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RESEARCH ARTICLE

Differences in injectable platelet-rich fibrin fraction of peripheral blood on the release of TGF-β1 and PDGF-AB

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ABSTRACT

Injectable platelet-rich fibrin (i-PRF) refers to second-generation platelet concentrate. In this study, the results of i-PRF centrifugation were fractionated into three layers: yellow i-PRF, buffy coat, and red i-PRF. Injectable platelet-rich fibrin fractions used in this study were yellow i-PRF, red i-PRF, and a mix of both. This study aimed to examine the level of growth factor release of transforming growth factor beta 1 (TGF-β1) and platelet-derived growth factor (PDGF) in yellow i-PRF, red i-PRF, and a mix of yellow i-PRF and red i-PRF with the ratio of 1:1. A total of 10 ml of peripheral blood from healthy female donors was centrifuged (at 700 rpm in 3 minutes) to obtain i-PRF and fractionated into three layers. The upper yellow layer was taken as yellow i-PRF, while the bottom red layer was taken as red i-PRF and was taken together with the middle layer (buffy coat). The release of TGF-β1 and PDGF in each of i-PRF fractionation method, i.e. yellow i-PRF, red i-PRF, and a mix of yellow i-PRF and red i-PRF with a ratio of 1:1 was measured with ELISA. The measurement was observed for 24 hours, 3 days, 7 days, 10 days, and 14 days. Data analysis used the two-way ANOVA test with a significance level of 0.05 and a post hoc LSD analysis to establish group significance. The group of yellow + red i-PRF significantly released PDGF-AB ($p < 0.05$). TGF-β1 was the highest of all groups on day 14. All groups showed an increase in growth factor release from time to time. The fractionation method of injectable platelet-rich fibrin affected the release of growth factor of PDGF-AB and TGF-β1. The highest release of PDGF-AB and TGF-β1 was found in the yellow + red i-PRF group with a ratio of 1:1 in the 14-day group, which was significant with the other two groups ($p < 0.05$).

Keywords: fractionation; injectable platelet-rich fibrin; platelet-derived growth factor; transforming growth factor beta 1

INTRODUCTION

Periodontal disease is a chronic infectious and inflammatory disease of the oral cavity mainly caused by gram-negative anaerobic bacteria, and characterized by the decay in the supporting tissues of the teeth, including the alveolar bone and connective tissue of the periodontium.[1] Therapy of periodontal disease aims to prevent any disease by controlling infection and inflammation. Its goal is also to maintain and improve the health, function, comfort, and aesthetics of all periodontal structures and tissues.[2]

The final purpose of periodontal treatment is to regenerate any missing periodontal tissue.[2] The new periodontal therapy has included gene, protein, and cell-based tissue regeneration supported with resorbable or non-resorbable scaffolds and biomaterials. The main focus of this therapy is on bone regeneration to stabilize the tooth or implant. In addition, soft tissue regeneration becomes necessary, particularly for aesthetic purposes.[3]

Polypeptide growth factors are a group of natural biological mediators that control the key cellular events in tissue repair, including cell proliferation, chemotaxis, differentiation, and matrix synthesis by binding specific cell surface receptors. This group consists of fibroblast growth factors (FGF), platelet-derived growth factor (PDGF), insulin-like growth factors (IGF), transforming growth factors (TGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and certain proteins that play an important role in periodontal tissue regeneration and wound healing.[4]

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Platelet concentrates are the suspensions of growth factors concentrated in platelets that act as bioactive additives locally applied to induce wound healing. Platelet-rich fibrin (PRF) refers to a second-generation platelet concentrate in which platelets and leukocytes are found in a complex fibrin matrix. Cytokines and growth factors in PRF include interleukin 1β (IL-1β), IL-4, TNFα, PDGF A and B, TGF-β1, IGF-1, and VEGF. In PRF, the growth factors TGF-β1, PDGF-AB, and VEGF are the highest growth factors. All growth factors play a role in increasing the soft tissue and hard tissue healing by stimulating collagen production, and increasing wound strength and callus formation.[5]

Platelet-rich fibrin is divided into two: solid platelet-rich fibrin and liquid platelet-rich fibrin, called as injectable platelet-rich fibrin (i-PRF). The main difference between PRF and i-PRF is the speed of blood centrifugation. During the making of i-PRF, the centrifugation speed is lower than that of PRF to make the material richer in leukocytes, platelets, VEGF, TGF-β1, and PDGF, compared to PRP. The results of i-PRF centrifugation have 3 layers: upper liquid yellow zone, buffy coat, and red blood cell containing lower part.[6] In the study by Thanasrisuebwong et al., the upper liquid yellow zone as yellow i-PRF had some stronger physical properties than red i-PRF due to a denser fibrin network, while red i-PRF had several better biological properties due to more growth factors.[6] In this study, the measurement of growth factor release consisting of TGF-β1 and PDGF-AB in yellow i-PRF, red i-PRF, as well as a mixture of yellow i-PRF and red i-PRF with a ratio of 1:1 will be carried out.

