Comparative histologic evaluation of titanium platelet-rich fibrin and platelet-rich fibrin in hypertensive and smoker participants: A cell cytology study

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Abstract:

Introduction: In recent years, a growing interest has emerged with the use of platelet-rich products for the treatment of many clinical conditions in dentistry. Objective: The present study aimed to define the structural characteristics of titanium platelet-rich fibrin (TPRF) and PRF in hypertensive and smoker participants. Materials and Methods: Ten milliliters of blood samples was drawn using a syringe from ninety participants (healthy, hypertensive, and smokers). Five milliliters of blood was transferred to each of dry glass tube and titanium tube. The clot obtained after centrifugation from each tube was processed for light microscopy analysis. Results: The TPRF samples have demonstrated a highly organized and thicker fibrin network with continuous integrity as compared to PRF in healthy participants. The hypertensive and smokers showed less prominent fibrin border between the cellular structures in both the platelet concentrates, but sufficient fibrin mesh network was found in TPRF clot as compared to PRF clot in the test participants. Conclusion: This is the first human histologic study to define the fibrin meshwork in both TPRF and PRF clots in hypertensive and smokers. The platelet activation by titanium offered high characteristics to fibrin network.

Key words: Hypertension, platelet-rich fibrin, smokers, titanium platelet-rich fibrin

INTRODUCTION

The ultimate goal of treating a diseased condition is to achieve the healing of the lost structure through the process of regeneration. The greatest challenge encountered in the medical and dental field is the challenge in the development of biological surgical additive for regulating inflammation, healing, and to gain regeneration.[1] Decade back, the important role of platelet in homeostasis and wound healing process has been established. The platelets have pool of protein storage which is essential to wound healing, along with various growth factors. The alpha-granules of the platelets fuse with the platelet cell membrane after activation. In this process, some secretory proteins are transformed to a bioactive state. The secretion of active proteins allows them to bind to transmembrane receptors of the target cells. Once bound, intracellular signal proteins are activated. The signaling pathway enables the expression of a gene sequence that directs cellular proliferation, collagen synthesis, and osteoid production.[2]

The evolution of platelet concentrates started from the development of fibrin sealant to the first generation of platelet-rich plasma (PRP). Due to certain limitations of PRP, the introduction of the second-generation platelet concentration came into existence.[3]

The second generation of platelet concentrate known as platelet-rich fibrin (PRF) was developed in 2001 by Choukron et al. As per Khorishidi et al. 2015, the platelet concentrate exhibits a good tensile strength, toughness, and stiffness and has successfully and widely been used in regenerative field.[4] In spite of the success of PRF, various modifications have been made in
PRF preparation. The introduction of the third generation of platelet concentrate evolved by changing the structural design of tube, using a biocompatible material titanium known as titanium PRF (TPRF).6,7

The various generations of the platelet concentrates mainly focus for regeneration of lost soft and hard tissue as it is mainly derived from the fibrin clot present in the middle of the tube which contains the maximum growth factors.7

Variation in fibrin network can be contemplated due to the difference in the entrapment of white blood cells (WBCs) and platelets because of change in vascularity. Various factors affect the fibrin matrix formation, and the entrapment of WBC and leukocytes changes due to change in vascularity which may be due to genetic factors and acquired factors (abnormal concentration of factor XIII and thrombin in plasma, hypertension, blood flow, platelet activation, oxidative stress, hyperglycemia, hyperhomocysteinemia, medications, and cigarette smoking).8,9

Thus, the present study aimed to evaluate histologically the variation in fibrin network pattern in hypertensive and smoker participants.

MATERIALS AND METHODS

The research was conducted in Oxford Dental College and Hospital, Bengaluru. A sample size of ninety participants was obtained with a power of the study of 90%.

