use of PRF membrane as a barrier membrane. However, it has also been found that PRF membrane has shown higher degradability (approx. 1-2 weeks) and less rigidity as compared to commercially available barrier membranes. Tranexamic acid, is a synthetic derivative of the amino acid lysine, that blocks lysine binding sites on plasminogen molecules and exerts an anti-fibrinolytic effect. Thus, we need a substance that can prolong the stability of PRF.

Methods: Comparison of degradability of PRF membrane between Group A-PBS (n = 20), Group B-PBS containing 150 mg of Tranexamic acid (n = 20), Group C-PBS containing 200 mg of Tranexamic acid (n = 20), PRF procured from each donor was measured. After 1 week of storing in the CO_2 incubator, the PRF pieces were retrieved and the percentage of remaining PRF was calculated. Tensile strength analysis was carried out after treating the PRF membrane for 15 minutes with the solution according to their respective group.

Results: Tranexamic acid at different concentration delayed the degradability and increased the tensile

strength of PRF efficiently, the most effective concentration was found to be 200mg of TXA.

Conclusion: Tranexamic acid serves as an economical, user-friendly, promising agent to enhance the physical properties of PRF.

Keywords: Guided tissue regeneration, Platelet-rich fibrin, Tranexamic acid.

Introduction

Platelet-rich fibrin (PRF) is a second generation platelet concentrate as described by Dohan et al., which consists of a high-density fibrin matrix polymerized in a tetramolecular structure, with the incorporation of platelets, leukocytes, cytokines, and circulating stem cells.[1] The platelet-rich fibrin under physiological condition releases multiple growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF-β), which are known to promote cellular proliferation and differentiation and, can facilitate wound healing, regeneration, and repair.[2] The PRF can be used as a biocompatible and biodegradable material in a raw clot form or an easily compressed membrane-like form. It has been applied in various surgical and post-surgical procedures such as in the repair of tympanic membrane perforations,[3] dental implant surgery, treatment of bony defects and recession defects,[4] post-extraction healing, or as a post-surgical wound dressing.[5] Since it is an autologous material which is derived from the patient's own whole blood without any additives like animal-derived thrombin or any other gelling agents or coagulants, makes it is cost-effective and devoid of any immunological risks.[6] However, when compared to the commercially available biodegradable membranes in an in-vitro study conducted by Sam et al., the PRF membrane degrades faster and has less rigidity as compared to collagen membrane of fish origin and bovine origin, indicating that the collapse of PRF membrane and its inability to maintain space can impact the outcome of periodontal regeneration procedures.[7] So there exists a need to increase the degradation time and rigidity of PRF to use it as a substitute for commercially available membranes.

Tranexamic acid (TXA) is a synthetic amino acid lysine-analogue antifibrinolytic agent, that reduces the local degradation of fibrin plasmin by blocking lysine-binding sites on plasminogen.[8] Studies have shown that systemic or topical application of Tranexamic acid is equally effective for controlling surgical site bleeding.[9] Tranexamic acid has also been widely used in dentistry and minor oral surgical procedures for inhibition of the degradation, stabilization of existing clots, and to limit bleeding.[10]

Hence, we hypothesize that tranexamic acid may help in enhancing the rigidity and prolong the degradation time of the PRF membrane. In this in-vitro study, we have aimed to compare the tensile strength and degradability PRF membrane treated with 150mg of TXA and 200 mg of TXA with PRF membrane alone against a negative control of phosphate buffer solution (PBS)

Materials and Methods

This study was conducted with the approval of the Institutional Ethical Committee and, written consent was taken from all the participants of the study. Blood samples were obtained from 40 adult, male, student volunteers, age ranging from 20 to 25 years. The participants were systemically healthy, non-smokers, without any symptoms of infection and no intake of any drug or antibiotics for at least 3 months before experiments began.

Commercially available ampoule of Tranexamic acid (500 mg / 5 ml) was used for the preparation of various concentrations (200mg, 150 mg) of Tranexamic acid. Three groups (n=20) were made Group A (control)- PBS, Group B (test group 1)-PBS with 150mg concentration

TXA, Group C (test group 2)- PBS with 200mg concentration TXA. The TXA solution was measured and 1.5 mL of TXA and 2 mL of TXA were mixed in 8.5 ml, 8 ml of PBS solution respectively in a separate sterile petri-dish.

Preparation of PRF was done according to the protocol developed by Choukron et al.,[11] 10 mL of blood was drawn from each participant by venipuncture of the right antecubital vein. The blood samples were collected in 2 different 6mL capacity test tubes without any anticoagulants (5 mL in each tube) and immediately centrifuged (REMI, R-303) at 3000 rpm for 10 min. After centrifugation, the PRF clot was removed from the tube using sterile tweezers and separated from the RBC base using sterile scissors. Each PRF clot was compressed between two sterile pieces of gauze for 15s and a membrane form was achieved. All the PRF membranes were measured and cut into the same size of 10 x 7 mm.

The procured pieces of PRF membrane were immediately hydrated with a solution of one of the three groups for 15 minutes in the petri-dish and then retrieved and subjected to tensile strength analysis using a Universal testing machine at Fan Services - Advanced Materials Testing & Research Lab, Nashik, India.

For degradation analysis, each PRF membrane pieces of equal sizes were dried with blotting paper and weighed in a micro-weighing machine (SHIMAZU AUW 120D) before immersing in the solution of either of the three groups and kept in a CO_2 incubator for 7 days. At the end of one week, the pieces were retrieved and dried with the blotting paper and weighed again. The degradation was then calculated in terms of the percentage weight of the remaining PRF.

Results

The data was compiled and analysed using GRAPH PAD software Inc.11452 EI Camino Real #215, San Diego 92130, USA. One-way ANOVA with Tukey post hoc test was used to statistically compare this data between the groups. P=0.05 or less was considered for statistical significance.

