Jurnal Riset Veteriner Indonesia
Journal of the Indonesian Veterinary Research
P-ISSN: 2614-0187, E-ISSN:2615-2835
Nationally Accredited Journal, Decree No. 36/E/KPT/2019.
Volume 8 No. 2 (July 2024), pp. 33-39
journal.unhas.ac.id/index.php/jrvi/

Radiographic Image of Fracture Healing with Bone Graft Equine Hydroxyapatite (BGEH) Implantation in Rabbits

Muhammad Zulfadillah Sinusi[a]*, Dian Fatmawati[a], Rini Amriani[a], Muhammad Dirga Gifardi[a], Ulfah Desianti Liding[a]

[a]Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Makassar 90245

*corresponding author: muhammadzulfadillahsinusi@unhas.ac.id

Abstract

Fractures refer to the obstruction of bone continuity due to trauma, stress, or pathological abnormalities. *Bone graft* is a material that have function to accelerate fracture healing through implantation. Horses are animal that commonly utilized by society such as sport animal, their meat for consumption and leather for textile industries, but horse bones are not beneficial and resulting in waste. The calcium and phosphorus content in horse bones are quite high, making it a potential material for bone grafting due to their more compact and hard structure. Making a bone graft from horse bones are called Bone Graft Equine Hydroxyapatites (BGEH) and evaluate its effectiveness for fracture healing. The sample in this study was used eight rabbits and divided into two groups. The first group was the control group by doing defect in femur rabbit without any implantation, while the second group was treatment group with horse bone graft (BGEH) implantation. The research procedure consisted of two stages: the creation of horse bone grafts and testing on experimental animal. In two weeks, the control group showed the formation of a soft callus characterized by low density at the edges of the fragments. In contrast, the treatment group with BGEH showed the formation of a thin callus distributed evenly across the entire bone defect area provided by high opacity in whole defect of bone. At six weeks, the control group showed that the bone area was not yet completely covered but had reduced in defect diameter, whereas the treatment group had achieved complete closure due to the formation of a more organized hard callus and new bone formation. The results indicated that the use of horse bone grafts could be an alternative for fracture management as it could accelerate bone healing

Keywords: *BGEH, bone graft, equine, fracture, hydroxyapatite*

Introduction

Bone graft is a material that functions to accelerate the bone healing process because it can provide mechanical support and signals to body molecules to maximize tissue growth. The main function of bone graft is to provide cells for osteogenesis, bone

production through osteoinduction and act as a mechanical support or osteoconduction (Oryan et al., 2014; Markel, 2020; Habibah et al., 2020). According to Herkowizt et al. (2004), bone grafts should ideally have bioabsorbable properties, be easy to use clinically, have an effective structure so that they can be adjusted to all clinical situations, do not transmit disease, and are inexpensive. Bone graft materials are divided into autografts, allografts, xenografts, and alloplasts (Greenwald et al, 2008). One of the most commonly used is the type of hydroxyapatite (HA) ceramic. Ceramic materials can also be obtained from the synthesis of organic materials, especially from animal bones (Markel, 2019).

Hydroxyapatite (HA) is a component of inorganic bone material in high quantities so it is widely used as an implant material because it has similarities with the composition of natural bone. In terms of chemical structure, HA has a stoichiometric formula of $Ca_{10}(PO_4)_6(OH_2)$ and a molar ratio of Ca/P=1.67, this is the material that is most similar to the inorganic part of bones and teeth (Boutinguiza et al., 2012). Good bone graft material must have good biocompatibility properties, do not cause reactions, are non-toxic, do not cause infection, are easily adaptable, and can stimulate new attachments (Maulidah et al., 2018), while Aisyah et al. (2012) added that HA has perfect biocompatibility properties and has good stability because it will not be damaged under physiological conditions when implanted in bones. HA material is mostly developed from animal bones such as pig bones, cow bones (Ayatollahi et al., 2015) and horse bones (Zecha et al., 2011). This study attempts to develop HA from horse bone waste using the wet method and high temperature heating. Synthesis using the wet method has the advantage of being able to produce HA yields with a high level of purity and cheaper manufacturing costs compared to other methods (Yuliana et al., 2017; Darwis and Warastuti, 2008). High temperature heating is known to affect the results of HA synthesis. Heating temperatures above 1000oC can produce purer HA with good bioactive properties (Jang et al., 2014; Kim et al., 2004).

Horse bones contain high calcium and phosphorus compositions so they have the potential to be developed as bone grafts. Research conducted by (Cooper et al., 2001) showed a calcium composition of around 42% and phosphorus of around 14% in the ribs and metacarpals. Calcium plays a role in controlling chemotaxis, proliferation and differentiation of osteogenesis cells and accelerating angiogenesis (Dvorak et al, 2004). Wathi et al. (2014) added that the levels of calcium phosphate in HA have the same bioactive, biocompatible and osteoconductive properties as natural bone minerals.

