

# The effect of compression to platelet rich fibrin towards fibroblast proliferation of periodontal ligament: An in vitro study



Vincensia M. Karina,\* Sri P. Lastianny, Soetomo Nawawi

## Abstract

**Objective:** The aim of this study was to determine the effect of compression to PRF towards the proliferation of periodontal ligament fibroblasts.

**Material and Methods:** In this study, periodontal ligament fibroblasts were divided into four groups: periodontal ligament fibroblasts, periodontal ligament fibroblasts with platelet rich fibrin non-compressed, periodontal ligament fibroblasts with platelet rich fibrin compressed, periodontal ligament fibroblasts with platelet rich fibrin releasate. Each group consisted of 6 samples. The examination of fibroblast proliferation was done using MTT Assay

on day 3 and 7.

**Results:** Analyzed using ANOVA test followed by post hoc test with at least significant difference. Based on the result, non-compressed PRF showed the highest fibroblast proliferation. It also showed a significant difference between groups of periodontal ligaments, non-compressed PRF and compressed-PRF. Meanwhile, groups of non-compressed-PRF and PRF-releasate showed insignificant difference.

**Conclusion:** This study showed that non-compressed PRF and PRF releasate is more effective to increase the periodontal ligament proliferation.

**Keywords:** Compressed-PRF, Non compressed PRF, Periodontal ligament fibroblasts, Platelet rich fibrin, PRF releasate  
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Department of Periodontology,  
Faculty of Dentistry, Universitas  
Gadjah Mada, Yogyakarta, In-  
donesia

## Introduction

Periodontal disease is an induction towards inflammation process resulting in periodontal ligament damage, tooth-supporting-bone loss and tooth loss if not treated properly.<sup>1,2</sup> The treatment of periodontal tissue damage is regeneration process. The meaning of regeneration is a reproduction or reconstruction of a wounded or lost part of the body, including the damaged or lost tissue function and shape. The aim of regenerative therapy is to restore the periodontal tissue structure and function such as gingival structure, alveolar bone, cementum and periodontal ligament.<sup>2</sup>

The healing of periodontal tissue after operation needs cell interaction between epithelial cell, gingival fibroblast, periodontal ligament fibroblast and osteoblast. Vessel damage may cause fibrin formation, platelet aggregation and releasing of some growth factors.<sup>3</sup> Periodontal ligament fibroblast is the key cell of regeneration process. If the lost periodontal tissue regeneration needs to be constructed, periodontal ligament fibroblast must be recruited and populate back to the root surface of the tooth.<sup>4</sup>

Platelet rich fibrin (PRF) is the new generation of platelet concentrate used to ease blood preparation treatment without biochemical properties.<sup>5</sup> Platelet rich fibrin contains glycosaminoglycan (heparin, hyaluronic acid), transforming growth factor  $\beta$ -1 (TGF  $\beta$ -1), platelet derived growth factors (PDGFs),

insulin-like growth factor (IGF), interleukin-1b (IL-1b), interleukin 6 (IL-6), tumor necrosis factor a (TNF-a), interleukin 4 (IL-4), vascular endothelial growth factor (VEGF).<sup>6</sup>

Platelet rich fibrin (PRF) can be immediately use either after centrifugation in the form of clot or after compression as a strong membrane. In periodontics, PRF is usually used in the form of membrane as guided tissue regeneration. Compression towards PRF cause fibrin network to condense and bind to each other, but the condensation effect of PRF towards healing process still needs to be reviewed. By product of compressed-PRF in the form of liquid is called platelet rich fibrin releasate (PRFR). Releasate is rarely used and often thrown away by clinicians. Platelet rich fibrin releasate contains PDGF, TGF  $\beta$ -1, VEGF, basic fibroblast growth factor (b-FGF).<sup>8</sup> However, no research about the releasate effect towards periodontal ligament fibroblast proliferation is found.

## Material and Methods

This research was validated by Ethics and Advocacy Unit Universitas Gadjah Mada with the number 00375/KKEP/FKG-UGM/EC/2015. The type of this research is quasi-experimental with: Independent variable: Platelet rich fibrin releasate

\*Correspondence to:  
Vincensia M. Karina, Department of  
Periodontology, Faculty of Dentistry,  
Universitas Gadjah Mada,  
Yogyakarta, Indonesia  
[vincensia.maria.k@mail.ugm.ac.id](mailto:vincensia.maria.k@mail.ugm.ac.id)

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(PRFR), non-compressed Platelet rich fibrin (PRF), compressed Platelet rich fibrin (PRF) and examination day on 3rd and 7th day; Dependent variable: the proliferation of periodontal ligament fibroblast.

