EVALUATION OF EFFECT OF THE STORAGE TIME ON PLATELET CONCENTRATION IN PLATELET RICH FIBRIN- BIOCHEMICAL AND MICROSCOPIC ANALYSIS

Clinical Science

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ABSTRACT

Aim of the present study was to evaluate the effect of storage time on platelet-rich fibrin (PRF) and the stability of the fibrin clot over a period of time. Three blood samples were drawn from participants in sterile blood sampling tube. Two blood samples drawn in sterile glass tubes were centrifuged to form PRF. Third non-centrifuged sample drawn in EDTA-containing tube was used to measure baseline blood platelet concentration. After PRF had formed, it was removed from respective test tubes at different time intervals i.e. immediately after centrifugation- PRF A (Group A) and after 60 min of storage time in the blood collecting tube- PRF B (Group B). Residual blood from each group was tested for platelet concentration and compared with baseline reading. Similarly, PRF membranes were studied microscopically immediately after centrifugation and other after 60 minutes. Platelet concentration of PRF in blood and PRF membrane for both groups was calculated using the difference between baseline and residual platelet concentration- biochemically and microscopically. A paired t-test showed a statistically significant difference between the two groups ($p < 0.005$).

KEYWORDS

Platelet rich fibrin, Centrifugation, Platelet concentration, Storage Time, Growth factors

INTRODUCTION

Wound healing is a complex biological process where many cellular events taking place simultaneously leading to the repair or regeneration of damaged tissues.¹⁻⁴ Many attempts have been made in the field of tissue regeneration with the aim of predictably repairing, regenerating, or restoring damaged and diseased tissues.¹⁻⁴ These include strategies with foreign materials often derived from allografts, xenografts, or synthetically produced alloplasts to regenerate host tissues.¹⁻⁴ While many of these materials have shown promise in various aspects of regenerative medicine, it is important to note that all create a "foreign body reaction," whereby a foreign material is introduced into human host tissues. Platelet concentrates collected from whole blood was first introduced over 20 years ago. The concept was developed with the aim of utilizing human blood proteins as a source of growth factors capable of supporting angiogenesis and tissue ingrowth based on the notion that blood supply is a prerequisite for tissue regeneration.⁵

CELLS	EXTRACELLULAR MATRIX	GROWTH FACTORS
<ul style="list-style-type: none"> • PLATELETS • LEUKOCYTES • RED CELLS 	<ul style="list-style-type: none"> • FIBRIN MATRIX (FIBRONECTIN+ VITRONECTIN) 	<ul style="list-style-type: none"> • PDGF • VEGF • IGF • EGF • TGFB • BMP2

Figure 1: Three main components of PRF⁶

MATERIALS AND METHODS

Three blood samples were drawn from the participants in sterile blood sampling tubes. Two blood samples drawn in glass test tubes were centrifuged to form PRF (Figure 2). A total of 30 participants (20 females and 10 males) were included in the study. The mean age of the participants was 22 years. The participants with any significant hematological disease, aspirin intake or any other medication that might interfere with the coagulation over the previous 2 weeks were excluded. Third non-centrifuged sample drawn in EDTA-containing tube was used to measure baseline blood platelet concentration.

Preparation of PRF- 10ml of blood was withdrawn in a glass test tube without any additives and was centrifuged at 3000 rpm for 10 minutes.⁷

After PRF had formed, it was removed from respective test tubes at different time intervals i.e. immediately after centrifugation (Group A) and after 60 min of storage time in the blood collecting tube (Group B). Residual blood from each group was tested for platelet concentration and compared with baseline reading (as an indirect measure of the platelet concentrate of PRF) and PRF membranes were also studied microscopically immediately after centrifugation and 60 minutes after its centrifugation. Platelet concentration of PRF in blood and PRF membrane for both groups was calculated using the difference between baseline and residual platelet concentration- biochemically and microscopically.

Figure 2: REMI R-4C Centrifuge

Figure 3: Prepared PRF

RESULTS

Platelet concentration of PRF in blood and PRF membrane for both groups was calculated using the difference between baseline and residual platelet concentration- biochemically and microscopically (Figure 4 and 5). A paired t-test showed a statistically significant difference between the two groups ($p < 0.005$) (Table 2). Platelet concentrations obtained from analysis of all 30 participants were within the normal laboratory reference range of $170400 \times 10^9/L$ of circulating blood. The mean blood platelet concentration was $1.6 \times 10^9/L$ (Table 1). There was no significant difference between the genders.

