

RESEARCH ARTICLE

The Role Of Calcium In *Anadara granosa* Shell Graft To Osteoblast Cells In Bone Healing

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ABSTRACT

Background: Angiogenesis and osteogenesis are pairs that occur in bone healing process. Blood vessels provide oxygen. Bone is constantly being remodelled process where osteoblasts are responsible for bone formation and osteoclasts for its resorption. Osteoblast function in the process of bone formation and an early activity can be seen in reparative bone healing phase. The use of *Anadara granosa* shell graft in which there are more calcium, is able to provide the same inorganic component of bone, and will influence osteoblast in bone healing. **Purpose:** To determine the effect of application *Anadara granosa* shell graft with content the large of calcium on the increased amount of osteoblast in the process of bone healing. **Materials and Methods:** Experimental animals used in this study were 21 male Wistar rats, which were than divided into 3 groups, namely the negative control group K(-), the positive control group K(+), and the treatment group P. In the negative control group without the application of graft, in the positive control group with the application of bovine graft and in the treatment group with the application of graft *Anadara granosa* on the femur rats. **Results:** Based on the result test showed a significant differences between K(-) with K(+), K(+) with P and K(-) with P. **Conclusion:** Calsium in *Anadara granosa* shell graft application effective to increased of osteoblasts in the process of bone healing.

Keywords: *Anadara granosa* shell, calcium, osteoblast, bone healing

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INTRODUCTION

Bone is a special formation of connective tissue composed of microscopic crystals calcium phosphate especially hydroxyapatite in the collagen matrix. Bone damage is a pathological condition the loss of bone structures caused by an increase in the normal resorption, decreased bone formation during a normal resorption

and increased resorption combined with a reduction in the formation tulang.¹ Periodontal disease is a disease caused by a bacterial infection and can lead to periodontal tissue destruction. Factors involved in bone destruction in periodontal disease is bacterial and host. Treatment of bone destruction caused by periodontal disease is done with the purpose of (1) eliminate the periodontal lesion, (2) obtain forms a

network that allows the patient to control plaque, and (3) get the reconstruction of bone and connective tissue to improve support for the tooth. Methods of treatment can be done with full thickness flap surgery by administering a bone graft using bone graft, according to indications.² Graft is a part of tissue taken from one place and transplanted elsewhere, either in the same individual or different. The goal is to repair a defect caused by disease, accident, or anomalies of growth and development in the oral cavity. Bone graft is a choice that is widely used to repair bone damage resulting from periodontal disease. Provision of bone graft material is expected to improve the clinical state of the periodontal bone, it is better when compared to the usual manner without additional cleaning surgical graft material.^{1,3} According to sources, the graft material is divided into four types: an autograft from the same individual, allograft from the same species, xenograft from different species and alloplast from synthetic material.^{4,5}

Biological requirements for bone regeneration. Surgical procedures for ridge augmentation are designed based on biological principles of bone regeneration. First, space-maintenance where new bone formation is needed is achieved by use of grafts. In order for bone formation to occur, grafts need to be osteoconductive acting as a scaffold onto which bone resorption and deposition occurs. Most graft materials allow for their resorption by osteoclasts prior to bone deposition by osteoblasts. Since the turnover rate of soft tissues is higher than that of bone, grafts are used alone when their surfaces have low resorption rates, or in combination with membranes that separate the graft from soft tissues, when their surfaces have

high resorption rates; This approach ensures that soft tissues are prevented from occupying the space where bone formation is intended.^{6,7}

Anadara granosa is one of the valuable economic resource and a source protein can used as a bone graft material. It contain minerals such as calcium, sulfur, aluminum, iron, copper, and iodine. Calcium levels as much as 98 % which is the largest content, because it contains essential minerals calcium for bone growth and teeth on the remineralization process gigi.^{8,9}

Anadara granosa shell graft expected to assist the process of bone formation and help the bone healing process, because *Anadara granosa* has a high calcium content, which can synthesize hydroxyapatite, are most of the bone elements inorganic filler, that are osteoconductive and osteoconductive and as a bone graft scaffold.^{10,11} In the process of bone healing, osteoblasts need a scaffold to reach the area of the defect, with the help of growth hormone that serves to stimulate osteoblasts to migrate to the area of the defect and make osteoblasts proliferate more in the defect area. The increased number of osteoblasts in the defect area will be able to support the healing process tulang.¹² Osteoblast activity appears first on the reparative phase in the process of bone healing. Osteoblasts will be seen in the cortex of the bone, a few millimeters from the area defects.¹³ Initiation of new bone formation and secretion of bone matrix will take place on the 7th day, in which osteoblasts only on the surface of the bone matrix.^{14,15}

Based on the above background, the author is interested in studying the effectiveness application *Anadara granosa* shell graft to increase the

number of osteoblasts in the healing process of bone is seen on the 7th day after such defects.

