BONE CHANGES AROUND HYDROXYAPATITE AND TITANIUM IMPLANTS AFTER ABUTMENT PLACEMENT IN RABBITS–OBSERVATIONS USING HISTOLOGICAL AND THREE-DIMENSIONAL EXAMINATIONS

Hideo Kanitani, DDS, PhD
Yoritoki Tomotake, DDS
Rudi Wigianto, DDS, PhD
Naeko Kawamoto, DDS, PhD
Tetsuo Ichikawa, DDS, PhD

KEY WORDS

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Hideo Kanitani, DDS, PhD, Yoritoki Tomotake, DDS, *and* ***Naeko Kawamoto, DDS, PhD,*** *are Instructors of Removable Prosthodontics at Tokushima University School of Dentistry, Tokushima, Japan.*

Tetsuo Ichikawa, DDS, PhD, *is Professor and Chairman of Removable Prosthodontics at Tokushima University, School of Dentistry. Correspondence should be addressed to Dr. Ichikawa, Department of Removable Prosthodontics, The University of Tokushima, School of Dentistry, 3-18-15, Kuramoto-cho, Tokushima-770, Japan.*

Rudi Wigianto, DDS, PhD, *is instructor of Preventive Dentistry at Mahasaraswati University School of Dentistry, Bali, Indonesia.*

We have previously developed a computer-aided system for examination of the three-dimensional bone structure around implants and observed the bone changes in the healing period after implant placement. This paper describes the bone changes around hydroxyapatite (HA) and titanium (Ti) implants after abutment placement using histological and three-dimensional examinations. Twenty-four HA and Ti implants were embedded in the tibias of adult male New Zealand white rabbits. After 8 weeks, the abutment had passed through periosteum and was placed under the skin. Rabbits were sacrificed 4 and 8 weeks following abutment placement. In conclusion, histological examination showed that, at 4 weeks after abutment placement, bone resorption around the implant neck was seen in both HA and Ti implants, and at 8 weeks, excessive bone formation was seen around the implant neck. Three-dimensional bone examination showed that abutment placement may affect bone formation and cause additional bone hypertrophy in the bone marrow area.

INTRODUCTION

Pure titanium (Ti) and titanium alloy are widely used as compatible implant materials because of the contact between the material surface and bone through so-called osseointegration.[1] In cases of low bone density, osseointegration is often not possible when primary fixation of the implant is not obtained during the bone healing period. Materials containing calcium, such as hydroxyapatite (HA) are also used as implant material because of the chemical bond between the HA and bone, through so-called biointegration.[2–4] It has been reported that biointegration has some advantages over osseointegration in

