

The Efficacy of Advanced Platelet-rich Fibrin (A-PRF) on Fibroblast Cell Regeneration

Diananda¹, Kamizar^{2*}, Anggraini Margono², Dini Asrianti², Ratna Meydiawati²

1. Conservative Dentistry Residency Program, Faculty of Dentistry, Universitas Indonesia.
2. Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia.

Abstract

One of the most distinct signs of irreversible pulpitis is the presence of permanently damaged fibroblasts, which are the primary component of dental pulp. The low compliance nature of the tooth affects its ability to self-repair and leads to the disruption of the extracellular matrix. Growth factors are a substantial part of cell regeneration. It has been acknowledged that advanced platelet-rich fibrin (A-PRF) can promote the healing process in injured fibroblasts. The objectives of this study were performed to evaluate the capability of various concentrations of A-PRF. An experimental design was carried out in serum-starved fibroblasts. Methods: Fibroblasts were cultivated for three weeks and divided into two groups. Group I consisted of serum-starved fibroblasts. Group I was divided into three subgroups that were treated by various concentrations of A-PRF (50%, 25%, and 12.5%). Group II consisted of normal fibroblasts, serving as the positive control. Fibroblast proliferation was calculated by the Luna-II™ automated cell counter to evaluate the fibroblasts' ability to regenerate after A-PRF application. PRF was demonstrated to stimulate cell regeneration on serum-starved fibroblasts. As a result, the highest proliferation level was shown in the 25% group. It was concluded that A-PRF may be a promising therapeutic autologous biomaterial.

Experimental article (J Int Dent Med Res 2017; 10(Special Issue): pp. 789-792)

Keywords: Serum-starved fibroblasts; irreversible pulpitis; growth factors; A-PRF; regenerative endodontic.

Received date: 18 August 2017

Accept date: 20 September 2017

Introduction

One of the most distinct signs of irreversible pulpitis is the presence of permanently damaged fibroblasts, the primary component of dental pulp. The low compliance nature of the tooth affects its ability to self-repair and leads to the disruption of the extracellular matrix. In irreversible pulpitis, conventional endodontic—complete removal of infected dental pulp is mandatory to achieve a favorable outcome.¹

While promoting a resolution of the pathological condition and impeding future infections, conventional endodontic therapies fail to heal dental pulp cells, leaving the tooth pulpless and susceptible to other pathological

conditions that could lead to a more severe condition, including tooth loss.² Stated as “an appropriate scaffold for regenerative endodontics”, platelet-rich fibrin (PRF) includes all the properties needed for a scaffold. Often regarded as Choukroun's PRF, PRF is a second-generation platelet concentration that does not require the addition of any anticoagulants. It contains growth factors that are involved in wound healing and immunity, and it has been postulated as a promoter of tissue regeneration.

PRF deserves further prospective research for its possibility for regenerative endodontic therapy, as well as its potential scaffold-containing growth factor.³⁻⁵

To optimize the amount of growth factor in PRF, a pilot study was conducted to modify PRF by decreasing the rotational speed (revolutions per minute/RPM), while increasing the centrifugation time in the A-PRF group. This allowed for the amplification of neutrophilic granulocytes in the distal part of the clot.

*Corresponding author:

Kamizar
Department of Conservative Dentistry
Faculty of Dentistry, Universitas Indonesia
E-mail: kamizar_kz@yahoo.com

Capturing the total number of monocytes in PRF may make PRF become more active in stimulating bone grafts and increase the transformation of monocytes into macrophages, hence amplifying the bone stimulation effect. In the S-PRF group, neutrophils were mostly found at the red blood cell (RBC) and BC interface. Neutrophilic granulocytes contribute to monocyte differentiation into macrophages. Accordingly, a higher presence of these cells might be able to influence the differentiation of host macrophages and macrophages within the clot after implantation. Thus, A-PRF might influence bone and soft tissue as well as pulp tissue regeneration, especially with the presence of monocytes or macrophages and their growth factors.⁶

Material and methods

The A-PRF was isolated as follows: ten milliliters of whole blood were centrifuged at 1500 rpm for 14 minutes. Fibrinogen was initially concentrated at the highest part of the tube before the circulating thrombin transformed it into fibrin. A fibrin clot was then obtained in the middle of the tube, just between the red corpuscles at the bottom and cellular plasma at the top. Theoretically, platelets should be trapped in large numbers in the fibrin meshes.