MATERIALS AND METHODS

This *in vitro* study is a pure laboratory experiment with a quantitative design. This research was conducted at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada. All procedures have been approved by the Ethics Committee of the Faculty of Dentistry at Gadjah Mada University (project number 00528/KKEP/FKG-UGM/EC/2020).

Blood samples were taken from healthy female donors aged 25-26 years. They had no history of taking antiplatelet or anticoagulants, no symptoms of COVID-19, taken a non-reactive rapid test maximum one week before blood sampling, and did not travel out of town 14 days prior to blood sampling. The participating donors gave informed consent to be research subjects for peripheral blood collection. The researchers and donors were required to use level 1 Personal Protective Equipment (PPE). Ten ml of peripheral blood from the donors was taken and put into a sterile plastic tube. The blood sample was then centrifuged using a centrifuge (Eppendorf centrifuge 5430R, Germany) at 700 rpm for 3 minutes at room temperature.

The results of i-PRF centrifugation were fractionated into yellow i-PRF, buffy coat, and red i-PRF. Each yellow i-PRF and red i-PRF was taken using a blue tip and inserted into two different tubes. The top yellow layer of yellow i-PRF above the buffy coat layer was taken, while for the red i-PRF it was done for the third or lowest red layer. The buffy coat layer was also taken in the red i-PRF.[6]

Growth factor release was measured with TGF-β1 and PDGF-AB ELISA kit (Cusabio Technology LLC, USA). Injectable platelet-rich fibrin of each of protocols (yellow i-PRF, red i-PRF, mix of yellow i-PRF and red i-PRF with the ratio of 1:1) was inserted into 6 well plates in the DMEM media of 350 µl. The plate was placed in a 5% CO2 incubator at a temperature of 37 ºC until clotting. In the period of 24 hours, 3 days, 7 days, 10 days and 14 days, 5 ml of culture media was taken and frozen at a temperature of -20 ºC. The concentrations of TGF-β1 and PDGF were measured according to the instructions of the manufacturer and the optical density was assessed using a 450 nm microplate reader. Measurements were carried out through duplication. There were 72 samples which were divided into time groups (24 hours, 3 days, 7 days, 10 days, and 14 days) and treatment groups (yellow i-PRF, red i-PRF, mix of yellow i-PRF and red i-PRF with the ratio of 1:1).

The research data were analyzed using IBM SPSS version 24.0. The Saphiro-Wilk test was used to assess the data distribution and the Levene Test was used to test the data homogeneity. Data

analysis was carried out using parametric analysis with two-way ANOVA test with a significance level of 0.05 and followed by a post hoc Least Significant Difference (LSD) analysis to determine the difference in significance between each group.

RESULTS

The release of growth factors of PDGF-AB and TGF-β1 was observed within 24 hours, 3 days, 7 days, 10 days, and 14 days. Table 1 and Table 2 showed that there was an increase in PDGF-AB and TGF- β1 levels at 24 hours, 3 days, 7 days, 10 days, and 14 days in all groups; however, the amount of the increase was different. Both growth factor release patterns showed almost similar pattern (Figure 1 and Figure 3). Yellow i-PRF, red i-PRF, and a mix of yellow and red (yellow+red) i-PRF (1:1) increased during the observation time. The concentration of PDGF-AB in the red i-PRF group showed the highest significance at 10 days, and on day 14 the mixed yellow and red i-PRF group released the highest significant growth factor PDGF-AB. The concentration of TGF-β1 on day 14 was found to have the highest significance in the mix of yellow and red i-PRF. The total accumulated growth factor release was also calculated up to 14 days (Figure 2 and Figure 4) and it was found that both PDGF-AB and TGF-β1 showed a higher total accumulation of growth factor release in the mix of yellow and red i-PRF, followed by red i-PRF, and yellow i-PRF.

