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The Ability of Lysate-PRF Induces Proliferation of Fibroblast Cells in Endodontic Regenerative Therapy

Ratna Meidyawati, Endang Suprastiwi

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
Email: esuprastiwi@yahoo.co.id

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Abstract

Lysate-platelet rich fibrin (Lysate-PRF) is a scaffold that contains growth factor. **Aim:** To analyze the ability of two types of Lysate-PRF in inducing the proliferation of fibroblast cells. **Material & Methods**: Microplate with 24 wells each filled 200 µl suspension DMEM + 10% FBS and 10 × 104 fibroblasts. Then FBS is replaced with a concentration of 1%, for serum starvation process. Each 3 wells were exposed with 50%, 25%, 12.5% Lysate-PRF and 50%, 25%, 12.5% Lysate A-PRF respectively. Three other wells are exposed to 10% FBS. Microplate was incubated for 24 hour at 37˚C with 5% CO_2. The growth of fibroblast was calculated by automatic cell counter. **Results**: The highest mean value of the Lysate-PRF group was at 12.5% (312.833) while the Lysate A-PRF group at 25% (303.500). If all the groups compared did not show any significant differences. **Conclusions**: Lysate-PRF and Lysate A-PRF have the same ability as 10% FBS in inducing fibroblast cell proliferation or the same as physiological condition.

Keywords

Lysate-PRF, Proliferasi, Fibroblast, Regenerative, Endodontic

1. Introduction

Fibroblasts of the pulp are mostly present in the cell-rich zone and can differentiate according to the received signal. The odontoblast cell is one of the results of fibroblast cell differentiation; because of this ability, the use of fibroblast in the study may present a biological response [1] [2]. Regeneration is a dynamic process including inflammatory phases, granulation, re-epithelialization and tissue remodeling. Each phase involves cytokines, endogenous growth factors

and proteases, so that pulmonary regeneration also requires cytokine sources and growth factor [3].

Platelet rich fibrin (PRF) is a blood-processed product with one centrifugation step and no additional anticoagulants and bovine serum [4]. Lysate-PRF is part of a PRF containing cytokines, structured glycoproteins, and glican chains that are natural initiators in angiogenesis. Growth factors present in PRF are platelet derived growth factor (PDGF), transforming growth factor β1 (TGF β1) and insulin like growth factor (IGF). PRF can serve as a biological mediator that controls the proliferation, differentiation, and synthesis of extracellular matrix. The use of PRF as a scaffold and media containing growth factor is ideal for endodontic regenerative care [5] [6]. The form of the PRF gel may act as a viable barrier that limits the healing area to the outside environment that does not support the healing process [7]. In 2010, Huang *et al.* examined the effect of PRF on human pulp cells. The results showed that the growth factor of PRF can induce the proliferation and differentiation of pulp cells associated with secondary dentin formation [8].

Advances in Lysate-PRF manufacturing technology provide opportunities for endodontic regenerative care. This study will test and compare Lysate-PRF and Lysate A-PRF capabilities in fibroblast cell proliferation using a PBS 10% control group that can describe physiological processes. The results of this study can be developed and utilized for endodontic regeneration treatments.

2. Materials & Methods

2.1. Preparation of Lysate PRF

Human blood is taken intravenously from a healthy 32 year old man, inserted into 2 tubes each of 20 ml. In lysate-PRF the blood was centrifuged for 14 minutes with a spin speed of 1500 rpm and lysate A-PRF for 12 minutes at a speed of 2700 rpm on normal temperature. The results of centrifuge form three layers of platelet poor plasma (PPP) at the top layer, PRF in the middle, and red blood cells at the bottom (**Figure 1**). The PRF layer was taken and then incubated for 24 hours at 4˚C. After incubation, the remaining fibrin will settle at the bottom of the tube and its supernatant is called lysate-PRF. Lysae-PRF is inserted into a 2 ml eppendorf tube and stored at −20˚C. Before use each diluted to concentrations of 50%, 25% and 12.5%.

2.2. Fibroblast

The fibroblast cells are obtained from cultured crystalline fibroblast cells. 200 µl of 10 × 104 fibroblast/ml suspension in DMEM + 10% FBS were placed into each well at 24 microplate wells (Iwaki™), in incubation for 24 hours at 37˚C with 5% CO_2. The media on the well was aspirated and rinsed with PBS then replaced with a DMEM medium containing 1% FBS for serum starvation [10].

