The Efficacy of Platelet-Rich Fibrin Lysate (PRF-L) for Fibroblast Cell Proliferation

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Abstract

Background: One of the most distinct signs of irreversible pulpitis is the presence of permanently damaged fibroblasts, which is are the primary components of dental pulp. Regeneration of pulp tissue has been difficult, as the tissue is encased in dentin without and lacks a collateral blood supply except from at the root apical end. Growth factors play an important role to in promoting the healing process. It is acknowledged that Platelet-rich fibrin lysate (PRF-L) release contains growth factors and have has the potential ability to promote healing process of the of damaged fibroblasts. Objectives: To evaluate the proliferation capability of serum-starved fibroblasts by using treated with various concentrations of PRF--L. Methods: Human dermal fibroblasts were divided into two groups: Group I consisted of serum-starved fibroblasts and treated by with various three concentrations of PRF-L (50%, 25%, and 12,.5%). Group II consisted of normal fibroblasts as a positive control. , meanwhile s Serum-starved fibroblasts without any PRF-L treatment served as a negative control. Fibroblast proliferation capability was evaluated after PRF-L application and compared to control groups. Results: Serum-starved fibroblasts treated with inside 25% PRF-L had showed significantly ($Pp<0.05$) increased proliferation rates when compared to control fibroblasts. Fibroblasts proliferation is was lowest in the 12,.5% group but was not significantly different compared to the other PRF-L treatments with other groups. Conclusions: In this study that A 25% PRF-L treatment provided give an optimal result in fibroblast cell proliferation.

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Introduction

Irreversible pulpitis is a severe inflammatory condition characterized by extensive damage to where fibroblasts, as the most common cell type in the pulp were extensively damaged. Regeneration of pulp tissue has been is difficult because the tissue is encased in dentin without and lacks a collateral blood supply except from at the root apical end. Growth factors play an important role to in promote promoting the healing process.[1]

Platelet lysate is a human blood component that contains high concentrations of growth factors. This product can be obtained from platelet-rich fibrin (PRF), which is already used in many regenerative dental procedures.

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PRF is a second-generation platelet concentrate that has the capability to accelerate wound closure and mucosal healing by the formation of a fibrin bandage and release of growth factors. It releases platelet cytokines, such as VEGF, PDGF, TGF-β1 and IGF, that potently control local properties, such as cell migration, cell attachment, cell proliferation, and cell differentiation that perpetual to the healing process. PRF can also play an important role in tissue engineering, as it can serve as a scaffold to support cell growth and differentiation. PRF can be considered to represent an ideal scaffold because it contains necessary growth factors while also providing a three dimensional architecture that can selectively bind and localize cells and then undergo slow biodegradation overtime. [2-5]

Platelet The lysate obtained from PRF proved to release(PRF-L) contains a large number of growth factors at and the higher concentrations is higher than those can be

collected from PRPPRF alone. When the Incubation of a PRF gel was incubated in at a temperature of 4°C for 24 hours causes, the gel will to deflate and the supernatant, called a lysate, will then contains a variety range of platelet growth factors called lysate.[6] This occurs because the release of growth factors from PRF progresses slowly, but the level is higher than found in PRF itself. Although PRF is already used in many regenerative dental procedures, but the use of PRF-L has not been widely reported. This study was conducted to evaluate the proliferation capability of serum-starved fibroblasts after following application of different concentrations of PRF-L in various concentration.

Material and methods

Samples Twenty millimeters of venous blood (20 ml) were collected without any anticoagulant and. According to the standard Choukroun's protocol, tubes were immediately centrifuged at 2,700 rpm (approximately 400g) for 12 minutes according to the standard *Choukroun protocol*. A fibrin- dense clot was obtained formed in the middle of the tube, between the red blood cells at the bottom and the liquid serum (called platelet poor plasma) at the top. This fibrin-dense clot from PRF in a tube glass was transferred to a glass tube and incubated at 4°C for 24 hours to produce the PRF-L, according to the a previously described method.[7] After the residual fibrin attached to the bottom of the tube, during the incubation. The supernatant (the PRF-L) was aspirated, and transferred into a 2 ml Eppendorf tube, and stored and stored at -20°C, known as PRF-L.

Cryopreserved fibroblasts were obtained from cryo in Dermama Biotechnology Lab (Dermama, Indonesia). The cells were derived from human foreskin dermis and cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, New York, NY, USA). The fibroblasts were divided into two groups: Group I consist of serum-starved fibroblasts and were treated by with different various concentration of PRF-L (50%, 25%, and 12,.5%). Group II consisted of normal fibroblasts as a positive control and s Serum-starved fibroblasts without any treatment served as a negative control. Fibroblast proliferation capabilities were evaluated after PRF-L application and compared to the control groups.

