



RESEARCH ARTICLE

ASSESSMENT OF EFFECT OF STORAGE TIME ON PLATELET CONCENTRATION OF PRF: IN VITRO STUDY

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Abstract

Introduction: The ability of PRF to sustain its platelet concentrate over the tested time may have significant clinical implications. Blood drawn before the start of the procedure and stored as PRF improves patient comfort and saves operator time, since no specialised equipment or storage facilities are required.

Aim: To determine whether storage time has a significant effect on the platelet concentration of platelet-rich fibrin (PRF).

Materials and Methods: A total of 10 healthy participants were enrolled into the study. From all the participants, three separate blood samples were collected by venepuncture. Two of the blood samples were centrifuged to form PRF. Formed PRF was removed from the respective test tubes at different time intervals - immediately after centrifugation (Group A) and after 60 min of storage time in the blood collecting tube (Group B). The residual blood from each group was tested for platelet concentration and comparison was made with non-centrifuged baseline sample (Group C).

Results: The residual mean platelet concentration of Group A was higher than that determined for Group B. The results from this study indicate that there was a significant difference in the mean platelet concentration of PRF when stored for 0 and 60 minutes.

Conclusion : The study concluded that clot disintegration does not take place after 15 minutes of storage, but rather remains stable over a period of at least 60 minutes and may in fact increase in platelet concentration during that time.

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Introduction:-

The development of bioactive surgical additives, which are being used to regulate the inflammation and increase the speed of healing process⁽¹⁾, is one of the great challenges in clinical research. In this sense, healing is a complex process, which involves cellular organization, chemical signals, and the extracellular matrix for tissue repair⁽²⁾. The understanding of healing process is still incomplete, but it is well known that platelets play an important role in both haemostasis and wound healing processes⁽³⁾.

Platelets' regenerative potential was introduced in the 70's⁽⁴⁾, when it was observed that they contain growth factors that are responsible for increase collagen production, cell mitosis, blood vessels growth, recruitment of other cells that migrate to the site of injury, and cell differentiation induction, among others⁽⁵⁾.

The most common platelet concentrate used in these procedures is platelet-rich plasma (PRP) and by the early part of the 21st century, its use in various surgical procedures was commonplace. Whitman et al⁽⁶⁾, in 1997, were the first to introduce the use of platelet-rich plasma in oral surgical procedures. However, using it also presents risk because bovine thrombin, which is used to handle PRP, may generate antibodies to factors V, XI, and thrombin that could cause coagulopathies that may endanger life⁵. On the other hand, PRF was first used in 2001 by Choukroun et al.⁽⁷⁾, specifically in oral and maxillofacial surgery, and is currently considered as a new generation of platelet concentrate. It consists of a matrix of autologous fibrin and has several advantages over PRP⁽¹⁾, including easier preparation and not requiring chemical manipulation of the blood, which makes it strictly an autologous preparation⁽⁵⁾. It is now regarded as a biological scaffold different to PRP. However, even though the use of PRF is gaining widespread clinical acceptance, several questions regarding its biological stability remain unanswered. The aim of this study was to determine whether storage time has a significant effect on the platelet concentration of platelet-rich fibrin (PRF).

Materials and Methods:-

The present study is cohort analytical study carried in department of Periodontics, M.G.Vs KBH Dental College, Nashik. The study was explained to the patients, informed consent was obtained. The study protocol was approved by the Institutional Ethical Committee of M.G.Vs KBH Dental College, Nashik.

A total of 10 healthy participants were enrolled into the study. From all the participants, three separate blood samples were collected by venepuncture. Two of the samples were acquired using tubes containing clot activators, i.e., VacuetteR 10ml serum tubes with Z Serum Clot Activator (Greiner BioOne International AG, Germany), whereas the remaining blood sample was drawn into a 10ml BD VacutainerR tube that contained dipotassium-EDTA, an anticoagulant (BD Diagnostics, New Jersey USA).

