Basic Medical Research

Bone growth evaluation in collagen-hydroxyapatite implant locations using digital radiography: an animal model

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ABSTRACT

BACKGROUND Digital radiography has been used to evaluate the progress of bone growth with a collagen-hydroxyapatite implant in rabbit tibias. This study aimed to introduce digital radiography methods that provide comprehensive data availability for continuous information retrieval from the implant preparation to the cultivation period.

METHODS 38 digital radiographs were divided into 3 treatment groups, namely a single defect without implant (control), single-implant, and three-implant. Radiographic acquisitions were performed at preparation time and post-implantation from 0 to 56 days. Observations were concentrated on the implantation site, followed by creating a lateral profile. The prediction of implantation growth was determined using relative bone density (RBD) percentage.

RESULTS Based on the profile, the recovery process consisted of implant absorption and new bone tissue deposition. The absorption process was highly influenced by the defect size. In the control and single-implant groups, regardless of the different recovery processes, similar recovery results were observed 56 days post-implantation, with an RBD value of approximately 90%. Meanwhile, the three-implant group only had an RBD value of 62%.

CONCLUSIONS Radiography can evaluate absorption and new bone growth during implantation in New Zealand white rabbits. Radiographs, which can be obtained at any time during cultivation, offered more information on the recovery implantation process than the other method that relies on data obtained after sacrificing the animals.

KEYWORDS bone growth, bone implant, collagen, digital radiography, hydroxyapatite

Biomaterials are synthetic materials that can be used as bone implants.[1] They are expected to have osteoconductivity properties that can induce bone growth naturally and support structural, functional, metabolic, and biomechanical recovery similar to natural bone.[2] Autograft is the safest method to repair bone defects using the patient's own bone. However, this method is challenging due to the limited availability of usable bone and the potential for morbidity at the donor site, including pain, loss of function, and injury during surgical procedures.[3] An allograft, obtained from a bank of bones collected from cadavers, can be used for patients with large bone defects. Alternatively, xenografts, consisting of other species of bone, can be used to repair large bone defects.[4]

Bone substitutes is important for treating bone defects, leading to increased public demand for implants. Therefore, biomaterials must be made from more affordable and biocompatible materials.[5] Based on the constituent materials, biomaterials are grouped into three types: metals, ceramics, and polymers.[6] Metal materials are strong and resistant to loads, though they lack bioactivity and cannot interact with surrounding tissues; therefore, they do not support the osteointegration process and require complicated operations for removal and installation.[7] In contrast, ceramic materials, especially calcium phosphate, can facilitate bone progenitor and crest cells for attachment, survival, migration, proliferation, and differentiation.[3] Other material implants, such as hydroxyapatite (HA), have been widely used for smaller-volume bony defects. This material has chemical and structural similarities with bone minerals.[8,9] Unfortunately, HA is brittle; therefore, a collagen-HA composite is more suitable for bone implants.

Most *in vivo* studies on new bone growth tissue in collagen-HA implant locations obtained data after sacrificing the animals; therefore, the data are limited.[10] Yang et al[11] studied bone growth at the collagen-HA implant location using radiography and bone growth samples obtained every 4 weeks by sacrificing the animals. Purwanti et al[12] studied the bone growth via radiography and observation when a different HA-chitosan and HA-tricalcium phosphate implant was used. Therefore, this study aimed to evaluate the bone growth process using a collagen-HA implant and obtain preparation, directly post-implantation (Day 0 post-implantation), and post-implantation data using digital radiography.

METHODS

This study included 38 digital radiographs of rabbit tibias after collagen-HA composite implantation. The radiographs were obtained at 55 kV, 30 mAs, and 0.16 sec. These radiographs were the results of *in vivo* study provided by Biophysics Laboratory, Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Indonesia.

Animals

Male New Zealand white rabbits (*Oryctolagus cuniculus*) with a body weight of 3–3.5 kg and age of approximately 7 months were obtained from Biofarma, Bandung, with ethical code no 190 2021 IPB. A single defect of approximately 5 mm in diameter and 10 mm in height was created in the tibia of each rabbit. A cylindrical scaffold material implant with a 3 mm diameter and 10 mm in height was used. The rabbits were divided into three groups: single defect without implant (control, n = 2), single-implant (n = 2), and three-implant (n = 2). In the three-implant group, the size defect was 3 times larger than a single-implant rabbit tibial bone, with defect location was close to each other.

