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Efficacy of Platelet Rich Plasma on Micrografting Towards Epithelization Process

Regine Viona[1], Herman Yosef Limpat W.[2]
[1] *General Surgery Resident of Brawijaya University, Faculty of Medicine, Saiful Anwar General Hospital, Malang, Indonesia*
[2] *Plastic and Reconstructive Surgery Consultant of Brawijaya University, Faculty of Medicine, Saiful Anwar General Hospital, Malang, Indonesia*
Corresponding Author: Regine Viona

Objectives*: Wound healing process especially for burn injury had been talked about recently, pain burden and disability of burn injury encouraged for an effective treatment, one of the treatment include micrografting. The application of Platelet Rich Plasma on micrografting are able to speed up wound healing in burn injury.*
Methods*: This study is a clinical experimental study, nine patients with burn injury who underwent micrografting are treated in two different manner, first half of total area covered with micrografting are given platelet rich plasma, while the other half are left as it is. Epithelization are measured histologically by the appearance of macrophage, fibroblast and collagen; clinically and percentage on graft take.*
Results: *Significant increased p<0,05 on histology appearance; macrophage in platelet rich plasma group are 11.222 and 4,111 in non platelet rich plasma group; fibroblast in platelet rich plasma group are 16,444 and 6,556 in non platelet rich plasma group respectively; and collagen in platelet rich plasma group account for 6,778 and 1,994 in non platelet rich plasma group. The result on clinical evaluation on the 5th and 10th day are -2,236 (p=0,025) and-2,000 (p=0,046) respectively. Graft take are also increasing significantly in platelet rich plasma group with 82,222 and non platelet rich plasma group 72,778 (p=0.001). The application of Platelet Rich Plasma on micrografting demonstrate significant difference histologically and clinically.*
Conclusion: *Platelet rich plasma are efficacious towards wound healing process through increasing of macrophage, fibroblast and collagen that can speed up epithelization.*
Keywords: *micrografting, platelet rich plasma, macrophage, fibroblast, collagen, epithelization, graft take*

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I. Introduction

Wound healing in burn injury still remain a trending topic in many studies in the field of plastic surgery, especially burn injury with wide burn surfaces. Because of its high morbidity on the pain burden and disability this matters need to be taken care of seriously. Many studies are carried out to encourage researcher to discovered new technique that are more effective to manage burn injury. This study applied the used of *Platelet Rich Plasma* on *micrografting*, that has never been done before in Indonesia.

One of the operative techniques for burn injury with wide burn surfaces is micrografting, which is using smaller size skin donor to covered wide recepient areas. Micrografting has many advantages compared to to other graft techiques, including smaller size (up to square centimeters) with minimum bleeding and injury, this technique is very effective for wide burn surfaces with least donor area. In micrografting epithelization occurred similarly with even distribution of the graft that can speed up wound coverage. Risk of failure for micrografting is very low, usually occurred because of technical errors, bad vascularization, contaminations or infections from recepient wound site, edema, trauma and bad nutritions. Failure only occured on the effected graft, meanwhile surrounding epithelization process continues, without effecting the graft aestethically and functionally[1]. Good micrografting technique makes wound healing faster, shorter hospital stay for only 14 to 15 day for micrografting compared to other graft techiques that can take up to 21 days[2].

Platelet Rich Plasma is plasma with higher content of platelet. Plasma with platelet concentrates contain 3 to 5 times platelet concentration in peripheral blood[3,4]. Platelets are very important in haemostasis and wound healing[5,6]. Wound or tissue injury can trigger activation and agregation of platelet that can released more than 30 types of growth factors or known as secretory proteins. *Growth factors* that are release into the blood stream including *platelet-derived growth factor* (PDGF), *transforming growth factor-β1* (TGF-β1), *epidermal growth factor* (EGF), *fibroblast growth factor* (FGF), *platelet-derived angiogenesis factor 4* (PF4) and *platelet-activating factor* (PAF)[7.] Growth factors are chemotaxis to macrophage, induce fibroblast, endotel and osteoblast production. Fibroblast produces glycosaminoglican, elastin fibers dan glycoprotein to synthesize extracellular

matrix (collagen) and vascularization[8].

