Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2021; 21:e5890
https://doi.org/10.1590/pboci.2021.043
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Comparison of Transforming Growth Factor 1 (TGF-β1) Expression in Various Lysate Platelet-Rich Fibrin (L-PRF) Concentrations on Human Dental Pulp Stem Cell Differentiation

Muhammad Zaal Haq[1], Anggraini Margono[2], R. A. H. Nilakesuma Djauharie[3], Dini Asrianti[3], Indra Topanesa[3], Indah Julianto[3]

[1]Conservative Dentistry Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
[2]Conservative Dentistry Department, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.
[3]Dermatology Department, Faculty of Medicine, Universitas Sebelas Maret, Jakarta, Indonesia.

Correspondence: Anggraini Margono, Conservative Dentistry Department, Faculty of Dentistry, Universitas Indonesia, Salemba Raya No. 4, Jakarta, 10430, Indonesia. E-mail: margonodewi@yahoo.com

Academic Editor: Alessandro Leite Cavalcanti

Received: 12 January 2020 / **Review:** 19 June 2020 / **Accepted:** 23 August 2020

How to cite: Haq MZ, Margono A, Djauharie RAHN, Asrianti D, Topanesa I, Julianto I. Comparison of transforming growth factor 1 (TGF-β1) expression in various lysate platelet-rich fibrin (L-PRF) concentrations on human dental pulp stem cell differentiation. Pesqui Bras Odontopediatria Clín Integr. 2021; 21:e5890. https://doi.org/10.1590/pboci.2021.043

ABSTRACT

Objective: To compare Transforming growth factor-β1 (TGF-β1) expression in various L-PRF concentrations on the hDPSC differentiation process. **Material and Methods:** hDPSC cell cultures were subjected to serum starvation by reducing FBS levels in the hDPSC culture media. Lysate PRF was obtained from the PRF gel, which was then incubated at 4°C for 24 h. The supernatant was dried, transferred to a 2-ml Eppendorf tube, and stored at −20°C. The evaluation of TGF-β1 expression in 1%, 5%, 10%, and 25% L-PRF samples and 10% FBS (control) during the process of hDPSC differentiation was quantified using an ELISA reader on day 7. The expression of TGF-β1 was subjected to a one-way ANOVA test, followed by Bonferroni's post hoc test with significant values ($p<0.05$). **Results:** Significant differences were noted in TGF-β1 expression between 1%, 5%, 10%, and 25% L-PRF and the control group (10% FBS). The highest TGF-β1 expression occurred with 25% L-PRF (0.645 ± 0.048), followed by 10% L-PRF (0.461 ± 0.035), 10% FBS (0.374 ± 0.013), 5% L-PRF (0.275 ± 0.045), and the lowest expression was with 1% L-PRF (0.160 ± 0.045). **Conclusion:** The best result of TGF-B1 expression in hDPSC differentiation was in the 25% L-PRF group.

Keywords: Platelet-Rich Fibrin; Cell Differentiation; Dental Pulp; Culture Media.

Association of Support to Oral Health Research - APESB

Introduction

Regenerative endodontic treatment (RET) has led to a paradigm shift in endodontic treatment through pulp regeneration. It was reported that RET was based on the basic principles of tissue engineering, which involves three main components: stem cells, a scaffold comprising an extracellular matrix framework, and growth factors as signaling proteins [1-3]. Transforming growth factor-β1 (TGF-β1) is a polypeptide member of the beta cytokine transformation superfamily. This protein is secreted to perform many cellular functions, including cell growth, proliferation, differentiation, senescence, and apoptosis [4]. TGF-β1 can initiate the differentiation of human dental pulp stem cells (hDPSCs) into several other cell types through limited lineages or multipotent, such as odontoblasts. TGF-β plays an important role in reducing the number of DNA (Id) binding protein inhibitors, known as inhibiting factors for protein differentiation. Moreover, TGF β1 also regulates the transition process, from proliferation to differentiation, so that the process will be stopped and continue to differentiation process [5].