Radiographs were obtained during the preparation, directly post-implantation (Day 0 post-implantation), and at 7, 14, 28, 42, and 56 days post- implantation for the control and single-implant groups. Rabbits in the three-implant group underwent radiography during preparation, post- implantation, and at 14, 28, and 56 days post- implantation.

Radiographic evaluation

The radiographs were evaluated using ImageJ software (National Institute of Health, USA). Initially, the radiograph was cropped to contain the part of the tibia that underwent implantation in a 350 × 380-pixel image. A sample lateral image profile obtained from the trabecular to the cortical direction is shown in Figure 1a and is presented in graphs illustrating the pixel value and distance.

The process of bone absorption and growth at the implantation site was evaluated using the relative bone density (RBD) percentage, represented as the ratio of the mean implantation site area (pixels) or defect area to the mean area of the surrounding normal bone (pixels). RBD percentage is a modified region of interest (ROI) calculation first reported by Geiger et al.[13] The RBD percentage represents the similarity between the implant or defect area and the

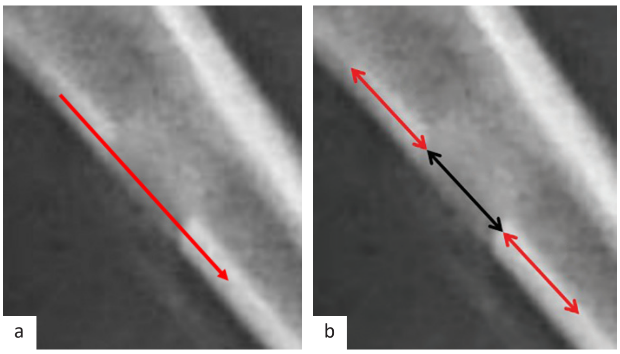

Figure 1. ROI analysis of tibia in rabbits. (a) ROI for lateral profile (red line arrow); (b) ROI for RBD percentage from each region (the red double line arrows were the natural bones around the defect [references], while the black line arrow was the implant/defect area [interesting area]). RBD=relative bone density; ROI=region of interest

area of natural bone. The profile and measurement of the mean gray value were conducted using ImageJ software (National Institute of Health). First, the measurement parameter was prepared in ImageJ (open the ImageJ-Analyze-set measurement [check area, standard deviation, and mean gray value]). Then, the radiograph image was added to the software. The radiographs were cropped to 350 × 380 pixel rectangles using the rectangle tool, and the images were duplicated. The freehand line tool was used to create an ROI for the implant or defect area and the natural bone area. Then, the profile was created, analyzed, and plotted (Figure 1a). The mean gray value was determined, analyzed, and measured (Figure 1b). The results were transferred directly to a statistical worksheet.

RESULTS

In the control group, the recovery process primarily relied on the deposition of bone tissue covering the defect and was completed 56 days after cultivation (Figure 2), with an RBD percentage of approximately 90%. The recovery in the single-implant group included implant absorption, which occurred 7 days after implantation, and new bone growth (Figure 3). After 56 days, the implantation recovery was nearly complete, with an RBD percentage of 91% (Figure 4).

In the three-implant group, the absorption was still in progress at 28 days after implantation (Figure 5). New bone growth occurred within 28 days after the absorption process, with an RBD percentage of 62% (Figure 6).