The samples of this research were periodontal ligament fibroblast derived from periodontal ligament of premolar root surface and platelet rich fibrin from 6 donors. Samples were divided into 4 groups, Control Group: fibroblast cell in DMEM, PRF I Group: fibroblast cell in DMEM + non-compressed PRF, PRF II Group: fibroblast cell in DMEM + compressed PRF, PRF III Group: fibroblast cell in DMEM + PRF Releasate.

Ten ml of intravenous blood was taken using syringe then moved into a tube to be centrifuged at 2700 rpm for 12 minutes in order to produce platelet rich fibrin. As a result, three layers were obtained, the upper layer contained acellular plasma, the middle layer contained PRF and the lower layer contained erythrocyte. Afterwards, platelet rich fibrin was separated from those two layers.

Three kinds of Platelet rich fibrin were used in this research, they were: non-compressed platelet rich fibrin, blood samples after being centrifuged at 2700 rpm for 12 minutes; compressed Platelet rich fibrin obtained after PRF underwent compression using PRF Box for 10 minutes until membrane was formed. Later on, platelet rich fibrin was cut using punch biopsy with a diameter of 5mm; Platelet rich fibrin releasate, which is the by-product of compressed PRF in the form of serum liquid called releasate PRF.

The examination of fibroblast proliferation was done using MTT assay on 3rd and 7th day at wavelength of 570 nm. The reading result of the examination showed number of proliferations of periodontal ligament fibroblast. In order to know the exact number of fibroblast proliferation, the data was processed using software curve expert.

## Results

The research was taken a place at Dermatology and Venereology Laboratory of Medical Faculty, Universitas Gadjah Mada. The samples of this research were periodontal ligament fibroblast and platelet rich fibrin.

Table 1 shows the mean and standard deviation of the proliferation of periodontal ligament fibroblast cells on baseline (day 0), third day, and the seventh day. The lowest mean of periodontal ligament fibroblast cells proliferation was present in all groups at baseline which is  $2.500.00 \pm 0.00$ . The highest mean of periodontal ligament fibroblast cells proliferation was found in the periodontal ligament fibroblast group

with non-compressed platelet rich fibrin which is  $34.171,69 \pm 3.941,92$ . Table data shows that there is an increase in proliferation of fibroblast cells in line with observation time and in each group as shown in the graph.

The graphic shows increments of periodontal ligament fibroblast proliferation. The lowest increment was showed by control group contained periodontal ligament fibroblast only, while the highest increment was showed by a group of periodontal ligament fibroblast and non-compressed platelet rich fibrin.

Table 2 showed that ANOVA test result showed that  $p = 0,000$  which is  $< 0,005$ . This indicates that there is a statistically significant difference between group and time.

Table 3 showed that there is no significant difference between groups on day-0, between periodontal ligament fibroblasts + non-compressed PRF group and periodontal ligament fibroblasts + PRF releasate group on the 7th day. There is a significant difference between group on day 7 and also for each group between measurement time.

## Discussion

The observation shows that there is an increase in proliferation of fibroblast cells of the periodontal ligaments in each group. This is in line with Chandran et al.<sup>9</sup> view, which states that PRF induces periodontal ligament cell proliferation. Platelets contain  $\alpha$ -granules that contain growth factors such as platelet derived factor (PDGF), transforming growth factor-b (TGF-b), and vascular endothelial growth factor (VEGF). The PDGF receptor is present in the periodontal ligament and will activate fibroblasts. PDGF also serves as a chemoattractant for the activation of periodontal ligament fibroblasts.

The greatest growth between the third and seventh days was in the fibroblast group of periodontal ligaments with platelet rich fibrin releasate. This is probably due to the basic form of releasate in the form of a liquid so that the growth factor released more easily to trigger the proliferation of fibroblasts. Groups of periodontal ligament fibroblasts with compressed platelet rich fibrin and non-compressed platelet rich fibrin had a smaller growth of periodontal ligament fibroblasts compared to groups with platelet rich fibrin releasate. This is probably due to the structure of the fibrin network that retains growth factor release causing the proliferation of fibroblast cells to not be high enough. This is in line with Chandran et al.<sup>9</sup> view, which states that the cytokines trapped within the fibrin network will be released only when appropriate, hence having a long-term effect. The result of LSD test of mean