The inclusion criteria included individuals with age from 20 to 60 years, normal platelet count of 150,000–450,000 per microliter, smokers with a history of smoking for the past 3 years or more and 5–6 cigarettes per day, hypertensive individual with antihypertensive medication with a blood pressure recording of >150/95 mmHg but without the habit of smoking, minimum of ten teeth should be present, should not have chronic periodontitis, without antibiotic medication administration in the past 6 months, had not undergone systemic or periodontal surgery past 1 year. The exclusion criteria included individuals suffering from hypertension and diabetes, decrease or increase in platelet count, and bleeding disorders and pregnant and lactating mothers.

Ninety participants were recruited following the fulfillment of inclusion and exclusion criteria. The healthy participants included 22 males and 8 females. Hypertensive individuals included 18 males and 12 females. All male participants were recruited in smoker group. The individuals were explained of inclusion and exclusion criteria. The healthy participants included 18 males and 12 females.

The steps followed for obtaining the PRF and TPRF clot slides by this method were as follows [Figure 5]:

Step 1: Fixing – In this process, the PRF and TPRF clots were transferred into a perforated stainless steel cassette in which a small chit for ease of identification of patient was written with a lead pencil and was transferred into 10% formalin-containing container. These were then fixed for 24 h to prevent autolysis and to preserve the biological tissues in life-like state.

Step 2: Tissue processing – After 24 h of fixation, the cassette-containing clots were transferred into a perforated stainless steel cylindrical container where it was subjected to dehydration, clearing, and infiltration of wax into the clots as they passed through various processing solutions such as 10% formalin, 60%, 70%, 80%, 90%, and 100% isopropanol alcohol, xylene (two changes), and paraffin wax in an orderly manner. This process was continued for 16 h in an automated tissue processor (Leica) which enable to remove the water from tissue and replace in a medium that after solidification will allow thin sections to be cut.

Step 3: Embedding – In this step, it enabled tissue sectioning with Leuchars blocks by embedding tissue using paraffin wax. It enabled the tissue section for sectioning and in preparation of the slide.

Step 4: Tissue sectioning – 4 µm thickness of tissue slice was obtained using Leica microtome.

Step 5: Dewaxing – The deparaffinization of the slides was done by heating it for about 55°C, followed by dropping into xylene to eliminate wax enabling the tissues to be stained.

Step 6: Tissue staining – Hematoxylin and eosin stain sections were used for staining. Staining provides contrast to the tissue as well as highlights particular features of interest.

Step 7: Slide numbering – The slides were numbered based on the order for record.

Step 8: Histological slide analysis – Following the preparation of stained section of PRF and TPRF clot from Group A (healthy participants), Group B (hypertensive...
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**Figure 1:** The study design with the distribution of age and gender in Groups A, B, and C

**Figure 2:** (a) Blood collection; (b) centrifugation machine

**Figure 3:** (a) Glass Vacutainer; (b) platelet-rich fibrin clot

**Figure 4:** (a) Titanium tube; (b) titanium platelet-rich fibrin clot

**Figure 5:** Platelet-rich fibrin and titanium platelet-rich fibrin clot preparation for histological analysis (a) fixing and labeling steps (b) tissue processing (c) embedding (d) prepared slide

**Figure 6:** Healthy participants (a) platelet-rich fibrin clot: (F) dense fibrin network with entrapment of platelets and white blood cells (arrow mark); (b) titanium platelet-rich fibrin clot: (F) thicker prominent fibrin border (arrow mark)

**Figure 7:** Hypertensive participants (a) platelet-rich fibrin clot: (F) loose fibrin network with less amount of cellular (C) component present in the clot (arrow mark); (b) titanium platelet-rich fibrin clot: (F) thin fibrin border and less dense fibrin network with cellular (C) component in the clot (arrow mark)

**Figure 8:** Smoker participants: (a) platelet-rich fibrin clot: (F) loose fibrin network mesh with diffusely arranged (C) cellular component and febrile border (arrow mark); (b) titanium platelet-rich fibrin clot: (C) dense meshwork with (F) thick fibrin border (arrow mark)
participants), and Group C (smoker participants); the slides were assessed with compound microscope at ×20 and ×40 magnifications to evaluate:

1. Presence of dense fibrin network
2. Presence of loose fibrin network
3. Enmeshment pattern of platelets and WBC cells within the dense and loose type of fibrin network.