Of 24 wells used only 21 wells and each test group each uses 3 wells *i.e.* group 1; 50% lysate-PRF, group 2; 25% lysate-PRF, group 3; 12.5% lysate-PRF, group 4;

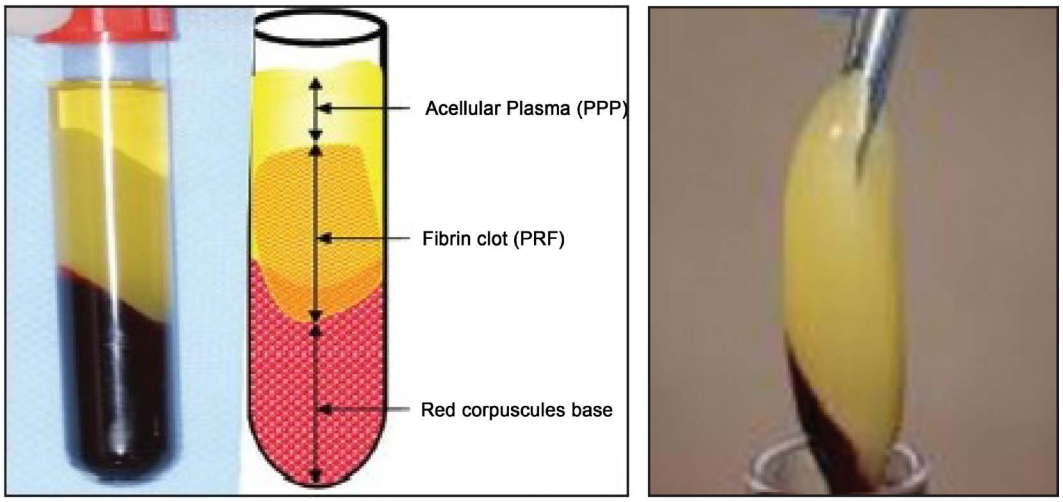

Figure 1. Left: Blood on the vacutainer tubes after centrifugation, divided into 3 layers (lower layers of red blood cells, middle layer of fibrin clumps, upper layer of the aselular plasma); Right: PRF [9].

50% lysate A-PRF, group 5; 25% lysate A-PRF, group 6; 12.5% lysate A-PRF and 3 other wells for control group with 10% PBS, then incubated for 48 hours at 37˚C with 5% CO_2. The growth of fibroblasts was calculated by duplo in each group by using automatic cell counter (Luna-II™, Logos Biosystems).

2.3. Data Analysis

Data were analyzed using SPSS 22 with One Way ANOVA statistic test.

3. Results

In Table 1, the highest mean values in the Lysate-PRF group were at a concentration of 12.5% while in the Lysate A-PRF group at 25% concentration. Average values in all groups showed no significant difference.

In Table 2, if the between group is compared with either control or with the treatment group, the result is no significant difference. This indicates that both Lysate-PRF and Lysate A-PRF capabilities are similar to the control group or 10% PBS.

4. Discussion

In this study used fibroblasts as objects because fibroblast is a major component of pulp tissue constituents that have the activity of proliferation and differentiation are easily observed [11].

In addition, the use of fibroblasts from standardized ready-to-use preparations may represent general tissue conditions because they share biological properties.

To attenuate fibroblast, a serum starvation method is used, which is a standard microbiological procedure for synchronizing phases in cells. Serum starvation is performed for 48 hours for fibroblast cells to undergo cell arrest conditions and cannot replicate DNA for cleavage. Conditions in prolonged G0/G1

Table 1. Mean and standard deviation of total cells (cell/mL) in the control group, Lysate-PRF and Lysate A-PRF.