II. Methods

This is an experimental study, 9 patients with burn injury underwent micrografting, each patients are treated in two different manner. Burn injury patients with micrografting are then devided into two side, half side of the micrografting are given PRP (intervention group) and on the other side are left as it is (control group). This study took place in Burn Care Unit at Saiful Anwar General Hospital, Malang, Indonesia. The making of Platelet Rich Plasma took place in Central Laboratory of Saiful Anwar General Hospital, Malang. While histology examination took place in Pathology Anatomy Laboratory at Faculty of Medicine, Brawijaya University. All patients are treated based on Ethical Clearance Committee published by Saiful Anwar General Hospital, Malang, Indonesia.

Histologic variable are evaluated on the 5th day, clinical variable are evaluated on the 5th and 10th days and the percentage of graft take are evaluated on the 10th days.

Histology. Speciments are taken from every wound by punch biopsy size 6. Speciments are taken on the 5th day (proliferation phase), sent to Pathology Anatomy Laboratory to be painted using HE and examine under microscope using 400 x and 1000 x power. Macrophage and fibroblast are measured by counting the number, while collagen are measured using micrometer.

Clinical Variable (*Wound Assestment Tools*). On the 5th and 10th day, clinical evaluation are made using clinical indicators on both control and intervention side, to determined speed and successfull rate of wound healing.

***Graft Take*.** Evaluation on the percentage of graft take are carried out on the 10th days on both side to evaluate the speed and successfull rate of epithelization.

Data for macrophage, fibroblast, collagen and percentage of graft take are numerik data and normality test are carried out using *Saphiro-wilk* and then analyzed using *paired t-test.* Meanwhile the data for clinical variable *(Wound Assestment Tools)* are categorical data, that are devided into 4 category, including *minimal, mild, moderate* and *extreme severity*, and analyzed using *Wilcoxon test*. A value of $p<0,05$ was considered as statistical significance.

III. Results

Histological

Significant increased $p<0,05$ on histology appearance; macrophage in platelet rich plasma group are 11.222 and 4,111 in non platelet rich plasma group; fibroblast in platelet rich plasma group are 16,444 and 6,556 in non platelet rich plasma group respectively; and collagen in platelet rich plasma group account for 6,778 and 1,994 in non platelet rich plasma group

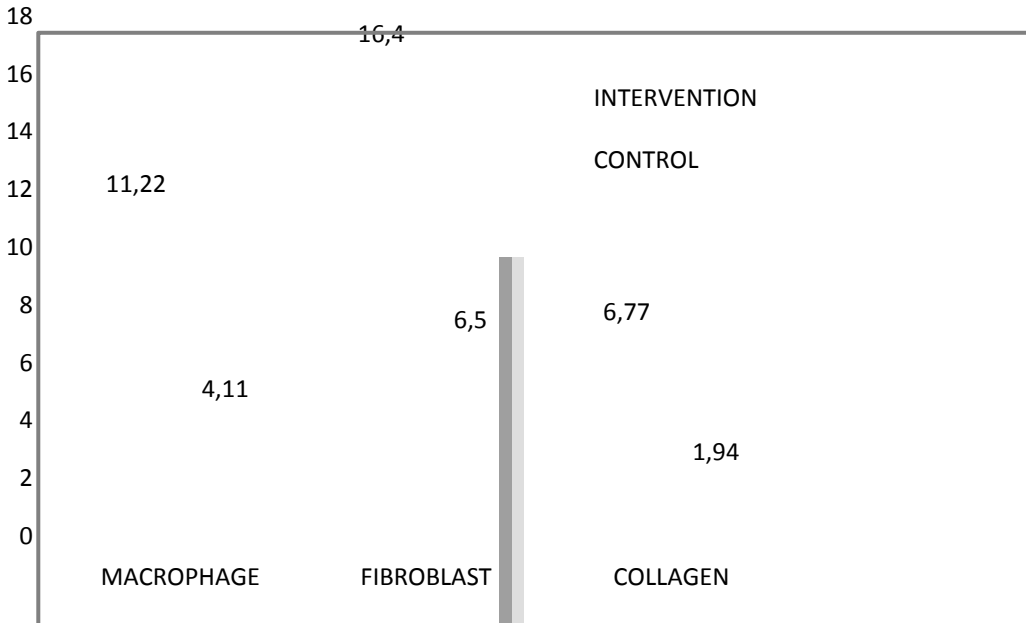

Table 1 : Mean of Macrophage, Fibroblast, Collagen count in each group. Intervention Group has higher Macrophage, Fibroblast and Collagen than Control Group on the 5th day.

