

## Comparison of Platelet Rich Plasma and 10%Dextrose Solution Effect Towards Muscle Healing Process

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### ABSTRACT

Both Platelet Rich Plasma (PRP) and Dextrose 10% have proliferated effect by promoting some growth factors. Using PRP and dextrose 10% intralesional injection in muscle injury were estimated faster and promote muscle quality. The experimental comparative study was conducted on 27 rats that have muscle injury grade 2, then divided into 3 groups. The first group was injection with PRP, the second group with dextrose 10%, the third group with NaCl 0.9% as a control group. After one week, the subjects were sacrificed, and their gastrocnemius muscle was examined to see the level of myoblast through the immunohistochemical technique. The results show an increased level of myoblast cell in PRP and a 10% dextrose group than control, and the level of myoblast cell was higher in the PRP group than the 10% dextrose group (PRP:10% Dextrose:0.9%NaCl=12,33:8,00:5,67). In conclusion, using PRP and 10% dextrose intralesional injection increase the level of myoblast cell in muscle injury grade 2, and using PRP was better than 10% dextrose.

**Keywords:** Platelet rich plasma; 10%Dextrose; Myoblast, Prolotherapy; Muscle healing

### INTRODUCTION

Muscle injury is a quite common injury often occurred in acute or recurrent situation and leads to a decreased ability of activity performance. It could be due to a direct impact of a blunt trauma, punctured wounds, or excessive use in an exercise [1,2].

Management of muscle injury conservatively includes five steps known as PRICE: Protect Rest, Ice, Compression, and Elevation. PRICE method effectively carried out for the first 1-2 days to reduce inflammation and edema at the site of injury. Start from the 3rd day, physiotherapy can be done by Trans Electrical Nerve Stimulation (TENS) or with a neodymium-YAG laser. Isometric contractions can also be started and continued with concentric and eccentric contractions gradually [3]. Surgery might be required in certain patients, such as in athletes with large intramuscular hematoma, grade III muscle injury where the muscle cannot contract, grade III injuries when the rupture of the muscle more than 50%, and there is persistent pain in more than 6 months [3-5].

Normal movement can usually be achieved at week 4. The formation of connective tissue because of myofibroblast will hinder the movement and increase the risk of recurrence. Recurrence within 2 months after returned to daily activities

shows that rehabilitation program does not progress properly. This becomes a problem for professionals such as injured athletes who need a good recovery faster. Therefore, a variety of methods and modalities are developed to improve muscle healing [3-5].

In the last decade, prolotherapy by using some growth factors was developed as therapeutic modality in the treatment of muscle injuries. Administration of growth factors can be directly, for example by the administration of platelet rich plasma (PRP) which contains a variety of growth factors, or indirectly by administering growth factor stimulants that can stimulate the body to produce growth factors [6]. Platelet rich plasma has been applied locally on diabetic ulcers to speed up the healing process [7,8]. However, direct use of growth factors is actually impractical because it requires special tools and preparation [9].

Dextrose is a simple carbohydrate which is an indirect growth factor. It increased 12-lipoxygenase pathway (12-LO) on arachidonic acid metabolism which has the effect of angiogenesis and increase the level of growth factors on muscle cell [10]. 10% dextrose fluid is easily to use and more practical.

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Therefore, this research of comparing the effects of platelet rich plasma and 10% dextrose towards injured muscle healing is proposed.

## METHODS

This is a comparative experimental study using 27 wistar strain rats then divided in to 3 groups. The first group was injection with PRP, the second group with 10% dextrose, and the third group with 0.9% NaCl as a control group, were compared.

Inclusion criteria were rats which meets the following requirements: male, age 12-16 weeks, weight 180-250 grams and in healthy conditions. Exclusion criteria were as follows: infected during adaptation or during the study, died during adaptation or during the study.

### This research was conducted with several stages as follows:

Subjects were quarantined for 7 days for adaptation, then the rats were randomized into 3 groups, each group consisting of 9 rats, and were put into a cage with certain treatment. Platelet rich plasma was prepared by Messori et.al method [11], which is a blood sample is taken from the four rat's heart, 3.5mL each using a 5mL sterile syringe. The blood sample then was put into a 4.5 mL tube Vacutainer BD-Citrate. The tube containing the blood is then taken to the Clinical Pathology Laboratory of Molecular Biology department for the manufacturing of platelet rich plasma. By using a centrifuge machine MRi 23-Jouan, the blood tube was centrifuged 160xg for 20 minutes at 22°C. After centrifuged, the blood will be separated in three layers: plasma, platelet concentrate and red blood cells. Then the top phase (at the top mark of 1.4mL) was transferred into a new tube. The tube is then centrifuged again at 400xg, for 15 minutes at 22°C. After centrifuged, the blood will be separated in two layers: the supernatant phase - platelet poor plasma (above the mark of 0.35mL) and platelet rich plasma which are below the line. Platelet-poor plasma inside the tube was removed to left only platelet rich plasma [11].

