ORIGINAL ARTICLE

Donor Compatibility on Crossmatch-test Result of Freeze-dried Homologous Platelet-rich Plasma

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ABSTRACT

Introduction: Homologous platelet-rich plasma (hPRP) is made from unused products from healthy blood donors and used for periodontal tissue regeneration therapy. This study tried to explore the benefits of this unused product to enhance community dental health services. The Fresh hPRP (liquid form) is obtained from the blood by centrifuge or sedimentation. The red blood cells could be separated from the plasma to sedimentation for a few hours and let the cells settle by gravity. Another type is Freeze-dried hPRP (FD-hPRP) (powder form) has been proposed as a consistent result for product standardization and fabrication of a ready-to-use product for feature uses. The hPRP must be passed from the crossmatch test to prevent an immune reaction. This research aimed to determine the difference of donor compatibility on the crossmatch result between single centrifugation and sedimentation methods. **Methods:** Twenty samples of blood type O were divided into four groups; group I was fresh hPRP centrifugation, group II was fresh hPRP sedimentation, group III was FD-hPRP centrifugation, and group IV was FD-hPRP sedimentation. The crossmatch test was done to determine donor compatibility utilizing the gel-test method. **Results:** The data were interpreted using the chi-square test, and it showed that all four groups in the crossmatch test were 100% compatible. **Conclusion:** The conclusion indicated that FD-hPRP groups event obtained by both methods and fresh hPRP were efficiency is guaranteed, as sedimentation and freeze-dried products decrease the need for specialized facilities and staff even are readily obtainable on request.

Keywords: Homologous, Freeze-dried platelet-rich plasma, Crossmatch-test, Compatibility

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INTRODUCTION

Platelet-rich plasma was a biological mediator for periodontal regeneration and one of the procedures for regulating and increasing wound healing. Generally, PRP is an additional ingredient in the graft to increase new bone formation and periodontal regeneration, one of the materials for treating periodontal disease. It has been encouraged that PRP usage might increase bone deposition and bone quality in such dental treatments as periodontal surgery (1,2). Many regenerative treatments continue to be developed in the addition of biological mediators that can help tissue regeneration, such as giving growth factors in PRP. Platelet-rich plasma was chosen because it has several advantages, including the content of high growth factors, and recreates a part in wound healing and bone remodeling (3,4).

Platelet-rich plasma (PRP) is a product made from a patient's blood (autologous) and blood donors (homologous). Platelet homologous PRP (hPRP) content is higher than autologous PRP (A-PRP). Fresh hPRP is PRP obtained after blood is processed in liquid form (5). Fresh hPRP was taken from healthy donor blood at the PMI blood bank based on the standard of blood transfusion services. The half-life of growth factor in fresh hPRP is short and the length of time for PRP storage is 48 hours at room temperature (6). Due to overcoming the storage time of hPRP can be stored for a long time and can be used if needed; then the freeze-drying method is recommended (powder form)(7).

The process of obtaining PRP with many growth factors has varied techniques. The technique commonly used is to take some blood and then centrifugation (single centrifugation) to separate platelets from red blood cells and white blood cells(8). According to Dohan et al.(9), the general concept for producing PRP is taking venous blood and then mixing it with anticoagulants to prevent platelet activation and degranulation. The most commonly used anticoagulant is citrate dextrose formula A (ACD-A), the standard anticoagulant currently accepted. Platelet-rich plasma varies greatly in platelet count, concentration, and growth factor activity. The PRP preparation procedure is thought to have contributed to this variation, including the condition of centrifugation, the type of reagent for blood collection, and platelet activation. Centrifugation machine is available in laboratories and several clinics. Still, centrifugation machines are unavailable in rural areas or clinics with insufficient space. Red blood cells could be separated from the plasma in sedimentation for a few hours and let the cells settle by gravity (8,10).