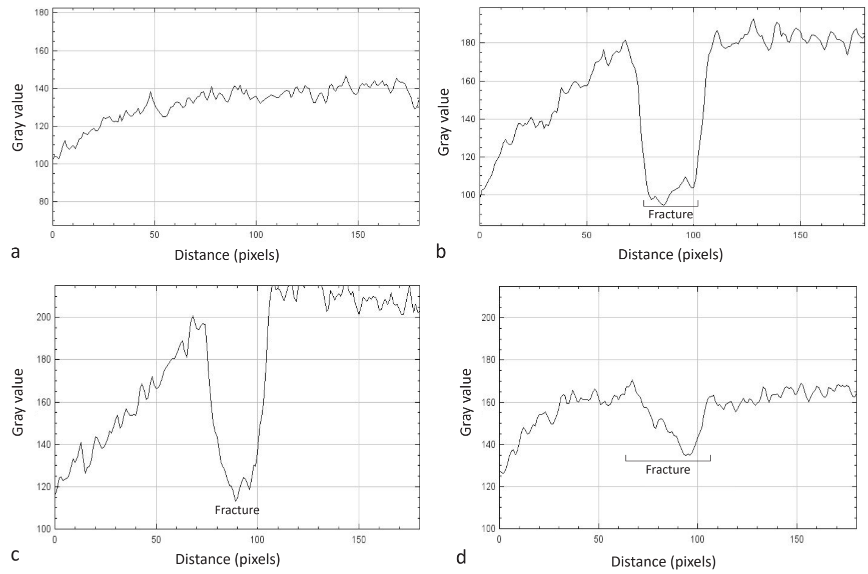

Figure 2. Lateral profile of the control group. (a) During preparation; (b) Day 0 post-fracture; (c) 7 days post-fracture; and (d) 56 days post-fracture

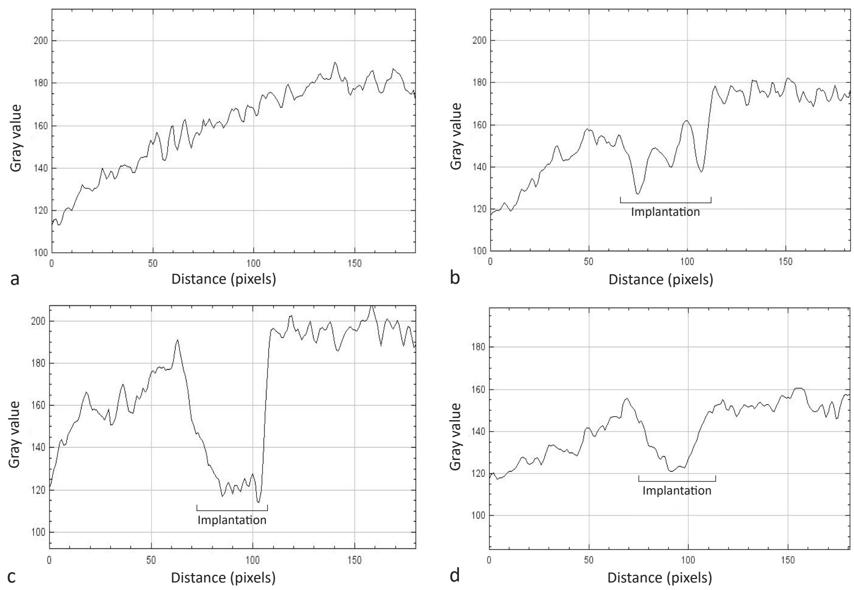

Figure 3. Lateral profile of the single-implant group. (a) During preparation; (b) Day 0 post-implantation; (c) 7 days post-implantation; and (d) 56 days post-implantation

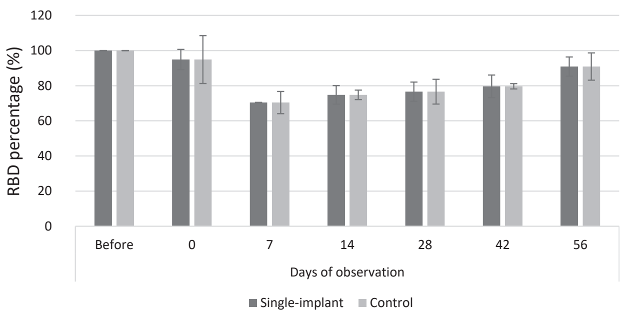

Figure 4. Relative bone density (RBD) percentage results for the control and single-implant groups

DISCUSSION

This study investigated the effects of a collagen-HA implant in rabbit tibias using radiography. Implant absorption in the single-implant group occurred after 7 days. The actual maximum absorption could not be predicted. However, the absorption process probably occurred within less 7 days, indicating that the bone tissue growth started within 7 days, while absorption in the three-implant group occurred after 28 days. Bone growth was slower in the three-implant group than in the single-implant group. The absorption and bone growth rates depended on the defect size. Furthermore, the radiographs used in this study provided quantitative data regarding the implant process using RBD percentage, which may serve as a tool to evaluate bone growth when distinguishing between the density of the implanted area and the natural bone is challenging.

