# Differences of Post-Placement Bone Implant Contact (BIC) Value of Dental Implant Coated and Not Coated With Platelet Rich Plasma (PRP)

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# Abstract

**Objective:** The success of a dental dental implant treatment focuses on a phenomenon called osseointegration. Evaluation of Bone Area (BA) and Bone-Dental implant Contact (BIC) through histomorphometric analysis is the most widely used parameter to measure osseointegration. The aim of this study was to see post-placement Bone Dental implant Contact (BIC) value of dental implant coated and not coated with PRP.

**Materials and Methods:** This study was an experimental laboratory conducted at Laboratory of Veterinary Faculty, Hasanuddin University. The sample was baby buck rabbit, aged 4-8 months old, weight 1500–2000 gram, divided into 2 groups each group consist of 12 rabbit, control group not coated with PRP and treatment group coated with PRP. Data analysis using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

**Results:** There was a significant difference between the mean BIC values and the 20% increase in BIC values in LP1, LP2 and LP3 between treatment and control group on day 0, 3, 7, and 14.

**Conclusions:** There was a difference in the average of post-placement BIC value of dental implant coated and not coated with PRP.

Keywords: Bone implant Contact (BIC), post-placement, Platelet Rich Plasma (PRP)

# 1. Introduction

The success of a dental implant treatment focuses on a phenomenon called osseointegration which was first introduced by Branemark, the microscopic characteristic of bone formation on the surface of the dental implant. Surface composition and roughness are parameters that may play a role in the interaction of dental implanted tissue and osseointegration. Evaluation of Bone Area (BA) and Bone-Dental implant Contact (BIC) through histomorphometric analysis is the most widely used parameter for measuring osseointegration. Platelet-rich plasma (PRP) is an easy and inexpensive way to obtain growth factors in physiologic proportions that might favour the regenerative process. (A.Roffi et al., 2013; Subhaini & Herda, 2008; Elias et al., 2010; Kurniati, 2012)

One of the best sources of growth factors in the body is blood platelets. Growth factors such as platelet-derived growth (PDGF) and transforming growth factor- $\beta$  (TGF- $\beta$ ), which are present in  $\alpha$ -grains of platelets and released in the wound area, have proven to be of vital importance in healing bone, gingiva, and skin. Some researchers have tried to increase osteogeneration rates in peri-implant bone by using biological factors, especially PDGFs, commonly known as platelet rich plasma (PRP) or plasma rich growth factor (PRGF). Platelet-Rich Plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline .(Thor, 2006; Civinini, 2011; Kundu et al., 2014)

In recent years PRP has widespread diffusion in the treatment of soft tissue and bone healing. Used ordinary dental implants are made of titanium only or titanium alloys with the addition of surface modifications of the dental implant to enhance osseointegration.(Carl, 2007; Malik et al., 2011; Palwinder, 2011) The scientific basis for this success is the occurrence of osseointegrated dental implants with bone and patient clinical conditions that include

adequate quality and quantity of bone.(Garcia, 2010)

Hence, the authors are interested to see the value of post-placement BIC dental implant coated and not coated with PRP.

#### 2. Material and Method

This study was an experimental laboratory conducted at Laboratory of Veterinary Faculty, Hasanuddin University. The sample was baby buck rabbit, aged 4-8 months old, weight 1500–2000 gram, divided into 2 groups each group consist of 12 rabbit, control group not coated with PRP and treatment group coated with PRP.

Prior to treatment, all rabbits were adapted and kept in groups (2 rabbits per cage) for 7 days to condition animals in good health.Furthermore, rabbits enter the surgical stage of the femur bone to insert dental implants coated and not coated with PRP. Dental implant used in this study was from Osstem Implant with SA Surface (Sandblasted acid etched) 3.0x8.5 mm. Dental implant installation procedure according to the installation instructions of the Osstem dental implant.

Specimens of os femur were taken from rabbits that have been euthanized using xylazine 1.5 cc intra cardiac. Bone specimen fixated in 10% formalin solution for 5 days, then rinsed under running 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 hrs) repeatedly by replacing the new solution each day until the decalcification process is completed. The decalcification process depends on the size of the specimen. After the decalcification process is completed, the specimens were rinsed under running water followed by soaking the specimen in ammonia solution for 30 minutes to neutralize the acids from the combination of 8% hydrochloric acid solution and 8% formic acid. Rinse the specimen under running water for 24 hours then proceed with paraffin embedding process.

