

# Systemic Assessment of Calcium and Phosphorus Level after Implantation of Porous Iron in Rats

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**Abstract.** One of important aspects in bone healing process is physiological level of calcium (Ca), and phosphorus (P) that can be altered by implantation of biodegradable porous iron. Therefore, this study aims to investigate the concentration of Ca, P and Ca/P ratio in the peripheral blood during the implantation period up to 4 months. Forty adult male Sprague Dawley rats were used and divided into 3 groups receiving different pore size of iron implants (pore size 450, 580, 800 $\mu$ m) and one group of sham. The implants (5x2x0.5mm) were inserted into flat bone defects at latero-medial of femoral bone. Blood sample was taken from ventral tail artery before and after 4 month of implantation. Calcium and P concentrations in the blood were determined by BA-88A Semi-Auto Chemistry Analyzer. Results showed that concentration of Ca and P are slightly higher after implantation than before implantation, except for the 450 $\mu$ m group. The Ca/P ratio before and after implantation was increased in the sham group, and decreased in the 450 and 800 $\mu$ m groups. Concentration of Ca, P and Ca/P ratio insignificantly change between before and 4 months after surgery in some groups.

## 1. Introduction

Porous biodegradable metal made of pure iron has been considered as ideal material for developing bone scaffolds. Porous structure of sintered iron foams allowed increase in degradation rate [1]. Although iron utilizing as bone material implant is still debated due to its cytotoxicity [2], but iron as bone metal implant has proven non toxic to rats [3]. Iron is considered an essential mineral as the central element in oxygen transport and utilization [4]. Study about Porous iron as bone metal implantation already done in mice. This study showed that the porous iron implant have minimal inflammation effect through peripheral white blood cell examination [5].

Iron as biodegradable metal frequently combined with other material and also increased the number of porous surface of the implant to increase degradation rate [6]. Materials that commonly combine with iron are poly (lactic-co-glycolic acid), hydroxyapatite (HA), tricalcium phosphate (TCP) and biphasic calcium phosphate (BCP) [6,7]. Increasement of the degradation rate of iron implant is expected to accelerate the bone healing processes. Bone scaffold frequently use in bone disorders to restore the damaged bone [8].

In human, 99% of calcium and 85% of phosphorus are found in bone [9]. Calcium, together with phosphorus, constitute the main component of bones [10] as hydroxyapatite. One of important aspects in bone healing/growth is the in-vivo physiological level of calcium (Ca) and phosphorus (P) that can be altered due to the implantation of a scaffold. The skeleton is the body's principal reservoir of



calcium and phosphorus. Contrary to its appearance, bone is a dynamic tissue, and calcium and phosphate are continuously being deposited and released [11].

Several studies of porous iron implant had been done, but only a few study discussed the correlation between the porous iron implant and systemic of bone mineral such as Ca and P. Previous study has been discussed about this correlation just in 1 month observation [3]. Therefore, this study aims to investigate the concentration of Ca, P and Ca/P ratio in the peripheral blood during the implantation of porous iron in rats in long period 4 month observation.

## **2. Materials and Methods**

### *2.1 Specimens preparation*

Pure porous iron specimens (Alantum, Korea) with pore size of 450, 580 and 800  $\mu\text{m}$  were cut from commercial product to 5 x 2 x 0.5 mm of size without any prior treatments. Specimens were sterilized using an autoclave with high pressure saturated steam at 121°C, and followed by UV ray for 1 hour.

### *2.2 Implantation process*

This study was approved by Animal Care and Use Committee, Bogor Agricultural University (12-2015 RSHP FKH IPB). Forty adult male Sprague Dawley rats were used and divided into 3 groups receiving different pore size implants (450, 580, and 800 $\mu\text{m}$ ) and one group of sham as control. After 12 hours of fasting, the animals received premedication (Atropine<sup>®</sup>, Indofarma, Indonesia). All rats were implanted under general anesthesia ketamin (Ketamil<sup>®</sup>, Illium, Australia) and xylazin (Illium xyla<sup>®</sup>, Illium, Australia) influenced. After anaesthetized, femoral hair was shaved and the skin was disinfected with 70% alcohol and 10% iodine before implantation surgery. The rats were place in left lateral recumbence on the operation table. The implantation surgery procedure was started with skin incision in the femoral area, the muscle was then retracted until the femur bone reached. The implants (5 x 2 x 0.5mm) were inserted into flat bone defects at latero-medial of femoral bone of the rats. Sham treatment was done to all the procedure, but inserted the material implant. Femoral muscle and skin were sutured using absorbable 4/0 polyglactin suture (Hinglact, HiCare, India). To prevent infection, rats were treated with general antibiotic amoxicillin and clavulanic acid (claneksi<sup>®</sup> dry syrup, sanbe, Indonesia) for 5 days post surgery, orally.

