Peri-Implant Bone Density in Senile Osteoporosis-Changes from Implant Placement to Osseointegration

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ABSTRACT

Purpose: The aim of this study was to examine healing over time after implant body placement in a senile osteoporosis model and a control group.

Materials and Methods: In this study, 16-week-old male mice were used. The senile osteoporosis model consisted of senescence-accelerated prone 6 mice and the control group consisted of senescence-accelerated resistant 1 mice. Titanium-coated plastic implants were used as experimental implants whose dimensions were 3.0 mm in length, 1.1 mm in apical diameter, and 1.2 mm in coronal diameter. Bone samples were collected at 5, 7, 14, 21, and 28 days after implant placement. A micro-quantitative computed tomography (QCT) system was used to scan these samples and a phantom in order to quantitate bone mineral measurements. Bone mineral density (BMD) of each sample was measured. Each sample was also examined by light microscopy after QCT imaging. At 14 and 28 days after implant placement, the bone-implant contact (BIC) ratios were calculated from light microscopy images and were divided into cortical bone and bone marrow regions.

Results: When BMD was compared between the osteoporosis and control groups using micro-QCT, the osteoporosis group had a significantly lower BMD in the region 0–20 μm from the implant surface in the bone marrow region at 14 days onward after implant placement. Compared with the control group, the osteoporosis model also had significantly lower BMD in all regions 0–100 μm from the implant surface in the bone marrow region at 14 days after placement. However, in the cortical bone region, no statistically significant difference was observed in the regions at the bone-implant interface. Light microscopy revealed osseointegration for all implants 28 days after implant placement. The osteoporosis model tended to have lower BICs compared with that of the control group, although this did not reach statistical significance.

Discussion: Our results showed that osseointegration was achieved in the osteoporosis model. However, the BMD was 30–40% lower than that of the control group in the region closest to the implant surface in bone marrow region. Peri-implant BMD was lower in a relatively large area in the osteoporosis model during an important time for osseointegration. Therefore, this result suggests that osteoporosis might be considered as a risk factor in implant therapy.

Conclusion: The osteoporosis model had a lower BMD than the control group in the region closest to the implant during an important time for osseointegration. This result suggests that senile osteoporosis might be a risk factor in implant therapy. However, the osteoporosis model and the control group had no difference in peri-implant BMD in the cortical bone region. This suggests that risk might be avoided by implant placement that effectively uses the cortical bone.

KEY WORDS: bone mineral density, micro-QCT, osseointegration, osteoporosis, quantitative computed tomography, titanium coating

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INTRODUCTION

Osteoporosis is defined as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture".[1] Bone structure and mass are maintained by the equilibrium of bone absorption and formation. Osteoporosis occurs when this equilibrium is disrupted and bone resorption exceeds bone apposition. This disease is increasingly seen in aging societies.

One of the risk factors for implant therapy is poor bone quality. Osteoporosis is a disease in which bone mineral content is reduced and the trabecular microstructure changes, which leads to reduced bone strength. Osteoporotic patients are expected to have worse outcomes for implant therapy compared with healthy patients. However, there are relatively few reports on osteoporosis and the outcomes of implant therapy, and the results of reports are not consistent with each other.[2–5] In basic research, there are studies on high-turnover osteoporosis using ovariectomized animal models, but there are no reports on reactions of peri-implant tissues in low-turnover osteoporosis as seen in the elderly.

In the present study, we placed experimental implants in a senile osteoporosis animal model to examine peri-implant tissue reaction and to evaluate the level of osseointegration over time. First, micro-quantitative computed tomography (QCT) was used to measure changes in peri-implant bone mineral density (BMD) over time and the effects of osteoporosis were examined in peri-implant tissues. Next, peri-implant tissue reaction was examined using light microscopy

MATERIALS AND METHODS

Experimental Animals

A senile osteoporosis model was used, which consisted of 16-week-old male senescence-accelerated prone 6 (SAMP6) mice and senescence-accelerated resistant 1 (SAMR1) mice (Japan-SLC, Shizuoka, Japan). Fifteen SAMP6 mice (experimental group) and 15 SAMR1 mice (control group) were used in this study. This study was conducted with the approval of the Animal Experimentation Committee at Fukuoka Dental College (approval number: 08003).