Previous authors developed second-generation platelet concentrates without anti-coagulant ingredients [6]. These platelets can be concentrated in the top layer of a tube after a single centrifugation process at a speed of 2,700 rpm (750 g) for 12 minutes [7]. This autologous concentrate is known as PRF, which has a high fibrin matrix content. PRF also contains a large number of white blood cells, which can support tissue healing by increasing immune responses and securing a large number of growth factors for a long time, between 7 and 14 days [8]. Modifications to the process for manufacturing PRF can be carried out by performing a freezing liquefaction process, which aims to lyse platelets and collect the growth factors released upon their lysis. This modification results in PRF lysate (L-PRF) [9].

Saeed et al. [8] revealed the effectiveness of 1% PRF exudates in hDPSC differentiation on day 7 have the superior ability for osteo differentiation by Alizarin Red staining and calcium mineral deposits. Abuarqoub et al. [9] used lysate derived from PRP as a culture medium for hDPSC and SCAP differentiation. His study showed that 5% PRP lysate had the highest DSPP expression among all groups. DSPP is a non-collagen protein that induces mineralization in the extracellular matrix and is not found in other tissues; therefore, it can be used as a phenotype marker of odontoblast activity [9].

Another study evaluated the platelet distribution pattern and the release of different growth factors (VEGF, TGF-β1, and EGF) in three PRF matrices (PRF, A-PRF and A-PRF+); it was concluded that the release of growth factors was significantly higher on days 7 and 10 [10]. The expression of TGF-β1 in other stem cells' differentiation process has been previously analyzed; the present study was conducted to analyze differences in TGF-β1 expression in various concentrations of L-PRF.

Material and Methods

Laboratory Procedures

All work procedures were performed in a biohazard cabinet under sterile conditions. Samples of hDPSCs were obtained from previous research (no. 35/ethical approval/FKGUI/V/2018, no. Protocol: 090250218), and serum starvation was performed by reducing FBS levels in the hDPSC culture media.

Initially, the culture medium was supplemented with 10% FBS; following the second and third passage, the cells were harvested and the culture medium was replaced with DMEM, which was added together with 1% FBS for 24 hours. Using a disposable syringe, 10 ml of blood was taken from three different donors from the cubitus vein. Within 2 min of collecting the blood, it was centrifuged at 2700 rpm for 12 min.

This resulted in two layers: the PRF gel at the top and red blood cells at the bottom. The PRF gel layer was removed using sterile tweezers and placed in a sterile tube.

To obtain L-PRF, the PRF gel was incubated for 24 h at 4°C after it was confirmed that the remaining fibrin was attached to the bottom of the tube. The supernatant was dried, transferred to a 2 ml Eppendorf tube, and stored at -20°C. The supernatant comprised platelet lysate. Lysate platelets were made in four concentrations consisting of 1%, 5%, 10%, and 25%. Concentration dilution was performed using DMEM (Dulbecco's Modified Eagle Medium, Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) with a comparison according to the expected percentage of L-PRF.

Induction was performed using differentiation media consisting of nutritional media (DMEM), 50 μm/ml ascorbic acid-L diphosphate, 10 mM sodium glycerophosphate-β salt, and 10 μM dexamethasone. This differentiation medium was then mixed with 1%, 5%, 10%, or 25% L-PRF, or 10% FBS as a control, into wells containing 1×10^5 hDPSCs.

The process of changing cells from hDPSCs to odontoblasts was characterized by TGF-β1 expression, with cell morphology measured on day 7 in each test and control group. The amount of TGF-β1 expression was measured using an ELISA reader.

Data Analysis

Retrieval of cell images was carried out using a microscope at 10× magnification before and after being treated for 7 days. Quantitative data from the results were statistically analyzed. The expression of TGF-β1 was subjected to a one-way ANOVA test, followed by Bonferroni's post hoc test with significant values ($p<0.05$).