Clinical

Clinical indicators including edge of wound, exudate type, exudate amount, granulation tissues and epithelization. Using *Wound Assetment Tools*, those indicators are categorized into 4 category: *minimal*, *mild*, *moderate* and *extreme severity*. Data form will be in ordinal scale and analyzed using *Wilcoxon test*. The result on clinical evaluation on the 5th and 10th day are -2,236 (p=0,025) and-2,000 (p=0,046) respectively.

Graft Take

Graft take are also increasing significantly in platelet rich plasma group with 82,222 % and non platelet rich plasma group 72,778% (p=0.001).

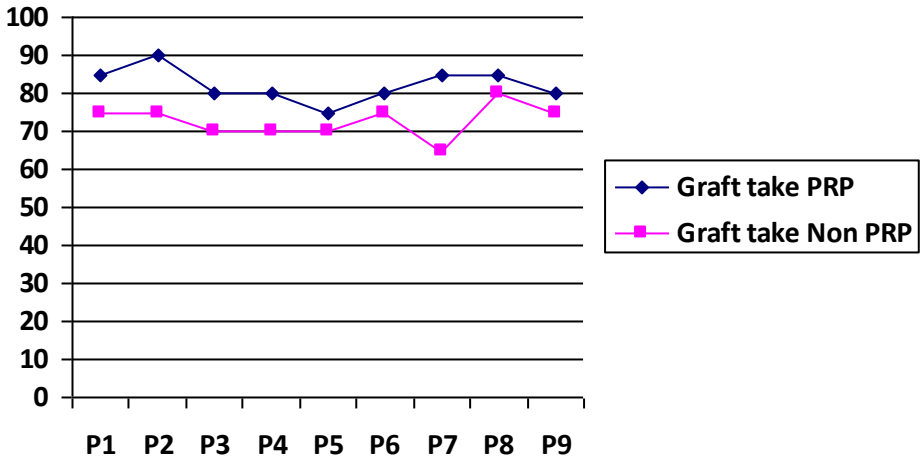

Table 2. Graft Take comparison between Intervention Group (PRP) than Control Group (Non PRP)

IV. Discussion

Microscopic appearance on PRP group using 400x and 1000x power shows more fibroblast and macrophage compared to Non PRP group. Also the density of collagen are more intense in the PRP group compared to the Non PRP group.

Platelet Rich Plasma as parts of blood cells contain platelet concentrate above normal[9,10].