Experimental Implants

The experimental implants were plastic implants coated with a thin titanium film. The thickness of the titanium coating was 100–150 nm as measured by transmission electron microscopy.[6–8] The titanium coating without the incorporation of impurities was confirmed in previous studies.[7,9] These implants were similar to the ones used for rats by Okamatsu and Morinaga et al. but were tapered to attain better adaptation to the implant sockets for mice. The implant body surface was made smooth and even by treatment with a surface smoothing agent for dentistry (Dynaseal® Kuraray Medical, Tokyo, Japan). The final dimensions of the plastic implant were 3.0 mm in length, 1.1 mm in apical diameter, and 1.2 mm in coronal diameter. The implant body surface was coated with titanium using the methods of Watazu et al.[6,9] and a DC-magnetron sputter system (L332S-FHS, ANELVA, Tokyo, Japan) (Figure 1). A titanium film was deposited by sputtering a titanium target (99% purity) for 10 minutes under an argon atmosphere (0.22 Pa) at room temperature (Figure 2). These implants were then placed in the experimental animals.

Surgical Treatment

The experimental animals were placed under general anesthesia by inhalation of isoflurane (Forane® Abbott Japan, Tokyo, Japan). Hair was shaved around both knee joints and an incision of approximately 12.0 mm was made from the knee joint along the anterior border of the tibia to expose the bone surface. A 1.0 mm diameter drill was used to prepare an implant socket from the medial to the lateral side of the tibia. The socket was prepared 5.0 mm distal to the apex of the tibia at the knee joint. Titanium-coated plastic implants were placed on the left and right tibias. The periosteum and skin were repositioned and closed using absorbable sutures (VICRYL®, Johnson & Johnson, New Brunswick, NJ, USA) (Figure 3).

Sample Preparation for Micro-QCT Examination

An experimental implant was placed in both tibias of the mice. Three mice were sacrificed at 5, 7, 14, 21, and 28 days after implant placement. Therefore, six specimens were obtained at each period after implant placement. Specimens were collected for examinations of experimental implants and peri-implant bone. The samples were fixed in half strength Karnovsky fixative.

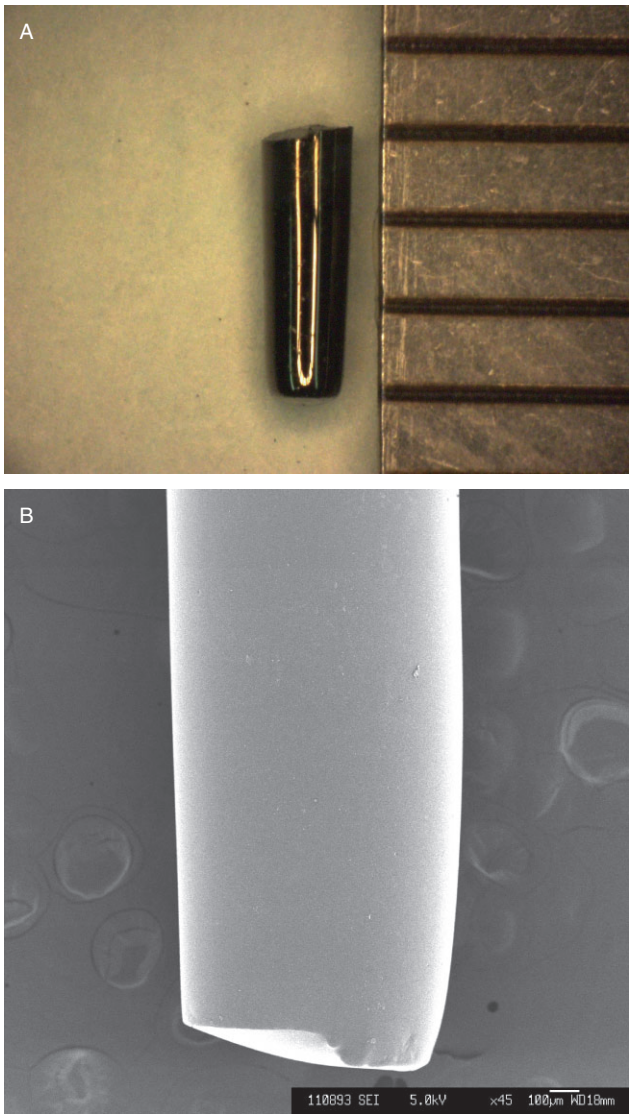

Figure 1 (A) Implant body after titanium coating. (B) Scanning electron microscopy image after titanium coating of implant body surface.