Then, the platelet rich plasma was given to gastrocnemius muscle of the subject (group 1). And the second group was injection with 10% dextrose, and the last group was injection with 0.9% NaCl.

Before injection, the subject was prepared in grade II muscle injury condition manually by several stage as follow: The sample rats were sedated with HCl ketamine intramuscularly. The hair of rat's lower limbs were shaved. Aseptic and antiseptic procedure with 70% alcohol and 10% povidone iodine was done. Cutis and subcutis incisions were made at the posterior part of the lower leg rats for 2cm. Diameter of the rat's gastrocnemius muscle was measured. Incision was done perpendicularly towards the gastrocnemius muscle fibers by 50% of the diameter [12].

After receiving treatment, the wound is closed using 4/0 nylon thread and immobilized by a circular cast.

In the first 3 days, the rats were given antibiotics cefazolin 200mg intramuscularly.

After 1 week, the sample material of injured muscle will be taken. The sample material with a size of 0.5x0.5x0.5 cm was put into a tube containing formalin. The sample is then brought to the Laboratory of Pathology for preparations. The sample preparation mixture will be added with reagent myoD1, to perform the binding with myoblast cell. Then under the microscope CX-21, the intensity of MyoD1 reagent binding with myoblast cells and myoblast cells distribution formed in each preparation will be counted.

The data were analyzed with univariate analysis by SPSS 18.0. This analysis is done to get a general idea of frequency distribution by describing each of the variables used in the research. Then the normality of the data is tested with the Shapiro-Wilk test. Then proceeded with the homogeneity test using Levene test. Then proceeded with post hoc analysis using difference test and Fisher's Least Significant Difference (LSD).

## RESULTS

One week after treatment, samples were taken from the rat's gastrocnemius muscle with 0.5x0.5x0.5cm lesion and immunohistochemical examination using myoD1 reagent was done, and the myoblast distribution was calculated with Histoscore method by scoring the intensity and myoblast cells distribution [13].

Myoblast cell intensity with MyoD1 divided into three scoring: (+)weak with score of 1, (+)moderate with score of 2, (+)strong with score of 3. Myoblast cell distribution was divided into four scoring: <20% with a score of 1, 20-50% with a score of 2, 51-80% with a score of 3, >80% with a score of 4 [13].

The immunohistochemical examination results were scored and compared between two groups, where the value of the score is based on Hscore scoring system with formula:  $Hscore = (intensity + 1) \times myoblast\ distribution$  [13].

Assessment was given in the form of ordinal scale scores with a total score ranging from 2-16.

Figures 1-3 shows a picture of the myoblast cell anatomical pathology binded to the reagent myoD1 at various intensities.

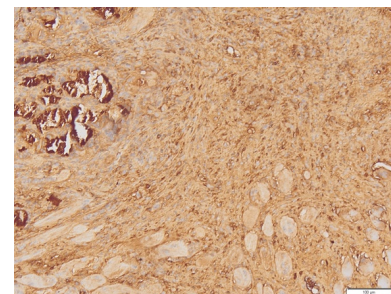
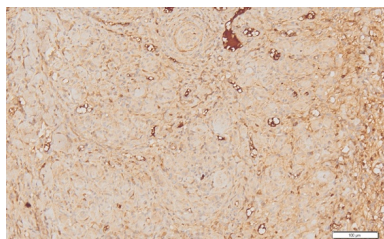
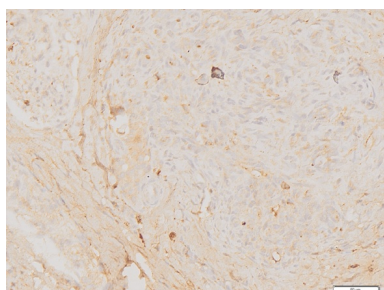


Figure 1: Picture of myoblast cells that bind myoD1 on strong intensity.



**Figure 2:** Picture of myoblast cells that bind myod1 at moderate intensity.

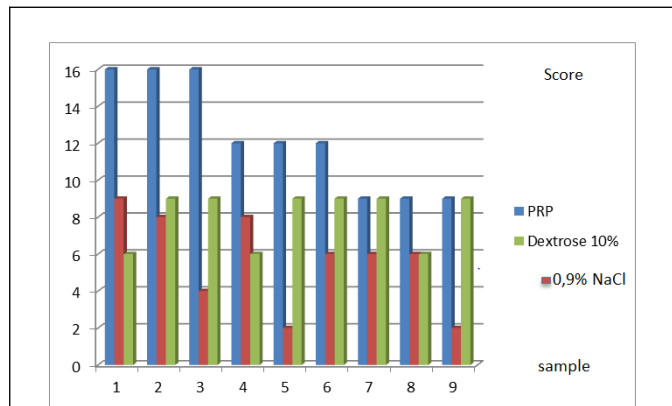


**Figure 3:** Picture of myoblast cells that bind myod1 on weak intensity.

Research results for the three groups of rats were descriptively shown in the following tables and figures (Table 1, Figure 4).

