

Comparison of Fibroblast amount on the Gingival Healing Process in the Wound Applied by Platelet-rich Plasma (PrP) and chlorhexidine (cHX)

by Irene Edith 16

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Comparison of Fibroblast amount on the Gingival Healing Process in the Wound Applied by Platelet-Rich Plasma (PRP) and Chlorhexidine (CHX)

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ABSTRACT

Aim: This study was aimed to compare the amount of the fibroblast on the gingival healing process using platelet-rich plasma (PRP) and chlorhexidine (CHX).

Materials and Method: Fifteen homogen mice were included in this study. Five mice in PRP group, five in CHX group and four in control group. One mice was sacrificed for PRP preparation. 10 mm incision was made in mandibular gingiva and applied with PRP and CHX according to their group. The fibroblast of the healing process were analyzed with electron microscopic at the 1st, 3rd, and 5th day.

Result: In histopathological analysis, the healing process was greater in the experimental group than in control group on 1st and 3rd day, with the increasing amount of the fibroblast. The fibroblast enhanced significantly with $P > 0.009$, but on the 5th day the healing process was average at the experimental and control group.

Conclusion: The addition of PRP significantly increased gingiva healing process. PRP were important because of the growth factor and may be useful in the future to increase the healing process.

Clinical Significance: The goal of the periodontal treatment are enhance periodontal health and obtain gingival aesthetic. All these goals are depend on the success of the healing process which can be obtained from the periodontal surgery. Nowadays, clinician use Platelet- Rich Plasma (PRP) to enhance the healing process.

Keywords: Periodontal healing, PRP, Wound Healing

Introduction

The goal of periodontal treatment is to improve periodontal health and likewise the patient's need for aesthetic importance and functional requirements. To achieve this goal, periodontal treatment should be able to handle or increase attachment levels, reducing probing depths because these parameters are used as an illustration of increased dental retention.^{1,2}

For more than three decades, the main goal of periodontal treatment has shifted from improving to rebuilding periodontal tissue by repairing the damage caused by the disease process.^{2,3}

Various animal studies have been conducted to examine the regeneration of periodontal tissues

including insulin-like growth factor, fibroblast growth factors, epidermal growth factor, platelet-derived growth factors, vascular endothelial growth factors, parathyroid hormone, transforming growth factor- β and bone morphogenic proteins.^{4,5}

The main function of platelet is to prevent acute blood loss and repair the vascular wall and maintain tissue after injury. During the healing process, platelets are activated through contact with collagen, exposed the bloodstream after endothelial injury. Platelet products include Platelet Rich Plasma (PRP) which can be used without previous platelet activation. PRP applications in different tissues have yielded promising results in different pathologies such as acute and chronic injuries to bone and cartilage.^{6,7}

The aim of this study was to evaluate the healing process of gingiva and fibroblast formation on the wound that has been applied with platelet rich plasma.

Method

This study was a laboratory experiment conducted at the Laboratory of Department of Biology, Faculty of Mathematics and Natural Sciences, Makassar State University; Veterinary Center Maros; and Patology Anatomy Laboratory, Faculty of Medicine, Hasanuddin University. Study sample were mice that selected based on the inclusion criteria and exclusion criteria in this study. Inclusion criteria were male healthy mice weighing 300-400 grams, and age between 4-8 months. Exclusion criteria were no weight loss more than 10% after the adaptation period in the laboratory.

Prior to the experiment, mice were kept and adapted in animal experimental enclosures made of plastic basins and wires as a cover. Adaptation and pretreatment performed for one month to be checked for health to ensure their good health. Given a special feed for and water, that was also kept on room temperature in the scientific range.

One mice was sacrificed to have blood taken on the heart, and the blood was taken using 3 ml syringe and put in the tube. The tube was centrifuged for 10 minutes at first and another 8 second at 2000 rpm to obtain the poor plasma platelet in the first layer, rich fibrin platelets in the second layer and red blood cloth on the third layer. When

RBC was separated, PPP and PRF were centrifuged again for 8 minutes at 1000 rpm. From the second centrifuge, we obtained platelet rich plasma without coagulant that will be applied to the wound. 14 mice were divided into 3 groups, group I (five mice) will be applied with platelet rich plasma (PRP), Group II (5 mice) will be applied with Chlorhexidine gel, and group III (4 mice) were not applied anything. Operative area were disinfected with povidone iodine, then local anesthetic solution were deposited and incision were made using a scalpel on vestibulum region. Histopathologic examination to calculate the number of fibroblasts were done on the 1st, 3rd, and 5th day after treatment. The tissue were taken and fixed on 10% formaline solution and given HE staining to checked under microscope with 40x viewing field.