A micro-QCT system (SMX-100S, Shimadzu, Kyoto, Japan) was used to measure the BMD of each sample, which was simultaneously scanned with a phantom in order to quantitate bone mineral measurements (TRI/3D-BON-BMD-PNTM, RATOC System Engineering Co. Ltd, Tokyo, Japan). Imaging was performed under the following conditions: tube voltage of 100 kV, tube current 100 μA, and slice width of 10 μm. Three-dimensional bone morphometric software was used for micro-QCT data analysis (TRI/3D BON, RATOC System Engineering Co. Ltd, Tokyo, Japan). Micro-QCT data was reconstructed three-dimensionally using the software and color coding was performed by the BMD level calculated from the QCT results. A more detailed

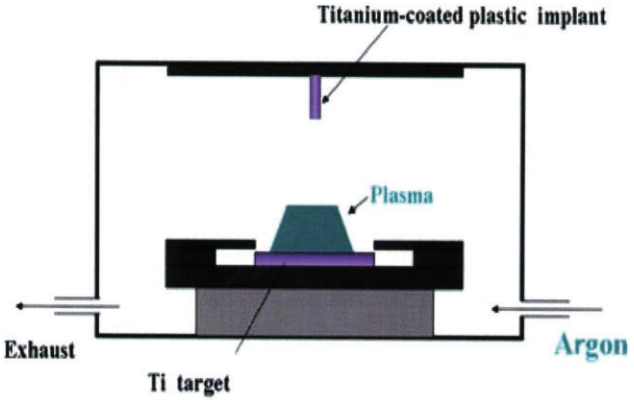

Figure 2 Schematic of the DC-magnetron sputter system.[7] A titanium film was deposited by sputtering a titanium target (99% purity) for 10 minutes under an argon atmosphere (0.22 Pa) at room temperature. The DC power was 300 W, the distance between cathode and dish was 90 nm, and pre-sputtering time was 5 minutes.

examination of BMD was performed for peri-implant tissues. The peri-implant area was divided into 20-μm thick tube-shaped regions, which were 0–100 μm from the implant surface. BMDs were calculated in regions 200 μm high in the cortical bone and bone marrow (Figure 4).

Sample Preparation for Light Microscopy

Left and right tibias with implants were harvested, immersed in half strength Karnovsky fixative (2.5% glutaraldehyde/2% paraformaldehyde solution) for 24 hours and then decalcified with 10% ethylenediaminetetraacetic acid for 2 weeks. They were post-fixed in 2% osmium tetroxide, block stained in 0.25% uranyl acetate, and dehydrated with ethanol, which was then substituted with propylene oxide. The samples were embedded in epoxy resin (Epon 812, Taab, Aldermaston, UK) and cured in a curing unit at 38°C for 24 hours and at 60°C for 48 hours. The cured samples were sectioned (slice

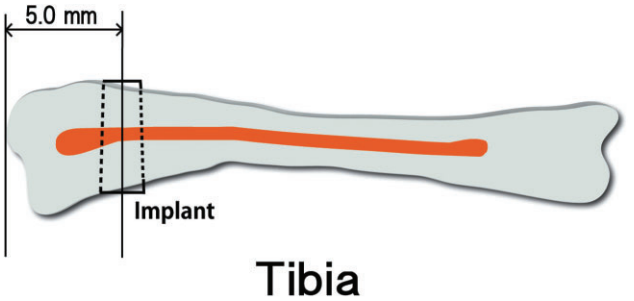

Figure 3 Schematic diagram of implant body placement. Implants were placed bicortically 5.0 mm distal to the apex of the tibia at the knee joint.

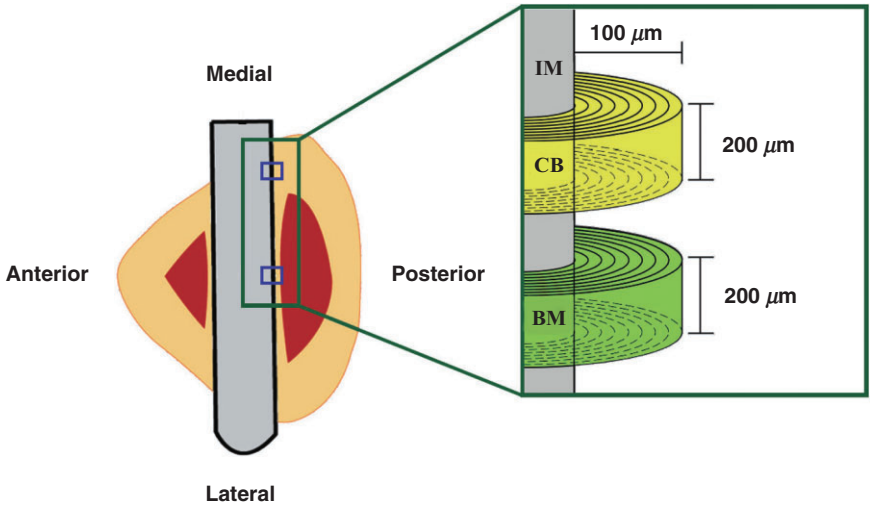

Figure 4 Evaluation using micro-quantitative computed tomography. Bone mineral density was measured 0–100 μm from the implant surface in the cortical bone and bone marrow regions. IM = implant; CB = cortical bone; BM = bone marrow.

thickness: 0.70 μm) with a microtome (REICHERT-NISSEI ULTRACUT-S, Leica, Solms, Germany) for examination by light microscopy. Toluidine blue was used for staining and then a light microscope was used for examination. The samples used in QCT measurements were also examined by light microscopy after QCT imaging. In all implants, contact between implant and bone tissue (osseointegration) was observed.