RESULTS

The histologic section processed from both PRF and TPRF clots in all the participants showed an outermost layer of RBC presence of by the dense fibrin network layer with maximum number of platelet and WBCs entrapped, and the inner layers were dominated by loose fibrin network with entrapment of platelets and WBC.

Platelet-rich fibrin and titanium platelet-rich fibrin clot in healthy participants

The age group of healthy participants included in the range of 20–60 years of age. In the PRF clot, the presence of both dense and loose fibrin network was seen. The percentage of dense fibrin network was more as compared to loose fibrin network, and entrapment of platelets and WBC cells was layered within the dense fibrin network whereas less cells were diffusely arranged in loose fibrin network. A fibrin border was appreciated too as depicted in Figure 6a.

The TPRF clot showed highly organized dense fibrin network as compared to normal PRF clot with less percentage of loose fibrin network. A prominent fibrin border of considerable thickness was appreciated in Figure 6b.

Platelet-rich fibrin and titanium platelet-rich fibrin clot in hypertensive participants

In Group B, the hypertensive participants were enrolled in the study with the age group of 50–60 years and the histologic stained slides in both PRF and TPRF clots showed the followings:

The PRF clot was occupied with higher percentage of loose fibrin clot, and platelet and WBC were seen at the periphery near to the dense fibrin network which was present in a reduced quantity as illustrated in Figure 7a.

In TPRF clot, though the dominancy of loose fibrin network was more, dense fibrin network was present in a reduced quantity which showed a packetizing pattern of entrapment of cellular components, and a greater amount of cellular components were diffusely arranged all through the clot. The fibrin border could not be appreciated in both the clots of hypertensive participants as depicted in Figure 7b.

Platelet-rich fibrin and titanium platelet-rich fibrin clot in smoker participants

The smoker participants’ age group ranged from 20 to 40 years. In the PRF clot, distribution of both dense and loose fibrin networks was present. However, compared to the dense fibrin mesh, the loose fibrin mesh was dominant as seen in Figure 8a. The WBC and platelet aggregates were seen more in the dense fibrin network and the rest part of the clot; the enmeshment of cells was diffusely scattered.

In the TPRF clot, the presence of both dense and light fibrin networks was homogeneously present. However, as compared to PRF clot of the same smoker individual, TPRF clot showed maximum amount of entrapment of platelets and WBC in the dense fibrin network as compared to loose network and the presence of a fragile fibrin border which was completely absent in PRF clot as seen in Figure 8b.

DISCUSSION

The present clinical research demonstrated the variation in fibrin meshwork pattern of PRF and TPRF clot in systemic and environmentally exposed individual through histological analysis. As per our knowledge of the literature, this study was first of its own kind which deviated the thinking of understanding the pattern of fibrin clot in a compromised individual. The present study thus histologically evaluated the change in fibrin network pattern in both smokers and hypertensive participants.

The importance of understanding the variation in the fibrin clot in hypertensive and smoker participants in the platelet concentrates could be because it contains highest concentration of growth factors in the first 1 mm of the yellow clot, just above the red clot which plays an immense role in the regeneration of the lost tissues. Moreover, the accumulation of platelets in significant amount is seen at any vascular injury when the subendothelium layer gets exposed to bloodstream. Platelet thrombus formation is triggered by the presence of fibrillar collagen component. Platelet thrombus formation is dependent on platelet glycoprotein which binds to fibrinogen and causes cross-linking of the platelets. Hence, the influence of systemic and environmental conditions was a necessity to evaluate the quality of fibrin meshwork.