The results of the two-way ANOVA test showed p=0.000 with the level of significance of 0.05 in the application group and time group. This indicated a significant effect between the application group and time group towards the release of PDGF-AB and TGF-β1. The interaction of the application group and time group showed $p < 0.05$ meaning that both had a significant effect on the release of growth factor.

DISCUSSION

Platelet concentrate is an autologous source that has been used in dentistry for over three decades as a regenerative material. It is capable of releasing a number of growth factors for promoting

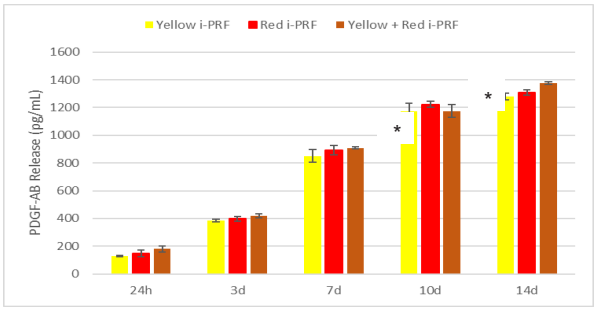

Figure 1. Graph of growth *factor* PDGF-AB release (*p < 0.05)

Table 1. The Mean of Growth Factor PDGF-AB by Group and Time

		Mean ± SD PDGF-AB (pg/ml)				
Groups	N	24 hour	day 3	day 7	day 10	day 14
Yellow i-PRF	4	128.115 ± 5.078	384.846 ± 10.107	851.000 ± 47.297	1173.115 ± 57.995	1278.692 ± 25.028
Red i-PRF	4	147.346 ± 25.336	398.500 ± 16.478	893.115 ± 35.174	1222.538 ± 22.214	1306.769 ± 19.445
Yellow + Red i-PRF	4	181.384 ± 22.334	419.846 ± 13.152	909.269 ± 6.976	1174.461 ± 47.528	1375.615 ± 8.164

Table 2. The mean of growth factor TGF-β1 by group and time

		Mean ± SD TGF- β1 (pg/ml)				
Group	N	24 hour	day 3	day 7	day 10	day 14
Yellow i-PRF	4	5.510 ± 0.399	17.127 ± 0.577	21.227 ± 0.599	26.174 ± 0.724	32.983 ± 1.192
Red i-PRF	4	6.101 ± 0.185	17.725 ± 0.294	22.448 ± 0.600	26.533 ± 0.469	32.380 ± 0.306
Yellow + *Red* i-PRF	4	6.835 ± 0.436	18.003 ± 0.564	22.866 ± 0.555	26.354 ± 0.523	34.223 ± 0.174

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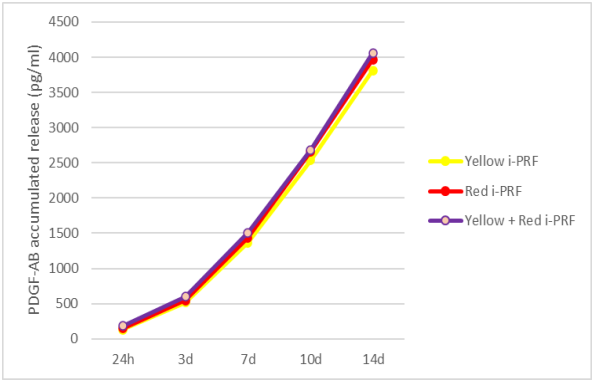

Figure 2. Graph of total accumulation of the release of growth factor PDGF-AB

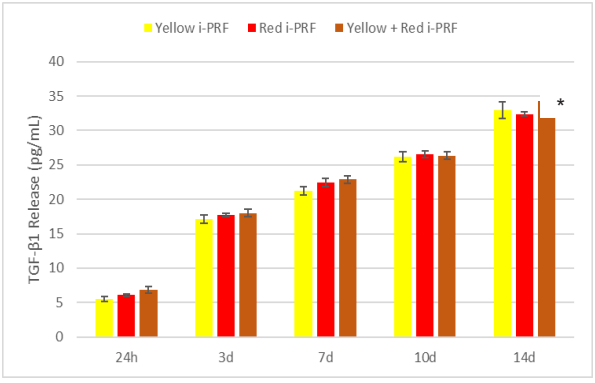

Figure 3. Graph of release of growth factor TGF-β1 (*p < 0.05)