For the cell culture, human dermal fibroblasts were used. They were cultured in a Dulbecco-modified Eagle medium with 4.5 g/L glucose, 2mmol/L L-glutamine, 2.5mg/L amphotericin B, 100µg/L streptomycin, 10IE/L

penicillin, and 10% fetal bovine serum. Human dermal fibroblasts were placed in a 96 well with a 100.000 density/well.

After dermal fibroblasts grew to 70% in DMEM containing % FBS, the DMEM and 10% FBS were replaced with DMEM and 1% FBS (low serum) for 48 hrs. Serum-starved fibroblasts were trypsinized and prepared for A-PRF application in three different concentrations (50%, 25%, and 12.5%). After 48 hrs., each group was removed and washed twice with a sterile phosphate-buffered saline (PBS) solution, and proliferation was evaluated using the Luna-II™ automated cell counter.

Results

According to the results, A-PRF treatment induced statistically significant proliferation in serum-starved fibroblasts. Statistical analysis was performed using Kruskal-Wallis, followed by Mann-Whitney multiple comparisons. For these tests, P-values of <0.05 were considered statistically significant. The highest proliferation level was shown in the 25% group. Compared to all other groups, the 25% group was proven to be statistically significant (p<0.05). Meanwhile, there was no significance between the 12.5% and 50% group. The microscopic effects of A-PRF on the proliferation of human fibroblasts cells at 48 hrs. after treatment are shown in Fig. 1. The data obtained from the Luna-II™ automated cell counter (Fig. 2 and Table 1) confirmed the microscopic results.

Table 1. Post hoc analysis comparison of serum-starved fibroblasts proliferation between groups.

	50% A-PRF	25% A-PRF	12.5% A-PRF
Control	0.024	0.004	0.004
50% A-PRF		0.002	0.650
25% A-PRF			0.040

Post hoc Mann Whitney; p<0.05 = significant.

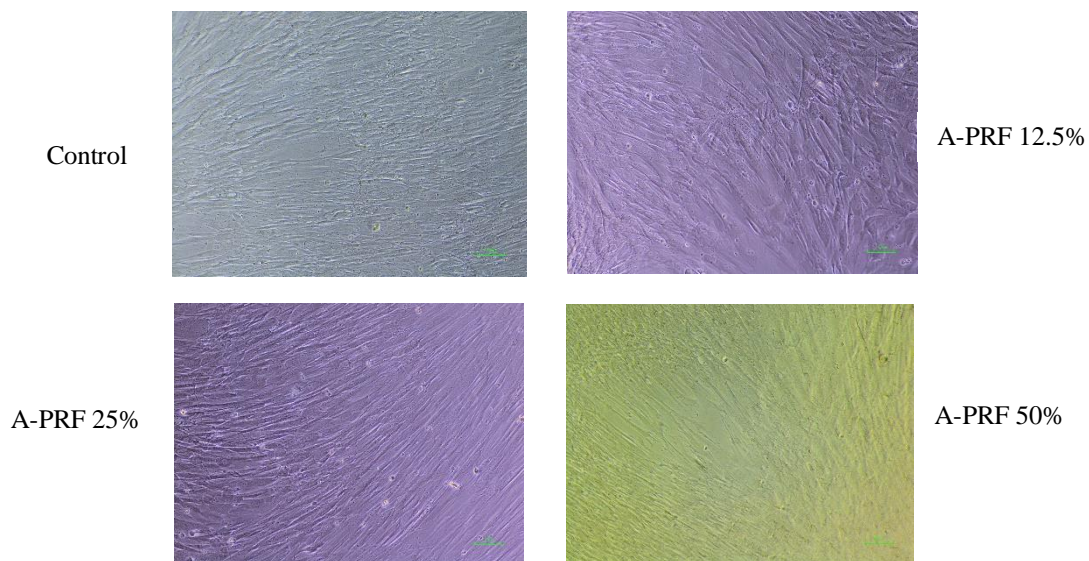


Figure 1. Microscopic analysis of human fibroblast (HF) cells in four different conditions: HF+ medium + 1% FBS (control group), HF+ medium + 50% A-PRF, HF+ medium + 25% A-PRF, and HF+ medium + 12.5% A-PRF. Assessments were done at 48 hrs. after treatment.