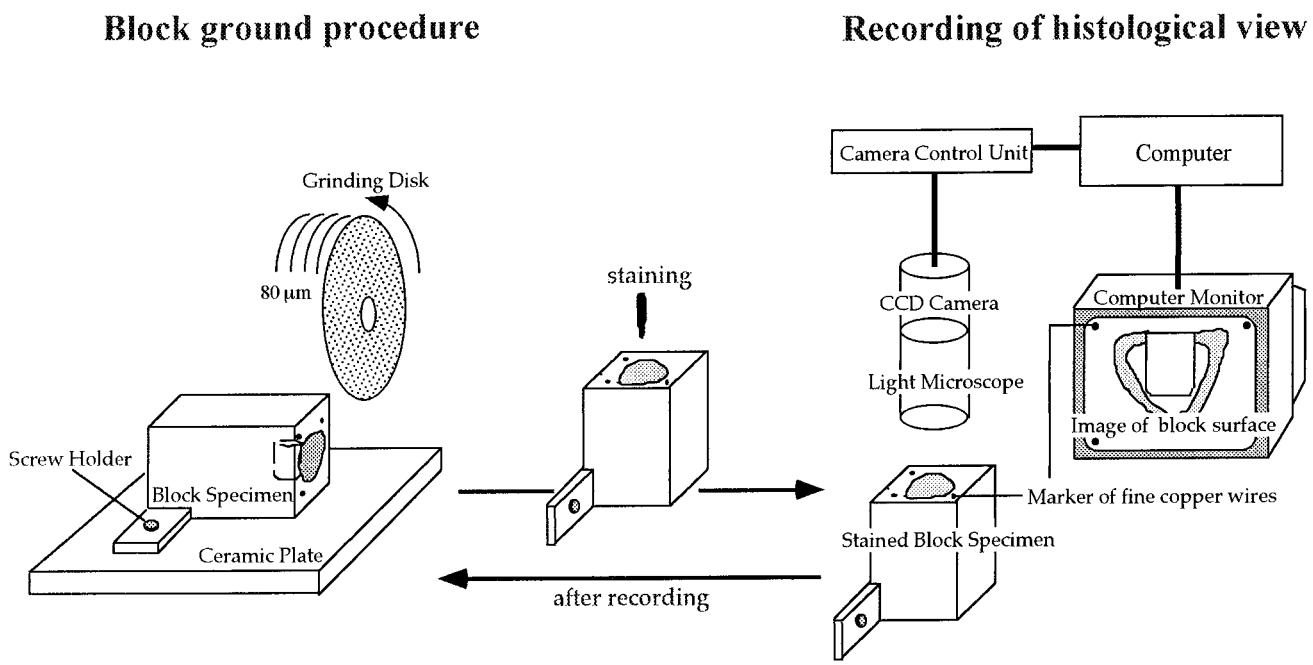

FIGURE 1. Schematic illustration of the procedure for the three-dimensional bone examination.

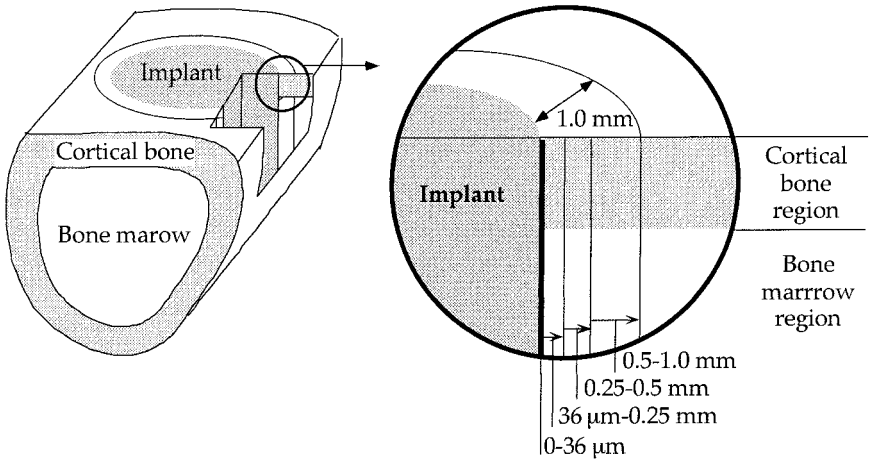

FIGURE 2. The percent bone volume was examined for the region up to 1 mm from the implant surface and categorized into 0–36-μm, 36-μm–0.25-mm, 0.25–0.5-mm, and 0.5–1.0-mm regions. The percent bone volume of each was also divided into two regions: cortical bone and cancellous bone regions. The percent bone volume (%) was obtained using the equation (bone volume/volume to be examined) × 100.

animal studies. The bone healing period following HA implants is shorter than that following titanium implants.[5,6] Moreover, the bond strength between the HA implant and bone is higher than that using Ti.[7,8] On clinical application, however, there are occasions when the tissues surrounding the HA implants are confronted with fatal conditions, such as periimplantitis of HA with rapid bone resorption.[9] These reports may suggest that it is insufficient to understand only the difference of the bone–implant interface between HA and Ti implants.