Evaluation of Bone-Implant Contact (BIC) Ratio

For light microscopy samples at 14 and 28 days after implant placement, the images were magnified on the monitor using a digital microscope (VHX-100, KEYENCE, Osaka, Japan). A measuring tool included in the microscope was used to measure the length of the peri-implant in the bone. The same method was used to measure the length of the area with contact between the implant surface and bone tissue. The measurements were used to calculate the BIC ratios, which were expressed as percentages. The results of the measurements were separated into the cortical bone region and the bone marrow region.

Statistical Analysis

Results were expressed as means ± standard deviations. Differences between the control and experimental groups were assessed using a parametric Student *t*-test or nonparametric Wilcoxon test depending on the distribution of data. Statistical differences were considered significant at $p < .05$.

RESULTS

Evaluation of Peri-Implant Tissue Using Micro-QCT

At 7 days after implant placement, the micro-QCT results showed that both experimental and control groups had peri-implant new bone formation. Both groups had sparse new bone formation in the bone marrow region, but the experimental group had slightly more bone formation.

In the experimental group, peri-implant new bone formation was seen around the entire implant, but the BMD was low (shown in blue and purple color). In the control group, there was relatively thick peri-implant new bone formation, although new bone mass was low in some areas. There were high BMDs (shown in yellow) in plural areas relatively far from the implant surface (Figure 5).

At 14 days after implant placement, the micro-QCT results showed that the experimental group had a wider area of new bone formation compared with the control group. However, the experimental group had sparse, mesh-like bone formation in the bone marrow region. The experimental group also had a relatively low BMD and the majority of the areas were blue. The control group had relatively thin new bone that covered the entire area surrounding the implant. On the side of the cortical bone, there was a region of high BMD (yellow) with plate-like new bone formation (Figure 6).

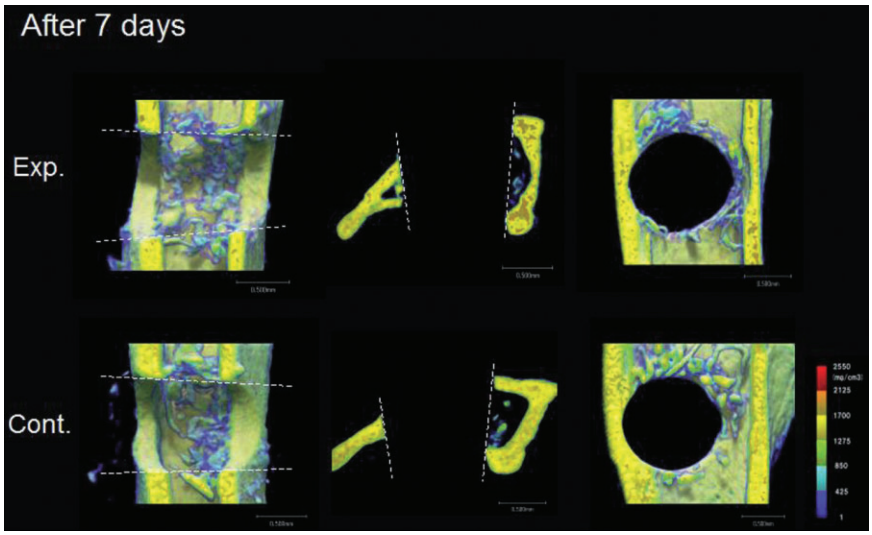

Figure 5 Three-dimensional image 7 days after implant placement. In the experimental group, peri-implant new bone covered the entire area surrounding the implant but bone mineral density was low. In the control group, new bone mass in some peri-implant areas was low but the layer was relatively thick. Bone mineral density was high in an area relatively far away from the implant surface. The white dotted line is the outline of the implant. Exp. = experimental group; Cont. = control group.

At 28 days after implant placement, the micro-QCT results showed that the experimental group had more sparse bone formation in the bone marrow region compared with the control group. The experimental group had a mixture of areas with lamellar and mesh-like bone formation. This group also had a larger area of low BMD (blue). The control group had lamellar bone formation around the entire implant. Much of the bone covering the implant had a higher density (yellow) than in the experimental group (Figure 7).