Organ samples were cut along the dental implant site with vertical and horizontal directions so that histopatological observation can be done through two aspects. Furthermore, organ samples were immersed in a stratified alcohol solution (dehydration) starting from concentrations of 70%, 80%, 90% and 95% for one day (24 hours) respectively, dehydration followed by 100% glow concentration (two immersions ) with the same concentration, each for one hour. The dehydrated organ sample was subsequently cleared in xylol (clearing) which was made glow (two immersions) each for 15 min. Before it is finally grown in paraffin, the tissue in the paraffin blocks is sliced with a thickness of 5 $\mu$ m using a microtome (Indoexim, India), then placed on the object glass, and stored in an incubator with a temperature of 40 °C for 24 hours.

The result of incision was stained with the raw stain of eosin hematoxylin (HE). HE staining is used to look at tissue structures that allegedly have pathological changes. Furthermore, the tissue is removed before it is covered with a glass cover (mounting). The observations were performed under a microscope with 10x and 16x subjective lens enlargements as well as 10x, 40x, and 100x objective lenses. The shooting is done using a digital camera at 100x magnification with emersion oil.

Using Optilab Image Raster v3, the average BIC value was seen on days 0, 3, 7 and 14 after dental implant placement. The results of the examination were recorded and data analysis using SPSS program version 20.0 (SPSS Inc., Chicago, IL, USA).

## 3. Result

Day	Field of View 1		Field of View 2		Field of View 3	
	Mean $\pm$ SD	p value	Mean $\pm$ SD	p value	Mean <u>+</u> SD	p value
0	$0.0000 \pm 0.0000$	0.029	$0.0000 \pm 0.0000$	0.029	$0.0000 \pm 0.0000$	0.029
3	$0.0000 \pm 0.0000$		$0.0000 \pm 0.0000$		$0.0000 \pm 0.0000$	
7	$21.5733 \pm 0.50738$		$20.5033 \pm 0.34948$		21.8133± 0.75162	
14	55.7933± 0.31943		54.8167± 0.38799		$55.0700 \pm 0.85767$	

Table 1. Comparison of BIC values in control group on LP1 (Field of View 1), LP2 (Field of View 2), and LP3 (Field of View 3)

Normality data test: *Shapiro-Wilk test; p* <0.05; not normally distributed.

\**Friedman* test; p < 0.05; significant.

Based on the results of statistical tests, we obtained p value = 0.029 (p <0.05) which mean there was a significant difference in LP1, LP2 and LP3 between BIC values on days 0, 3, 7, and 14 after dental implant placement without PRP. It can also be seen from the results of histologic examination on day 0, treatment of non PRP dental implants and with PRP has not shown the presence of osteoblast cells and osteocytes (Figures 1 and 2).

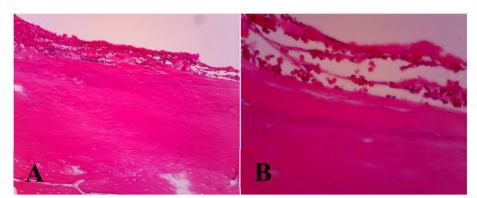


Figure 1. Non PRP dental implant on day 0 shows no new osteoblasts and osteocytes to form as a sign of new bone formation. Magnification A: 10x10, B: 10x40. HE staining.

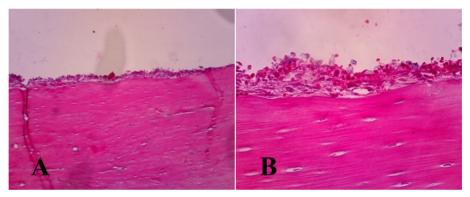


Figure 2. Dental implant with PRP on day 0 did not show the presence of new osteoblasts and osteocytes as a sign of new bone formation. Magnification A: 10x10, B: 10x40. HE staining

Table 2. Comparison of BIC value in treatment group on LP1 (Field of View 1), LP 2 (Field of View 2) and LP 3 (Field of View 3)