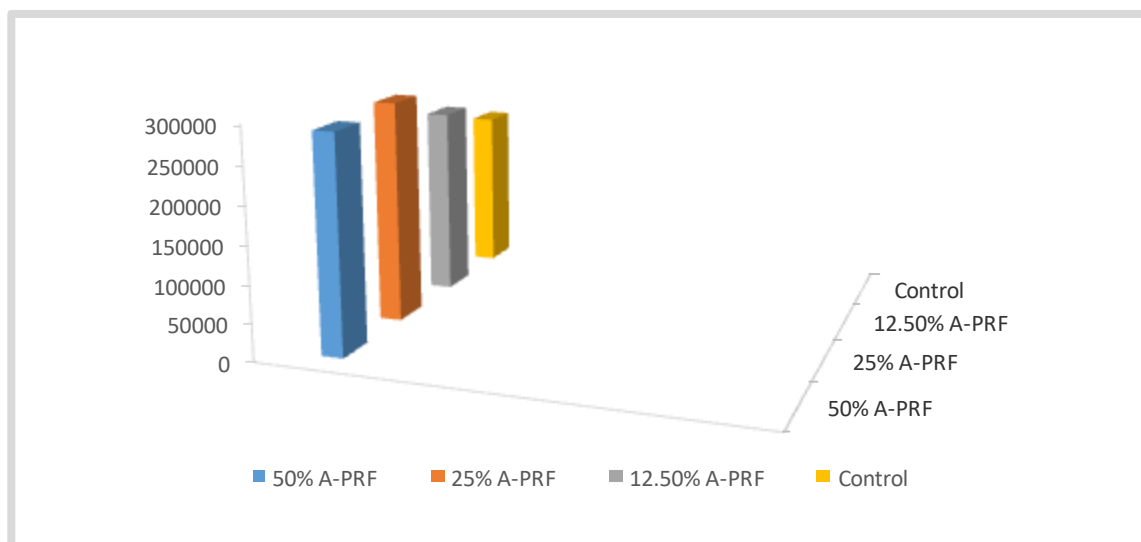


Figure 2. The effects of A-PRF on fibroblast proliferation compared to the control. The negative control contained a culture medium supplemented with 1% FBS, and the positive control contained a culture medium supplemented with 10% FBS. The results were calculated as cell counts compared to the negative control.

Discussion

The current study indicates the ability that A-PRF has in promoting cell proliferation in serum-starved fibroblasts. It was also reported that gingival fibroblasts, periodontal ligaments, and osteoblast proliferation were stimulated by PRF. This phenomenon can be explained by the following: PRF can release several kinds of

growth factors, such as PDGF and TGF- β , which modulate fibroblast proliferation through TGF- β -related mechanisms.⁷⁻⁹

The results may have differed because of the individual divergence in platelet count and the time intervals between blood sample centrifugation, fibrin membrane collection, and cell culture treatment.

In addition, previous studies enrolled multiple volunteers to prepare PRF. Even among individuals with similar platelet counts, it is possible for them to have different growth factors and concentrations of, for example, TGF- β , PDGF, VEGF, and bFGF; this might interfere with the results. Therefore, in the current study A-PRF was obtained from the same donor to eradicate the confounding effect.¹⁰

The growth factor in platelet concentrates vary highly with highly various effects. For example, platelet granules include angiogenesis stimulators with both bFGF and VEGF and angiostatic factors, such as endostatin and thrombospondin-1; each has confounding effects. The inconsistency of the obtained results can be explained by the level dissimilarity of these factors. The mitogenic activity of platelets is not strictly determined by the platelets' growth factors and particles, and membrane fragments also play an important role here.¹¹

Studies have shown that the effect of fibrin plasma on cell proliferation rate is not correlated with a higher concentration of platelets. In previous studies, a range of an optimal concentration of platelets ideal for cell proliferation was equivalent to 2.5 times the normal amount in blood, ranging from 150,000 to 400,000 platelets per microliter. However, the number of platelets in PRF cannot be counted because the platelets are trapped within the dense fibrin network of PRF. The lower proliferation has a high dependency on the pH level, and the high platelet concentration resulted in pH changes that have negative effects on fibroblast proliferation.¹²⁻¹⁴

Conclusion

A-PRF is a promising therapeutic autologous biomaterial. In the current study, the highest proliferation level was shown in the 25% group. However, despite the evident regenerative benefits of A-PRF, validation of its clinical applications is still limited. Consequently, there is a constraint for the rationalization of its use. Additional randomized, controlled clinical trials are needed to test the long-term benefits and ultimate outcomes associated with A-PRF.