Indicators of successful treatment in fracture cases are by looking at radiographic images to observe callus formation, fusion between fragments and the degree of bone tissue density. This study aims to see radiographic changes in rabbit femur bones implanted with HA from horse bones. The benefits of this study are that the HA that has been made is expected to be an environmentally friendly alternative bone graft and can be used in handling fracture cases.

Meterials and Methods

Materials

This research was conducted in two activities: (1) The preparation of bone graft material from horse bone waste which obtained in slaughter horses and (2) Implantation of bone grafts on experimental animals, specifically rabbits.

Preparation of Horse Bone Graft

Knife, saw, furnace machine (@Controlab), electronic blender, automatic stirrer, pestle, mortar, digital scale (@Mettler Toledo), and measuring glass. Horse bone waste, aquabidest, phosphate, and H_2O_2.

Implantation on Experimental Animals

Shaving tools, stethoscope, thermometer, bone drill kit, orthopaedic set, minor surgical set, syringe, scale, gloves, ruler. Eight 7-month-old male New Zealand White rabbits, ketamine, xylazine, atropine sulphate, tampon, catgut chromic 3/0 (®Gea), silk 3/0 (®OneMed), anticoagulant tubes, povidone iodine, antibiotics, and EDTA tubes.

Research Procedure

This research has received approval from the research ethics committee with number *69/UN4.6.4.5.31/ PP36/ 2023.*

Preparation of Horse Bone Graft Bone Preparation

The demineralization process involved cleaning horse bone waste from remaining tissue. The bones were then dried by sun exposure for 1-5 days, cleaned, and cut into 0.5 x 0.5 cm pieces.

Deproteinization

The bones were then soaked in *hydrogen peroxyde* H2O2 solution to remove remaining fats and proteins. This process was repeated until the bones turned clean white. The H2O2 solution also acted as an oxidizer to oxidize surface contaminants and kill bacteria adhering to the bones.

Sintering and Synthesis

The deproteinized horse bones were then sintered using a furnace machine at 1000°C for 2 hours. Subsequently, the bones were crushed and sifted to obtain calcite powder that obtain *calcium carbonate* (CaCO3). The calcite powder was then synthesized by mixing with phosphate at 70-80°C, followed by a second sintering at 1000°C for 2 hours. This heating process produced hydroxyapatite powder, referred to as *Bone Graft Equine Hydroxyapatite* (BGEH). The bone graft was then placed and stored in tubes until it was applied to the test animals.

Implantation on Experimental Animals

Preparation of Animals

Eight male rabbits were adapted to food and environment for one week before surgery. The rabbits were fasted for 8-10 hours before the surgery. Anesthesia injection was then performed with a combination of ketamine and xylazine, each at doses of 10 ml/kg body weight and 3 mg/kg body weight, respectively, via intramuscular injection.

Implantation Surgery

Surgery involved making an incision on the skin in the lateral femur area, approximately 3 cm long. The musculus vastus lateralis and musculus biceps femoris were retracted to expose the femur bone. The diaphysis of the femur was drilled with a bone drill with a diameter of 0.3 mm until it penetrated the medullary cavity. The drilling technique was carried out by penetrating perpendicularly to the periosteum. During drilling, the bone surface was irrigated with physiological NaCl to prevent heat damage. The rabbits were divided into 2 groups, with each group consisting of 4 rabbits. Group I was the control group that did not receive any implant material in the drilled area of the femur diaphysis. Group II was the treatment group that received BGEH implant material in the drilled area of the femur diaphysis. The dosage of the bone graft was adjusted according to the size of the bone defect created.

Sample Collection

For two weeks post-surgery, radiographic X-ray images of the rabbits in each group were taken, focusing on the femur bone where the implantation had been performed. The same procedure was conducted at six weeks post-surgery for each treatment group.

Results and Discussion

The results The results of radiographic examinations at each evaluation period showed varying outcomes. The two-weeks radiographic observations revealed a round-shaped bone defect (Figure 1). It was due to the loss of some bone fragments caused by drilling during the implantation process and . The control group showed a round radiolucent area because it was not filled with an implant, while the treatment group appeared radiopaque with high tissue density due to the presence of the BGEH implant.

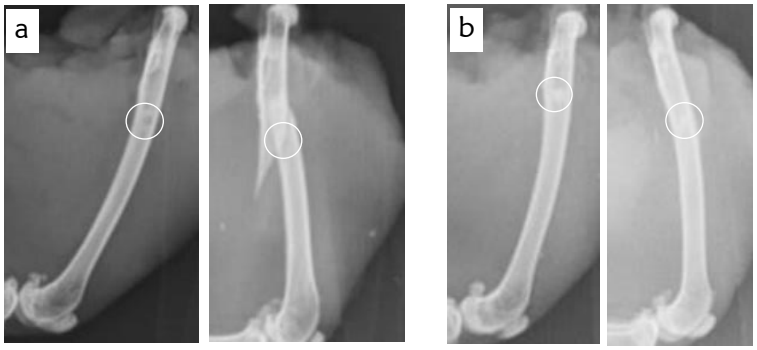

Figure 1. Radiographic examination results of the right rabbit femur at two weeks. (a) Control group without implant. (b) Group with BGEH implant. The white circle indicates the area of the bone defect.