Results

Table 1: Average and standard intersections of fibroblasts on each group

Day	Group	Mean	Std. Deviation
Day 1	CHX	25.500	7.7782
	CONTROL	25.500	2.1213
	PRP	29.500	2.1213
Day 3	CHX	22.000	8.0416
	CONTROL	23.250	5.0580
	PRP	34.500	6.3640
Day 5	CHX	18.500	6.3640
	CONTROL	21.000	1.4142
	PRP	15.000	2.1602

Table 2: Data Analysis

	DfSum	SqMean	SqF	value	Pr(>F)
Day	2	381.1	190.56	6.537	0.00909 **
Group	2	50.9	25.43	0.872	0.43818
Day:group	4	250.6	62.65	2.149	0.12480
Residuals	14	15	437.2	29.15	---
Signif. codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' ' 1

Table 1 showed the average mean number of fibroblast on the three groups The highest rate of fibroblasts was on the third day of PRP application. Diagram 1 showed the average value of the number of fibroblasts by group and by day. In the control group, the average number of fibroblasts on the first day was 25, 23 on the third day, and

21 on the fifth day. In the group given Chlorhexidine, the average number of fibroblasts on the first day was 25, then decreased to 22 on third day and decreased again to 18 on the fifth day. In the group given the PRP, on the first day the average number of fibroblasts was 29, then increased to 34 on the third day and decreased to 15 on the fifth day.

Based on Table 2, obtained p-value for the day effect on the difference of the amount of fibroblasts was 0.00909, that mean there was a significant difference in the number of fibroblasts on the 1st, 3rd and 5th day.

In the treatment group, p-value was 0.43818 that mean there was no significant difference in the amount of fibroblasts between the control group, the chlorhexidine group, and PRP group.

In the day-interaction and treatment group, p-value was 0.12480, this value was not significant at 95% significance level (p-value = 0.12480; p-value > 0.05). Thus, there is no interaction effect between day and treatment on the amount of fibroblast. For this interaction group further analysis is done to ascertain whether or not there is a significant interaction.

Discussion

From the results of fibroblast examination in all groups, the highest amount of fibroblast was found in platelet rich plasma group on the third day, which was 34. Platelets are activated for aggregation by open collagen (intrinsic system). At the same time, the injured vein vasoconstricted, triggered by platelets, to reduce blood loss and fill tissue gaps with blood clots consisting of cytokines and growth factors. Furthermore, blood clots contain fibrin molecules, fibronectin, vitronectin and thrombospondine, forming a temporary matrix as a scaffold structure for migration of leukocytes, keratinocytes, fibroblasts and endothelial cells and also acts as a reservoir growth factor. Vasoconstriction occurs by coagulant formation leading to local perfusion failure with successive oxygen depletion, increased glycolysis and pH change. Vasoconstriction is then followed by vasodilation in which traumatized tissue undergoes a reperfusion phenomenon. Both platelets and leukocytes release cytokines, chemokines and growth factors to activate inflammatory processes, stimulate collagen synthesis, activate fibroblast transformation into myofibroblasts, initiate angiogenesis and support the process of re-epithelization.^{8,9}

Neutrophil formation is very important on the first days after injury because of its ability in protease secretion and phagocytosis kill local bacteria and help reduce necrotic tissue. Neutrophil initiates debridement by releasing highly active antimicrobial agents i.e. cationic and eicosanoid peptides, and proteinases, namely

elastase, cathepsin G, proteinase 3 and urokinase-type plasminogen activator. Approximately 3 days after the injury, macrophages enter the injury zone and support the ongoing process by performing pathogenic phagocytosis and the remnants of cell formation and secreting growth factors, chemokines and cytokines. Macrophages have many functions including defense response, promotion and inflammatory resolution, apoptotic cell transfer and cell proliferation support and tissue recovery after injury.⁸

Compared with the other treatment groups, PRP group had the highest mean number of fibroblasts though only occurred on the third day after treatment. Platelet rich plasma achieves homeostasis through the formation of fibrin clots initiated by platelet activation and aggregation. The freeze is produced fibrin polymerization of fibrinogen monomers in the presence of calcium and thrombin. The results of platelet aggregation in platelet platelets are held in place by blood clots and inhibit blood flow. In addition to maintaining homeostasis, the fibrin clot then produces a matrix for the migration of tissue-forming cells, including existing fibroblasts responsible for the synthesis of collagen and endothelial cells involved in angiogenesis. The traction force generated by these migratory cells in fibrin clots can assist wound contraction. This migration of cells together is also responsible for overhauling blood clots into tissue repairment.^{10,11,12}

