ORIGINAL ARTICLE

Effect of Implantation Ti-6Al-4V ELI in femoral bone defect regeneration of Sprague Dawley rat

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ABSTRACT

Ti-6Al-4V ELI is one of the most commonly used dental implant restore function. The solution treatment temperature variation can significantly increase the strength, but it is not yet known the effect of these temperature variations on the alloy's biocompatibility properties. Twelve female Sprague Dawley rats were divided into six groups as follows: the treated group, the control group, and the defect group without implant material. In the treated group, the femur bone defect was implanted with as-cast Ti-6Al-4V ELI, 850°C, 950°C, and 1050°C heat-treated Ti-6Al-4V ELI implant material. The rats were euthanized after 30 days postimplantation and evaluated histologically. The results show that the histological scoring of the specimen for femur defect without implant material is 2 (fibrous union and fibrocartilaginous), score with implant as-cast is 2.5, the sample with 850°C heat treatment material is 2.5, 950°C is 2.5, and the temperature at 1050°C is 2.5. The score of 2.5 is between score 2 and score 3: hemorrhage, fibrous union, fibrocartilaginous microhemorrhage, and mineralized cartilage union. In conclusion, there is no effect of different heat treatment temperatures for Ti-6Al-4V ELI implant material in rat bone regeneration's maturation level.

Key words: Bone regeneration, heat treatment, Ti-6Al-4V ELI, titanium implant

INTRODUCTION

Dental implants need to restore the function of mastication in partial or full dentures. These are recognized as a predictable treatment modality with a high clinical success rate so that this field develops rapidly and dynamically.[1,2] The American Dental Association outlines some acceptance guidelines for dental implants, including the following: evaluation of physical properties that ensure sufficient strength; demonstration of the ease of fabrication and sterilization potential without material degradation; safety and biocompatibility evaluation, including cytotoxicity testing and tissue interface characteristics; and freedom from defects and at least two independent longitudinal prospective clinical studies demonstrating efficacy.[3]

Ti-6Al-4V alloys have been widely used for implant materials due to its good biocompatibility, excellent mechanical properties, and good corrosion resistance.[4] Based on the high demand for medical devices, especially implants in the country and most of them are still filled with imported products, the Center for Material Technology – BPPT Indonesia conducts research and development of Ti-6Al-4V ELI alloy material for medical implant applications. A

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previous study shows that heat treatment resulted in better mechanical properties of castings.[5]

The price of titanium alloy is quite high, and its production also requires high costs. Therefore, net-shape or near-net-shape technology gets much attention. One type of net-shape or near-net-shape technology is the casting process.[6] To reduce the production costs of implant manufacturing with investment casting, a method of integration of Ti-6Al-4V ELI has been carried out using local raw materials. Increasing the strength of as-cast material is done by solution treatment and the aging process. The solution treatment temperature variation can significantly increase the strength, but it is not yet known the effect of these temperature variations on the alloy's biocompatibility properties. Material production is carried out with local raw materials in vacuum kitchens and investment casting technology. Increasing the hardness of the combined results of Ti-6Al-4V ELI is carried out with a solution to heat treatment and aging to improve microstructure properties. Our previous research results show that the solution treatment temperature variation affects the ELI Ti-6Al-4V alloy's mechanical strength and reduces its corrosion rate.[6]

MATERIALS AND METHODS

This research was conducted according to established animal welfare guidelines and followed protocols approved by the Ethical Committee of Health Research, Faculty of Medicine, Universitas Indonesia, Indonesia (No. 665/UN2.F1/ETIK/VI/2018). Twelve adult female Sprague Dawley rats were used in this experiment. These rats received *ad libitum* food and drink. These rats were randomly divided into six groups: controls, bone defect controls, implanted with Ti-6Al-4V ELI as-cast alloy, implanted with 850°C heat-treated Ti-6Al-4V ELI, implanted with 950°C heat-treated Ti-6Al-4V ELI, and implanted with 1050°C heat-treated Ti-6Al-4V ELI.