The increase in density in the defect area of the treatment group indicates that the BGEH implant material can act as a mediator for fracture healing. Hydroxyapatite bone grafts have osteoconductive properties that facilitate the formation of connective tissue and trabeculae throughout the bone defect area, resulting in the appearance of smooth callus growth (Markel, 2020). The presence of callus in radiographic examinations indicates a good bone healing process. Normal callus formation occurs over 1 to 3 weeks, beginning with irregular mineralization, trabecula formation, and then transitioning to more organized callus. Increased opacity is observed during

mineralization events due to the injury, including mineralization of hematoma from fractures, bone fragments, or cancellous graft material. Furthermore, at ten days post-fracture, the fracture line becomes less distinct as the ends of the bone fragments undergo resorption. The fracture gap decreases in size, and some free bone fragments show reduced opacity from day five to twenty post-fracture (Henry, 2013).

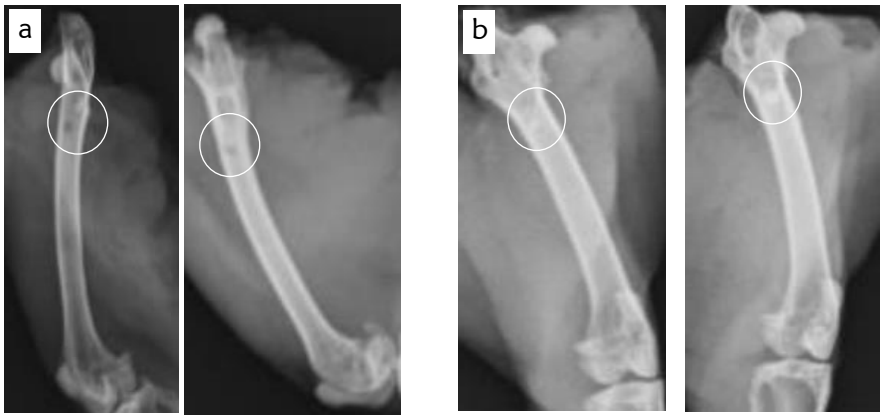

Figure 2. Radiographic examination results of the right rabbit femur at six weeks. (a) Control group without implant. (b) Treatment group with BGEH implant. The area of bone defect is indicated by the white circle.

Radiographic examination at six weeks showed that the treatment group with the BGEH implant material had achieved complete closure due to the formation of a more organized hard callus and new bone formation (Figure 2). In contrast, the control group exhibited an area where the bone was not yet fully covered but had reduced in diameter. The treatment group showed reduced opacity at the edges of the bone defect, indicating that the BGEH bone graft had started to be absorbed by the body (Figure 2). This finding aligns with the view expressed by Herkowizt et al. (2004) that an ideal bone graft should have bioabsorbable properties, meaning it should be absorbed by the body, easy to use clinically, and have an effective structure adaptable to various clinical situations. Huang et al. (2009) added that bone grafts should also allow for faster mineralization compared to normal bone repair and support the formation of new bone.

Fracture healing with stable internal fixation will lead to primary healing, where osteogenesis cells directly respond to restore bone continuity. This results in faster healing time by minimizing inflammation and excessive callus formation. Bone graft implantation also plays a role in primary healing as it can mediate osteogenesis cells to accelerate bone healing. The horse bone waste-derived bone graft (BGEH) created in this study has shown potential for osteoinductive and osteoconductive properties, as evidenced by better bone healing in six weeks compared to the non-implant group. Oryan et al. (2012), Markel (2020), and Habibah et al., (2020) noted that bone grafts should possess osteoinductive properties for bone cell production and conductiveness to support mechanical processes. This study demonstrates that hydroxyapatite biomaterial bone grafts made from horse bones have potential as an alternative graft to accelerate fracture healing while being environmentally friendly. This research was

still basic and need more measurement for validation. For further research are needed to see the effectiveness of bone grafts from horse bones, such as testing graft materials, pathological and toxicity examination for animals.

Conclusion

The result from this reaseach showed that Bone Graft Equine Hydroxyapatite (BGEH) can be mediated bone formation by acclelerated callus formation. These results can be a potential reason that horse bone can be another alternative in making bone graft biomaterials.

Acknowledgments

The authors would like to thank PDPU school of Hasanuddin University for funding this research. The author states there is no conflict of interest with the parties concerned in this research.

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