Differences in PRP Group and Chlorhexidine Group: On histopathological examination between groups treated with PRP and chlorhexidine, there was a difference in the number of fibroblasts on the first day, this is because on the first day the most widely formed are inflammatory cells resulting from the inflammatory response. When the inflammatory blood vessels becomes leaky, it releases plasma and neutrophils into the surrounding tissues. As neutrophils digest bacteria and debris, it will die and release intracellular enzymes in the surrounding matrix. Furthermore, fibrin is broken down as part of the phagocytic process, degradation products attract the subsequent involved cells such as fibroblasts and epithelial cells.^{13,14,15}

On the third day, there was a significant difference on the amount of fibroblast between groups that was given PRP and chlorhexidine. This is because PRP has growth factors that is important in wound healing processes, such as platelet-derived growth factor (PDGF), transforming growth factor β 1, β 2, β 3 (TGF- β 1, β 2, β 3), platelet

3 derived angiogenesis factor, insulin-like growth factor 1 (IGF-1), platelet factor 4 (PF-4), epidermal growth factor, epithelial cell growth factor (VGA), vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF) and other cytokines.^{16,17,18}

The second group which is also important from growth stimulation² molecules is growth-derived platelets (PDGFs). These molecules are contained in platelet granules, and the release of PDGF at the injury sites during formation of clots are essential for the repair process. PDGF is closely related to the oncogene (v-sis) virus. Thus, molecules such as growth factor are involved in controlling the growth of transformed cells. Like EGF, the response to this stimulus is determined by the presence of certain PDGF receptors. PDGF is produced in two isoform molecules.^{16,17}

2 Fibroblast growth factor refers to a group of proteins that interact with one or more receptors in different cell types. FGF-4 and -2 (FGF acid and base) are important because of their ability to stimulate cell growth and capillary cell invasion in various models. Most forms of FGF have growth factor, causing many cell populations, but endothelial cells seem to have greater sensitivity.¹⁸

2 Growth factors 1 and 2 that specifically stimulate proliferation and differentiation. The process of healing epithelial cell² wound due to the unique receptor on these cells. Both growth factors have a classic paracrine action because they are produced in the dermis and act on the epidermis. Vascular endothelial growth factor (VEGF) or vascular permeability factor is the most selective growth factor for endothelials.^{19,22,23}

In chlorhexidine group, the number of fibroblasts on 1st, 3rd, and 5th day is relatively in the same quantity⁵, this is because chlorhexidine is an antiseptic that after up to 24 hours there is an increase in new connective tissue cells, just below the surface layer of inflammation and necrosis. The connective tissue grows to the coronal surface, creating new gingival margin⁵ and sulcus. The epithelial activity on the margin peaks at 24 to 36 hours. New epithelial cells⁵ originate from the basal layer and epithelial spine layer from the wound margins, migrating toward the wound on the fibrin layer which is then absorbed and replaced by the connective tissue layer. Epithelial cells progressed in an unorganized motion, with cells mounted on the substrate by hemidesmosome and new basal lamina.²⁰

This study was different of than previous study, whereas this study directly applied CHX after gingivoplasty and then evaluated on day 1, 3 and 5, while the other study CHX was given on day 7 and 14 after gingivoplasty.¹⁹ Under normal circumstances, fibroblast division activity is rarely seen, but cell injury appears to be more active in producing³² extracellular matrices. Proliferation of fibroblasts¹⁵ the wound healing process is naturally stimulated by interleukin-1b (IL-1b), platelet derived growth factor (PDGF), and fibroblast growth factor (FGF). In addition, another study revealed that fibroblast²⁹ migration in the area of the injury is stimulated by transforming growth factor (TGF), a growth factor produced by granulation tissue formed during the inflammatory process. The process of wound healing is strongly influenced by the role of migration and proliferation of fibroblasts in the injury site.¹⁷ The more specific function of collagen is to form a new connective tissue matrix and by removing the substrate by fibroblasts, indicating that macrophages, new blood vessels and fibroblasts as a unit.^{19,20} The limitation of this study was antiseptic and PRP application were given on anesthetized sample with short duration.

Conclusion

16 Platelet-rich plasma (PRP) is an autologous blood derivative that contains supraphysiological platelet²³ concentrations that aid in tissue regeneration as it is rich in growth factors and other cytokines. Platelet-rich plasma administration on the wound can increase the number of fibroblasts that will accelerate tissue healing process.²² Platelet Rich Plasma (PRP) is rich in growth factor,³ such as platelet-derived growth factor, transforming growth factor β 1, β 2, β 3 (TGF- β 1, β 2, β 3), and platelet derived angiogenesis factor (PDAF) new cells on wound healing process.

21 **Conflict of Interest:** There is no conflict of interest in this study.

Source of Funding: Domestic government

Ethical Clearance: This study obtained a label of ethics escaped by the number: 0066/PL09/KEPKFKG - RSGMUNHAS/2018 and register number UH 17120068 on Oktober 9, 2018.

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