The variation in PRF clot at various age groups was evaluated, wherein older age group period, loose fibrin network and scanty entrapment of WBC and platelets. Till date, research on age or any systemic conditions has not been evaluated for TPRF clot. TPRF fibrin histomorphometrically has shown a larger fibrin network area than leukocyte- and platelet-rich fibrin network, and also, fibrin seemed thicker in the TPRF samples. Thus, platelet activation by titanium tubes has shown higher characteristics.

Dohan et al. observed the potential benefits of using platelet concentrate in regeneration process due to the cytokines present and fine flexible fibrin network and stabilization of the wound. Laurens et al. stated that the importance of wound healing depends greatly depends on the fibrin structure, such as the thickness of the fibers, the number of branch points, the porosity, and the permeability. It also depends on platelet concentrates and their functions. Hence, the present study focused on the histologic evaluation of fibrin mesh pattern and its interaction with platelets and WBC in hypertensive and smoker participants.

The result obtained from the healthy participants in the present study was in accordance with the histologic evaluation performed by Tunali et al. in 2014 in healthy individuals where a denser fibrin mesh with entrapment of cellular component was appreciated from TPRF clot.
The smoker participants in general were known to cause downregulation of immune response. Rival et al. in 1987 observed a decrease in platelet aggregation which further affected the clot formation.\[^{[19]}\] In vitro study done by Eichel and Shahrik\[^{[20]}\] in 1977 has shown an alteration in chemotaxis, phagocytosis, and killing activity of neutrophil when exposed to nicotine or whole tobacco smoke. Thus, the present observations in the PRF and TPRF clot in smoker participants could be explained due to changes in the cellular component on chronic exposure of nicotine.

In hypertensive participants, both the clots histologically have shown a dominancy of loose fibrin network more but in a reduced quantity of dense fibrin network with interspersed loose fibrin pattern with the entrapment of cellular components. The fibrin border could not be appreciated in both the clots of hypertensive participants. Where in hypertensive subjected had shown an elevated WBC count leading to a chronic low grade inflammation. This further caused alteration in endothelial function, affecting nitrous oxide and prostaglandin production and consequently loss of vasodilatation, aortic thrombotic and atheroma formation in vascular endothelium. This phenomenon causes an increase adherence of stimulated leukocytes to the endothelium of the blood vessel causing capillary leukocytosis and subsequent increased vascular resistance. Variation in function of platelet too has been evaluated by El Haouari and Rosado et al. 2009\[^{[22]}\] where platelets from hypertensive patients showed increased sensitivity to agonists and have high intracellular-free calcium(2+) (Ca[2+]) concentration. Furthermore, in hypertension, platelets show enhanced endogenous production of reactive oxygen species and a reduced antioxidant status which increases protein tyrosine phosphorylation, enhances Ca(2+) mobilization, and attenuates nitrous oxide bioavailability. The diminished cellular component and presence of increase loose fibrin mesh could also be attributed to the age group of participants recruited in hypertensive group which was in accordance with study by Yajamanya et al. in 2016.\[^{[15]}\]

This research work emphasized the influence of systemic and environmental condition which could alter the fibrin clot formation and its interaction of cellular component further delaying the regeneration process.

To our understanding, certain limitations of the study included wide variation in age group between smokers and hypertensive participants. Evaluation of fibrin clot of individual having hypertension and smoking could have yielded a better validation. The sex distribution in all the groups was heterogeneously distributed. Histologically, correct appreciation of the changes in the individual fibrin strand morphology and thickness, difficulty in identifying and separating out the platelets and WBCs from each other represented by hematoxylin staining, and exact counting of the platelets entrapped within the fibrin meshwork were difficult.

