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

S F Siallagan1, F Amelia1, N D Utami1, M F Ulum1, A Boediono1, S Estuningsih1, H Hermawan2,* and D Noviana1,*

1Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia
2Department of Mining, Metallurgical and Materials Engineering & CHU de Quebec Research Centre, Laval University, Quebec City, Canada

* Email: deni@ipb.ac.id

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 μm) 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 450μm group. The Ca/P ratio before and after implantation was increased in the sham group, and decreased in the 450 and 800μm 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]. Increaseamet 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 phosphor 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 longe 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 μm 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 μm) and one group of sham as control. After 12 hours of fasting, the animals received premedication (Atropine®, Indofarma, Indonesia). All rats were implanted under general anesthesia ketamin (Ketamil®, Illium, Australia) and xylazin (Illium xyla®, 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® 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±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 μm group. The significant changes in Ca and P concentration are showed in 800μm group. Significant changes concentration also can be seen between control and 800 μm groups. The Ca/P ratio before and after implantation was insignificantly increased in the sham group, and insignificantly decreased in the 450 and 800 μm groups.
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 μm. 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 μm. 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 potently 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 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, osteoconductive 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
[1] Oriňáková R, Oriňák A, Bučková L M, Giretová M, Medvecký L, Labbanczová E, Kupková M, Hrubovčaková M, Koval K 2013 Iron Based Degradeable Foam Structures for Potential Orthopedic Applications Int. J Electrochem Sci 8 12451–12465
[2] Jia P, Xu Y J, Zhang Z L, Li K, Li B, Zhang W, Yang H 2012 Ferric Ion Could Facilitate Osteoclast Differentiation and Bone Resorption through the Production of Reactive Oxygen Species J Orthop Res 30 1843–1852
[3] Ulum M F, Paramitha D, Siallagan S F, Sariningrum A, Maharani A D K, Harjo F A, Nashrulloh M F, Laily R, Rahmadani D, Wulansari R, Maheswari H, Hermawan H, Noviana D 2016 Local and systemic analysis of porous iron implantation in femoral bone of rats 10th World Biomaterials Congress 2970
[4] Wang J and Pantopoulos K 2011 Regulation of cellular iron metabolism Biochem J 434 365–381
[5] Paramitha D, Noviana D, Estuningsih S, Ulum M F, Nasution A K, Hermawan 2015 Proc Int Conf on APF Conf Proceedings 1677
[6] Yosop A H, Daud N M, Nur H, Kadir M R A, Hermawan H 2015 Controlling the degradation kinetics of porous iron by poly(lactic-co-glycolic acid) infiltration for use as temporary medical implants Scientific Reports 5 11194
[7] Ulum M F, Arafat A, Noviana D, Yusop A H, Nasution A K, Abdul K M R, Hermawan H 2014 In vitro and in vivo degradation evaluation of novel iron-bioceramic composites for bone implant applications Mater Sci Eng C Mater Biol Appl 1 336–44
[8] Bose S, Roy M, Bandyopadhyay A 2012 Recent advances in bone tissue engineering scaffolds Trends Biotechnol 30 546-554
[9] Grossman J 2014 Disorder of Fluids and Electrolyte Balance Porth’s Pathophysiology ed 9th ed Grossman S C and Porth C M Chapt 39 1019-1059
[10] Christy L 2014 Structure and Function of the Musculoskeletal System Pathophysiology The Biologic Basis for Disease in adult and children ed 7th, ed K L McCance, S E Huether, V L Brasher, N S Rote (Missouri: elsevier) chapt 43 pp 1515-16
[11] Bhagavan N V and Ha C-E 2015 Essential of Medical Biochemistry 2nd ed (Elsevier: San Diego) pp: 511-516
[12] Schenck P A 2010 Electrolyte Disorders : Ca-P and Mg Textbook of Veterinary Internal Medicine Vol 1, ed Ettinger S J, Feldman E C (Missouri: Elsevier) Chapt 79 Sect 2 pp 308-313
[13] Medeiros D M, Plattner A, Jennings D, and Stoeker B 2002 Bone Morphology, Strength and Density Are Compromised in Iron-Deficient Rats and Exacerbated by Calcium Restriction. J. Nutr 132 3135–3141
[14] Boonrungsiman S, Gentleman E, Carzaniga R,Evans N S, McComb D W, Porter A E, Stevens M M 2012 The role of intracellular calcium phosphate in osteoblast-mediated bone apatite formation PNAS 109 (35) 14170-14175
[15] Park J-W, Suh J-Y,Chung H-J 2008 Effects of calcium ion incorporation on osteoblast gene expression in MC3T3-E1 cells cultured on microstructured titanium surfaces J Biomed Mater Res 86A 117–126
[16] Matassi F, Botti A, Sirleio L, Carulli C, and Innocenti M 2013 Porous metal for orthopedics implants Clin Cases Miner Bone Metab 10 (2) 111-115
[17] Fan X, Roy E, Zhu L, Murphy T C, Ackert-Bicknell C, Hart C M, Rosen C, Nanes M S, Rubin J 2004 Nitric oxide regulates receptor activator of nuclear factor-kappaB ligand and osteoprotegerin expression in bone marrow stromal cells Endocrinology 145 (2) 751-759

[18] Rubin J, Rubin C, Jacobs C R 2006 Molecular pathways mediating mechanical signaling in bone Gene 367 1-16

Acknowledgments
The authors acknowledge the support of Indonesian Ministry of Research, Technology and Higher Education in International research collaboration and scientific publication No 079/SP2H/LT/DPRM/II/ 2016. This work is part of an international research collaboration between Bogor Agricultural University, Indonesia and Laval University, Canada.