Group	n	mean (SD)	95 CI	p
10% PBS	3	260.500 (18.187)	215.322 - 305.678	0.315
50% Lysate-PRF	3	259.333 (25.477)	196.045 - 322.621	
25% Lysate-PRF	3	305.667 (19.902)	355.106 - 256.228	
12.5% Lysate-PRF	3	312.833 (25.320)	375.731 - 249.936	
50% Lysate A-PRF	3	285.333 (40.646)	386.303 -184.363	
25% Lysate A-PRF	3	303.500 (57.765)	446.995 - 160.005	
12.5% Lysate A-PRF	3	261.167 (44.501)	371.713 - 150.620	

*test of meaning with Anova one way, $p < 0.05$.

Table 2. P-value between control group and Lysate-PRF (50%, 25%, 12.5%) with Lysate A-PRF roup (50%, 25%, 12.5%).

	10% PBS	50% Lysate A-PRF	25% Lysate A-PRF	12.5% Lysate A-PRF
10% PBS		1.00	1,.00	1.00
50% Lysate-PRF	1.00	1.00	1.00	1.00
25% Lysate-PRF	1.00	1.00	1.00	1.00
12.5% Lysate-PRF	1.00	1.00	1.00	1.00

*Post Hoc Tamhane significance test with $p < 0.05$.

cell phases in cell arrest may cause apoptosis. According to Liu *et al.* (2004) the occurrence of DNA fragmentation as an early sign of cell apoptosis performed serum starvation for 48 hours [12].

To produce 2 types of lysate-PRF centrifugation with different times and speeds taken from the same donor, so that the results are not biased. This has an impact on the composition and concentration of growth factors such as TGF-B, PDGF, VEGF and bFGF [13].

Ghanaati *et al.* (2014) states that the difference in speed and time of centrifugation will produce different PRFs. In A-PRF sizes, the shape and density of the particles is smaller with the distribution of platelets and neutrophil granulocytes more evenly compared with PRF [14].

Incubation on PRF for 24 hours with a temperature of −20˚C aims to obtain platelet lysate. According to Anitua *et al.* (2010) the lysate form has a higher growth factor concentration than PRF. Growth factors of PDGF and TGF-β in regeneration play a role in forming collagen which plays a role for soft tissue healing and callus formation in healing of hard or bone tissue [15]. PDGF synchronizes migration and proliferation of mesenchymal cells, whereas TGF-β stimulates matrix synthesis such as fibronectin and collagen type I According to Vahabi *et al.* (2015) the supernatant (lysate) of the PRF has the ability to increase the proliferation of human gingival fibroblasts [16].

In Table 1 and Table 2, average values in all groups were similar and compared between 10% PBS, 50%, 25% and 12.5% lysate PRF and lysate A-PRF groups did not differ significantly. These results indicate that both the PBS 10% group, the lysate PRF group and the A-PRF lysate group have the same ability against the proliferation of fibroblast cells. PBS 10% is a physiological fluid that can present physiological conditions. So the conclusions lysate PRF and lysate A-PRF have the same ability with 10% PBS or physiological conditions. This result is consistent with research conducted by Huang *et al.* (2010) on pulp cells, that PRF is a mitogen that can increase pulp cell proliferation. 8 Whereas in previous studies, it was stated that PRF can trigger the proliferation of fibroblast cells in gingival tissue, ligament periodontium, and osteoblast cells [17].

The concentration of lysate PRF and lysate A-PRF has no effect on the effect of fibroblast cell proliferation, this is in accordance with previous studies which proved that cell proliferation rates are not correlated with increased concentration. According to Vahabi (2015) the ideal optimal concentration for cell proliferation is 2.5 times the platelet count in the blood, which normally ranges from 150,000 to 400,000 per microliter [16]. The proliferation rate is related to pH and platelet concentration. In growth media has a standard pH ranging from 7.2 - 7.8, pH for optimal cell growth [18].

In this study, the proliferation of fibroblast cells in 50% lysate A-PRF did not give optimal results because of high platelet concentration, a decrease in pH of the media was marked by the change of color toward clear white opaque to yellowish color different from 25% concentration and 12.5%. The value of fibroblasts at 25% lysate PRF concentration is not higher than 12.5% although this is not influenced by pH, but this is probably due to an adequate number of growth factors for reduced regeneration. This is thought to lead to not seemingly statistically significant differences.

5. Conclusion

The ability of Lysate-PRF and Lysate A-PRF in inducing fibroblat cell proliferation is equal to 10% FBS or has the same ability as physiological conditions.

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