Day	Field of View 1		Field of View 2		Field of View 3	
	Mean <u>+</u> SD	p value	Mean $\pm$ SD	p value	Mean $\pm$ SD	p value
0	$0.0000 \pm 0.0000$		$0.0000 \pm 0.0000$		$0.0000 \pm 0.0000$	0.029
3	$98.7833 \pm 0.77009$	- - 0.029	$83.7833 \pm 0.49813$	0.029 	$89.2967 \pm 0.85500$	
7	$237.8933 \pm 2.33329$	- 0.029	$199.8300 \pm 1.70473$		$188.3433 \pm 1.04314$	
14	$246.1400 \pm 0.49427$		$238.0933 \pm 0.91920$		$246.1400 \pm 0.49427$	

Normality data test: *Shapiro-Wilk test*; p < 0,05; not normally distributed.

\**Friedman* test; p < 0.05; significant.

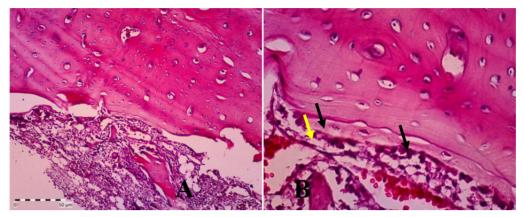


Figure 3. Non PRP on day 14 showed some new bone layers characterized by the presence of osteocytes (black arrows), osteoblasts (yellow arrows). Magnification A: 10x10, B: 10x40. HE staining. Bar 50 μm

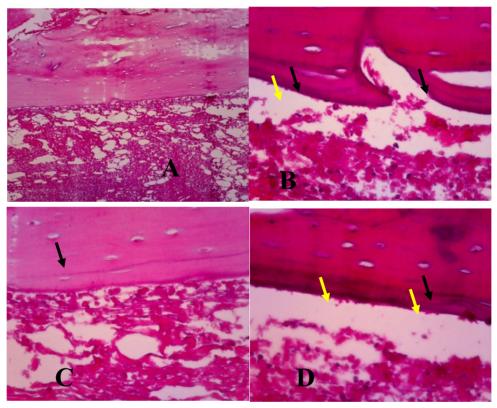


Figure 4. PRP dental implant on day 14th showed a new bone layer characterized by osteocytes (black arrows), osteoblasts (yellow arrows). Magnification A: 10x10, B, C and D: 10x40. HE staining

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Day	Field of View 1		Field of View 2		Field of View 3	
	Mean <u>+</u> SD	p value	Mean $\pm$ SD	p value	Mean <u>+</u> SD	p value
0	$0.0000 \pm 0.0000$	0.001	$0.0000 \pm 0.0000$		$0.0000 \pm 0.0000$	0.029
3	$49.3917 \pm 54.10805$		$41.8917 \pm 45.89110$	0.001	44.6483 ± 48.91279	
7	$129.7333 \pm 118.49297$		$110.1667 \pm 98.22743$		$105.0783 \pm 91.21586$	
14	$147.9717 \pm 100.97656$		$146.4550 \pm 100.38675$		$161.6050 \pm 103.55980$	

Table 3. Comparison of BIC value in treatment and control group on LP1 (Field of View 1), LP2 (Field of View 2), and LP3 (Field of View 3)

Normality data test: *Shapiro-Wilk test;* p < 0.05; not normally distributed.

\**Friedman* test; p < 0.05; significant.

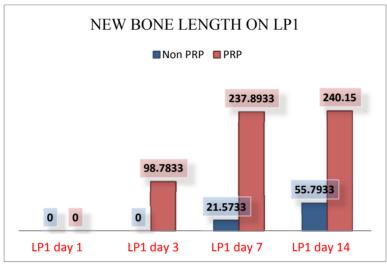


Diagram 1. Bar chart of BIC value comparison between treatment and control group on LP1 (Field of View 1)

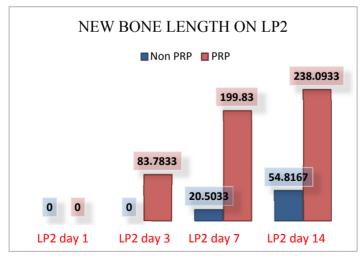


Diagram 2. Bar chart of BIC value comparison between treatment and control group on LP 2 (Field of View 2)

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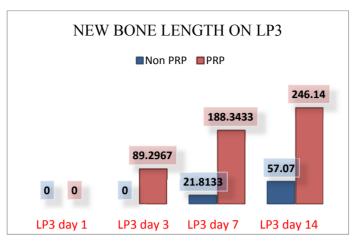