Serum starvation technique were was applied by replacing reducing the foetal bovine serum (Gibco, New York, NY, USA) in the culture medium from 10% to 1% within for 48 hours. After serum starvation, the Group I was treated with various concentrations of PRF-L diluted in DMEM, and one group with some cells left untreated as an the negative control. Meanwhile, the positive control groups (normal fibroblasts) were Group II was cultured in DMEM supplemented with 10% foetal bovine serum. The fibroblasts proliferation was counted determined by using an automated cell counter (ScepterTM Sensors, 60 µum, Mexico City, Mexico).

Triplicate experiments were performed to ensure reproducibility. Statistical analysis was performed using a one-way ANOVA, followed by post hoc Bonferroni's multiple comparisons and P values less than 0.05 were considered statistically significant.

Results

In this study showed that Application PRF-L improved the proliferation ability of serum-starved fibroblasts. The highest fibroblast cell proliferation was obtained with 25% PRF-L (Table 1), even exceeding the number of proliferation of normal fibroblasts (291,000 cells/ ml).

Table 2 showed shows that the fibroblast cell proliferation in PRF-L 25% was statistically significant higher ($p<0.05$) for fibroblasts treated with 25% PRF-L than for compared to the negative control (303,500 ± 57,892 vs. 218,750 ± 26,133 cells/ml), but no significant differences were noted for the proliferation ability among in response to the different concentrations of PRF-L was not statistically significant.

Table 1. Comparison of Proliferation of serum-starved fibroblasts proliferation between groups in response to treatment with platelet-rich fibrin lysate (PRF-L).

	Mean (SD)(cells/ml)	p-value
Control	218750 (26133.312)	
PRF-L 50%	285333 (44016.664)	
PRF-L 25%	303500 (57892.141)	
PRF-L 12,.5%	261167 (42527.246)	**0.019**

One-way ANOVA; p<0.05 = significant

Tabel 2. Post Hoc analysis comparison of serum-starved fibroblasts proliferation between groups

	Mean Difference	CI 95%		p-value
		Minimum	Maximum	
Control vs PRF-L 50%	-66583.333	-141117.10	7950.43	0.10
Control vs PRF-L 25%	-84750.000*	-159283.77	-10216.23	**0.02**
Control vs PRF-L 12,5%	-42416.667	-116950.43	32117.10	0.668
PRF-L 50% vs PRF-L 25%	-18166.667	-92700.43	56367.10	1.00
PRF-L 50% vs PRF-L 12,5%	24166.667	-50367.10	98700.43	1.00
PRF-L 25% vs PRF-L 12,5%	42333.333	-32200.43	116867.10	0.672

* The mean difference is significant at the 0.05 level.
Post hoc Bonferroni; p < 0.05 = significant

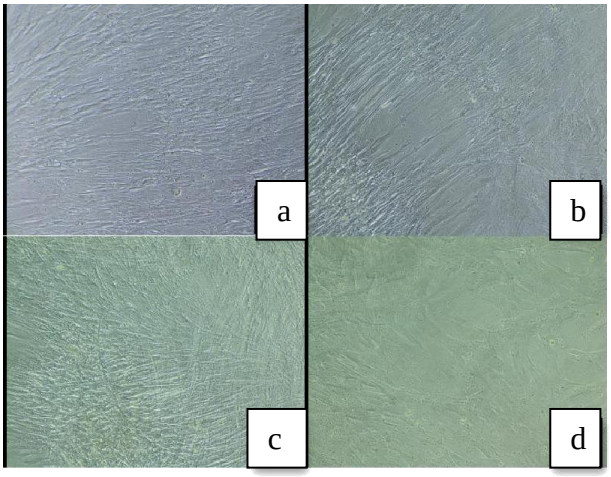

Figure 1. Microscopic analysis of fibroblast cells in cultured under four different conditions: (a) Control – serum-starved fibroblasts, (b) Fibroblasts in treated with 12.5% platelet-rich fibrin lysate (PRF-L) 12,5%, , (c) Fibroblasts in treated with 25% PRF-L 25%, (d) Fibroblasts treated with in 50% PRF-L 50%.