The application of hPRP to periodontal treatment is a form of transfusion of blood components by including some antigens in the recipient. Before the transfusion, a series of pre-transfusion tests are needed, namely blood type ABO, Rhesus examination and crossmatch test. Rhesus system antigens are not expressed, but there is a possibility that platelets are contaminated with components of red blood cells that can induce rhesus D alloimmunization during transfusion. Homologous PRP needs a crossmatch test to see that the donor PRP recipient does not provide an immune response to PRP to be given so that the goal of the hPRP administration can be beneficial for the treatment of periodontal tissue (11). This research aimed to determine the difference in donor compatibility on the crossmatch result between single centrifugation and sedimentation methods.

MATERIALS AND METHODS

Platelet-rich plasma preparation: This laboratory experiment was designed by the Ethics Committee, Faculty of Dentistry, Universitas Gadjah Mada, Indonesia, and has been approved with registration quantity 0029/KKEP/FKG-UGM/EC/2019. Homologous PRP was taken from the double bag on the Indonesian Red Cross (PMI). The donor's condition passed all selection criteria and assessments of the screening process at PMI. The blood was centrifugated (RC KUBOTA 9942) at 1000 rpm in 22 °C for 15 min to split platelet-rich plasma (PRP) and the red blood cells (RBC) fraction. We took 100-120 mL of PRP from blood type O. the other technique hPRP is by taking venous blood, after which blending it with anticoagulants (ACD-A) that is the usual anticoagulant in PMI, red blood cells could be separated from the plasma in the way of sedimentation for eight hours and allow the cells settled through the gravity. Homologous PRP divided into four groups; Group I fresh hPRP was acquired using centrifugation. Group II fresh hPRP by sedimentation. Group III FD-hPRP was acquired using centrifugation and Group IV FD-hPRP by sedimentation.

Freeze-drying Procedure: Homologous PRP was frozen at -40 °C for 12 hours the freeze-drying for 48h using freeze drier machine (Freeze Dryer Modulyo, Edwards). Then freeze-dried were mashed with a mortar and filtered using a 60-mesh filter inside the laminar flow hood station.

Crossmatch test: The recipient's blood cell samples had been taken from PMI, and all of the groups used twenty samples consisting of the blood types O. This research turned into examined for compatibility using the Gel method. The blood grouping and Rhesus test had been examined earlier than crossmatching. For crossmatching, serum of recipients with the same blood group had been used for the gel check methods. A 0.8 % suspension of the recipient's red cells was organized by mixing 10µl of red cells in one ml of LISS (I.D. diluents). 50 µl of the recipient's red cell suspension is added to the microtube, followed by 25 µl of the donor's serum. The card is incubated using an incubator (ID-Incubator 37 S II) at 37°C for 15 minutes, then centrifuged using centrifugation (Grifols Spin 12) in I.D. centrifuge and results can be seen. The donor's blood is compatible when no agglutination indicates that the recipient is suitable for transfusion. A negative reaction shows pellets of RBCs at the bottom of the microtube with no aggregates in the gel matrix.

RESULTS

The homologous PRP used in this study was fresh homologous and homologous, which is carried out by freeze-drying using centrifugation and sedimentation methods (8,10). Data fresh hPRP group in this study used twenty recipient blood samples summarized table I. Based on table I, the outcomes of donor compatibility show 100% compatible in both fresh hPRP groups.

Based on table II, the results of donor compatibility show 100% compatible in both methods in FD-hPRP.

Table III shows the descriptive results of the crossmatch test with the FD-hPRP and fresh hPRP treatment. Based on Table III, it was found that the fresh treatment data was 100% compatible, and the freeze-dried treatment data was also 100%. From the chi-square test results in Table III, significant values did not produce negative (-) results.

