

RESEARCH ARTICLE

Effect of freeze-drying process of collagen-activated platelet-rich plasma on transforming growth factor- β 1 level

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ABSTRACT

Periodontal tissue damage requires regenerative material to repair the damage. Platelet-rich plasma (PRP) is known as a regenerative material from blood which contains high level of growth factor that plays a role in wound healing and tissue remodeling. However PRP has a weakness, i.e. it is too watery so it is easily dissolved in the oral cavity, and should be used immediately after preparation. Therefore PRP storage method is needed to increase the benefits of PRP. The addition of collagen to PRP serves as a scaffold as well as an activator that stimulates the release of growth factors. One method of storing PRP is by freeze-drying process. The purpose of this study was to analyze the effect of freeze-drying process of collagen-activated PRP (PRP+C) on transforming growth factor- β 1 (TGF- β 1) levels. Transforming growth factor- β 1 is a cytokine content in PRP, that plays a role in bone remodeling and is an important stimulator for osteoblast formation, causing chemotaxis, osteoblast proliferation and differentiation. In this study, PRP was produced from peripheral blood probandus. Platelet-rich plasma was then activated with collagen (PRP+C), and divided into two groups: freeze-dried PRP collagen (FD PRP+C); and non freeze-dried PRP+collagen (PRP+C). Transforming growth factor- β 1 levels were measured using the ELISA method, followed by independent t-test. The TGF- β 1 level of FD PRP+C group was significantly higher than PRP+C group ($p < 0.05$). From this study it can be concluded that freeze-dried collagen-activated PRP has an effect to increase TGF- β 1 level.

Keywords: collagen; freeze-drying; platelet-rich plasma; TGF- β 1

INTRODUCTION

Periodontal disease is a multifactorial and complex condition that affects the periodontium. It is characterized by the loss of collagen membrane with the subsequent destruction of periodontal tissues. One of the consequences of periodontal disease (periodontitis) is periodontal intrabony defects. There is a growing interest in the use of platelet-rich plasma (PRP) for the treatment of intrabony pocket since it is a concentrated source of autologous platelets enriched with several growth factors.¹ Platelet-rich plasma (PRP) is an autologous source of several growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)- β , insulin growth factor (IGF), vascular endothelial growth factor (VEGF), platelet-derived

endothelial cells growth factor (PDECGF), platelet-derived angiogenesis factor (PDAF) and fibroblast growth factor (FGF).²

Autologous PRP is usually prepared before surgery and immediately used after preparation. This leads to longer duration of patient treatment and also requires more operators.³ Homologous PRP is a PRP prepared from donor blood which can be used by other patients after cross-matched processes.^{4,5} Human platelet concentrates are usually stored in a blood bag at 22 °C with a shelf life of 3-7 days, according to the standard of blood bank. This limits the availability of platelets to address emergency needs.⁶ It would be most effective if PRP can be isolated first and stored until the time of use.³

Platelet storage method at room temperature in the long term was introduced by lyophilization or freeze-drying techniques. The ability to store PRP for a long time and keep the cytokines therein contained will eventually expand its clinical use. This allows for the taking of a patient's blood sample then stored and used several times at later time.^{3,6} Shiga et al in their study concluded that PRP activated using calcium chloride (CaCl_2) + thrombin then freeze-dried and stored within eight weeks had the same growth factor as fresh PRP, whereas PRP stored in room temperature and frozen has decreased levels of growth factor significantly.⁷ Research by Pan et al concluded that PRP activated using CaCl_2 then freeze-dried and stored for four weeks at room temperature had higher levels of TGF- β 1 than PRP activated using CaCl_2 without freeze drying at 15 min incubation.⁶

Transforming Growth Factor (TGF)- β 1 is a growth factor contained in PRP, which is released in large amounts after activation.⁶ Measurement of TGF- β 1 levels is a representative way to assess the cytokine content present in PRP. Study on TGF- β 1 levels needs to be done to determine the effect of freeze-drying process on the cytokines contained in PRP.

MATERIALS AND METHODS

The protocol of this study was approved by the ethics committee of Faculty of Dentistry Universitas Gadjah Mada with registration number 001250/KKEP/FKG-UGM/EC/2017. This experimental study used blood from human subject of which the inclusion criteria were: having normal platelet count confirmed with routine blood test, and no abnormal bleeding history.

Forty mL of donor blood was taken from artery antecubital and 9 mL was separated into each vacutainer tube. One mL of 3.8% sodium citrate was added as anticoagulant. Platelet-rich plasma was prepared by differential centrifugation. An initial centrifugation was done at 1200 rpm for 10 minutes (EBA 200 Hettich, America). This first centrifugation was separated into two layers: platelet-poor plasma (PPP) in the upper layer and red blood cells in the

second layer. Platelet poor plasma was separated using 3 way stopcock (Onemed), followed by centrifugation at 3500 rpm for 10 minutes. The second centrifugation resulted in PRP on one-third of the bottom layers while PPP at two-third of the top layer. Three way stopcock was used to separate PRP.