Diagram 3. Bar chart of BIC value comparison between treatment and control group on LP3 (Field of View 3)

Comparison of BIC values in LP1, LP2, and LP3 between treatment and control group can be seen in Table 3. The p value is 0.001 (p < 0.05), meaning that there is a significant difference between the mean BIC values and the 20% BIC values increase in LP1, LP2 and LP3 between treatment and control group on day 0, 3, 7, and 14. From the bar chart shown in diagram 1, diagram 2 and diagram 3, it appears that the BIC value increase almost 5 times greater in installation with PRP than without PRP.

Because the authors used histopathologic analysis, in which the size for preparations of bars 50  $\mu$ m, for that examination was done in 3 fields of view ie the top, middle, bottom and then averaged, that can be seen on Table 1, 2 and 3. Referring to the period 6-21 days of bone calcification matrix, the authors evaluated just 2 weeks long to see the initial bone healing that can be seen on Figure 1-4, and the life probability of animal object were sometimes only 2 weeks.

## 4. Discussion

This study calculated the mean value of post-placement BIC dental implant coated and not coated with PRP. Dental implant BIC measurements are standard procedures for evaluation of bone formation on the surface of the dental implant. High BIC values are considered a prerequisite for dental implant stability, which is clinically feasible for functional dental reconstruction. Specifically, the difference in BIC values between the test and reference surfaces was statistically analyzed to compare the osteogenic potential of the dental implant surface (Harmon, 2013).

In PRP preparation, a 5ml blood volume was taken in accordance with Marx, RE, study which showed that to achieve maximum effectiveness at least a minimum platelet concentration of 1.000.000/L in 5 mL plasma volume. The platelets contained in this concentrate release alpha grains containing a mixture of growth factors that initiate proliferation, chemotaxis and cell differentiation, which are important for osteogenesis. In addition to its procoagulant effects, PRP is a source of growth factors involved in initiating and maintaining wound healing by accelerating bone repair, promoting fibroblast proliferation, and enhancing tissue vascularization.(Goel, 2014; Gruber, 2002).

The main effect of PRP comes from PDGF, which has been identified as an essential protein for healing of hard tissue and soft tissue. PDGF has been demonstrated to stimulate chemotaxis, mitogenesis and replication of stem cells at wound sites to areas of tissue injury. This leads to the formation of bone matrix and angiogenesis by stimulating increased levels of Vascular Endothelial Growth Factor VEGF. This in turn can lead to accelerate soft tissue healing due to vascularization. PDGF also stimulates the production of fibronectin, the cell adhesion molecule used in cellular.(Thor et al., 2006; Fogelman et al., 2012; Frenkel, 2002)

Regeneration of bone-forming cells in this study was seen from the BIC parameters, shown higher in the treatment group than the control group where the difference was statistically significant (P = 0.001). This proves that the use of PRP increases the initial reaction of bone-forming cells on the surface of the dental implant. This finding is in line with the results reported by Wojtowics et al. and Fuerst et al. But contrary to, Butter field et al. Schlegel et al. and Russy et al. whose reported different results that the use of PRP has not shown significant results. (Galli, 2005)

The use of PRP on the dental implant surface not only improved the healing process of bone dental implants compared with the control, but also increased the BIC in treatment group statistically significant (P = 0.029) than

that of control. This is in line with those reported in previous studies including Fontana et al. Kim et al. and Furest et al. The researchers placed an dental implant on two minipig jaw sides. In their study, PDGFs were applied to one dental implant while the other was installed without growth factor. They measured BIC after 4 to 8 weeks and reported a 55.3% BIC value for dental implants with growth factor and a BIC score of 38.91% for control. They reported that anchorage dental implants could be strengthened in the jawbone by applying PDGFs. However, other studies using PDGFs for bone grafts or bone replacements do not report considerable attainment in terms of bone osteogeneration or on the surface of the dental implant. (Wang, 2015; Bernhardt et al., 2012)

#### 5. Conclusion

Based on the results of this study it can be concluded that there is a difference in the average value of post-placement BIC of dental implant coated and not coated with Platelet Rich Plasma (PRP).

## **Competing Interests Statement**

The authors declare that they have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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