

# Platelet-rich plasma improves initial bone remodeling



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## Abstract

**Objective:** To compare the osteoblast cell formation process after installation implant with and without added of platelet-rich plasma (PRP).

**Methods:** Twenty-four male rabbit were selected by purposive sampling, divide into two groups. Group 1 implant with added PRP were installation in the distal part of the thigh bone 12 male rabbits, Group 2 implants without the addition of PRP mounted on the distal part of the thigh bone 12 male rabbits. All rabbits in euthanasia after 0,

7, 14, and 28 days were then analyzed histologically to determine the formation of osteoblast cells.

**Results:** There is increased formation of osteoblast cell formation in implant installation with the addition of PRP when compared with implants without PRP as time increases.

**Conclusion:** Administration of PRP in implants can speed up and increase osteoblast cell formation.

**Keywords:** Bone remodeling, Implant, Platelet-rich plasma

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## Introduction

Correction of bone defects in the installation of dental implants can be accelerated by adding platelet-rich plasma (PRP) material that can initiate wound healing by releasing locally employed growth factors.<sup>1-3</sup> Platelet-rich plasma is a protein taken autologous, using regeneration of the body itself, so it has the advantage that; PRP can prevent disease transfer and immunologic reactions, PRP is easy to obtain because it comes from self-administered animal blood, time is short, easy and safe to apply, PRP has high fibrin content, PRP accelerates epithelial, endothelial and epidermal regeneration which can promote tissue healing through angiogenesis and collagen synthesis, PRP can accelerate bone formation and maturation.<sup>4</sup>

Bone formation begins with the release of platelet derive growth factor (PDGF), TGF and IGF. Vascular endothelial growth factor increases endothelial growth proliferation. Platelet derived growth factor will bind to endothelial cells to initiate wound healing through collagenase activity. Transforming growth factor-beta (TGF-B) will bind to osteoblasts and stem cells to stimulate osteoid production resulting in bone resorption and woven bone formation.<sup>5</sup>

PRP is a platelet in gel form extracted through centrifugation of bovine thrombin mixture, 10% calcium chloride and venous blood. PRP also contains significant growth factor concentrations that will increase bone formation and

mineralization, induce stem cells to differentiate into osteoblasts, minimize bone resorption, increase angiogenesis and produce collagen through fibroblast activation.<sup>6,7</sup>

The purpose of this study was to analyze the effect of the addition of PRP on the installation of dental implants to the initial bone remodeling. This research is almost similar to the research done by Brunamelia de Oliveira on the evaluation of biomaterials with and without platelet-rich plasma: a histometric study using beagle dogs, but different in terms of methods, and research samples. In this study only use the time from the first day to the fourteenth day because only want to see the initial bone formation.

## Material and Methods

Twenty four New Zealand rabbits, aged 4-8 months, weight 1500-2000 grams maintained for seven days in clean, air-free cage, fed and drank three times a day. Divided into two groups, the first group contained twelve rabbits placed implanted with PRP on her thighbone, the second group containing 12 rabbits placed an implant without PRP on her thighbone. Furthermore each 3 rabbits will be sacrificed after 1, 3, 7, and 14 days after implant installation both in first and second group. Intake of bone tissue around the implantation area, making histologic preparations to calculate the number of osteoblast cells using an Optilab microscope Raster V3 image with magnification 10, 40 and 100.

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### Animal maintenance

Eligible rabbits are grouped in cages made of iron (two rabbits in one cage). Adaptation was performed for 7 days in the cage before the implant installation was done. Food is given 3 times each day in the form of a combination of carrots, kale, cabbage and chicory. The temperature and humidity of the room is left in the natural range of about 32 degrees Celsius. Before the intervention of each rabbit measured his weight, then divided into 2 groups, group 1 (implant installation without PRP) on the femur then labeled the numbers 1-12 and group 2 (installation of implants with PRP) on the femur is then labeled number 13 - 24.

### Preparation of platelet rich plasma

Set up non EDTA tubes and label them according to animal data. Blood collection in rabbits is done in a jugular vein as comfortable as possible as the syringe is inserted into a large vein on the neck where it was previously disinfected with a cotton swab moistened with alcohol. Needle direction 30-45° to vein, pin hole facing up. After obtained blood as much as 5 ml, pull needle and press with alcoholic cotton. Insert in the tube without coagulant. Blood sampling was performed on each animal sample just before surgery for PRP production.

Insert a tube containing the blood that has been taken from the jugular vein into the centrifuge. Blood is centrifuged at 3000 rpm for 15 minutes. Then take the tube from the centrifugation tool looks 3 layers of the upper part of the plasma, in the middle there is a white ring area is a buffy coat and the bottom is red blood cells.