**Table 1** Mean and standard deviation of periodontal ligament fibroblast cells by time and group

Group	Mean and Standard Deviation		
	Day 0 (n=24)	Day 3 (n=24)	Day 7 (n=24)
FLP	2.500.00 ± 0.00	6.225.08 ± 1.063.79	11.881.90 ± 2.823.07
FLP+	2.500.00 ± 0.00	11.055,67 ± 3.942,66	34.171,69 ± 3.941,92
Non-compressed PRF			
FLP+	2.500.00 ± 0.00	11.612,35 ± 3.125,51	26.207,72 ± 5.385,66
Compressed PRF			
Releasate	2.500.00 ± 0.00	8.745,96 ± 3.531,43	32.505,59 ± 2.951,23

**Table 2** ANAVA test result on mean periodontal ligament fibroblast proliferation

	F	Sig.
ANAVA	98.962	0.000

**Table 3** Least Significant Difference (LSD) test on mean fibroblast proliferation

	F3	F7	F+C 0	F+C 3	F+C 7	F+NC 0	F+NC 3	F+NC 7	F+R 0	F+R 3	F+R 7
F 0	0.029*	0.000*	1.000	0.000*	0.000*	1.000	0.000*	0.000*	1.000	0.000*	0.000*
F 3		0.001*	0.029*	0.002*	0.000*	0.029*	0.050	0.000*	0.029*	0.136	0.000*
F 7			0.000*	0.872	0.000*	0.000*	0.622	0.000*	0.000*	0.065	0.000*
F+C 0				0.000*	0.000*	1.000	0.000*	0.000*	1.000	0.000*	0.000*
F+C 3					0.000*	0.000*	0.740	0.000*	0.000*	0.91	0.000*
F+C 7						0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
F+NC 0							0.000*	0.000*	1.000	0.000*	0.000*
F+NC 3								0.000*	1.000	0.000*	0.000*
F+NC 7									0.000*	0.171	0.000*
F+R 0										0.000*	0.000*
F+R 3											0.000*

## Abbreviation:

F0	: periodontal ligament fibroblast day-0
F3	: periodontal ligament fibroblast day-3
F7	: periodontal ligament fibroblast day-7
F+C 0	: periodontal ligament fibroblast+ Compressed PRF day-0
F+C 3	: periodontal ligament fibroblast+ Compressed PRF day-3
F+C 7	: periodontal ligament fibroblast+ Compressed PRF day-7
F+NC0	: periodontal ligament fibroblast+ Non compressed PRF day-0
F+NC3	: periodontal ligament fibroblast+ Non compressed PRF day-3
F+NC7	: periodontal ligament fibroblast+ Non compressed PRF day-7
F+R 0	: periodontal ligament fibroblast+ PRF releasate hari ke-0
F+R 3	: periodontal ligament fibroblast+ PRF releasate hari ke-3
F+R 7	: periodontal ligament fibroblast+ PRF releasate hari ke-7

proliferation of fibroblasts by group on day 0 shows  $p > 0.05$  which is 1.000 in all groups because on day 0 all groups have same amount of periodontal ligament fibroblasts.

On the seventh day, statistically significant data were as follows: periodontal ligament fibroblasts with periodontal ligament fibroblasts + compressed platelet rich fibrin; periodontal ligament fibroblasts with periodontal ligament fibroblasts + non-compressed platelet rich fibrin; periodontal ligament fibroblasts with periodontal ligament fibroblasts +

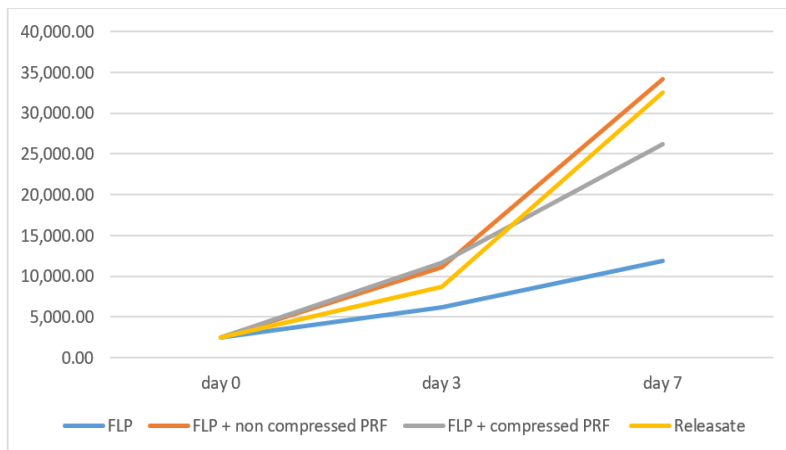
releasate; periodontal ligament fibroblasts + compressed platelet rich fibrin with periodontal ligament fibroblasts + non-compressed platelet rich fibrin; periodontal ligament fibroblasts + compressed platelet rich fibrin with periodontal ligament fibroblasts + releasate.