Surgical procedure

The anesthesia consists of 1.5 mL ketamine, and 0.5 mL xylazine was injected by intramuscular injection. The surgical area was sterilized, and the fur was removed until the femur visible and rinse out with povidone-iodine. An incision was made in the femoral condyle area, followed by the soft-tissue's open. A bone defect of 3 mm depth and 1 mm in diameter was made at the femur using bone diamond bur. The defect of treated rats received implant material. The wound was closed using catgut with an interrupted suture technique [Figure 1].

After 4 weeks, the rats were sacrificed, and their femur was taken. The femur was stored in 10% formalin solution (pH 7.2). Then, the femur was immersed in the decalcifying solution for 2 weeks until the bone softened. After that, the femur was neutralized with Na_2SO_4 for 24 h before being immersed in a 70% alcohol solution for 24 h and being cut. The sample was immersed in 70%, 80%, 95%, and 100% for 1 h each before the embedding process. The sample then embedded in the paraffin block and cut for microscopic evaluation. The hematoxylin and eosin were evaluated by a microscope at ×20 magnification. Salkeld Histological Scoring was used in order to determine the bone maturation.[7]

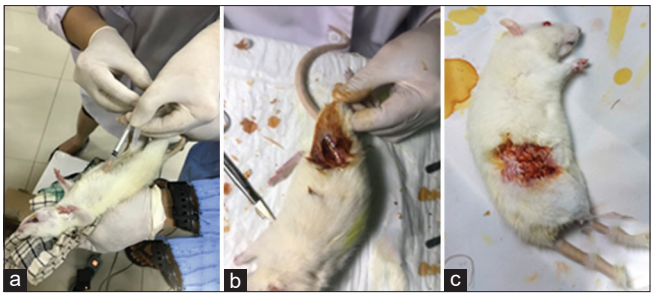

Figure 1: Surgical procedure. (a) Anesthesia; (b) Sample was inserted inside the defect; (c) Wound was closed using catgut

RESULTS

The histological evaluation was carried out after 30 days of implantation. We decided to finish the experiment earlier than other previous studies to prevent the animals' infection but still can determine the bone regeneration process by comparing the histological score to the control group. The histological view showed pale purple and dark purple appearances in one of the rat with an implant and two in control group, indicated mineralized bone and osteocytes in these area. In sample 1 and two defect group histological view, bone regeneration just reached fibrous union and fibrocartilage formation. Some lumps of blood were seen in the sample 2 defect group histological view [Figure 2]. The modified Salkeld histological score for this group is 2. A similar result to sample 2 defect group was found in sample 2 as-cast Ti-6Al-4V ELI, sample 2 850°C heat-treated Ti-6Al-4V ELI, sample 1,2 950°C heat-treated Ti-6Al-4V ELI, and sample 2 1050°C heat-treated Ti-6Al-4V ELI. Figure 2 shows, the histological analysis for sample 1 as-cast Ti-6Al-4V ELI, and sample 1 850°C heat-treated Ti-6Al-4V ELI. One of 1050°C heat-treated Ti-6Al-4V ELI sample histological views shows mineralized cartilage and more mature bone formation. The modified Salkeld histological score for this sample is 3 [Figure 3].

The result of the bone healing and regeneration of the animal is shown in Table 1.

DISCUSSION

This study described the evaluation of implantation implant material Ti-6Al-4V ELI with heat treatment 850°C, 950°C, and 1050°C in the femoral bone of Sprague Dawley rats using modified Salkeld histological scoring. There are seen