MATERIALS AND METHODS

The initial step of this study started with 21 male Wistar rats were used divided into three groups, each group using 7 rats. All rats will be adapted and adjusted, as well as experimental animals kept in a cage for one week. Day 8 all animal experiments would be making a defect in the femoral os dextra amounted to 2.5 mm. In K (-) group are not applied to graft on defect, the K (+) group HA graft was applied bovine on defect, and in P group applied Anadara granosa shell graft on defect.

First anesthesia with ketamine and xylazine 0,01mL 0,1mL mixed and injected with a dose of 0,11mL / 100gr BW on os femur dextra intramuscular.¹⁶ After the rats began unconsciously, that will be shaved defect. Then given 10% povidine iodine antiseptic of the area around a defect for five minutes.¹⁷ Next step by using a scalpel made incision, parallel to the femur of mice, along 2 cm in soft tissue then removed using a periosteal elevator on defect. After finding os femur dextra, then created a defect in the form of a half-deep hole about 5 mm using a round bur in a straight handpiece.

Preparation of applications using dappen glass upside down and mix the graft material and blood of rat in dappen glass. The graft material and blood of rat until they are well mixed. Put the powder material graft and the blood of rats in the defect using the excavator which has been made up of solid and close the defect surface. After implantation is done, plug the

membrane tape/guide tissue regeneration (GTR) and do suturing to close the skin and soft tissue on the femur os administration of 0,1cc/ 100gr BW analgesic novalgine needed to control swelling and sakit.^{18,19}

On seven days after the defect and treated proceed with the euthanasia of all animals using cloroform, which then continued with making preparations, namely the removal of tissue os femur dextra. The next process is a bone decalcification, followed by making preparations for HPA and observation to get results.

RESULTS

The results of research in negative control (K-) group, a positive control (K+) group and the treatment group (P) getting in the data :

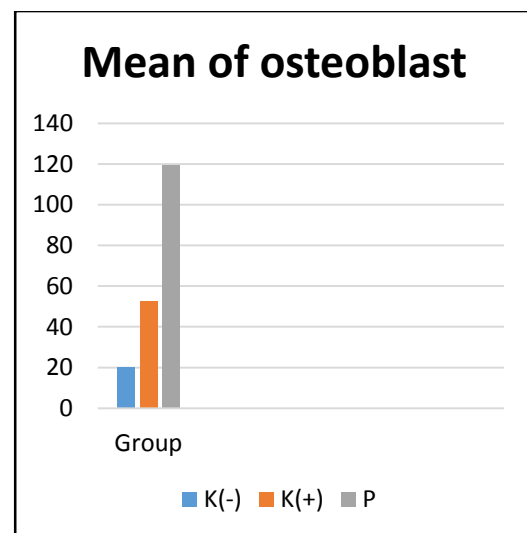


Table 1. The Mean Group

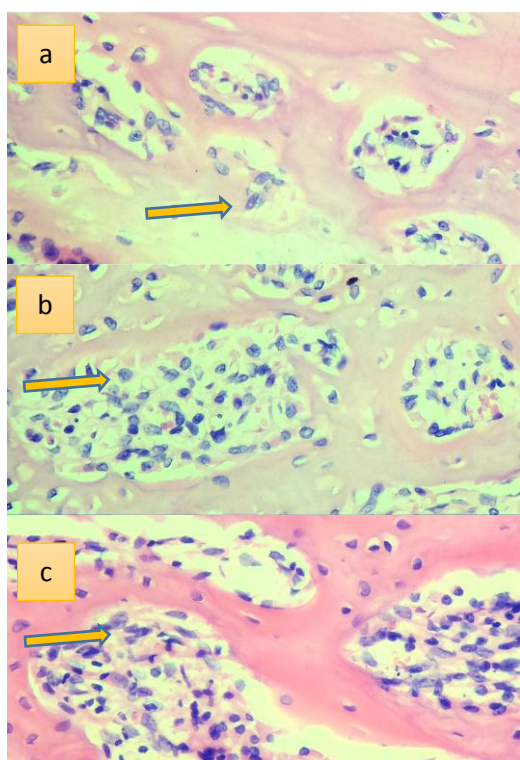


Figure 1. Osteoblast a) K(-)group/no grafts b) K(+) group/bovine grafts c) P group/*Anadara granosa* shell grafts

Figure 1 shows the osteoblast from each treatment group. The treatment (P) group with *Anadara granosa* shell grafts applications, scored highest compared to other treatment groups. While the positive control K(+) group with bovine grafts applications, osteoblast showed scores higher than the negative control K(-) group with no graft.