We previously reported a computer-aided system for examination of the three-dimensional (3D) bone structure around implants.[10,11] The study reported observations for the healing period only. The purpose of the present study was to clarify the bone changes around HA and Ti implants after abutment placement using the above-mentioned system and histological examination.

MATERIALS AND METHODS

Specimen preparation

Commercially pure titanium and dense hydroxyapatite implants were trial made (Pentax, Tokyo, Japan) for this experiment. The dense HA[12] (100% pure crystallized HA) implant was synthesized by the dry method, that is, the HA powder was passed through a sieve, cold-pressed, and then sintered at 1000–1300°C in air. Each implant was prepared as a cylinder 4.0 mm in diameter and 5.0 mm in length with a smooth surface, the surface of the Ti being turned and finished by a machine to the same smoothness as a Brånemark implant.

A commercially pure titanium abutment was also trial made for both implants. The abutment was prepared as a column 4.0 mm in diameter and 5.0 mm in length. The dimensions of the surface were the same as for the Ti implants.

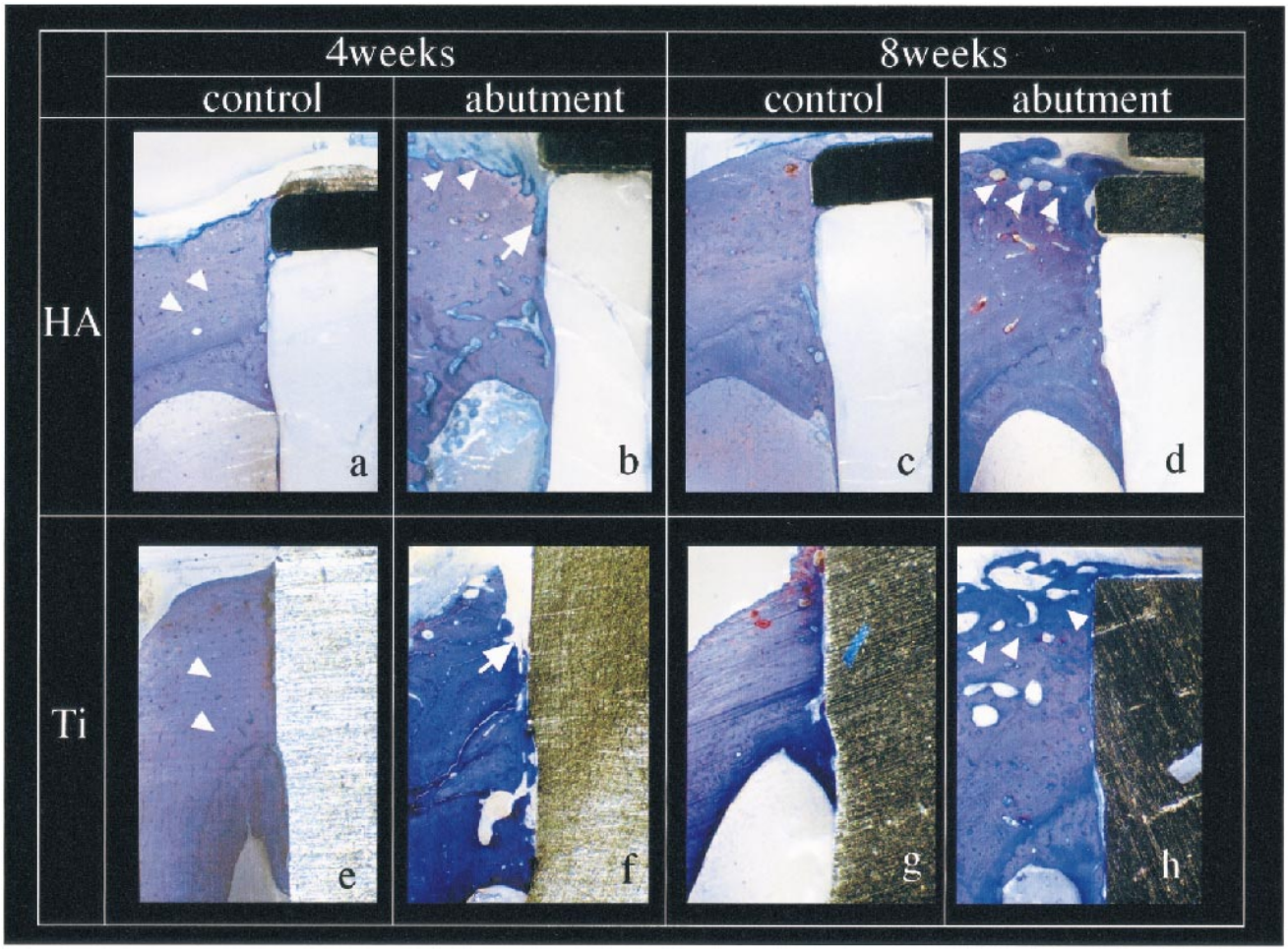

FIGURE 3. Histological views of bone around the implant neck. (a–d) HA implants. (a) Control after 4 weeks. (b) Four weeks after abutment placement. (c) Control after 8 weeks. (d) Four weeks after abutment placement. (e–f) Ti implants. (e) Control after 4 weeks. (f) Four weeks after abutment placement. (g) Control after 8 weeks. (h) Four weeks after abutment placement.

Twelve adult male (weight 3.0–3.5 kg) New Zealand white rabbits were used in the present study. Twelve HA implants were embedded in the tibias of six rabbits, and 12 Ti implants were embedded in the tibias of six rabbits, as in our previous reports.[10,11]