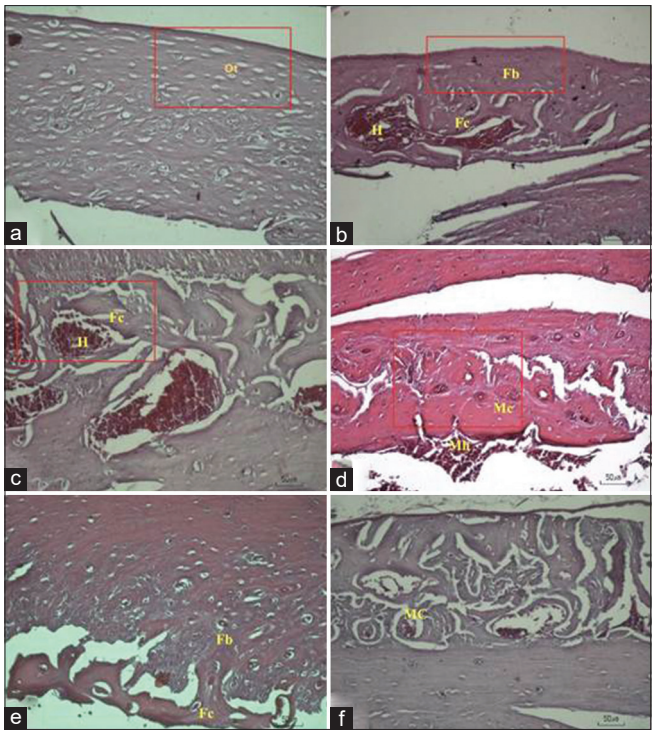

Figure 2: Histological observation at ×20 magnification of sample 1: (a) control; (b) defect; (c) as-cast Ti-6Al-4V ELI; (d) 850°C heat-treated Ti-6Al-4V ELI; (e) 950°C heat-treated Ti-6Al-4V ELI; 1050°C heat-treated Ti-6Al-4V ELI. Bone regeneration component such as fibrous tissue (Fb); fibrocartilage (Fc); (f) mineralized cartilage (Mc), and another component such as hemorrhage (H) or microhemorrhage (Mh) can be seen

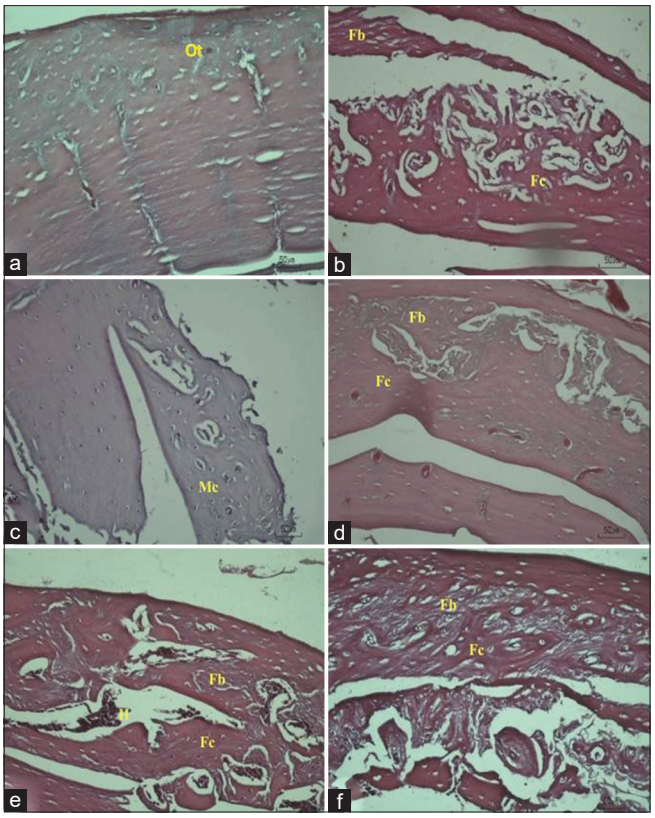

Figure 3: Histological observation at ×20 magnification of sample 2: (a) control; (b) defect; (c) as-cast Ti-6Al-4V ELI; (d) 850°C heat-treated Ti-6Al-4V ELI; (e) 950°C heat-treated Ti-6Al-4V ELI; 1050°C heat-treated Ti-6Al-4V ELI. (f) Bone regeneration component such as fibrous tissue (Fb); fibrocartilage (Fc); mineralized cartilage (Mc), and another component such as hemorrhage (H) or microhemorrhage (Mh) can be seen

osteocytes, which are mature bone cells that maintain their daily metabolism, such as cell exchange of nutrients.[8]