Activation of PRP was obtained by mixing the PRP supernatant and Collacure collagen sponge, the volume ratio was 1 mL:1 mg. Then an aliquot was divided into two groups (FD PRP+C and PRP+C), each was incubated for two hours.

Freeze drying procedure was done by freezing PRP+C at -40°C for 12 hours, then drying for 48 hours using freeze-drier machine (Freeze Dryer Modulyo, Edwards).³ The sample of FD PRP+C was resuspended using a sterile distilled water before ELISA procedure. After resuspension, FD PRP+C weight was adjusted to be the same as the weight before freeze-drying to avoid change of FD PRP+C component concentration.⁷

The concentration of TGF- β 1 was quantified by using Colorimetric Enzyme-Linked Immunosorbent Assay (ELISA) (Quantikine ELISA Human TGF- β 1, RnD System, USA) method at 450 nm wave length. The concentration of TGF- β 1 was obtained in units of pg/mL by using curve expert version 1.2 software. SPSS program version 22.0 was used to analyze data.

RESULTS

We quantified TGF- β 1 of FD PRP+C and PRP+C. Table 1 shows the mean and standard deviation of TGF- β 1 of FD PRP+C and PRP+C.

As presented in Table 1, the TGF- β 1 level of FD PRP+C group was higher than that of the PRP+C group. The results for the normality and homogeneity tests showed that the data of TGF- β 1 were normally distributed and homogenous ($p > 0.05$). In order to determine the mean differences between the two groups, Independent t-test parametric test was done. Table 2 indicates that the difference of TGF- β 1 levels between the FD PRP+C and PRP+C groups was significant.

Table 1. Level of TGF β 1 in FD PRP+C and PRP+C group

Group (n)	Number of samples	Mean and standard deviation (pg/mL)
FD PRP+c	12	3067.76 \pm 166.50
PRP+c	12	1590.47 \pm 128.87

Table 2. Result of Independent t-test of TGF- β 1 level between FD PRP+C and PRP+C groups

	T	df	Sig. (2-tailed)	Mean difference	Standard error difference
t-test for equal means	24.306	22	.000*	1477.29583	60.77863

*: p value significant

DISCUSSION

This study indicated that the mean TGF- β 1 growth factor in the FD PRP+C group was higher than that in the PRP+C group. This is supported by the statistical analysis of independent t-test which showed that there was a significant difference between the two groups. The results are in accordance with the proposed theoretical basis, that freeze-drying process can maintain the content of cytokines, as well as increase the levels of growth factor (in this study is TGF- β 1) in the group of FD PRP+C.

The possible cause of these outcomes is that in the group of FD PRP+C, platelets are activated biochemically and physically. According to Textor,⁸ platelet stimuli can be physical, chemical, or a combination of both. Biochemical activation occurs when collagen is incorporated into PRP. Collagen-induced platelet activation process begins with collagen bonding with a glycoprotein VI receptor. This bond induces several sets of intracellular signals, on one side activating the α 2 β 1 intergrins which then bind to other collagen molecules. The collagen bond with these two main receptors then induces an intracellular signaling cascade that leads to calcium release and activation of protein kinase C. Both are responsible for platelet response to signal sequence. The platelet response is in the form of aggregation and release of granular content through exocytosis.⁹ Fufa et al in their study concluded that the use of type I collagen to activate

PRP may be safe and effective, and have an equal release of growth factor compared with currently available methods of PRP activation.¹⁰

The freeze-drying process includes freezing PRP at -40 °C then sublimation by lowering air pressure and raising the temperature to 38 °C. This is a way to rupture cell membranes by thermal lysis method. Rupture of cell membrane occurs due to the formation of ice on the cell membrane, causing it to be easily broken, consequently platelets will release the growth factor contained in it.¹¹ Thus PRP will be physically activated because of the freeze-drying process. The combination of collagen-activated and freeze-dried PRP causes TGF- β 1 contained in the FD PRP+C group to be higher than that in the PRP+C group.

According to Nakatani et al the use of freeze-dried collagen-activated PRP in patients requiring surgical periodontal therapy can be done by considering several things, such as the sterility of the material to be used.³ In fact, during preparing and freeze-drying PRP, it is possible for bacterial contamination to take place. Sterilization should be performed before freeze-dried collagen-activated PRP is used for clinical purposes. Usri suggests the use of γ beam radiation at a minimum dose of 25 kGy for sterilization of graft material.¹²

CONCLUSION

Based on the research that has been done, it can be concluded that freeze-drying process of collagen-activated PRP has an effect to increase TGF- β 1 level.

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