Results

On day 1, before being supplied with culture medium, cells consisted only of a monolayer, whereas by day 7, hDSPCs were developed into multilayers. There were significant differences between the L-PRF treatment groups and the control group (Table 1).

Table 1. Average values of TGF-β1 expression (ng/ml) with different concentrations of L-PRF and the control group.

Culture Media	N	Mean	SD	p-value
FBS 10%	3	0.374	0.013	0.000*
L-PRF 1%	3	0.160	0.045	
L-PRF 5%	3	0.275	0.045	
L- PRF 10%	3	0.461	0.035	
L- PRF 25%	3	0.645	0.048	

*One-way ANOVA Test.

As seen in Table 2, according to the post hoc Bonferroni test, there were significant differences in average TGF-β1 expression (indicated by an asterisk). This showed that there was a significant difference between the control group (10% FBS) and the groups containing 1% and 25% L-PRF. Meanwhile, there were no significant differences between the control group (10% FBS) and the groups with 5% and 10% L-PRF. There were significant results among each group; 1% L-PRF and 10% L-PRF groups ($p=0.033$); 1% and 25% L-PRF groups ($p=0.00$); and 5% and 25% L-PRF groups ($p=0.001$).

Table 2. Analysis of TGF-β1 expression (ng/ml) with different L-PRF concentrations and the control group.

Culture Media	FBS 10%	*L*-PRF 1%	*L*-PRF 5%	*L*-PRF 10%
FBS 10%				
L-PRF 1%	0.033*			
L-PRF 5%	1.000	0.669		
L- PRF 10%	1.000	0.003*	0.076	
L- PRF 25%	0.007*	0.000*	0.001*	0.079

*Post hoc Bonferroni test.

Table 2 shows that an increase in the mean expression of TGF-β1 could be seen by day 7, with higher concentrations of L-PRF leading to greater expression of TGF-β1 being detected. The use of L-PRF with greater concentration produced TGF-β1 expression, which was better than 10% FBS as a control group. The highest TGF-β1 expression occurred with 25% L-PRF (0.645 ± 0.048), followed by 10% L-PRF (0.461 ± 0.035), 10% FBS (0.374 ± 0.013), 5% L-PRF (0.275 ± 0.045), and the lowest expression was with 1% L-PRF (0.160 ± 0.045).

Discussion

This experimental laboratory study aimed to analyze differences in TGF-β1 expression during the differentiation process of hDPSCs when using various L-PRF concentrations. This study is a continuation of previous studies conducted by Saeed et al., which examined the potential of exudate 1%, 5%, 10% and 25% PRF toward differentiation of hDPSCs [8]. Another study compared the 20% L-PRF potential and 25% in the proliferation of hDPSCs, 25% L-PRF proved better than 20% L-PRF [11]. A recent study of A-PRF also showed that A-PRF has superior potential for supporting the proliferation of hDPSCs. The highest proliferation rate was seen with 25% A-PRF, while the lowest was seen with 10% A-PRF [12]. From these previous studies, it can be concluded that there was an excellent potential ability to modify PRF, such as exudate PRF, L-PRF and A-PRF, in proliferation and differentiation of hDPSCs.

In this study, the potential ability of 1%, 5%, 10%, and 25% L-PRF in hDPSCs differentiation process was analyzed by examining TGF-β1 expression. The results of this study explained the transition from the process of proliferation to that of differentiation and how this was influenced by the expression of TGF-B1 by hDPSCs. It is assumed that the culture media used for hDPSC growth can influence TGF-β1 expression. In this study, therefore, the ability of various L-PRF concentrations related with TGF-β1 expression result was compared to find the optimal media for the differentiation process of hDPSCs [11].