BMD was examined over time in the cortical bone region within 20 μm of the implant surface. At 7 days after implant placement, bone density tended to be higher in the experimental group than in the control group, but no statistically significant difference was observed. BMD decreased over time in the control group, while it tended to peak 14 days after implant placement in the experimental group. However, no statistically significant difference was observed (Figure 8).

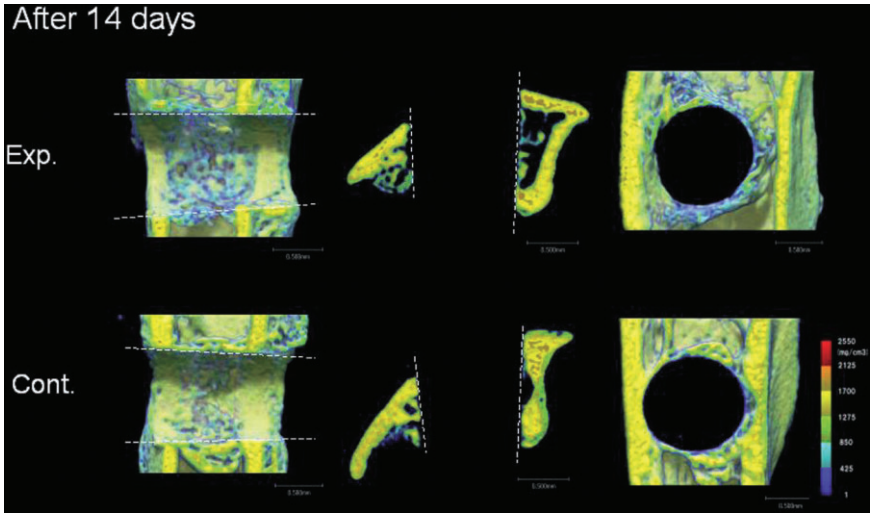

Figure 6 Three-dimensional image 14 days after implant placement. The experimental group had new bone formation in a wider area compared with the control group. However, bone formation was sparse in the bone marrow region and there was a wide region with relatively low bone mineral density. The white dotted line is the outline of the implant. Exp. = experimental group; Cont. = control group.

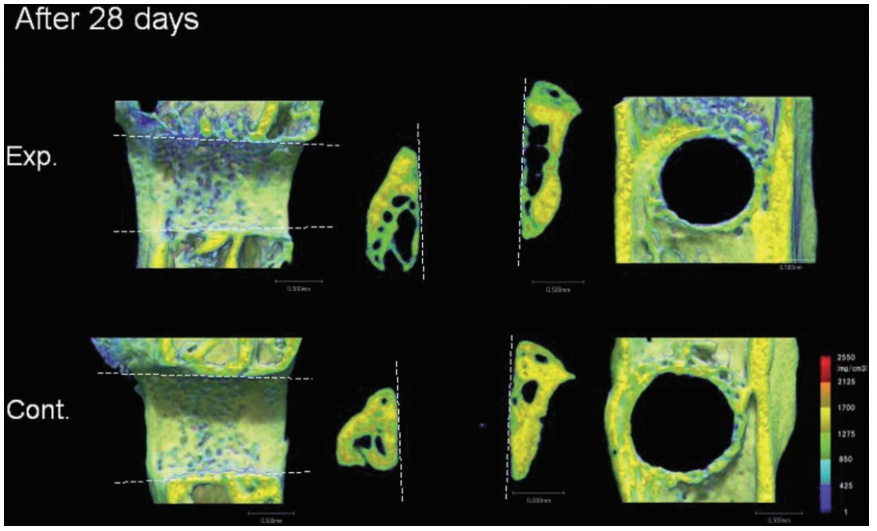

Figure 7 Three-dimensional image 28 days after implant placement. The experimental group had sparse bone formation in the bone marrow region compared with the control group. There was a mixture of areas with lamellar bone formation and mesh-like bone formation. The experimental group had a wider region with low bone mineral density compared with the control group. The white dotted line is the outline of the implant. Exp = experimental group; Cont. = control group.

BMD was examined over time in the bone marrow region within 20 μm of the implant surface. At 5 days after implant placement, BMD was significantly higher in the experimental group than in the control group. A similar trend was seen from implant placement to 7 days after placement. At 14 days onward, BMD was significantly lower in the experimental group and the difference was approximately 30–40% (Figure 9).

Changes in peri-implant BMD were measured from the distance from the interface of implant and bone in the cortical bone region. At 14 days after implant placement, BMD tended to be higher in the experimental group than in the control group in the region within 100 μm of the implant surface. However, no statistically significant difference was observed except in the region 60–80 μm from the bone-implant interface. The region closest to the implant (within 20 μm of the implant surface) had the largest difference. This difference in BMD became less noticeable the farther from the implant the measurements were taken (Figure 10).

