Preliminary Study of Scandium-46 Labeled Composite (Hydroxyapatite - Chitosan - Collagen) Biodistribution in Rats Bone Implant Model

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Abstract. Research related to bone fractures is currently focused on accelerating healing time with fewer complications. In some cases related to biological and mechanical factors that interfere with the healing process, it will take a longer time to heal. Hydroxyapatite (HA) is a promising material used as a scaffold for bone implants with various advantages. The in vivo biodistribution of Sc-46 labeled composite (HA-Chitosan-Collagen) remains unclear. In this research, Scandium-46 was prepared as a non-carrier free radioisotope solution by irradiating 100 mg Sc2O3 target in TRIGA 2000 Reactor Bandung. In vivo experiment was performed on Sprague Dawley rats weighing approximately 250 g. Rats bone implant model was divided into two groups with n = 3 per time point. The Sc-46 labeled composite (HA-Chitosan-Collagen) have implanted to rats femur 10 mg with radioactivity 10 μCi. Rats were euthanized using accepted protocol and all organs were counted for radioactivity using Wipe Test Counter with NaI(Tl) detector. The percent of radioactivity measured per gram of tissue weight (%ID/g). Biodistribution results showed that Sc-46 labeled composite (HA-Chitosan-Collagen) using the bone-implant method were significantly different compared with the normal bone for 1, 3, and 8 days of the time interval with p<0.05. These observations suggest that Composite (HA-Chitosan-Collagen) is available for bone implants and remains at the implant site until bone recovery.

1. Introduction
Research on bone fracture cases is oriented towards reducing healing time with fewer complications. Normally, a bone fracture will heal within 6-8 weeks. But in some cases, longer healing time would be taken associated with biological and mechanical factors that interfere with the healing process [1]. The number of bone fracture cases are high worldwide. Based on the research conducted by Kwok et al, the data from several countries in Asia (Hongkong, Thailand, Indonesia, and Japan) showed that the highest prevalence of vertebral fracture in men and women was found in Japan. Mostly it was suffered by the elderly person (above 65 years) [2].
There has been increased interest in scaffold-based strategies for the bone-implant as represented by the exponential rise in the number of research over the past decade [3]. Human bone consists of non-stoichiometric carbonated hydroxyapatite (HA) with trace amounts of other ions. Hydroxyapatite becomes promising material due to its non-toxicity and biocompatibility as a pharmaceutical or biological agent's delivery system, especially in orthopedics and dentistry for bone tissue regeneration [4]. Hydroxyapatite-chitosan-collagen composite scaffolding has potential as a maxillofacial reconstruction material [5].

However, the development of implant material and initial research for clinical use should be carried out on animal models. This is important because clinicians and pathologists understand the basic structure of bone even though there is no animal model that is physiologically, biomechanically and hormonally similar to humans [6]. Rodents are one of the most commonly used animal models considered useful in preclinical studies for testing biomaterials as bone substitutes, and regarded as one of the first choice models for in vivo test for the regeneration of the bone tissue [7].

This research was conducted to observe the biodistribution and stability of HA-Chitosan-Collagen composite on rat’s bone using radioisotope scandium-46. Our hypothesis is that radiolabeled composite (HA-Chitosan-Collagen) will remain in the implant site until the bone has recovered.

2. Materials and methods

2.1. Preparation of Sc-46 Labeled (HA-Chitosan-Collagen) Composite

The hydroxyapatite-chitosan-collagen composite was synthesized using the method described by Charlena et al [8]. Optimum conditions for hydroxyapatite synthesis of scandium-46 based were determined by three controlled variables i.e pH, the molar ratio of Sc-46: hydroxyapatite and mixing time. Sc-46 was prepared as non-carrier free radioisotope solution by irradiating 100 mg of Sc₂O₃ in TRIGA 2000 Reactor Bandung at 5 x 10⁵ n.s.1.cm⁻² neutron flux for 3 days. Irradiated Sc₂O₃ was dissolved in 3M HCl then the pH of the solution was adjusted to 4 by adding NaOH and phosphate buffer. The labeling process of HA composite was carried out by mixing 10 mg of HA composite solids with a volume of Sc-46 radioisotope solution (1:1 molar ratio of Sc-46: HA) for 5 minutes in 2000 rpm vortex mixer. Labeled HA composite was separated from its supernatant by centrifugation. Sc-46 content in labeled HA and supernatant was analyzed by Wipe Test Counter with NaI(Tl) detector in the Sc-46 gamma energy range.

2.2. Bone Implant Model

The animal model of bone-implant was approved by the Ethics Committee for The Care and Use of Laboratory Animal, National Nuclear Energy Agency with protocol number: 002/KEPHP-BATAN/III/2019. Rats were under anesthetized conditions using ketamine and xylazine. The right leg area was shaved for surgery procedure. Implantation was applied in the bone femur area and the implant material is inserted into the bone that has been prepared using a micromotor bone drill with the same depth and size. The composite (HA-Chitosan-Collagen) labeled Sc-46 material is inserted into the femur and covered using Ethicon bone wax.

2.3. Biodistribution Study

The experiment was performed on Sprague Dawley rats weighing approximately 250 g. Rats bone implant model were divided into two groups, control group and treated group (1, 4, and 8 days) with n = 3 per time point. The Composite (HA-Chitosan-Collagen) labeled Sc-46 was implanted to rats femur 10 mg with radioactivity 10 µCi. Then after the time interval, rats were euthanized using accepted protocol and the tissue of interest was collected. All tissue and blood were weighed using an analytical scale and counted for radioactivity using Wipe Test Counter with NaI(Tl) detector. The percent of radioactivity per gram of tissue weight (%ID/g) was determined using the following formula:
\[
%ID/g = \frac{\text{count per gram organ}}{\text{count dose given}} \times 100\%
\]  
(1)

**Data Analysis:** All values were presented as mean ± standard error of the mean. Statistical analysis was performed using One Way ANOVA. Values of \( p < 0.05 \) were considered being statistically significant.