The histological analysis of the treatment group showed that bone formation reached cartilage mineralization, similar to the research conducted by Berglundh *et al.* and Abrahamsson *et al.* This research evaluated the process of bone formation and osseointegration after implant material made of Cp titanium implantation in mandibular dogs, as referred to in the 4th week after the implantation, the large volume of the woven bone had been replaced by lamellar bone that was present in around implant surface and preexisting bone. There are trabeculae of woven bone around the primary bone marrow in the middle part of the defect area and a thin of mineralized tissue.[9-11]

The histological analysis of the treatment group based on the modified Salkeld histological scoring showed a different score between sample 1 and sample 2. Many factors influence the process of bone healing.[10,11] Heredity, gender, and age of animals are known to affect fracture healing. Female rats showed delayed bone healing compared with male rats, as did older animals compared to younger ages.[12] Sprague Dawley is rat colonies that are maintained by random breeding. Random mating means that the animals for breeding are chosen without regard to their relationships. So that these colonies have genetics that is more or less heterogeneous.[13]

This study shows that different heat treatment temperature does not affect the level of bone regeneration and maturation. The research conducted by Yi *et al.* sacrificed the experimental group at the 2 and 6 weeks after titanium implantation in the femur bone of Sprague Dawley rats to evaluate the initial healing status and the osseointegration process. The results showed that after 2 weeks of implant implantation, there is already a process of bone formation. After 6 weeks of implant placement, the procedure of implant processing is continual with more new bone formation and more robust integration of bone into implants' surface.[14]

Research conducted by Berglundh *et al.* and Abrahamsson *et al.* observed that there were gradually more mineralized tissues in the observation period of 6 weeks after implant placement.[9,10] In the observation period of 8 weeks and 12 weeks after implant placement, signs of remodeling can be seen in the tissue bone. This hard tissue was surrounded

Table 1: Comparison of each sample in the bone regeneration process and its modified Salkeld score

Sample	Microscopic evaluation in the defect area	Modified Salkeld histological scoring	Average score
Control-1	No abnormalities	-	Non
Control-2	No abnormalities	-	
Defect without implant-1	Fibrous union and fibrocartilaginous	2	2
Defect without implant-2	Hemorrhage, fibrous union, and fibrocartilaginous	2	
Implant as-cast-1	Microhemorrhage and mineralized cartilage union	3	2.5
Implant as-cast-2	Hemorrhage, fibrous union, and fibrocartilaginous	2	
Implant 850-1	Microhemorrhage and mineralized cartilage union	3	2.5
Implant 850-2	Hemorrhage, fibrous union, and fibrocartilaginous	2	
Implant 950-1	Hemorrhage, fibrous union, and fibrocartilaginous	2	2
Implant 950-2	Hemorrhage, fibrous union, and fibrocartilaginous	2	
Implant 1050-1	Microhemorrhage and mineralized cartilage union	3	2
Implant 1050-2	Hemorrhage, fibrous union, and fibrocartilaginous	2	

by a bone marrow containing adipocytes, vessels, collagen fibers, and some mononuclear leukocytes. This process indicates that it takes more than 4 weeks to observe until new bones' formation is perfect.[10,11] This study's limitation is the number of animals for the test and the experiment for just 4 weeks after titanium implantation in the femur bone. However, some studies reported that in 4 weeks after the bone defect experiments, the calcium deposition, bone density, and the increase in new bone volume were observed.[15-17]

CONCLUSION

Bone regeneration can be found in the surrounding implantation site of the heat-treated Ti-6Al-4V ELI. This condition was indicated by fibrous tissue formation, fibrocartilage, and mineralized cartilage in the surrounding implantation site. Therefore, heat-treated Ti-6Al-4V ELI has the same biocompatibility properties as normal bone regeneration.

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Conflicts of interest

There are no conflicts of interest.

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