Changes in peri-implant BMD were measured from the distance from the interface of implant and bone in the bone marrow region. At 14 days after implant placement, peri-implant BMD was significantly lower in the

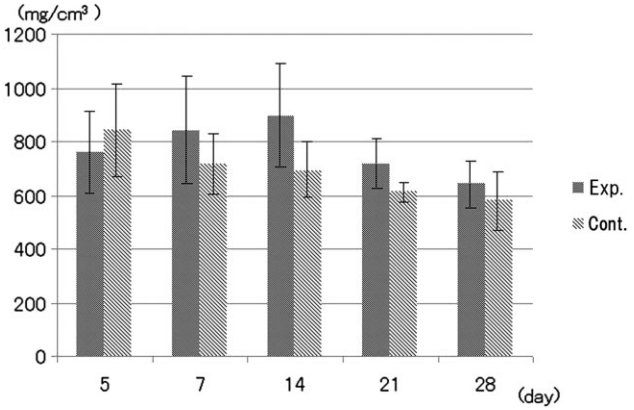

Figure 8 Cortical bone region changes in bone mineral density over time within 20 μm of the implant surface. A statistically significant difference was not observed between the experimental and control groups. Cont. = control group; Exp. = experimental group.

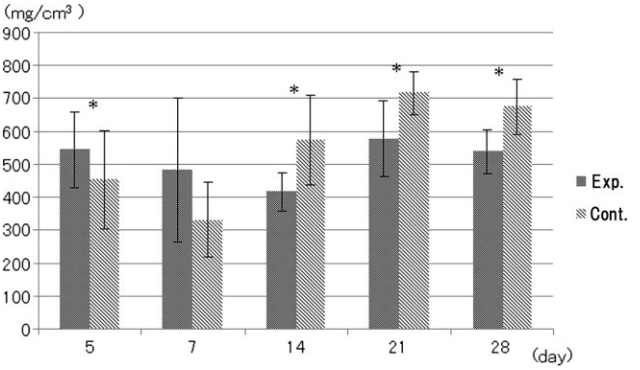

Figure 9 Bone marrow region changes in bone mineral density over time within 20 μm of the implant surface. Statistically significant differences were observed between the two groups, except at 7 days after implant placement (*$p < .05$). Cont. = control group; Exp. = experimental group.

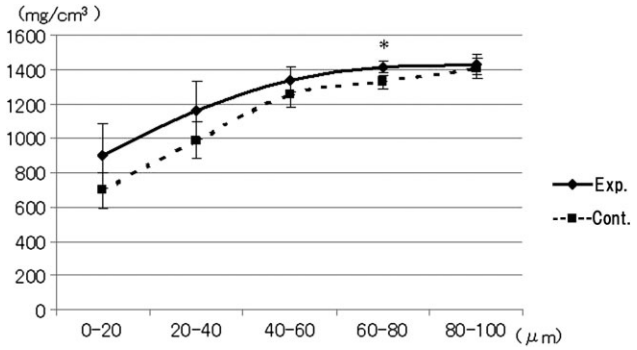

Figure 10 Cortical bone region peri-implant bone mineral density 14 days after implant placement. The differences in bone mineral density decreased between the two groups the farther away from the implant surface the measurements were taken (*$p < .05$). Cont. = control group; Exp. = experimental group.

experimental group than in the control group in all regions within 100 μm of the implant surface. The BMD difference between the experimental and control groups were almost constant regardless of the distance from the implant surface. BMD was approximately 30–40% lower in the experimental group than in the control group (Figure 11).

Examination of Peri-Implant Bone Using Light Microscopy

A light microscope was used to examine peri-implant bone in both experimental and control groups. Both groups had new peri-implant bone formation in the cortical bone and bone marrow regions 28 days after implant placement. Contact was observed between the implant surface and bone and thus osseointegration was achieved (Figure 12). Histological examination by light

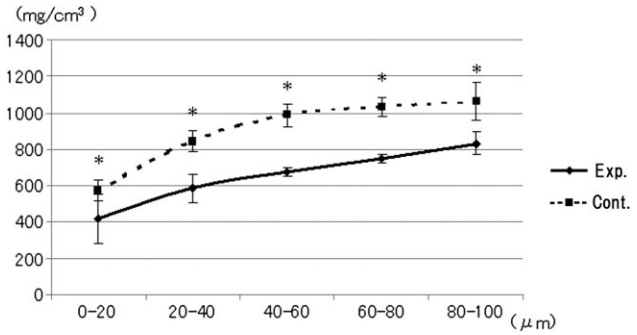

Figure 11 Bone marrow region peri-implant bone mineral density 14 days after implant placement. BMD was significantly higher in the control group than in the experimental group for all regions 0–100 μm from the implant surface (*$p < .05$). Cont. = control group; Exp. = experimental group.