Table I : Tabulation of the PRP collection methods by crossmatch test from Fresh Treatment

		Methods of fresh hPRP		Total
		Group I	Group III	
Blood cells agglutination (Fresh)	Compatible	5	5	10
		100.0%	100.0%	100.0%

Table II : Tabulation of the PRP collection methods by crossmatch test from Freeze-dried Treatment

		Methods of fresh hPRP		Total
		Group II	Group IV	
Blood cells agglutination (Freeze-dried)	Compatible	5	5	10
		100.0%	100.0%	100.0%

Table III : The *Chi-Square* Test

			Treatment		Total	Sig
			Freeze-dried	Fresh		
Blood Cells Agglutination	Compatible	N	10	10	20	0,500
		%	50.0%	50.0%	100.0%	
Total		N	10	10	20	
		%	50.0%	50.0%	100.0%	

DISCUSSION

The result showed 100% compatible each fresh hPRP group because the blood samples were acquired from the blood bank of PMI, and the recipients have been physically healthful in commonplace considering that they passed the standards selection of the blood bank. The usage of hPRP changed into a form of blood component transplantation. Periodontal treatment injected several antigens into the recipient's body. Platelet transfusion can increase the number of functional platelets, especially in wound healing periodontal treatment. Platelets are small no-nucleate cellular fragments whose principal physiological function is to prevent blood loss through maintaining hemostasis. Platelets possess-complex surface antigens: on the one hand, their membrane bears the typical immune molecules, human leukocyte antigen (HLA), and the blood group antigen ABO; alternatively, a particular sort of glycoprotein complicated with polymorphisms constitutes the human platelet alloantigen (HPA) and antigen of polysaccharide ABO blood type to some other, in which it was distinguished in blood transfusion system (12,13).

Immunocompetent recipients will provide an immune response to donor antigens (alloimmunization). Antigens that are involved in the immune response contained in platelets, HLA, HPA and ABO antigens. This can occur in the use of fresh homologous PRP, although the donor and recipient blood groups are the same, there is still a possibility of incompatibility. Incompatibility can be caused by errors in ABO blood group detection due to errors during the examination and when labeling blood bags and errors detecting ABO system donor blood groups because there is no detection of weak antigens in recipient red blood cells such as decreased antibodies or not producing antibodies. The detection of antigens can be caused by some conditions such as cell grouping patients,

old age, patients with malignancy, patients taking immunosuppressant drugs, bone marrow transplant patients (14,15).

Compatible result in both group FD hPRP are due to the freeze-dried hPRP process which can cause some protein content to decrease (16) because some plasma proteins are sensitive to heat, one of which is all proteins that have the potential for allergic processes to be denatured, and antibodies not detected. Proteins are embedded within the lipid bilayer membrane to preserve the biological and physiological features of the membrane. The membrane can alternate chemical physics (phase transition), while frozen platelet membrane lipids go through a phase transition; this may cause ion leakage throughout the membrane. Further to the transition phase, cold situations can induce the separation of lateral phases in membrane components and purpose aggregation of microdomains and platelets to end up activated. While drying proteins and membranes catch up on the lack of hydrogen bonds in water (H2O) by forming hydrogen bonds with different molecules. It is affected by intermolecular interactions. In proteins, lack of hydrogen bonds with water may be compensated by way of proteins interaction and for this reason can cause protein aggregation or denaturation (17). Implications for future research are comparing PRF with homologous platelet-rich plasma. This suggests that the crossmatch test in freeze-dried homologous platelet-rich plasma can increase donor compatibility results.

CONCLUSION

Concerning this, there is no agglutination in products from freeze-dried homologous PRP, indicating that the donor's blood is well-matched with the recipient and efficiency is assured. The sedimentation techniques and freeze-dried products lessen the need for precise facilities and a specialized workforce and are easily on demand.