After 8 weeks, the rabbits were sedated using Nembutal administered intravenously, and the abutment was connected to the implant in the tibia. The abutment passed through the periosteum and was placed under the skin. On the contralateral side, a sham operation was carried out as control. The flap with the skin and the periosteum was reflected and sutured without the abutment placement.

Rabbits were sacrificed at 4 and 8 weeks following abutment placement using an overdose of Nembutal. The bone columns containing the implants were immediately placed into a 10% buffer formalin solution. Following nondecalcifying histological procedures, the specimens were embedded in a polyester resin (Maruto Co, Tokyo, Japan). The specimens were divided into two groups for histological and 3D bone examination. Eight specimens were used for the histological observation. Resin blocks were sectioned at a thickness of 200–300 μm and the surfaces were polished for light microscopic observation. The sections were stained with toluidine-blue solution.

This experiment was approved by the animal ethics committee for the University of Tokushima School of Dentistry.

Observation by three-dimensional examination

The 3D bone structure around the implant was examined using the procedure of Wigianto *et al*[10,11] (Fig 1). The embedded specimen was placed on the ceramic plate of a grinding machine using screw holders and was ground at intervals of 80 μm along the long axis of the implant. The function of the screw technique is to standardize the position of the block specimen when the block is returned to the grinding machine after recording the image. The block was removed from the grinding machine and the block surface stained