All the blood samples collected in the anticoagulant containing tubes were used to measure baseline platelet concentrations fig. 2, whereas the remaining blood samples (collected in the Clot Activator containing tubes) were used to prepare PRF by centrifugation (400g for 12 minutes) in a standard benchtop centrifuge (PLC-03, Hicare International, Taiwan) (fig 1). Therefore, from each study participant, two samples of PRF were obtained. The two PRF samples were then randomly allocated into either Group A or Group B (fig.3 & 4), using a simple coin toss.

For Group A, the PRF was removed from the tube immediately after preparation (0min), whereas for Group B, the PRF remained in the tube and was only removed after 60 minutes. The residual blood from each group was tested for platelet concentration and comparison was made with non-centrifuged baseline sample (Group C).

We calculated the platelet concentration of PRF for each group indirectly, by determining the numerical difference between the residual platelet concentration (of the remaining serum after removal of PRF) and the baseline platelet concentration for each specific study participant. In this study, platelet concentration analysis was carried out using an electronic automated cell counter.

Data was collected and entered into a spreadsheet (Microsoft Office 2010 Excel, Microsoft Corporation, Washington). The results were compared and analysed statistically using SPSSR Version 13 for Windows.

Results:-

A total of 10 participants (5 females and 5 males) were entered into the study. The mean age of the participants was 40 years. The youngest participant was 20 years old and the oldest, 54 years old. Platelet concentrations obtained from analysis of all 10 participants were within the normal laboratory reference range of $170-400 \times 10^9/L$ of circulating blood. The mean blood platelet concentration was $280.4 \times 10^9/L$ (Table 1). There was no significant difference between the genders.

After removal of formed PRF from Group A, the residual serum yielded minimal concentrations of platelets. The mean concentration of remaining platelets was $7.5 \times 10^9/L$. (Table 2)

For Group B, serum platelet concentrations were also minimal after removal of the prepared PRF with the mean concentration of the platelets being $4.0 \times 10^9/L$. (Table 2)

The residual mean platelet concentration of Group A was higher than that determined for Group B with the mean difference in platelet concentrates between the two groups being $3.50 \times 10^9/L$. Using a non-parametric Signed Rank Test, the statistical significance of the differences in platelet concentrates between the Groups A and B was analysed. The difference between the two groups was statistically significant ($p < 0.001$). The platelet concentration of PRF for both groups was calculated using the difference between baseline and residual platelet concentrations. (Table 3) A paired t-test showed a statistically significant difference between the two groups ($p < 0.001$) (Table 4)

Discussion:-

The aim of this study was to determine whether storage time has a significant effect on the platelet concentration of platelet-rich fibrin (PRF). Structural properties of PRF does not allow for direct measurement of its platelet concentration.

We calculated the PRF concentration for each group indirectly, by determining the numerical difference between the residual platelet concentration (of the remaining serum after removal of PRF) and the baseline platelet concentration for each specific study participant. Higher residual values indicate that less platelets were present in the removed clot whereas lower residual values would indicate that more platelets were present in the clot. When differences between the test groups and the baseline blood sample were compared, it was clear that 97%-98% of the platelets were concentrated in the PRF clot. The results of present study are in accordance with study done by DohanEhrenfest et al (2010). PRF has a natural fibrin framework with growth factors within that may keep their activity for a relatively longer period and stimulate tissue regeneration effectively^(8,9).

Previous research has indicated that storing PRF under certain conditions may affect its ability to clinically yield positive results. In fact it is not recommended to store platelet concentrate in its blood collecting tube, as the serum has not been flushed away from the clot, the growth factor content remains stable. It is a good way to gain 5-15 minutes, but this is not a long-term solution, because the clot slowly starts to sink into the tube after centrifugation and to merge with the red blood cell base, leading to an unusable material loaded with red blood cells and with weak mechanical properties. Instead several authors propose to keep the clots in a sterile metal cup and to press them into membranes with a sterile metal spoon when needed. Data regarding the maximal storage time and the ideal storage temperature of PRF are however largely lacking⁽⁸⁾.

The present study used standard blood collecting tubes with clot activators to store PRF for at least 60 minutes at room temperature. As the 60 min. of duration time was chosen on the basis of average time of any periodontal surgical procedure at the faculty of dentistry. The results of present study showed that it was possible to concentrate more than 97% of available blood platelet into a readily usable form and also storing the concentrate for 60 minutes resulted in a form of PRF that had significantly higher concentrations of platelets compared with non-stored PRF.