Take it with the plasma fluid pipette and buffy coat and insert it into the new tube. Next put the tube containing the plasma fluid and the buffy coat into centrifugation and rotate at 3000 rpm for 15 minutes. Remove the tube from the centrifugation machine, it will appear the top of the liquid form of the plasma and at the bottom there is a pellet that is rich in platelet deposits. Take the supernatant (upper fluid) and reserve the plasma fluid along with the platelet-rich Pellet (PRP). Take the plasma and pellet liquid using the Pasteur pipette into the needle and then labeled, Implants ready to installation and injected PRP around the implant.

### Surgical operation

Shave the rabbit's fur on the femur and degrade the femur area that has been shaved with betadine and then the rabbit in the anesthesia using a combination of 100 mg / ml ketamine and xylazine 2%. Anesthesia in animals attempted intramuscularly according to the calculated dose. Rabbits are held and conditioned as comfortable as possible.

The syringe is filled with an anesthetic that has previously sprayed the part to be injected with 70% alcohol. Poke the needle perpendicular to the middle of the thigh. The anesthetic material is injected slowly. Perform an incision on the part of the femur that has been cleaned feathers. The incision on the skin is parallel to the femur, then making a hole in the bone using a bur drill up to 3.0 mm in diameter. Insert the implant on the femur bone. 12 implants without injected PRP were installed in 12 rabbits and 12 implants with injected PRP on 12 rabbits so each rabbit was installed with one implant.

### Histomorphometric study

After the installation of the implant and the prescribed day, further euthanasia for femur bone tissue removal. Network retrieval was performed using a sterile minor surgical instrument. The tissue is then inserted into a sample pot containing 10% formalin for 5 days. Then rinse with water for 30 minutes to remove residual formaldehyde. The decalcification process begins by immersing bone specimens in a combined solution of 8% hydrochloric acid and 8% formic acid for one day (24 hours) which is repeated by replacing the solution every day until the decalcification process is complete. After the decalcification process is complete, the specimen is rinsed with running water followed by immersion of the specimen in ammonia solution for 30 minutes to neutralize acids from a combined solution of 8% hydrochloric acid and 8% formic acid. Organ samples are cut along the location of the implant horizontally and vertically.

Research ethics This study has been conducted ethical review and approved by research ethics committee Faculty of Medicine Hasanuddin University with number 93 / H04.8.4.5.31 / PP36-KOMETIK / 2016.

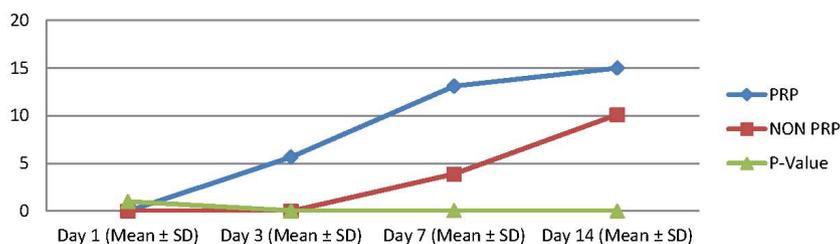
### Data analysis

In this study using Shapiro wilk test because the sample research below 50. The statistical test results of  $p < 0.05$  means that the data is not normally distributed or otherwise. For non-distributed data use the Mann Whitney Test to compare between the groups and the Friedman Test to test the changes that occur in each repetition of the observation. While the data is normally distributed using t-independent test Repeated ANOVA. Data processing is done electronically through SPSS program version 24.0 (SPSS Inc, Chicago, IL, USA).

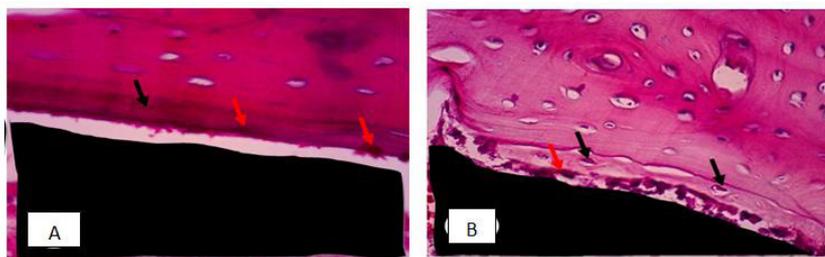
### Results

Table 1 illustrates the results of the study after implant installation of an increase in the number of

	N	Initial Bone Formation				p-value
		Day 1	Day 3	Day 7	Day 14	
		mean±SD	mean±SD	mean±SD	mean±SD	
PRP	12	0.00±0.00 <sup>a</sup>	5.67±0.33 <sup>a</sup>	13.11±0.69 <sup>a</sup>	15.00±0.33 <sup>b</sup>	0.029***
NON PRP	12	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	3.89±0.19 <sup>a</sup>	10.11±0.51 <sup>b</sup>	0.029***
P-value		1.000**	0.037**	0.046**	0.000****	



**Figure 1** The result of the study after implant installation with and without PRP



**Figure 2** Histological day 14, showed new bone layer which is characterized by osteocytes (black arrow), osteoblasts (red arrow) on the implants coated PRP group (A) and in the group not coated PRP also seen their osteocytes (black arrow), more osteoblasts (red arrows)

osteoblast cells that marked the beginning of bone regeneration with increasing time (from day one to day fourteen) both in implants with the addition of PRP, as well as to implants without PRP. There were significant differences in outcomes in both groups in the implants group with PRP, as well as implant groups without PRP (p = 0.029). In the results of the study there were also significant differences in outcomes between implant groups with PRP and implant groups without PRP (p = 0.001).