Table 1: Mean baseline platelet concentration of residual blood in cells $\times 10^9/L$ of blood

Mean Platelet Concentration in Residual Blood				
Group	Mean	N	Standard Deviation	Standard Error Mean
Group A	1.830	30	1.105	0.201
Group B	1.370	30	0.915	0.167

Table 2: Statistical analysis of PRF A and PRF B

Statistical Analysis of PRF A and PRF B								
Group	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Standard Deviation	Standard Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Group A-Group B	0.460	0.414	0.075	0.305	0.615	6.084	29	0.000

Figure 4: Cells In Prf Membrane Immediately After Centrifugation

Figure 5: Decreased cell count in PRF membrane 60 minutes after centrifugation

DISCUSSION

Because the structural properties of the PRF clot do not allow for a direct measurement of its platelet concentration, the residual platelet values left in the blood tube after removal of the PRF clot were used as an indirect measurement of the platelet concentration in the PRF clot. Higher residual values (as seen immediately after centrifugation) indicate that less platelets were contained in the removed clot whereas lower residual values (as seen after 60 minutes of centrifugation) would indicate that more platelets were transferred to the clot. The results from this study indicate that there is a significant difference in the mean platelet concentration of PRF when stored for 0 and 60 minutes.

From the platelet counts obtained in both test groups it was clear that a significant proportion of platelets had been removed from the blood after extraction of the PRF clots. The platelets get concentrated in the fibrin clot that is formed after the centrifugation. This acts as a reservoir for a concentration of growth factors (GFs) required in the initial stages of wound healing.⁸

Previous research has indicated that storing PRF under certain conditions may affect its ability to clinically yield positive results.⁹ In fact, it is not recommended to store the platelet concentrate in its blood collecting tube since it is assumed that it may disintegrate into an unusable form after about 15 minutes of storage.⁹

In this study, glass test tubes were to store PRF for at least 60 minutes at room temperature. This particular duration of time was chosen based on the average time of typical periodontal surgical procedures in the Department of Periodontology at College of Dental Sciences and Research Centre, Manipur, Bopal, Ahmedabad. The results of the study indicate that by using the presented protocol for platelet concentrate preparation, it was possible to concentrate maximum amount of the available blood platelets into a readily usable form.

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 ability of PRF to sustain its platelet concentrate over the tested time may have significant clinical implications.¹⁰⁻¹³

The release of growth factors is a significant property of blood platelets.¹⁰ Previous studies indicate that several of these factors play an essential role in osteogenesis and periodontal regeneration.^{16,17} When PRF is used, the release of these growth factors appears to be constant, and over a longer period of time when compared with that seen with PRP.¹² In a direct comparison between the two, PRP was shown to have an initial larger release of growth factors after activation. However, these high concentrations were not stable and decreased over time. On the other hand, PRF releases less growth factors initially, but sustains this release for a longer duration.¹² A number of authors speculate that this may be due to the fibrin clot that forms a network and acts as a reservoir for the trapped platelets.^{14,15,18} In the present study, it was shown that the platelet concentration of PRF improved over a period of 60 minutes of storage. This might 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.

Temperature may affect the storage potential of PRF and it has been suggested that storing it in near-freezing temperatures is not advisable. In this study, all the samples were stored at room temperature. It was clear that storage under these conditions had no detrimental effect on the platelet concentration of the PRF clot. Although the present study showed statistically significant differences between the two groups tested, some limitations were also evident. These include the limited number of study participants as well as the inability to directly measure the platelet concentration of PRF. Other factors include the small difference between the platelet concentrations of the groups tested. Although statistically significant, a mean difference of only 4.6×10^9 cells/L may not be clinically relevant and therefore further research is warranted to determine the clinical significance of these findings.

CONCLUSION

The viability of storing PRF in its own blood collecting tubes has not yet been reported. This study showed that by using the above method of platelet preparation, it is possible to concentrate maximum amount 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. Further investigations are warranted to determine whether this has any clinical implications.