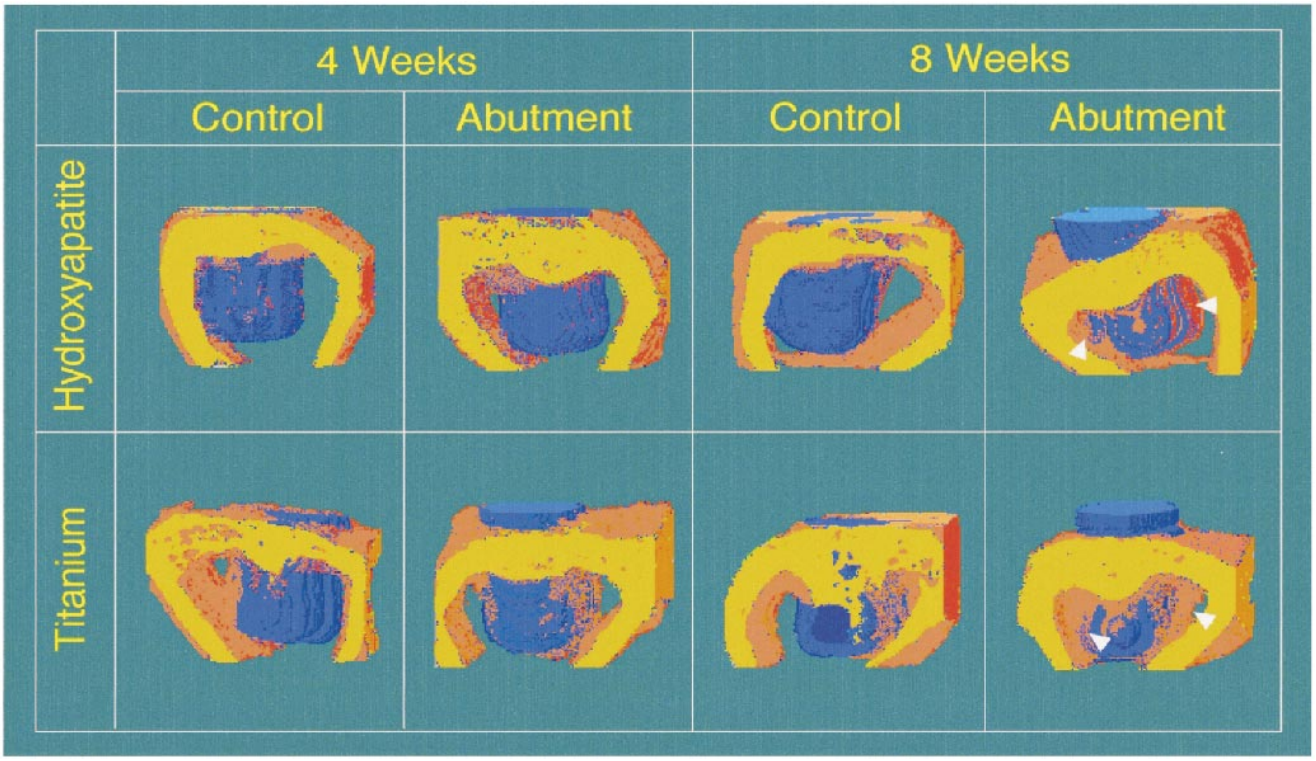

FIGURE 4. Three-dimensional graphics visualizing chronological change of bone structure around the HA and Ti implants (superior-anterior-medial view). The graphics make it easy to understand the changes to cancellous bone in the marrow cavity. Blue shows the implant. Yellow shows the surrounding bone. Arrowheads show the remarkable hypertrophic bone changes.

with Alizarin red S. This surface was examined under a light microscope and the view was recorded with a charge coupled device camera and a computer at a resolution of 36 μm. Block specimen grinding and image recordings were repeated.

Serial two-dimensional images were constructed into a 3D model using computer imaging software (Spyglass Dicer 1.0, Spyglass Inc, Mountain View, CA), and an objective evaluation was carried out using image analysis software (IP Lab Spectrum 3.1, Signal Analytics Co, Vienna, VA), as shown in Fig 2. Percent bone volume was divided into two regions: cortical bone and cancellous bone. In the region from the implant surface to each measurement, that is, 36 μm and 0.25, 0.5, and 1.0 mm, the percent bone volume was determined using the equation percent bone volume (%) = (bone volume/volume to be examined) × 100. Therefore, percent bone volume in the region from the implant surface to 36 μm distance was equal to the percent bone–implant contact.