**Table 1:** Intensity and distribution score of myoblast cells in each group based on the h-score scoring system.

Sample	Number	PRP	10%Dextrose	0.9%NaCl
H-Score	1	16	6	9
	2	16	9	8
	3	16	9	4
	4	12	6	8
	5	12	9	2
	6	12	9	6
	7	9	9	6
	8	9	6	6
	9	9	9	2
Mean data		12,33	8,00	5,67



**Figure 4:** H-Score scoring results of myoblast cells distribution in each rats.

Table 1 and Figure 4 illustrates the scoring of myoblast from muscle healing in rats. Based on these data it can be seen that the lowest score in the PRP group is 9, the highest score is 16 and the mean score is 12.33; lowest score at 10%dextrose group is 6, the highest score is 12 and the mean score is 8; whereas the lowest score in the control group is 2, the highest score is 9 and the mean score is 5.67, indicating that PRP group mean score was higher than 10%dextrose and control group, as well as the mean score of dextrose 10%group is higher than the mean score of the control group.

Then the Fisher Least Significant Difference (LSD) test was done to see whether there is significant difference in the number of myoblasts cells in the rat's muscles between the PRP, dextrose group and the control group (Table 2).

**Table 2:** Calculation results of fisher lsd test.

Group		Mean Difference	Std. Error	Sig.
0.9%NaCl	PRP	-6,66,667	1,15,470	,000
	dextrose	-2,33,333	1,15,470	,055
PRP	0.9%NaCl	6,66,667	1,15,470	,000
	10%dextrose	4,33,333	1,15,470	,001
10%Dextrose	0.9%NaCl	2,33,333	1,15,470	,055
	PRP	-4,33,333	1,15,470	,001

Based on the statistical calculation, it was obtained that M value between PRP and control groups of 6.67 with a p value of 0.0 M value between 10%dextrose group and control is 2.33 with a p value of 0.05. M values between groups PRP and dextrose 10% is 4.33 with a p value of 0.01. This statistical analysis showed that the value of p<0.05. Therefore, we can conclude that there are differences in the number of myoblast cells in the rat's

muscles in the PRP and 10%dextrose group towards the control group and there are differences in the number of myoblast cells in the rat's muscles in the PRP towards 10%dextrose group.

## DISCUSSION

These results shows that administration of intralesional injection of PRP and dextrose 10% on the injured muscle is proved to be effective by increasing the number of myoblast cells in the muscle ( $p < 0.05$ ) with a mean difference between groups PRP, dextrose and control groups is 12,33:8.00:5.67.

Increased number of myoblast cells in the muscles was injected with PRP and dextrose occurs because both PRP and dextrose are proliferant to the cell. PRP contains direct growth factors, while dextrose indirectly by stimulate increasing the level of growth factor that plays an important role in muscle cell migration and proliferation. The cells will produce growth factors in a few minutes to a few hours when exposed by dextrose with concentrations above 0.6% (the normal cell glucose concentration is 0.1%). Extracellular hyperglycemic condition will increase 12-lipoxygenase pathway (12-LO) on arachidonic acid metabolism which has the effect of angiogenesis and increasing the level of growth factors on muscle cells [10]. These growth factors, among others: Platelet Derived Growth factors, transforming growth factors beta, Epidermal Growth factors, Basic Fibroblast Growth factors, Insulin Like Growth factors and Connective Tissue Growth factors. All of these factors have been identified as the key to stimulate the healing of muscle injuries

10%dextrose injections induce cell proliferation without an increase in inflammatory reactions, so that it is suitable in cases of acute muscle injury that occurs when the inflammatory process increases significantly [14-19].

The results are consistent with other studies that stated that administration of Platelet Rich Plasma is proven to stimulate cell migration and myofibroblasts differentiation and enhance the healing process of muscle injuries [11]. Platelet rich plasma has been applied locally on diabetic ulcers to accelerate the wound healing process [7,8].

Increasing number of myoblasts cells in the administration of PRP is much higher than that of 10% dextrose, because PRP contains direct growth factors, so that it works directly to increase cell proliferation, whereas 10%dextrose works indirectly by stimulates increasing growth factors in cell.

Increasing number of myoblast cells in this study assessed based on scoring system of HScore. Hscore scoring system evaluates two important variables shown in healing of the injured muscle, the intensity and distribution of myoblast cells. Assessment was carried out by a specialist consultant of anatomical pathology to minimize the possibility of bias [13].

The weakness in this study was about the difficulties in terms of uniformity of rat's gastrocnemius muscle diameter, so there is a possibility of bias at the time of sampling for immunohistochemical examination.

## CONCLUSION

PRP and 10%dextrose intralesional injection on injured muscle may increase the number of myoblast cells and improve muscle healing. But the administration of PRP on injured muscle is more effective than the administration of 10%dextrose to improve muscle healing.

## SUGGESTIONS

PRP and 10%dextrose intralesional injection may become additional modalities recommendation in treatment of muscles injury grade 2. Both maybe can be applied to human, but another study was needed to know the effective doses to human's muscle injury.

## CONFLICTS OF INTEREST

The author declares there is no conflict of interest in this research.

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