The reason for this phenomenon may be related to clot activators. Due to the lack of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. The contact with a silica surface is required to activate the clot polymerization process⁽¹⁰⁾. Finally, platelets seem to be trapped massively in the fibrin meshes. Therefore, for successful preparation of PRF, speedy blood collection and immediate centrifugation before the initiation clotting cascade is absolutely essential. Quick handling is the key to achieve a clinically usable PRF clot⁽¹¹⁾. Another reason for the variation in platelet concentration may be that the recommended centrifuge time is too short to allow for complete clot formation to occur. Therefore, allowing the PRF clot to remain in the tube for a period longer than the recommended time, may result in a more complete physiological reaction taking place.

The ability of PRF to sustain its platelet concentrate over the tested time may have significant clinical implications. Instead of drawing the blood during surgical procedure, it allows for blood to be drawn before the start of procedure this improves the patients comfort and save the operators time. To store the PRF in same tube avoid its exposure to the environmental factors. It is also cost-effective, since no specialised equipment or storage facilities are required. This may be necessary in resource-poor settings.

The release of growth factors is a significant property of blood platelets. Some studies showed that PRP had limited effect to stimulate bone regeneration^(12,13). It is hypothesized that PRP releases growth factors quickly, just before the cell outgrowth from the surrounding tissue⁽¹⁴⁾. Platelet-rich fibrin is prepared naturally without addition of thrombin, and it is hypothesized that PRF has a natural fibrin framework and can protect growth factors from proteolysis. Thus, growth factors can keep their activity for a relatively longer period and stimulate bone

regeneration effectively. The platelet counts of PRF and PRP were similar, it is shown that PRF can release growth factors gradually and keep their activity to a relatively long period compared with PRP⁽¹⁵⁾.

In the present study, it was shown that the platelet concentration of PRF improved over a period of 60 minutes of storage. We assume that this may be due to prolonged fibrin clot maturation. It is therefore reasonable to speculate that if PRF is stored until optimal fibrin formation is achieved, then higher concentrations of growth factors may be available from the PRF during wound healing. Whether this has any clinical significance requires further investigation.

Limitation:

1. Limited number of sample size.
2. The inability to directly measure the platelet concentration of PRF
3. The small difference between the platelet concentrations of the groups tested. Although statistically significant, a mean difference.

Future perspect:

1. Therefore further research is warranted to determine the clinical significance of these findings.

Conclusion:-

This study showed that it is possible to concentrate more than 97% of the available platelets into a PRF clot. The study also indicated that, unlike in previous reports, clot disintegration does not take place after 15 minutes of storage, but rather remains stable over a period of at least 60 minutes and may in fact increase in platelet concentration during that time.

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Result Tables:

Table 1. Mean baseline platelet concentration in cell $\times 10^9$ /L of blood

	mean	SD
Male	2.75	40
Female	2.85	65

Table 2. Mean baseline platelet concentration of residual blood in cell $\times 10^9$ /L of blood

	Mean	N	SD	Std. error mean
Group A	7.5	10	3.01	0.55
Group B	4	10	1.90	0.33

Table 3. Mean calculated PRF platelet concentration in cell $\times 10^9$ /L of blood

	Mean	SD
PRF A	2.72	57
PRF B	2.76	58

Table 4. Statistical analysis of PRF A and PRF B (Paired t test, P value is significant)

Mean	SD	STD error mean	95% confidence interval		t	df	Significance
			L	U			
-3.9	3.13	0.57	-5.0	-2.7	-6.18	29	0.001

Figure 1. PRF Preparation



Figure 2. Baseline platelet concentration



Figure 3. Group A platelet concentration immediate after PRF after storage of PRF

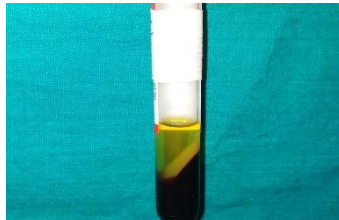


Fig.4 Group B platelet concentration 60 min.