Several studies have reported bone biomaterial implantation with composite HA nanoparticles.[9,14] Generally, adding HA nanoparticle to natural or synthetic polymers improves the osteoconductivity, absorption, and tissue bone growth of the biomaterial. Most previous studies regarding collagen-HA implant composites were observation studies. The animals were sacrificed to obtain the implantation bone samples and analyzed using scanning electron microscopy, histomorphometry analysis, or fluorescence microscopy.[14,15] Two previous studies[14,16] reported new bone growth 6 weeks after implantation in New Zealand white rabbits. However, Hoshi et al[15] reported new cranial bone growth 8 weeks after implantation. Animal sacrifice was not required in the current study, which provides detailed data regarding the implantation process.

The threshold volume, where implantation might not be needed, was determined in this study. Radiography allows for visualizing fracture dislocations, heterogenic ossification, or implant failure localization.[17] In the present study, radiographs were used to monitor the absorption process of the implant without triggering an inflammatory response. Additionally, this method of observation is cost-effective as it utilizes free, easy, and fast processing software.[13]

In conclusion, radiography was effectively evaluated absorption and new bone growth during implantation in New Zealand white rabbits. This method allowed for data collection over time during cultivation and monitoring dynamic changes during

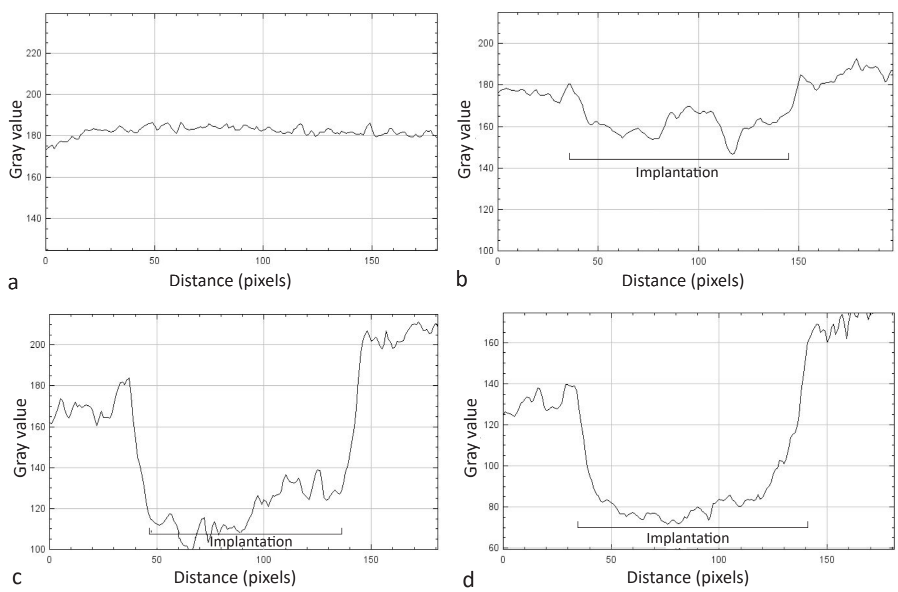

Figure 5. Lateral profile of the three-implant group. (a) During preparation; (b) Day 0 post-implantation; (c) 14 days post-implantation; and (d) 56 days post-implantation

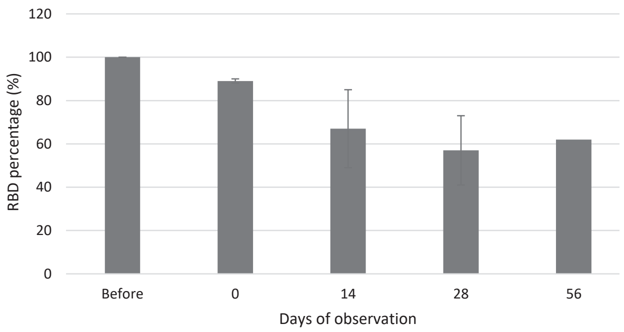

Figure 6. Relative bone density (RBD) percentage results of the three-implant group

implantation. The recovery process lasted 56 days in the control and single-implant groups, achieving an RBD percentage of approximately 90%. The recovery process was longer in the three-implant group, achieving an RBD percentage of 62%. The results from this study may be used to support future studies regarding the associations between absorption and bone growth over time.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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