3. **Result and Discussion**

**Bone implant models**

Bone Implant material was performed on the left femur bone as an implant model, and right femur bone for comparison. All tools used for surgical procedures are sterile and clean to avoid contamination. The research procedure for the implant process did not cause a secondary infection during observation.

![Figure 1. Bone implant method using Sc-46 labeled composite HA-Chitosan-Collagen. (a), Rats legs hair was shaved under anesthesia condition. (b), The surgical procedure continued with a small incision in the femur area with avoiding penetrated the large vein. (c), The femur bone in the diaphysis area was drilled using a micromotor bone drill, the drill was smooth and avoided to penetrate bone marrow. (d), Using forceps tweezers the muscle was opened and (e), Sc-46 labeled composite HA-Chitosan-Collagen were inserted into the bone and covered with bone wax. (f), The surgical procedure was ended with surgical suture with chromic catgut 4.0 for muscle and silk gut 4.0 for skin. Rats were recovered 3 hours post-surgery and there is no abnormal change.](image-url)
As shown in Figure 1, the Sc-46 labeled composite HA-Chitosan-Collagen implant procedure in femur has good results. It should be considered to increase the survival rate of the post-surgery rats model concerning the dose of anesthesia and the procedure of incising the area of the femur because it’s close to the blood vessels as shown in Figure 1b. Postoperative care is also optimized by maintaining body temperature from rats and to minimize the presence of secondary infections in the surgical area.

3.1. Biodistribution Study
Biodistribution study of Sc-46 labeled HA-Chitosan-Collagen composite was carried out at 3 intervals time of 1, 4, and 8 days. The results showed that the accumulation of Sc-46 labeled composite in the implant-bone compared to the non-implant bone showed a significant difference with $p<0.05$ in the accumulation of 1, 4, and 8 days, data were shown in Figure 2.

Accumulation focused on the physiological uptake in the implanted bone. Based on data in Figure 3 shown that accumulation at 1 day (51.03±6.49) %ID/g, 2 days (53.02±4.55) %ID/g, and 8 days (53.68±1.92) %ID/g post-implants. Based on the result, there were no significant differences ($p<0.05$) in bone-implant accumulation. The accumulation mechanism of the Sc-46 labeled composite at the time after implant will bind to the mineral layer on the bone, especially to the bone structure. The bone structure generally consists of mineral components that are non-stoichiometric carbonated hydroxyapatite (HA) with trace amounts of other ions ($\text{CO}_3^{2-}$, $\text{Na}^+$, $\text{Mg}^{2+}$, $\text{Fe}^{2+}$, $\text{F}^-$, $\text{Zn}^{2+}$, and silicate) [9]. The result in bone uptake was related to the ability of Sc-46 labeled composites binding with bone structure and lack of vascularization systems around the bones so that the material can’t be carried to other tissues or organs.

Evaluation in the circulatory system to see the physiological uptake of Sc-46 labeled composite was observed on blood, heart and lungs show low physiological uptake. The result respectively in blood 1 days (0.04±0.01) %ID/g, 4 days (0.19±0.17) %ID/g and 8 days (0.06 ± 0.00) %ID/g, hearth 1 days (0.04±0.00) %ID/g, 4 days (0.07±0.02) %ID/g and 8 days (0.06±0.00) %ID/g and lungs 1 days (0.04±0.00) %ID/g, 4 days (0.09±0.50) %ID/g and 8 days (0.06±0.00) %ID/g. The data are shown in the circulation system similar to background result by the wipe counter, it’s known that the possibility of an Sc-46 labeled composite distributed to the circulating organ is not found.
The digestive system in the intestine, liver, stomach and spleen, the excretory system in the kidneys and bladder also showed low physiological uptake. The use of animal models in bone material research is very important to know osteoconductivity, biocompatibility, mechanical properties, degradation, and interaction with host tissues of the material used [7]. The use of rats as a model for bone implants in research is still necessary and relevant. Until now there has not been a single animal model that represents all aspects of human disease, tissue architecture and healing and aging processes [10].

Likely the other research using -Sc, application of -Sc -Bleomycin and -Sc-NOTA as candidates for radiotherapy and radiodiagnostic agents [11][12]. The advantages of scandium-46 are longer half-life, which can be used for biological analysis in a long period of time .The method of giving implants material to the bone changes the biodistribution pattern of Sc-46, giving the preparations intravenously shows high accumulation in the liver. [11]. In this research, high accumulation in the liver is not found with the implant method. The use of HA-chitosan-collagen composite material certainly has advantages compared to composites that use HA alone, this is also related to the ability of HA to regenerate bone, and collagen which has the property of inhibiting the growth of pathogenic microbes, while chitosan is expected to be able to increase the strength of the composite because biodegradable, non-toxic, and biocompatible [8]. Labeling with Sc-46 also provides advantages because composite materials remain in composite form, compared to labeling using other radioisotopes.

4. Conclusion
We examined the biodistribution of Sc-46 labeled HA-Chitosan-Collagen composite using a bone implant model. The result proved that physiological uptake at bone has the same uptake at 1, 4 and 8 days of observation. This method was able to prove that the composite has a potential for bone implants and remains at the implant site until bone recovery. Future effort need to identify the molecular mechanism of Sc-46 labeled HA-Chitosan-Collagen composite binding with bone due to fracture case and prolonged the time interval.

Acknowledgment
The authors express their sincere thanks to the Center for Applied Nuclear Science & Technology and Center for Application of Technology of Isotope & Radiation, National Nuclear Energy Agency for financial support.

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