Acknowledgement

This article was approved and funded by HIBAH PITTA from the Directorate Research and Community Engagement Universitas Indonesia No. 1943 / UN2.R12 / HKP.05.00 / 2016. The authors also thank to Dr. Indah Julianto, SpKK, from Dermama Biotechnology.

The publication of this manuscript is supported by Universitas Indonesia.

References

1. Rôças IN, Lima KC, Assunção IV, Gomes PN, Bracks IV, Siqueira JF Jr. Advanced Caries Microbiota in Teeth with Irreversible Pulpitis. *J Endod.* 2015;41(9):1450-5.
2. Bansal R, Bansal R. Regenerative Endodontics: A State of the Art. *Indian J Dent Res.* 2011;22(1):122-31.
3. Hotwani K, Sharma K. Platelet Rich Fibrin - A Novel Acumen Into Regenerative Endodontic Therapy. *Restor Dent Endod.* 2014;39(1):1-6.
4. Sunitha Raja V, Munirathnam Naidu E. Platelet-Rich Fibrin: Evolution of A Second-Generation Platelet Concentrate. *Indian J Dent Res.* 2008;19(1):42-6.
5. Dohan DM, Choukroun J, Diss A, et al. Platelet-Rich Fibrin (PRF): A Second-Generation Platelet Concentrate. Part I: Technological Concepts and Evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006;101(3):37-44.
6. Ghanaati S, Booms P, Orlowska A, et al. Advanced Platelet-Rich Fibrin: A New Concept for Cell-Based Tissue Engineering by Means of Inflammatory Cells. *J Oral Implantol.* 2014;40(6):679-89.
7. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow Release of Growth Factors and Thrombospondin-1 in Choukroun's Platelet-Rich Fibrin (PRF): A Gold Standard to Achieve for All Surgical Platelet Concentrates Technologies. *Growth Factors.* 2009;27(1):63-9.
8. Tsai CH, Shen SY, Zhao JH, Chang YC. Platelet-Rich Fibrin Modulates Cell Proliferation of Human Periodontally Related Cells in Vitro. *J Dent Sci.* 2009;4(3):130-5.
9. Huang FM, Yang SF, Zhao JH, Chang YC. Platelet-Rich Fibrin Increases Proliferation and Differentiation of Human Dental Pulp Cells. *J Endod.* 2010;36(10):1628-32.
10. Weibrich G, Kleis WKG, Hafner G, Hitzler WE. Growth Factor Levels in Platelet-Rich Plasma and Correlations with Donor Age, Sex, and Platelet Count. *J Craniomaxillofac Surg.* 2002;30(2):97-102.
11. Italiano JE, Richardson JL, Patel-Hett S, et al. Angiogenesis is Regulated by A Novel Mechanism: Pro- and Antiangiogenic Proteins are Organized into Separate Platelet A Granules and Differentially Released. *Blood.* 2008;111(3):1227-33.
12. Liu Y, Kalén A, Risto O, Wahlström O. Fibroblast Proliferation due to Exposure to A Platelet Concentrate in Vitro is pH Dependent. *Wound Repair Regen.* 2002;10(5):336-40.
13. Gassling VL, Açil Y, Springer IN, Hubert N, Wiltfang J. Platelet-Rich Plasma and Platelet-Rich Fibrin in Human Cell Culture. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009;108(1):48-55.
14. Vahabi S, Vaziri S, Torshabi M, Rezaei Esfahrood Z. Effects of Plasma Rich in Growth Factors and Platelet-Rich Fibrin on Proliferation and Viability of Human Gingival Fibroblasts. *J Dent (Tehran).* 2015;12(7):504-12.