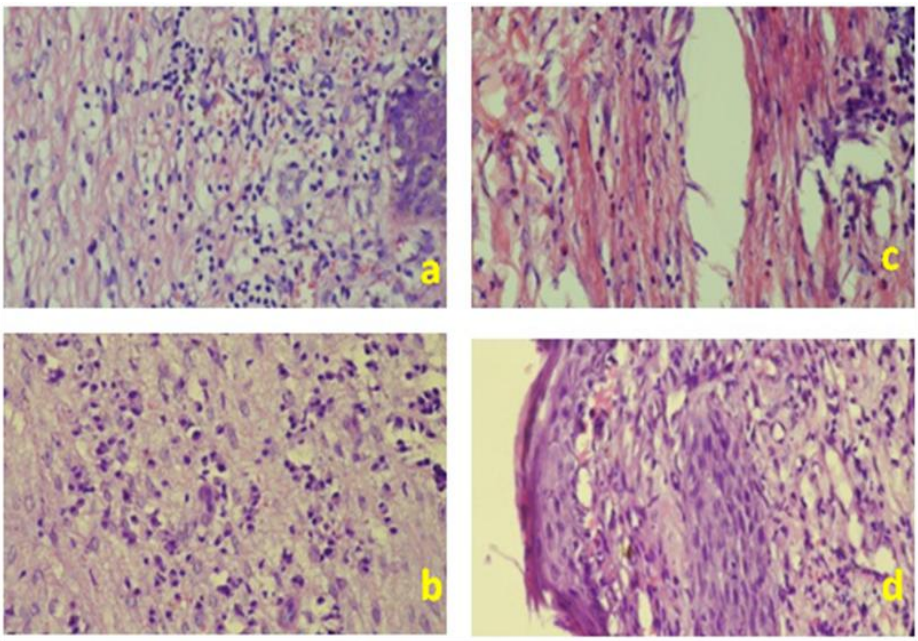

Figure 1. Magnification under microscope 400x; a and b are hystological feature without PRP; c and d are hystological feature using PRP

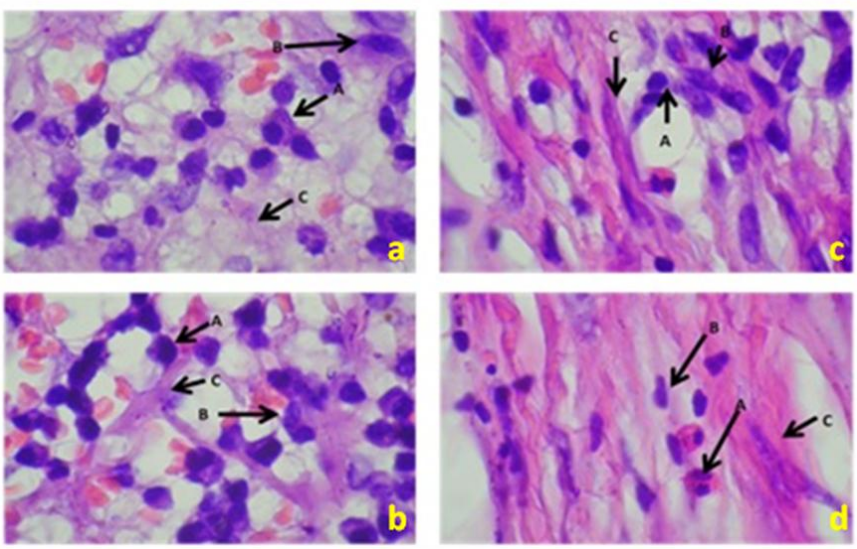

Figure 2. Magnification under microscope 1000 x on 5th day; a and b are hystological feature without PRP; c and d are hystological feature using PRP; arrow (**A)**: Macrofag ; **B**: Fibroblast; **C:** Collagen.

Platelet contain *alpha granule* that are released when activated. *Alpha granule* itself contain many secretory proteins (Growth Factor) including *platelet-derived growth factor* (PDGF), *transforming growth factor-β1* (TGF-β1), *fibroblast growth factor* (FGF), *epidermal growth factor* (EGF), *factor platelet factor 4* (PF4) dan *platelet-activating factor* (PAF), *interleukin* (IL-1), *platelet derived angiogenesis factor* (PDAF), *vascular endothelial growth factor* (VEGF), *platelet derived endothelial growth factor* (PDEGF), *epithelial cell growth factor* (ECGF), *insulin growth factor* (IGF), osteocalcin, osteonectin, fibrinogen, and thrombospondin[11,12].

Platelet Rich Plasma influence wound healing process and stimulate inflamatory respons[13]. Secretory protein released by platelet are chemotaxis to monocyte that will invaded wound site and differentiate into macrophage. Beside its phagocytic nature to pathogen and debris, macrophage also function in wound healing process. Macrophage interfere during inflammatory process, releasing growth factor that will induces fibroblast to synthesize extracellular matrix[14]. *Growth factors* induces fibroblast to proliferate and migrate and also to form extracellular matrix[15].

Fibroblast produces collagen, glycosaminoglycan, elastin fiber and glycoprotein that produces extracellular matrix. Type III collagen are formed on the 1st to 3rd days post trauma and reached the peak on the first week. Type III collagen will be degraded by metaloproteinase enzyme and replaced with stronger type I collagen when wound healing process enter remodelling phase in about three weeks. Platelet take parts in defense mechanism around wound edges by *signaling protein* that will destroyed macrophage[16]. Soon after wound healing process started, when collagen synthesize overwhelm its degradation, it will reached balance at the end of wound healing process. New tissues on the wound site will be dominated by collagen[17].