Table 2. Significant difference test

Groups	K (+)	P
K (-)	.000*	.000*
K (+)		.000*
P		

* Significant

Based on the result test showed a significant differences between K(-) group with K(+) group, K(+)group with P group and K(-)group with P group.

DISCUSSION

The ideal bone graft or bone substitute should provide three essential : osteoconductive matrix, osteoinductive factors and osteogenic cells.²⁰ *Anadara granosa* shell grafts have a osteoconductivity potential, so it can be as a scaffold or matrix. Pure chemically prepared and fabricated bone materials like calcium phosphate, calcium sulfate or coralline carbonate and phosphate grafts, are suitable as osteoconductive implants, since they provide stability to the damaged bone. They act osteoconductivity and to some also osteointegrativity, but they are lacking of any osteoinductive properties. The fabricated scaffold designed to mimic the natural inorganic/organic 3D bone structure must follow the nanofibrous architecture and must be engineered with high porosity in order to allow an ingrowth of cells and an efficient transport of morphogens, cytokines, growth factors and also nutrients, oxygen as well as waste products.^{20,21,23} *Anadara granosa* shell grafts processed with high temperature, in a tissue bank RSUD Dr. Soetomo has contents of 44% calcium carbonate, 23% calcium hydrogen phosphate and 33% calcium hydroxyl. Research has shown that ceramics such as hydroxyapatite and other types of calcium phosphate materials can promote formation of bone like mineral surface leading to increased interface between bone and the implanted material. Hydroxyapatite (HA), which comprises about 70% of bone, is an osteoconductive. Synthetic bone scaffolds have theoretically significant with allogeneous bone grafts because they are not fraught with uncertainties disease transmission or risk of infection or immunogenicity. The prerequisites of synthetic bone

scaffolds, to be effectively used for biomedical repair of bone defects, are to the physiochemical characteristics of the bone and to be associated with the properties to actively attract the bone constructing cells, either the progenitor cells or the functionally active terminally differentiated bone-forming cells.^{21,22} Bone repair materials like calcium phosphate, calcium carbonate, calcium sulfate and coralline carbonate grafts are characterized by good mechanical properties. They can be used as osteoconductive implant materials. They also may show osteointegrative properties.²³

This study was conducted to propose a *Anadara granosa* shell grafts as an alternative source of calcium by the calcination process. Calcination of calcium carbonate, calcium hydrogen phosphate and calcium hydroxyl are a process of producing CaO which is subjecting a substance to the action of heat.

Coralline calcium carbonate is obtained from natural coral. *Anadara granosa* shell likes natural coral with the highest content calcium carbonate (44%), and is composed primarily of aragonite, it is similar to that present in cancellous bone, hence it can potentially initiate new bone deposition rapidly. Calcium phosphate based delivery systems results in accelerated bone healing, because based BMPs delivery systems have tremendous potential for tissue engineering based bone constructs as an alternate to autogenous bone grafting procedures.^{20,22} Calcium phosphates which constitute the major inorganic phase of human hard tissues like the bone and teeth are bioactive and can be rapidly integrated into the human body. There are many bioceramic materials based on calcium such as hydroxyapatite (HA), tricalcium

phosphate (TCP), calcium aluminate (CA) and dicalcium phosphate dehydrate which have been used as fillers to repair bone defects.²⁴ Depending on the calcium phosphate reactivity osteoblast function can be affected, in their proliferation, their differentiation and their maturation phenotypes.²⁵ Calcium hydroxide in contact with bloody tissue generates a necrobiological zone. A calcite membrane develops, which reduces a deep acid burning, preventing a deeper penetration. This is an aqueous CaOH solution, which has a necrobiological effect and chance that the oily CaOH suspension had a beneficial bone regeneration effect.²⁶ So the initial step of bone remodelling is removal of old bone to provide places for new bone formation. Osteoblast play an essential role in osteogenesis, and are also responsible for differentiation and maturation of osteoclast. Bone is constantly being remodelled process where osteoblasts are responsible for bone formation and osteoclasts for its resorption. Osteoblast function in the process of bone formation and an early activity can be seen in reparative bone healing phase.

CONCLUSION

Anadara granosa shell graft has contents higher calcium in the form of calcium carbonate, calcium hydrogen phosphate and calcium hydroxyl. Calcium in *Anadara granosa* shell graft application effective to increased of osteoblasts in the process of bone healing.

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