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REFERENCES

1. Creeper F, Ivanovski S. Effect of autologous and allogenic platelet-rich plasma on human gingival fibroblast function: Effect of autologous and allogenic PRP on fibroblasts. Oral Diseases. 2012;18(5):494–500. doi: 10.1111/j.1601-0825.2011.01897.x.
2. Markopoulou CE, Markopoulos P, Dereka XE, Pepelassi E, Vrotsos IA. Effect of homologous PRP on proliferation of human periodontally affected osteoblasts. In vitro preliminary study. Report of a case. J Musculoskelet Neuronal Interact. 2009;9(3):167–72. Available from: https://ismni.org/jmni/pdf/37/07MARKOPOULOU.pdf
3. Giannobile WV, Al-Shammari KF, Sarment DP. Matrix molecules and growth factors as indicators of periodontal disease activity: Matrix molecules and growth factors as indicators. Periodontology 2000. 2003;31(1):125–34. doi: 10.1034/j.1600-0757.2003.03108.x
4. Bashutski JD, Eber RM, Kinney JS, Benavides E, Maitra S, Braun TM, et al. Teriparatide and Osseous Regeneration in the Oral Cavity. N Engl J Med. 2010;363(25):2396–405. doi: 10.1056/NEJMoa1005361
5. Prabhu R, Vijayakumar C, Bosco Chandra AA, Balagurunathan K, Kalaiarasi R, Venkatesan K, et al. Efficacy of Homologous, Platelet-rich Plasma Dressing in Chronic Non-healing Ulcers: An Observational Study. Cureus. 2018;10(2):e2145. doi: 10.7759/cureus.2145
6. Shiga Y, Orita S, Kubota G, Kamoda H, Yamashita M, Matsuura Y, et al. Freeze-Dried Platelet-Rich Plasma Accelerates Bone Union with Adequate Rigidity in Posterolateral Lumbar Fusion Surgery Model in Rats. Sci Rep. 2016;6:36715. doi: https://doi.org/10.1038/srep36715
7. Pan L, Yong Z, Yuk KS, Hoon KY, Yuedong S, Xu J. Growth Factor Release from Lyophilized Porcine Platelet-Rich Plasma: Quantitative Analysis and Implications for Clinical Applications. Aesth Plast Surg. 2016;40(1):157–63. doi: 10.1007/s00266-015-0580-y
8. Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. J Cutan Aesthet Surg. 2014;7(4):189. doi: 10.4103/0974-2077.150734
9. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang C-Q, Pinto NR, Bielecki T. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. Muscles Ligaments Tendons J. 2014;4(1):3–9. doi: 10.11138/mltj/2014.4.1.0013
10. Chang, Jae-hyung R., and Intini, G., Accelerated Method For Preparing Platelet Rich Plasma; 2019. Available from http://www.freepatentsonline.com/y2019/0134293.html
11. Yuan Shan., Pretransfusion Compatibility Testing; 2011. Available from http://pathology.ucla.edu/workfiles/Education/Transfusion%20Medicine/Pretransfusion-Compatibility-Testing.pdf,
12. Zhang Z-Y, Huang A-W, Fan JJ, Wei K, Jin D, Chen B, et al. The potential use of allogeneic platelet-rich plasma for large bone defect treatment: immunogenicity and defect healing efficacy. Cell Transplant. 2013;22(1):175–87. doi:

10.3727/096368912X653183
13. Rozman P. Platelet antigens. The role of human platelet alloantigens (HPA) in blood transfusion and transplantation. Transpl Immunol. 2002;10(2–3):165–81. doi: 10.1016/s0966-3274(02)00063-1
14. Syafitri R., Kasus-kasus Rujukan Imunohematologi;2016. Available from: http://ppds.fk.ub.ac.id/wp-content/uploads/2016/04/Pem-Uji-Silang-Serasi-Permasalahannya-presentasi-terbaru-24-Sept-12-presentasi-revisi.pdf,
15. Fung MK, Downes KA, Shulman IA. Transfusion of platelets containing ABO-incompatible plasma: a survey of 3156 North American laboratories. Arch Pathol Lab Med. 2007;131(6):909–16. doi: 10.5858/2007-131-909-TOPCAP
16. Bode AP, Fischer TH. Lyophilized Platelets: Fifty Years in the Making. Artificial Cells, Blood Substitutes, and Biotechnology. 2007;35(1):125–33. doi: 10.1080/10731190600974962
17. Crowe JH, Tablin F, Wolkers WF, Gousset K, Tsvetkova NM, Ricker J. Stabilization of membranes in human platelets freeze-dried with trehalose. Chemistry and Physics of Lipids. 2003;122(1–2):41–52. doi: 10.1016/s0009-3084(02)00177-9