### Discussion

This study has been conducted on the effect of additional PRP injection during implant installation on initial bone formation acceleration. The results showed an increase in the number of osteoblast cells in the implant added PRP compared to implants without PRP. This occurs because in PRP there is a growth factor that can be obtained from centrifuged platelets that will result in Platelet-Rich Plasma (PRP). PRP is a plasma volume that has platelet concentrations above the limit and there are seven growth factors. The normal platelet count in

human blood is between 150,000 /  $\mu$ L and 200,000 /  $\mu$ L with an average of about 200,000 /  $\mu$ L. Scientific evidence suggests an increase in bone and tissue healing using PRP with a concentration of 1,000,000 platelets /  $\mu$ L.<sup>8</sup> PRP injection was performed during implant installation in the hope that it triggers the acceleration of early bone formation so that the primary stability of the implant is quickly formed. The addition of PRP to the surface of the implant may increase the amount of osteoblast formation.<sup>9</sup> Platelet rich plasma contains granule- $\alpha$  consisting of a number of growth factors that stimulate healing of soft tissue and bone. The growth factor is PDGF, TGF, VEGF, EGF, ILGF.17 concentration of platelets to produce a positive biological effect on bone healing 1,000,000 / ml in 5 ml plasma.<sup>10-11</sup> Active secretion of growth factors occurs 10 minutes after coagulation. The growth factors found in PRP regulate cellular processes such as mutagenesis, chemotaxis, differentiation and cell metabolism.<sup>12-13</sup> Initial release IGF from platelet granules as chemotaxis, activation of stem cells masenkim.<sup>14</sup> Activation of stem cells improve progenitor cells that will turn into osteoblast cells. Transforming Growth Factor-beta binds to osteoblasts to initiate mitosis and stimulate the production of osteoid Vascular Endothelial Growth, angiogenesis and collagenase activity and increasing proliferation of endothelial cell growth. PDGF binds to endothelial cells to initiate the growth of capillaries and activates intracellular signaling to improve the ability of cell proliferation, matrix formation, collagen synthesis and cellular differentiation. Platelet Derived Growth Factor also be a mitogen and chemotactic for fibroblasts and osteoblasts. Epidermal Growth Factor to accelerate the formation of epithelial and reduce the spread of the wound. Some other growth factors are also working on a level lokal.<sup>16</sup> Marx said, in clinical trials, the use of PRP is said to improve the amount and quality of bone defects.<sup>17</sup> The same is stated by Andreas Thor in his research found the effect of PRP can improve and accelerate the bone healing.<sup>18</sup>

According to Dee et al few seconds after implant placement, the entire surface of the implant is covered with a thin layer of the serum protein which is a growth factor, surface characteristics of materials has great influence on the serum protein

attachment. Serum proteins associated with the activation of the physiological process of platelets (thrombocytes) and the release of granule- $\alpha$ . The platelet degranulation release growth factors and triggers chemotactic signal. According Schliephake et al, Environmental baseline between the bone-implant when performed implant (lesion on the bone) to the hypoxic environment and sour, good for PMN activity and tissue macrophages, causing blood clots to form fibrin which is becoming kallus.<sup>19</sup> Fibrin matrix serves as a scaffold (osteon conduction) for migration of osteoblasts that are osteopenia cells and eventually undergo differentiation in the recovery room. Osteoblasts calcified newly formed woven bone (woven bone) and trabecular bone between bone and implant.<sup>20</sup>

PRP is a key cellular process mutagenesis, chemotaxis, angiogenesis, differentiation and cell metabolism. Platelet Derived Growth Factor is an agent that stimulates regeneration because it regulates adhesion, migration, proliferation and differentiation of connective tissue, and osteoblasts (bone cells). Platelet Derived Growth Factor is a mitogen and chemotactic for osteoblasts, TGF- $\beta$  stimulates the osteoblast precursor cells and stimulates collagen synthesis. Platelet rich plasma "star jump" (jump over the beginning) reaction of the physiological process of an event regenerative.<sup>16,20</sup> This discussion concluded that there are differences in the number of osteoblasts after the formation of an implant coated with PRP and uncoated with PRP.

## Conclusion

Platelet Rich Plasma may accelerate and enhance the formation osteoblasts. It is seen in this study, that implants with PRP gave higher results than the implants without PRP.

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## Conflict of Interest

The authors report no conflict of interest.

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