Growth factors activate the signaling sequences necessary for the proliferation process to occur. Once a cell has divided into daughter cells and exited the cell cycle, there are several possibilities that may occur, including whether the cell can divide again, die via the process of apoptosis, enter senescence, or enter the phase of differentiation. The change from proliferation into differentiation depends on the presence of proliferation and activation factors of specific cell genes that can lead to different cells' formation [4]. DNA binding protein inhibitors (Id), known as an inhibitor of protein differentiation, play an important role in the proliferation of differentiation into differentiation. TGF-β can reduce the number of Id; this can inhibit the proliferation process and support the differentiation process [13,14].

The culture medium used in this study was PRF, which was modified by a freeze-thaw process to lyse platelets and collect the growth factors released; the result of this modification was named PRF lysate (L-

PRF). L-PRF can release several growth factors, including VEGF, PDGF, TGF-β1, and EGF. TGF-β1 plays a vital role during the transition from proliferation to differentiation [9]. The results showed that more TGF β1 expression was detected at higher L-PRF concentrations.

In this study, serum starvation was introduced by replacing the cell culture media supplement with 1% FBS for 24 hours. This procedure was used to equalize the cells' conditions before treatment, so that they could be prepared in the desired condition. In current cell culture methods, basal media is provided with supplements containing growth factors, proteins, and enzymes that support cell proliferation and differentiation. FBS is the most commonly used supplement and has become the gold standard for culture media. FBS contains various substances dissolved in water, including nutrients, hormones, growth factors, protease inhibitors, and neutralizing toxins [15]. However, FBS has some deficiencies; e.g., it has been reported that FBS poses a risk of xenogenic reactions and may be also contaminated with bovine viruses that could cause an allergic response. This was supported by Asrianti et al. [16], who reported that human platelet lysate (hPL) might be an alternative culture media control in addition to FBS; it is shown that the proliferation of hDPSCs in 10% FBS compared with 5% hPL is comparable both showing good result. In this study, however, FBS was still used as the gold standard control [15,16].

Table 1 shows significant differences between 1%, 5%, 10%, and 25% L-PRF compared with the results of the control group (10% FBS). This result proved that the increase of L-PRF concentration was in line with the increase in TGF-β1 expression. TGF-β1 expression was directly proportional to the increase in L-PRF concentration. Conversely, compared with the 10% FBS control group, as seen in Table 1, TGF-β1 expression with L-PRF was 10% and 25% higher than the FBS control group 10%. However, TGF-β1 L-PRF expression was 1% lower than the 10% FBS control group. Based on Table 2, the expression of TGF-β1 in the 5% L-PRF group did not differ significantly compared with its expression in the 10% FBS control group.

Conclusion

There were significant differences in TGF-β1 expression among the 1%, 5%, 10%, and 25% L-PRF groups and the 10% FBS control group. The increasing concentration of L-PRF in line with the TGF-β1 expression means that the higher the L-PRF concentration, the greater the expression of TGF-B1. Therefore, the best result of TGF-B1 expression in hDPSCs differentiation was in the 25% L-PRF group.

Authors' Contributions

MZH	https://orcid.org/0000-0002-9414-3912	Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft Preparation and Writing - Review and Editing.
AM	https://orcid.org/0000-0003-1598-2599	Conceptualization, Formal Analysis and Writing - Review and Editing.
RAHND	https://orcid.org/0000-0003-4404-5967	Conceptualization, Formal Analysis and Writing - Review and Editing.
DA	https://orcid.org/0000-0001-7176-724X	Conceptualization, Formal Analysis and Writing - Review and Editing.
IT	https://orcid.org/0000-0002-7170-7834	Methodology, Formal Analysis and Writing - Review and Editing.
IJ	https://orcid.org/0000-0002-4246-4845	Conceptualization, Formal Analysis and Writing - Review and Editing.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

Financial Support

None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

Acknowledgments

We thank our colleagues, Yanni Dirgantara S. Si and Emilia Rahmadaniah Utami, S. Si, from ProSTEM Laboratory, and other collaborators for their excellent experimental assistance and discussions.