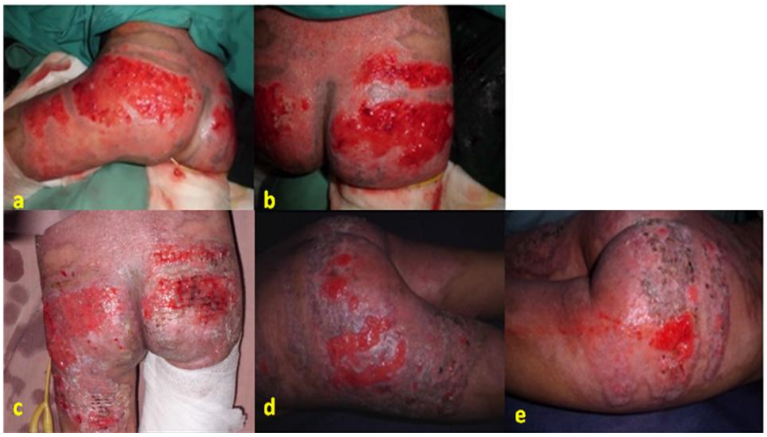

Figure 3. a. Pre operative clinical feature with PRP; b. Pre operative clinical feature without PRP; c. Clinical feature on the 5th day (left buttock using PRP and right buttock without PRP); d. Clinical feature on the 10th days (left buttock using PRP); e. Clinical feature on the 10th days(right buttock without PRP

	Assessment	Date Score	Date Score
1.Edges	1 = Indistinct, diffuse, none clearly visible 2 = Distinct, outline clearly visible, attached, even with wound base 3 = Well-defined, not attached to wound base 4 = Well-defined, not attached to base, rolled under, thickened 5 = Well-defined, fibrotic, scarred or hyperkeratotic		
2. Exudate Type	1 = None 2 = Bloody 3 = Serosanguineous: thin, watery, pale red/pink 4 = Serous: thin, watery, clear 5 = Purulent: thin or thick, opaque, tan/yellow, with or without odo		
3.Exudate Amount	1 = None, dry wound 2 = Scant, wound moist but no observable exudate 3 = Small 4 = Moderate 5 = Large		
4. Granulation Tissue	1 = No granulation tissue present 2 = Pink, &/or dull, dusky red &/or fills ≤ 25% of wound 3 = Bright, beefy red; < 75% & > 25% of wound filled 4 = Bright, beefy red; 75% to 100% of wound filled &/or tissue overgrowth 5 = Skin intact or partial thickness wound		
5. Epithelialization	1 = 100% wound covered, surface intact 2 = 75% to <100% wound covered &/or epithelial tissue extends >0.5cm into wound bed 3 = 50% to <75% wound covered &/or epithelial tissue extends to <0.5cm into wound bed 4 = 25% to < 50% wound covered 5 = < 25% wound covered		

Table 3: Wound Assestment Tools for clinical category, grouping on 4 category
5- 8 = minimal severity
9-12 = mild severity
13-16 = moderate severity
17-25 = extreme severity

Evaluation on the 5th day, in PRP group, 6 patients are categorized as mild severity and 3 patients are categorized as Moderate Severity. While in Non PRP group 1 patient is categorized as mild severity and 8 patients are categorized as moderate severity. Evaluation on the 10th days on PRP group all patients are categorized as minimal severity. Meanwhile in the Non PRP group, 5 patients are categorized as minimal severity and 4 patients are categorized as mild severity. Platelet Rich Plasma speed up epithelization process because platelet degranulation caused released of protein secretory (growth factor) that increased activation of macrophage and fibroblast that influence formation of collagen as main inggridients of extracellular matrix. Afterwards migration process of epidermal cell occurred from the edge of the wound to formation of connective tissues (extracellular matrix).

Evaluation on epithelization and graft take percentage on the 10th days. Group of wound with interventions underwent wider epithelization and clinically better results of wound healing compared to control group, so that interventional group has smaller wound compared to control group.

V. Conclusion

Platelet rich plasma are efficacious towards wound healing process through increasing of macrophage, fibroblast and collagen that can speed up epithelization.

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