The mean tensile strength is expressed in terms of N/mm^2 (Table 1.) Intergroup comparison for tensile strength analysis shown a high statistically significant difference between group A and B ($p < 0.001$) with group B showing better tensile strength. However, group C has shown the most superior tensile strength with high statistically significant difference when compared to group A ($p < 0.001$) and statistically significant when compared to group B ($p < 0.01$).

Table 1: Intergroup Comparision of Tensile strength

Group	Mean (SD)Tensile strength	p-value
A	0.0337 ± 0.008773	< 0.001**
B	0.0465 ± 0.004894	
C	0.0535 ± 0.0008	
Groups	Mean difference	p-value
A versus B	-0.01350	< 0.001**
A versus C	-0.02050	< 0.001**
B versus C	-0.007000	< 0.01*

Statistically significant difference*; High Statistically significant difference**

The mean percentage weight of remaining PRF (Table 2.) was shown high statistically significant difference on intergroup comparison between group A and group B ($p < 0.001$) and group A and group C ($p < 0.001$), group B and group C ($p < 0.01$) shown statistically significant difference.

Table 2: Intergroup Comparision of degradation analysis

Group	Mean (SD) percentage of remaining weight of PRF	p-value
A	44.32135 (2.834)	< 0.001**
B	73.07215 (1.280)	

C	75.76015 (2.632)	
Groups	Mean difference	p-value
A versus B	-28.751	< 0.001**
A versus C	-31.439	< 0.001**
B versus C	-2.698	< 0.01*

Statistically significant difference*; High Statistically significant difference**

Discussion

There are extensive studies carried out on clinical benefits of autogenous platelet-rich fibrin for their repair and regenerative potential, attributed to the presence of alpha granules that acts as a reservoir for various growth factors.[12] The purpose of this present study was to determine the effect of Tranexamic acid on PRF membrane in enhancing the rigidity and stability of PRF membrane, and how two different concentrations of TXA i.e 150 mg and 200mg respond to tensile strength analysis and time course degradation of the PRF membrane. The conception of using Tranexamic acid to enhance the physical properties of PRF membrane was based on the known mechanism of Tranexamic acid preventing break down of fibrin clot by inhibition of both plasminogen activator and plasmin activity, along with a positive effect on wound healing.[13] The results for tensile strength analysis showed a statistically significant difference by both the test groups when compared to the control group with superior results seen in the group that treated PRF membrane with the concentration of 200mg TXA. Similar results were observed in a study conducted by Aktaş et al.,[14] for an increase in tensile strength of PRF membrane when treated with ankaferd blood stopper, an agent similar to Tranexamic acid, that induces a haemostatic encapsulated protein network when comes in contact of blood. The higher tensile strength of the Tranexamic acid-treated PRF membrane will help in coverage and stabilization of graft particles at the site of the defect along with its use as a barrier membrane in guided tissue regeneration.

The barrier membrane is required to be preserved at the implantation site for 3–4 weeks for the enhancement of periodontal tissue regeneration and integration, hence better mechanical properties along with slower degradation rate is a desired feature of any bioabsorbable membrane to be used in a guided tissue regeneration procedure[15] However, the PRF membrane has the disbenefit of early loss of structural integrity at the implanted site. An in-vivo study in Wistar rats demonstrated that immunohistochemically the PRF becomes almost undetectable by the 28th day.[16] If a comparison is made between various absorbable membranes, those which are made of synthetic polymers such as polyglycolic acid, polylactic acid, copolymer, degrade at 12 months, whereas collagen-based membranes degrade faster and have been reported to remain stable for 16–38 weeks.[17] The non-crosslinked collagen membranes lose their structural integrity within 7 days.[18] Kawase et al.,[19] in their study has successfully attempted to prolong degradation of PRF membrane by similar cross-linking the fibres with the help of heat when compared to the gauze compressed PRF membrane.

The degradation analysis could be carried out for only 7 days since it would have been difficult to retrieve the pieces of PRF membrane from the solution and measure its weight after 7 days. In this present study, the PRF in the control group degraded faster than the PRF in both the test group, however, no statistically significant difference was seen for the degradation of PRF among both the test group. Around 27%-25% of PRF was degraded in the test

group as compared to 55% of PRF degradation in the control group. This could be attributed to the antifibrinolytic activity of Tranexamic acid and its effects on inhibition of binding of tissue plasminogen on the fibrin surface.[8] The solution in the control group turned cloudy compared to the solutions of the test group. The results of this present study are similar to a study conducted by Radha and Varghese,[20] 10%- 20% of degradation was observed with PRF treated with Tranexamic acid as compared to around 75% degradation observed with pure PRF.

In this present study, we tried to establish a simple and practical method that gives more rigidity and stability to the PRF membrane and to provide in-vitro evidence of its effectiveness. Tranexamic acid is quite economical and easily available commercially, the process of treating the PRF membrane with Tranexamic acid can be easily conducted chair-side, without much time consumption and applied as a barrier membrane. Future prospects to be considered with respect to the conduction of biological tests such as cell adhesion test of PRF treated with Tranexamic acid for evaluating its scaffolding properties and its in-vivo evaluation on a larger sample size.

Conclusion

The technique of treating the PRF membrane with Tranexamic acid successfully increased the tensile strength and delayed the degradation of the PRF membrane. The most effective concentration for imparting better physical properties is 200 mg of TXA. In addition, the protocol of this technique of treating the PRF membrane with Tranexamic acid is quite feasible and very user-friendly from a clinical perspective. Therefore, we believe that the Tranexamic acid-treated PRF membrane has the potential to be widely applied as a barrier membrane in guided tissue regeneration therapy.

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