References

[1] Chaparro O, Linero I. Regenerative Medicine : A New Paradigm in Bone Regeneration. In: Zorzi AR, Miranda JB. Advanced Techniques in Bone Regeneration. London: IntechOpen Limited; 2016. pp. 253-274. https://doi.org/10.5772/62523
[2] Žižka R, Šedý J. Paradigm shift from stem cells to cell-free regenerative endodontic procedures: a critical review. Stem Cells Dev 2017; 26(3):147-53. https://doi.org/10.1089/scd.2016.0264
[3] Backly RM El, Marei MK. Dental Pulp Stem Cells in Tissue Engineering and Regenerative Medicine: Opportunities for Translational Research. In: El-Badri N. Advances in Stem Cell Therapy: Bench to Bedside. Cham: Springer Nature Switzerland AG.; 2017. p. 171-196.
[4] Niwa T, Yamakoshi Y, Yamazaki H, Karakida T, Chiba R, Hu JC-C, et al. The dynamics of TGF-β in dental pulp, odontoblasts and dentin. Sci Rep 2018; 8(1):4450. https://doi.org/10.1038/s41598-018-22823-7
[5] Rosa WLO, Piva E, Silva AF. Disclosing the physiology of dental pulp for vital pulp therapy. Int Endod J 2018; 51(8):829-86. https://doi.org/10.1111/iej.12906
[6] Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. J Periodontol 2017; 88(1):112-21. https://doi.org/10.1902/jop.2016.160443
[7] Choukroun J, Aalam AA, Miron RJ. Platelet Rich Fibrin "PRF" and Regenerative Medicine: 'The Low-Speed Concept'. In: Tatullo M. MSCs and Innovative Biomaterials in Dentistry. Cham: Springer Nature Switzerland AG.; 2017. p. 21-42.
[8] Saeed MA, El-Rahman MA, Helal ME, Zaher AR, Grawish ME. Efficacy of human platelet rich fibrin exudate vs fetal bovine serum on proliferation and differentiation of dental pulp stem cells. Int J Stem Cells 2017; 10(1):38-47. https://doi.org/10.15283/ijsc16067
[9] Abuarqouba D, Awidi A, Abuharfeil N. Comparison of osteo/odontogenic differentiation of human adult dental pulp stem cells and stem cells from apical papilla in the presence of platelet lysate. Arch Oral Biol 2015; 60(10):1545-53. https://doi.org/10.1016/j.archoralbio.2015.07.007
[10] Bagdadi KE, Kubesch A, Yu X, Al-Maawi S, Orlowska A, Dias A, et al. Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)-based matrices: a proof of concept of LSCC (low speed centrifugation concept). Eur J Trauma Emerg Surg 2019; 45(3):467-9. https://doi.org/10.1007/s00068-017-0785-7
[11] Asri SR, Setiati HD, Asrianti D, Margono A, Usman M, Yulianto I. Optimum concentration of platelet-rich fibrin lysate for human dental pulp stem cells culture medium. J Int Dent Med Res 2019; 12(1):105-10.
[12] Illmilda, Asrianti D, Margono A, Julianto I, Wardoyo MP. Advanced platelet rich fibrin (A-PRF) supplemented culture medium for human dental pulp stem cell proliferation. J Int Dent Med Res 2019; 12(2):396-400.
[13] Berridge MJ. Cell Cycle and Proliferation. Cell Signal Biol 2012; 1-42. https://doi.org/10.1042/csb0001009
[14] Berridge MJ. Development. Cell Signal Biol 2012; 1-29. https://doi.org/10.1042/csb0001008
[15] Bieback K. Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. Transfus Med Hemother 2013; 40(5):326-35. https://doi.org/10.1159/000354061
[16] Asrianti D, Margono A, Swastiningtyas S, Asri ISR, Usman M, Yulianto I. Comparison of human platelet lysate and fetal bovine serum in culture media for human dental pulp stem cell proliferation. Int J Appl Pharm 2019; 11:157-59. https://doi.org/10.22159/ijap.2019.v11s1.16025