### *2.3 Blood examination*

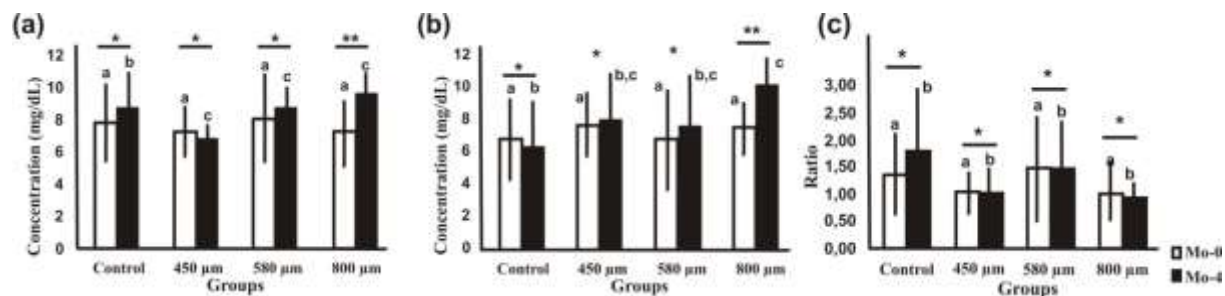
The peripheral blood was collected under general anesthesia influenced. Blood sample was taken from ventral tail artery before at 0 and 4 months after implantation. Calcium and phosphorus concentrations in the serum were determined by BA-88A Semi-Auto Chemistry Analyzer (Mindray Bio-Medical Electronics, China).

### *2.4 Data analysis*

Data were analyzed by one-way analysis of variance (ANOVA) with a post hoc Duncan test using SPSS software (SPSS Inc, USA) at the 95% confidence level, then expressed as mean $\pm$ standard deviation.

## **3. Result**

Figure 1 shows that concentration of Ca and P is slightly higher after implantation compared to before implantation, except for the 450  $\mu\text{m}$  group. The significant changes in Ca and P concentration are showed in 800 $\mu\text{m}$  group. Significant changes concentration also can be seen between control and 800  $\mu\text{m}$  groups. The Ca/P ratio before and after implantation was insignificantly increased in the sham group, and insignificantly decreased in the 450 and 800  $\mu\text{m}$  groups.



**Figure 1.** a Calcium concentration (mg/dL), b. Phosphorus concentration (mg/dL), c. Ca/P ratio after and before implantation of porous metal iron at femoral bone of rats. Different letters in superscript following values indicate statistical significant ( $P < 0.05$ ), \*represent  $P > 0.05$  indicate statistic insignificant, \*\* represent  $P < 0.05$  indicate statistical significant.

#### 4. Discussion

Calcium-phosphorus interaction is important. Fig 1a and b show an increase in Ca and P that indicate the mineralization process of bone healing [12] that regulated by parathyroid hormone (PTH) and vitamin D [9]. Iron, calcium and phosphorus deficient of rats have prevent decreased bone mass and increased fragility [13]. Fig 1.a. shows an increase of calcium concentration except the group 450  $\mu\text{m}$ . Bone mineralization is the final step in bone formation. Bone defect will stimulate PTH to increase calcium in extracellular fluid [9]. The higher concentration of calcium and phosphorus then will increase the number and function of osteoclast, then induce the osteoblast proliferation [14, 15]. Fig 1.a also shows that the concentration of Ca insignificantly decrease in group 450  $\mu\text{m}$ . The smaller pore size lead to the greater number of porous implant surface. Porous surface plays important role in cell growth and proliferation due to wider surface contact area and interconnectivity within the pores which lead to greater cell spreading [16] thus expected to reduce the requirement of calcium in mineralization processes. There is no direct interaction between iron degradation product of implant to calcium in bone remodelling.

Phosphorus exists in two forms within the body such as 10% inorganic (phosphate) which is routinely measured for laboratory purposes and 90% organic (e.g nucleic acids, phospholipids, ATP) [9]. Phosphorus is important in maintaining the structure of bones and teeth, maintaining of cell membranes, and supplying of energy. Most inorganic phosphorus is deposited in bone [9, 12]. Bone healing process will increased phosphate consumption that will increase the concentration of phosphorus in the fourth month after implantation except the control group (Fig 1.b). Insignificantly decreasing of phosphorus concentration in control group can be affected by a slight decrease in erythrocytes due to implantation process. Other groups have an indirect iron supplement from the material implant, that play role in oxygen transport and erythrocytes metabolism [11]. Also, there is no direct interaction between iron degradation product of implant to phosphorus in bone remodelling.

The result of Ca/P ratio is depend on the concentration of Ca and P. In all group Ca/P ratio insignificantly different at 0 and 4 months after implantation processes (Fig 1.c). Although insignificantly different but calcium and phosphorus concentration should be observed carefully. Nitric oxide synthase (NOS) is induced during fracture healing in rats and humans. Nitric oxide has pleiotropic effects in bone cell response, and potentially decrease resorption through decreasing osteoclast formation and activity [17]. Nitric oxide synthase is expressed at low level in their tissue of origin and their activity is mainly regulated by changes in free intracellular  $\text{Ca}^{2+}$  concentration. Rapid increase in intracellular calcium concentration could be occur due to phospholipase C activity and IP3 signaling resulting in release of calcium from intracellular stores in many cases [18]. As a free radical, smaller level of NOS will increase blood flow and induce bone healing but higher level of NO will increase oxidative stress probability and increase the activity of osteoclast [2]. Therefore, Ca, P, and Ca/P ratio examination with other examination such as blood examination should be investigated to determine the body condition during bone healing processes.

## 5. Conclusion

Bone healing involves complex interactions between mechanically stable environment, cell, osteo-conductive matrix with blood supply that affected the level of Ca and P. Concentration of Ca and P, and also Ca/P ratio insignificantly change between at 0 and 4 months after surgery in some groups. Iron supplement from the material implant play role in oxygen transport and erythrocytes metabolism, but there is no direct interaction between porous iron implant degradation product to Ca and P concentration. The concentration of Ca and P were affected by the porous size of iron implant, The smaller pore size will increase the surface contact area between the iron implant and surrounding cells.

## 6. References

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