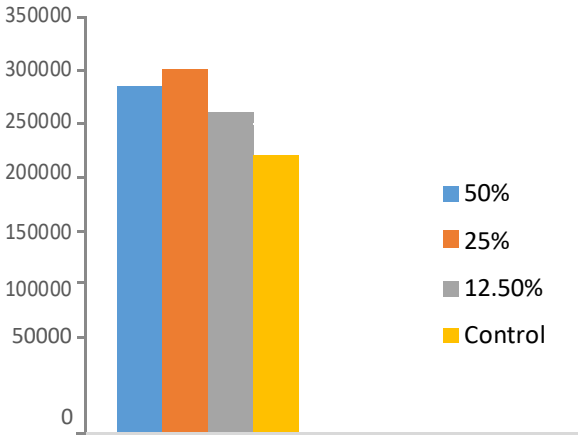

Figure 2. The effect of platelet-rich fibrin lysate (PRF-L) on proliferation of serum-starved fibroblasts proliferation compared to control. The control group contained culture medium was supplemented with 1% FBS.

Discussion

According to our results indicated that, treatment with 25% PRF-L can significantly improve the proliferation index of serum-starved fibroblasts ($p<0.05$) (Fig. 1) when compared to a negative control. The numbers of fibroblasts can, even exceeding the number of normal fibroblasts proliferating under similar culture conditions.

Our findings were different from those of a previous study which that showed that a significant induction of 50% PRF-L significantly induced the proliferation of fibroblast proliferation by 50% PRF-Ls when compared to control groups.[8] It may be discrepancy may reflect due to individual variations in terms of blood samples and treatment for the cell culture treatments. In the previous study, they previous study involved the use of were using artificially impaired fibroblasts that had been artificially impaired through by chronic UVA irradiation (total dosages of 10 J cm-2), whereas, serum-starved fibroblasts were used in this the present study.

Incubation of platelet concentrate at 4°C for 24 hours produces releases high concentrations of PDGF-BB and TGF-β1, and similar findings have been found with reported for PRF-L.[7,9] PDGF and TGF-β are the two main growth factors, which that encourages healing of soft tissue and bone through stimulation of collagen production, which to ameliorate promotes wound strength and initiation of callus formation. PDGF synchronize the migration, proliferation, and survival of mesenchymal cell lineages.

Among all the cytokines, TGF-β contains the most powerful fibrosing agent. It, as it induces enormous tremendous synthesis of matrix molecules, such as fibronectin and collagen-I, either by fibroblasts or osteoblasts.[10,11] Decreased in TGF-β1 receptor gene expression among in the serum-starved fibroblasts therefore leads to impaired TGF-β1 signal and impaired impairment of cellular proliferation, collagen synthesis, and cell migration. We assume is that the repaired restored proliferation index in response to PRF-L were was due to amelioration of TGF-β1 signal, based on the results of a previous study on dermal fibroblasts cells. On the surface of the dermal fibroblast membrane contains, the receptors for PDGF-BB and TGF-β1, and these both physically interact and ameliorate each other's signal and stability.[8]

The proliferative ability of 25% PRF-L on of the serum-starved fibroblasts is was higher in response to 25% PRF-L than to 50% PRF-L. It has been shown that previous studies have shown that increasing the concentration of platelets does not necessarily improve the proliferation effect instead, but there is a range of optimal concentrations of platelets is observed, with. As shown in previous studies, a platelet concentration equivalent to 2.5 times of the normal amount in blood is being ideal for cell proliferation.[12] A normal platelet count ranges from 150,000 to 450,000 platelets/ml per microliter of in blood. Therefore, in this the present study, 25% PRF-L has the optimal concentration for fibroblasts proliferation.

Another study proposed that the lower proliferation was pH dependent, and that a high platelet concentration resulted in pH changes that negatively affected fibroblast proliferation.[13] Platelet concentrates also contain different factors with that can have extremely diverse effects. Different levels of these factors may also explain the variability in results. Other studies have shown reported that the mitogenic activity of a platelet supernatant is not confined to its growth factors, as platelet particles and membrane fragments can also play an important roles in this regard.[14]

Conclusion

It can be concluded that Serum-starved fibroblasts treated with 25% PRF-L has better showed a higher proliferation potential ability on fibroblasts cell proliferation when compared to those treated with 12.5 or 50% PRF-L in 50% and 12.5%. . In the future, the use of PRF-L treatment can appears to be a good therapeutic approach for treating pulpitis in regenerative endodontic therapy fields. However, it is still needed more comprehensive studies ahead.

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Declaration of Interest

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Declaration of Interest

The authors report no conflict of interest.

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