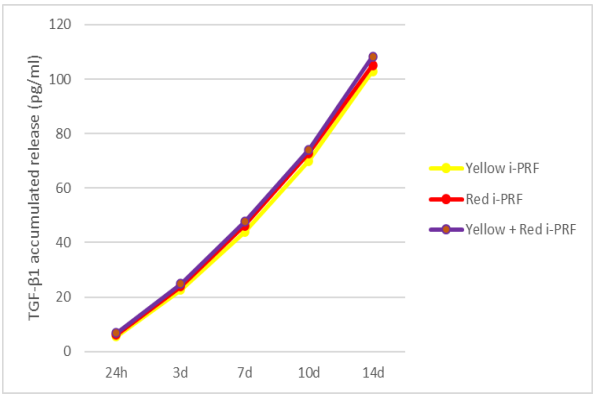

Figure 4. Graph of total accumulation of TGF-β1 release

tissue regeneration.[7] Platelet-rich fibrin (PRF) is the second generation of platelets that contains many growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF). Platelet-rich fibrin also acts as a drug delivery system for the growth factors, leading to the increase of neoangiogenesis.[8] Various types of PRF consist of solid PRF, fluid injectable PRF (i-PRF), advanced PRF (A-PRF), and advanced PRF+ (A-PRF+).[6,9] The use of solid platelet-rich fibrin (PRF) is restricted since it cannot be combined with bone replacement materials; consequently, PRF is produced in the form of a liquid that is referred to as injectable platelet-rich fibrin (i-PRF).[6]

By adjusting the different levels of spin centrifugation forces, injectable platelet-rich fibrin was developed in 2014 with the same level of quality of PRF. The low centrifugation and rapid duration on the i-PRF produce more leukocytes as well as growth factors and fibronectin, which are not found in PRF.[10] Injectable platelet-rich fibrin gives clinicians an advantage, i.e. the liquid formulation of i-PRF allows it to be used in periodontal tissue regeneration with or without the addition of other biomaterials. I-PRF has the advantage of exhibiting a continual release of growth factors and promoting cell migration by announcing the expression of type I collagen and transforming growth factor mRNA. This is accomplished by announcing the expression of both of these factors. Injectable platelet aggregates are used for the majority of procedures in the field of plastic and orthopedic surgery. It also lessens the likelihood of unfavorable reactions to the transplanted material in comparison to other grafting methods. In addition to this, it improves the viability of a

great deal of other procedures, such as those that involve regeneration. I-PRF has been shown to be beneficial and essential in periodontics for bone regeneration and wound healing.[7,8,11-14]

In this study, the tests on the release of growth factor of PDGF-AB and TGF-β1 were carried out at 24 hours, 3 days, 7 days, 10 days, and 14 days. The second highest release of growth factor occurred on day 14 in the group of yellow + red i-PRF. This mix of yellow + red i-PRF, to the best of the researchers' knowledge, has never been studied. In the study by Thanasrisuebwong et al., red i-PRF released higher PDGF and TGF-1 than yellow i-PRF.[6] In contrast to this study, the pattern of growth factor release showed a decrease on day 14 in comparison to the previous day. This study, on the other hand, found that growth factor release increased along with the observation time. This might be due to the differences in the blood samples which came from the Javanese race.

Yellow i-PRF has a dense fibrin matrix, while the red one has more growth factors.[6] Fibrin is a carrier of growth factors in a controlled system during the healing period.[8] Therefore, a mix of yellow i-PRF and red i-PRF released more growth factors compared to the yellow i-PRF or red i-PRF individually. The fibrin characteristics of the yellow i-PRF and the better biologic properties of the red i-PRF, if combined, can produce a higher release of growth factors, better than the individual one. Further research is deemed important to determine the decrease in growth factor because in this study the release of growth factor continued to increase on day 14.

CONCLUSION

The results of the fractionation of injectable platelet-rich fibrin of peripheral blood showed differences in the release of the amount of PDGF-AB and TGF-β1. The highest growth factor release was found in the mix of yellow i-PRF and red i-PRF with a ratio of 1:1 on day 14. This study is a preliminary study on growth factor release from the results of fractionation of injectable platelet-rich fibrin of peripheral blood with a span of 24 hours, 3 days, 7 days, 10 days, and 14 days. Further research could extend the observation period, followed by testing on experimental animals to observe cellular reactions, and then to be applied at clinical level.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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