TABLE 1 Bone-Implant Contact Ratio (%)

		14 Days	28 Days
CB	Cont.	52.6 ± 21.6	68.5 ± 16.8
	Exp.	49.6 ± 24.8	57.4 ± 11.5
BM	Cont.	53.9 ± 21.0	76.3 ± 12.0
	Exp.	33.8 ± 10.9	63.6 ± 7.0

BM = bone marrow; CB = cortical bone; Cont. = control group; Exp. = experimental group.

microscopy revealed no marked difference between the two groups in their bone-implant surface contact and in their peri-implant new bone. Samples used in QCT measurements were also examined by light microscopy after QCT imaging.

Evaluation of Bone-Implant Contact Ratio

In the cortical bone region, the experimental group tended to have lower BICs at 14 and 28 days after implant placement compared with those of the control group. A similar trend was seen in the bone marrow region, but there was no statistically significant difference between the experimental group and the control group (Table 1).

DISCUSSION

Elderly patients are often the target population for implant therapy. Such patients are more likely to have systemic diseases. Therefore, the effects of systemic diseases are an important issue that needs to be clarified to achieve good outcomes from implant therapy.

Alsaadi et al.[2] placed a total of 6,946 implants in 2004 patients and examined how general and local conditions of bone affected early loss of implants. They found a correlation between osteoporosis and implant loss. Slagter et al.[3] used the approach of Cochrane and examined implant therapy in osteoporotic patients. In four of 11 selected papers, they found significant bone density decrease, mineral content decrease, and implant loss in osteoporotic patients. As a result, they stated that they could not recommend dental implant therapy in osteoporotic patients. Holahan et al.[4] examined whether osteoporosis and osteopenia affect the survival rate of implants. They examined 746 women (3,224 implants), who were at least 50 years old at the time of implant placement. Their statistical analysis showed that patients diagnosed with osteoporosis or osteopenia were not significantly more likely to develop implant failure

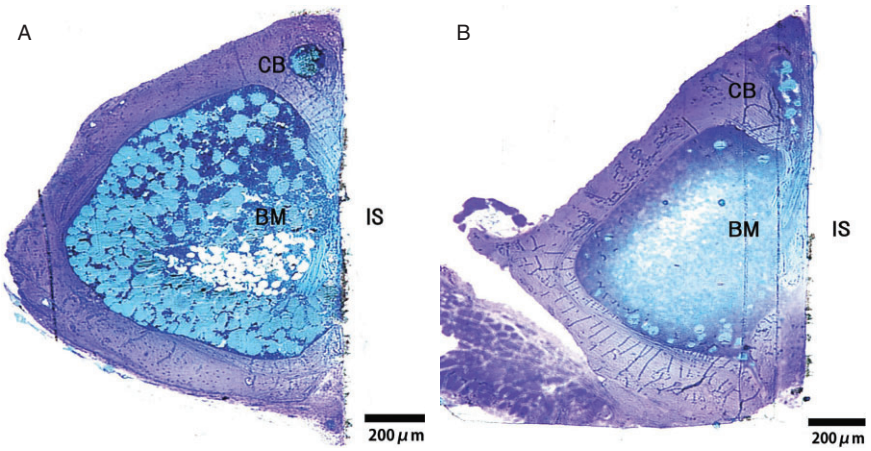

Figure 12 (A) Light microscope image of the experimental group 28 days after implant placement. New bone formation was observed in the cortical bone and bone marrow regions. (B) Light microscope image of the control group 28 days after implant placement. New bone formation was observed in the cortical bone and bone marrow regions. IS = implant space; CB = cortical bone; BM = bone marrow.

compared with those without such a diagnosis. Therefore, they concluded that a diagnosis of osteoporosis and osteopenia did not contribute to an increased risk of implant failure. Dao et al.[5] examined whether osteoporosis was a true risk factor for osseointegration in dental implant therapy. They found that osteoporosis was not a risk factor for implant failure and that there was no relationship between implant failure rate and age or sex. The conclusions from these previous studies are divided on whether or not osteoporosis is a risk factor for implant therapy and no consensus has been reached.