RESULTS

Histological examination

At 4 weeks after the sham operation, that is, at 12 weeks after implantation, the bone around the neck of both HA and Ti implants was compact, so-called cortical bone (Fig 3a, e, arrowheads). Both implants were directly in contact with the bone, but soft tissue with the lacunae of osteocytes or bone marrow was also seen. However, soft tissue contact was frequently seen on the surface of Ti implants, whereas little soft tissue contact was seen on the HA implant surfaces. In the bone marrow area, the HA implant surface was covered with a very thin layer of bone. In contrast, the surrounding bone on the surface of Ti implants showed a matured condition with osteon structure and was thicker than that on the surface of the HA implant. At 8 weeks after sham operation, the histological findings were similar to those at 4 weeks (Fig 3c, g).

The histological view of the HA implants at 4 and 8 weeks after abutment placement showed bone resorption around the neck of the HA implant (Fig 3b, arrow), and the condition of the cortical bone surface around the implants was rough, like a large-meshed net (Fig 3b, arrowheads). At 8 weeks after abutment placement, excessive cortical bone formation was seen as the reaction of fracture healing (Fig 3d, arrowheads) and invasion by soft tissue was minimal. In the bone marrow area, the surrounding bone was very thin. Similar findings were also seen around the Ti implants. At 4 weeks, bone resorption was seen around the Ti implants (Fig 3f, arrow). At 8 weeks, the implant was excessively covered with newly formed bone (Fig 3h, arrowheads). In the bone marrow area, the surrounding bone was