CONCLUSION

The fibrin clot pattern in hypertensive and smoker participant varied as compared to healthy participants in PRF and TPRF clot, but a better organization of meshwork and increased entrapment of cellular components were appreciated in TPRF clot. Considering the above-mentioned limitations, further research focusing on various systemic and environmental conditions should be taken into account.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Polson AM, editor. Early wound healing stability and its importance in periodontal regeneration. In: Periodontal Regeneration: Current Status and Direction. Chicago, Berlin: Quintessence Publishing Co., Inc.; 1994. p. 41-53.
2. Gassling VL, Açil Y, Springer IN, Hubert N, Wiltfang J. Platelet-rich plasma and platelet-rich fibrin in human cell culture. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:48-55.
3. Marx RE. Platelet rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;64:489-96.
4. Khorshidi H, Raoofi S, Bagheri R, Banhashemi H. Comparison of the mechanical properties of early leukocyte- and platelet-rich fibrin versus PRGF/Endoret membranes. Int J Dent 2016;2016:1849207.
5. O’Connell SM. Safety issues associated with platelet-rich fibrin method. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:587.
6. Tunali M, Ozdemir H, Kucukkocad Z, Akman S, Yaprac E, Firat E. In vivo evaluation of titanium-prepared platelet-rich fibrin (TPRF): A new platelet concentrate. Br J Oral Maxillofac Surg 2013;51:438-43.
7. Polson AM, Proye MP. Fibrin linkage: A precursor for new attachment. J Periodontol 1983;54:141-7.
8. Nunes CR, Roedersheimer MT, Simske SJ, Luttges MW. Effect of microgravity, temperature, and concentration on fibrin and collagen assembly. Microgravity Sci Technol 1995;8:125-30.
9. Patel J, Deshpande N, Shah M, Dave D, Shah S. PRF from self to self. Res Rev J Dent Sci 2013;1:304.
10. Baumgartner HR. The role of blood flow in platelet adhesion, fibrin deposition, and formation of mural thrombi. Microvasc Res 1973;5:167-79.
11. Turitto VT, Baumgartner HR. Initial deposition of platelets and fibrin on vascular surfaces in flowing blood. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, editors. Textbook of Hemostasis and Thrombosis. 3rd ed. Philadelphia, PA: Lippincott; 1994. p. 805.
12. Baumgartner HR. Platelet interaction with collagen fibrils in flowing blood. I. Reaction of human platelets with alpha chymotrypsindigested subendothelium. Thromb Haemost 1977;37:116.
13. Houdijk WP, Sakariassen KS, Nievelstein PF, Sixma JJ. Role of factor VIII von willebrand factor and fibronectin in the interaction of platelets in flowing blood with monomeric and fibrillar humancollagen types I and III. J Clin Invest 1985;75:53140.
14. Phillips DR, Charo IF, Parise LV, Fitzgerald LA. The platelet...
membrane glycoprotein IIb-IIIa complex. Blood 1988;71:831-43.

15. Yajamanya SR, Chatterjee A, Babu CN, Karunanithi D. Fibrin network pattern changes of platelet-rich fibrin in young versus old age group of individuals: A cell block cytology study. J Indian Soc Periodontol 2016;20:151-6.

16. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. 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:e45-50.

17. Laurens N, Koolwijk P, de Maat MP. Fibrin structure and wound healing. J Thromb Haemost 2006;4:932-9.

18. Tunali M, Özdemir H, Küçükdacı Z, Akman S, Yaprak E, Toker H, et al. A novel platelet concentrate: Titanium-prepared platelet-rich fibrin. Biomed Res Int 2014;2014:209548.

19. Rival J, Riddle JM, Stein PD. Effects of chronic smoking on platelet function. Thromb Res 1987;45:75-85.

20. Eichel B, Shahriz HA. Tobacco smoke toxicity: Loss of human oral leukocyte function and fluid-cell metabolism. Science 1969;166:1424-8.

21. Shankar A, Klein BE, Klein R. Relationship between white blood cell count and incident hypertension. Am J Hypertens 2004;17:233-9.

22. El Haouari M, Rosado JA. Platelet function in hypertension. Blood Cells Mol Dis 2009;42:38-43.