Data with no significant difference were found in the group of periodontal ligament fibroblasts + non-compressed platelet rich fibrin with periodontal ligament fibroblasts + releasate. Proliferation of periodontal ligament fibroblasts + compressed platelet rich fibrin is lower than that of non-compressed and releasate platelet rich fibrin which is supported by Ehrenfest et al.<sup>7</sup> which suggests that compression of the platelet rich fibrin causes the fibrin network structure to become denser and the release of growth factors become slower and gradual. PRF membrane releases growth factor continuously

and very slowly for at least one week.<sup>10</sup>

Increased proliferation of periodontal ligament fibroblasts between day 0, 3 and 7 have significant differences. This shows that there are proliferations of periodontal ligament fibroblasts. According to Manoranjan et al.<sup>4</sup> and Yaszemzki et al.<sup>11</sup> proliferation of periodontal ligament fibroblasts stimulated by PDGF reached a maximum response on day 3 and reached its peak on the seventh day.

The proliferation of periodontal ligament fibroblasts with the addition of compressed, non-compressed, and releasate platelet rich fibrin between days 0, 3, and 7 has significant differences. Burnouf et al.<sup>8</sup> and Kumar et al.<sup>6</sup> suggest that platelet rich fibrin contains growth factors such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor  $\beta$ -1 (TGF- $\beta$ 1), Vascular Endothelial Growth Factor (VEGF), basic fibroblast growth factor (b-FGF), Insuline-like growth factor (IGF). TGF- $\beta$ 1 functions to control the proliferation and differentiation of different types of cells; PDGF serves to promote proliferation and inhibit apoptosis; FGF serves to induce angiogenesis and proliferation



**Figure 1** mean graph of fibroblast proliferation according to group on day 0, 3 and 7

of fibroblasts; IGF acts as the mediator for other growth factors, is chemotactic to periodontal ligament fibroblasts, and is a regulator of proliferation and differentiation for most cell types.<sup>6,12-14</sup>

### Conclusion

Compressed-Platelet rich fibrin, non-compressed platelet rich fibrin and platelet rich fibrin releasate may increase periodontal ligament fibroblast proliferation. The highest proliferation of periodontal ligament fibroblast was showed by treatment group of non-compressed platelet rich fibrin and platelet rich fibrin releasate.

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### Conflict of Interest

The authors report no conflict of interest.

### References

- Guthmiller JM, Novak KF. Polymicrobial disease. ASM Press. Washington; 2002.
- Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work?. *Periodontol 2000* 2009;51: 208-219.
- Tsai CH, Shen SY, Zhao JH, et al. Platelet-rich fibrin modulates cell proliferation of human periodontally related cells in vitro. *J Dent Sci* 2009;4: 130-135.
- Manoranjan SJ, Faizuddin M, Hemalatha M, et al. The effect of platelet derived growth factor-AB on periodontal ligament fibroblast: an in vitro study. *J Indian Soc Periodontol* 2012;16: 49-53.
- 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: e37-44.
- Kumar R, Shubhashini N. Platelet Rich Fibrin: A new paradigm in periodontal regeneration. *Cell Tissue Bank* 2013;14: 453-463.
- Ehrenfest DMD, Corso MD, Diss A, et al. Three-dimensional architecture and cell composition of a choukroun's platelet-rich fibrin clot and membrane. *J Periodontol* 2010;81: 546-555.
- Burnouf T, Lee CY, Luo CW, et al. Human blood-derived fibrin releasates: composition and use for the culture of cell lines and human primary cells. *Biolog* 2012;40: 21-30.
- Chandran P, Sivadas A. Platelet-Rich Fibrin: its role in periodontal regeneration. *Saudi J Dent Res* 2014;5: 117-122.
- Dohan EDM, de PGM, Doglioli P, et al. 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: 63-69.
- Ichwana DL. Fiber composites as a method of treatment splinting tooth mobility in chronic periodontitis. *J Dentomaxillofac Sci* 2016;1: 190-192.
- Sánchez-González DJ, Méndez-Bolaina E, Trejo-Bahena NI. Platelet-rich plasma peptides: key for regeneration. *Int J Pept* 2012;2012: 1-10.
- Asrianti A, Nurhayaty N. Single-file reciprocating system for curved canals preparation: A case report. *J Dentomaxillofac Sci* 2018;3: 188-191.
- Fonny D, Lies WW. Reimplantation of avulsed teeth. *J Dentomaxillofac Sci* 2012;11: 115-118.



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