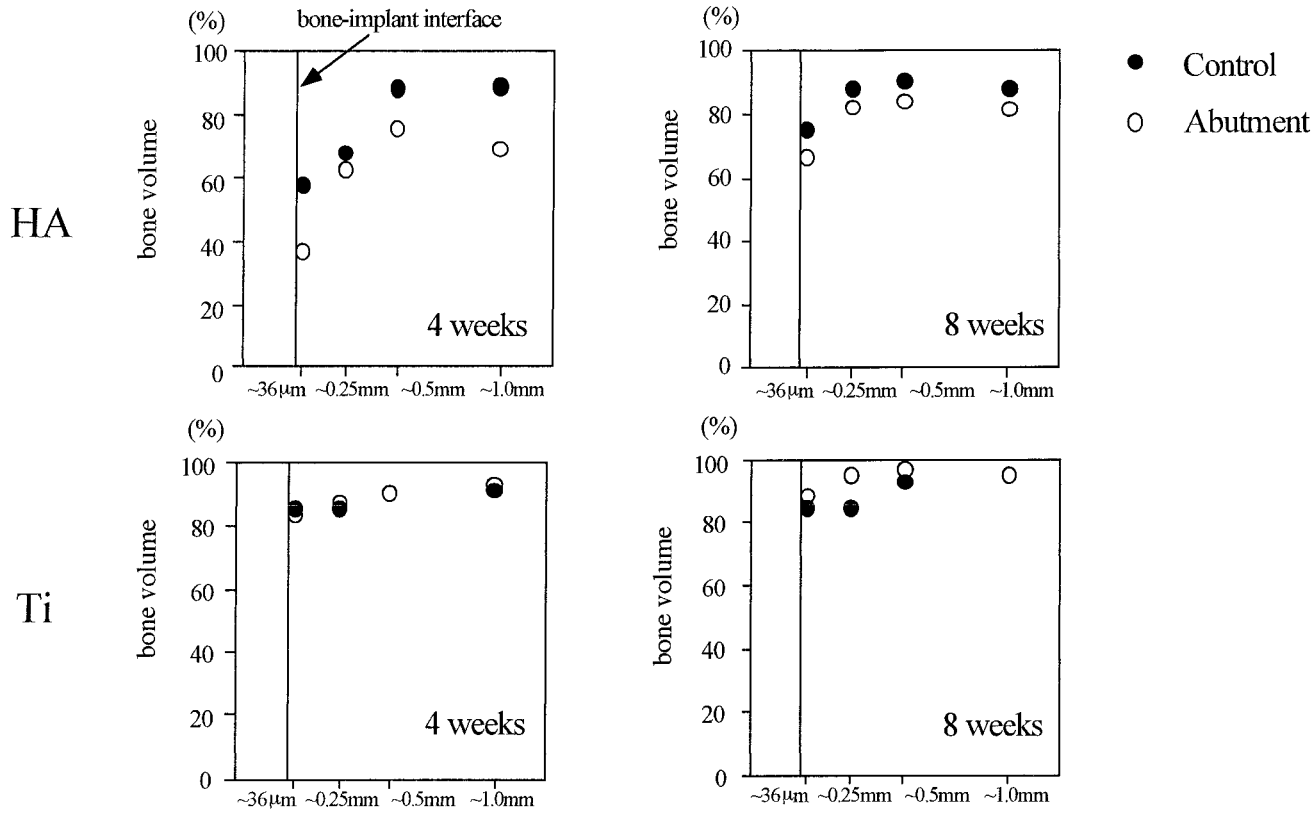

FIGURE 5. Chronological changes of the percent bone volume in the cortical bone area. ● or ○ was the average value of two samples.

thicker than that of HA implants and showed hypertrophic changes.

Three-dimensional bone structure

Figure 4 shows the chronological changes of the bone structure around the HA and Ti implants using the computer-aided system. Figures 5 and 6 show the bone volume ratio at each distance from the implant surface of the cortical bone and the bone marrow regions. In the cortical bone area, the percent bone volume was high at each distance from the implant surface and consistent regardless of implant material and implantation period. However, in the HA implants, the percent bone volume rate at 0–36 μm from the implant surface was very low, that is, only 36.1% (Fig 5). In the bone marrow area, no obvious features were seen. However, the percent bone volume rate in Ti implants was higher than that in HA implants. Moreover, in Ti implants, the percent bone volume rate at 0.25–0.5 mm from the implant surface was higher than that in HA implants (Fig 6). At 8 weeks after abutment placement, the bone volume in the bone marrow area was increased compared with the control (Fig 4, arrowheads).

DISCUSSION

Adequate bone thickness surrounding an implant is required to support the implant over the long term, and therefore 3D bone structure will be an important factor to investigate the aspects of bone remodeling around the implant. It has been reported that HA implants bind to the bone chemically,[2–4] whereas Ti implants are directly supported by bone contact without a chemical bond. Wigianto *et al*,[11] examined the 3D bone structure around pure Ti and dense HA implants in rabbits up to 8 weeks after implantation with calculations of percent bone–implant contact and percent bone volume. The results suggested that bone remodeling depended on the implant materials and implantation period and that more bone was observed around the HA implants in the early period but was decreased at 8 weeks, whereas around the Ti implants, the bone increased gradually up to 8 weeks. Similar results were seen in the control group of the present study. The bone reaction to an implant is pathologically recognized as a adaptation for a foreign body within the permissible range.[13] Therefore, it is explained that the survival of the implant is maintained by encapsulation of the surrounding bone. Pathological definitions of the bone response around HA and Ti must be different since the bone response differs between HA and Ti. The following hypothesis is proposed: The bone response to HA is a regressive change, as the surrounding bone becomes thinner due to so-called disuse atrophy, whereas the bone response to Ti is progressive, as the thickness of the surrounding bone increases gradually.[13] According to this hypothesis, the