REFERENCES

- Coury, A. J. (2016). Expediting the transition from replacement medicine to tissue engineering. *Regenerative Biomaterials*, 3(2), 111–113.
- Dai, R., Wang, Z., Samanipour, R., Koo, K., & Kim, K. (2016). Adipose-Derived Stem Cells for Tissue Engineering and Regenerative Medicine Applications. *Stem Cells International*, 2016, 1–19.
- Rouwkema, J., & Khademhosseini, A. (2016). Vascularization and Angiogenesis in Tissue Engineering: Beyond Creating Static Networks. *Trends in Biotechnology*, 34(9), 733–745.
- Zhu, W., Ma, X., Gou, M., Mei, D., Zhang, K., & Chen, S. (2016). 3D printing of functional biomaterials for tissue engineering. *Current Opinion in Biotechnology*, 40, 103–112.
- Upputuri, P. K., Sivasubramanian, K., Mark, C. S. K., & Pramanik, M. (2015). Recent Developments in Vascular Imaging Techniques in Tissue Engineering and Regenerative Medicine. *BioMed Research International*, 2015, 1–9.
- Miron, R. J., & Bosshardt, D. D. (2016). OsteoMac: Key players around bone biomaterials. *Biomaterials*, 82, 1–19.
- Dohan Ehrenfest, D. M., Del Corso, M., Diss, A., Mouhyi, J., & Charrier, J.-B. (2010b). Three-Dimensional Architecture and Cell Composition of a Choukroun's Platelet-Rich Fibrin Clot and Membrane. *Journal of Periodontology*, 81(4), 546–555. <https://doi.org/10.1902/jop.2009.090531>
- Gassling, V., Douglas, T., Warnke, P. H., Aqil, Y., Wiltfang, J., & Becker, S. T. (2010). Platelet-rich fibrin membranes as scaffolds for periosteal tissue engineering. *Clinical Oral Implants Research*, 21(5), 543–549.
- Choukroun, J., Diss, A., Simonpieri, A., Girard, M.-O., Schoeffler, C., Dohan, S. L., Dohan, A. J. J., Mouhyi, J., & Dohan, D. M. (2006). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 101(3), e56–e60.
- Hill, A. G., Hood, A. G., Reeder, G., Groom, R., Potter, P. S., Iverson, L. L., Keating, R., Speir, A., & Lefrak, E. (1993). Perioperative autologous sequestration II: A differential centrifugation technique for autologous component therapy: methods and results.
- Hood, A. G., Hill, A. G., Reeder, G. D., et al. (1993). Perioperative autologous sequestration III: A new physiologic glue with wound healing properties.
- He, L., Lin, Y., Hu, X., Zhang, Y., & Wu, H. (2009). A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 108(5), 707–713.
- Kang, Y.-H., Jeon, S. H., Park, J.-Y., Chung, J.-H., Choung, Y.-H., Choung, H.-W., Kim, E.-S., & Choung, P.-H. (2011). Platelet-Rich Fibrin is a Bioscaffold and Reservoir of Growth Factors for Tissue Regeneration. *Tissue Engineering Part A*, 17(3–4), 349–359.

14. Dohan Ehrenfest, D. M., Del Corso, M., Diss, A., Mouhyi, J., & Charrier, J.-B. (2010). Three-Dimensional Architecture and Cell Composition of a Choukroun's Platelet-Rich Fibrin Clot and Membrane. *Journal of Periodontology*, 81(4), 546–555.
15. Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J. J., Mouhyi, J., & Gogly, B. (2006). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 101(3), e45–e50.
16. Gassling, V., Douglas, T., Warnke, P. H., Açil, Y., Wiltfang, J., & Becker, S. T. (2010b). Platelet-rich fibrin membranes as scaffolds for periosteal tissue engineering. *Clinical Oral Implants Research*, 21(5), 543–549.
17. Del Corso, M., Sammartino, G., & Dohan Ehrenfest, D. M. (2009). Letter to the Editor: Re: "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." *Journal of Periodontology*, 80(11), 1694–1697.
18. Toffler, M., Toscano, N., Holtzclaw, D., et al. (2009). Introducing Choukroun's Platelet Rich Fibrin (PRF) to the reconstructive surgical milieu. *Journal of Implant and Advanced Clinical Dentistry*, 1(6), 21–31.