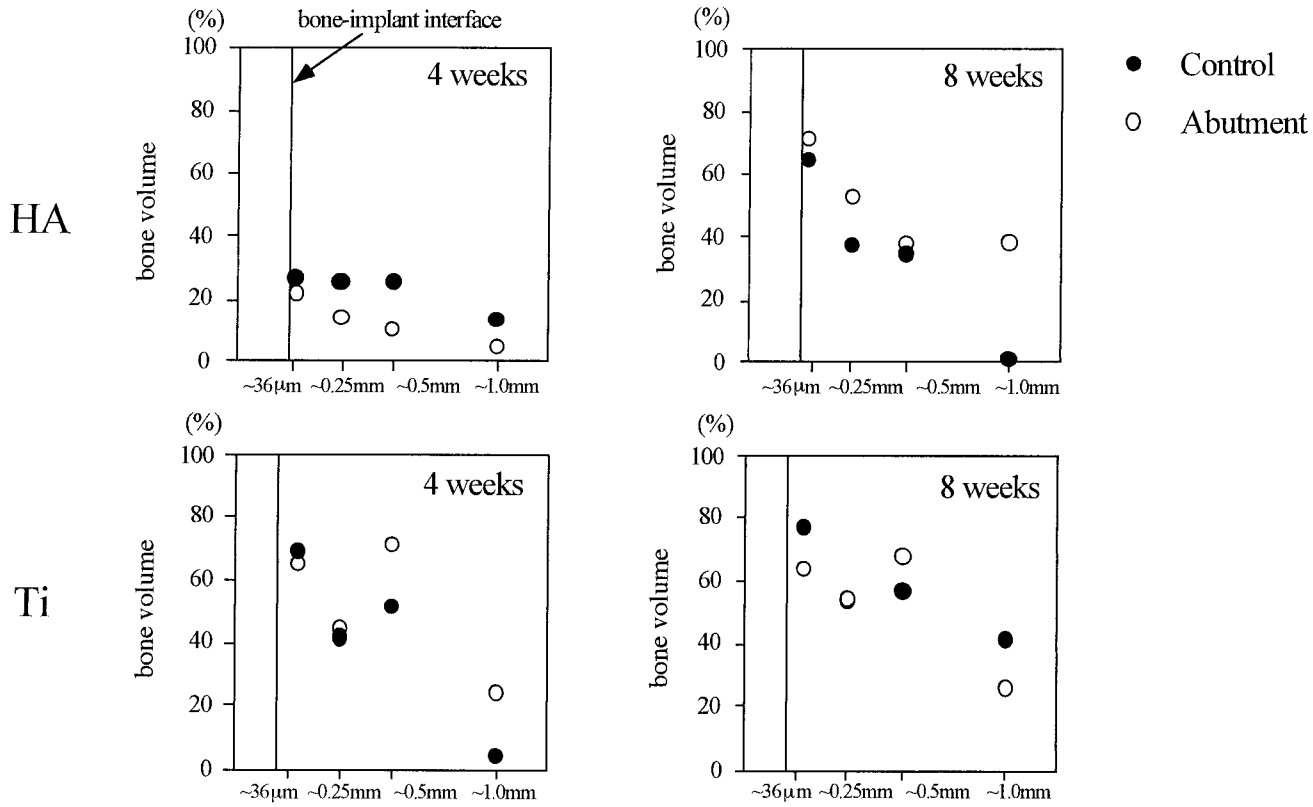

FIGURE 6. Chronological changes of the percent bone volume in the bone marrow area. ● or ○ was the average value of two samples.

results of the present study seem to be consistently explained.

The present study was conducted to clarify the influence of the abutment placement that generates mechanical stress on the bone around the implant. We initially established whether or not the abutment could pass through the skin. However, this was merely to ensure that the treated part remained clean and to prevent severe infection at the peripheral tissue. Abutments passing through the periosteum were placed under the skin. Therefore, mechanical loading was not able to be applied in this study. However, mechanical stress to the implant after the abutment placement is considered to be relatively larger than that before the placement.[14,15] In both implants, the abutment placement affects the surrounding bone, causing a slight resorption around the implant neck and additional bone hypertrophy in the bone marrow area.

In conclusion, histological examination showed that, at 4 weeks after abutment placement, bone resorption around the implant neck was seen in both HA and Ti implants, and at 8 weeks, excessive bone formation was seen around the implant neck. Three-dimensional bone examination showed that abutment placement may affect bone formation and cause additional bone hypertrophy in the bone marrow area.

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