Giro et al.[10] conducted an experiment involving implant placement in ovariectomized rats. They found that ovariectomy caused reduction in peri-implant BMD, particularly of the bone marrow region. Glösel et al.[11] searched for studies published between 1997 and 2008 in which implants were placed in osteoporotic or diabetic rats. They reported that ovariectomy reduced osseointegration of implants. Other reports have also indicated that osseointegration was inhibited in ovariectomized rats and that implant stability was decreased.[1,12–17]

The present study used a senescence-accelerated mouse model and examined changes in peri-implant BMD. We investigated the risk of implant therapy and the methods of risk avoidance. Senescence-accelerated mice (SAM) were introduced by the Jackson Laboratory, USA, in 1968. The Chest Disease Research Institute at the Kyoto University (Japan) developed SAM by selective inbreeding of the AKR/J strain to obtain mice with signs of premature senescence. The SAM consists of two strains: senescence-accelerated prone mice (SAMP) and senescence-accelerated resistant mice (SAMR) with a normal senescence profile. SAMP is further divided into nine substrains that exhibit distinct traits. One of the substrains is SAMP6, which is an animal model for osteoporosis and has been used in previous studies.[18–22] When changes in bone mass were compared between aging SAMP6 and osteoporotic human subjects, the changes in SAMP6 were consistent with those in human senile osteoporosis. These bone mass changes were characterized by slow loss and low peak as reported by Riggs and Melton[23] for human senile osteoporosis.[18] Silva et al.[21] reported, "From 4 to 12 months, there was evidence of age-related cortical thinning in SAMR1. By contrast, SAMP6 vertebrae were unchanged from 4 to 12 months." We thought that more aged SAMP6 might be proper for this study. However, if older mice had been used, we would have been concerned that SAMR1 as control would have also been old. Therefore, we used 16-week-old (4 months) SAM in this study.

In the present study, we prepared smaller experimental titanium-coated plastic implants for mouse tibias. When the experimental implants were placed in osteoporotic mice, peri-implant new bone formation and osseointegration were observed. These implants had a titanium layer of approximately 100–150 nm thickness and they were similar to the experimental implants used for rats by Okamatsu and Morinaga et al.[7,8] These implants are suitable for microscopy because the titanium layer is very thin. In previous studies, the implant bodies were metallic so artifacts were observed in the micro-QCT images, which hindered examination

of areas near the implants.[24–27] In the present study, the titanium layer of the implant was very thin, as in the report by Morinaga et al. and artifacts were not observed. Thus, areas adjacent to the implant surface could be examined and BMD could be measured.[8,28]

In our previous study, we reported that tissue with high BMD was observed by micro-QCT in the area furthest from the implant surface and we speculated that bone formation was initiated a short distance (30–50 μm) away from the implant surface.[8] In our other report, we found that BMD with the highest value was at a distance of 56 μm from the implant surface.[28] Therefore, we targeted an area 100 μm from the implant surface in this study. We calculated BMDs in regions 200 μm high in the cortical bone and bone marrow. Because that the thickness of cortical bone was 230–300 μm.

When BICs were examined in tissue sections by light microscopy, the ratios in the experimental group tended to be lower than those in the control group, but this did not reach statistical significance. In the bone marrow region, analysis using micro-QCT showed that the bone density of the experimental group was significantly lower than that of the control group. In the evaluation of peri-implant bone, a combination of tissue section evaluation and observations using QCT was thought to be effective. In the present study, peri-implant BMD was 30–40% lower in the experimental group (osteoporosis model) than in the control group for the bone marrow within 20 μm of implant surface.

In addition, peri-implant BMD was significantly lower in the experimental group (osteoporosis model) in a relatively wide area at a time important for osseointegration, that is, 14 days after implant placement. The results of this study cannot be directly applied to the clinical situation because the present study used mice. However, these results are consistent with clinical reports that found that implant treatment outcomes in osteoporotic patients were worse compared with the outcomes in healthy patients. Thus, these results suggest that osteoporosis should be considered as a risk factor in implant therapy. In the cortical bone region, there was no significant difference in peri-implant BMD between the experimental group (osteoporosis model) and the control group. This finding suggests that if the cortical bone region is effectively used in implant placement, there might be no difference in BMD between osteoporotic patients and healthy individuals.

In both the experimental and control groups, the bone density near the implant surface decreased in the bone marrow region from the time of implant placement to 7 days after placement. Thereafter the bone density increased. This change in bone density was greater in the control group than in the experimental group and the bone density of the experimental group was relatively constant. The difference in the bone density change may involve abnormalities of bone marrow stem cells, which would reduce osteogenic potential in the experimental group. The bone density of the experimental group was greater than that of the control group at 5 days after implant placement, but the cause is unknown. However, post-operative histological reactions may be involved.

In the present study, the experimental implants had mirror-like smooth surfaces. In contrast, various surface treatments have been used for clinical implants, which can improve osseointegration between the implant surface and bone. Therefore, the risk of osteoporosis might be reduced. However, the low peri-implant bone density shown in the present study can be a potential risk for any type of implant. Thus, implant therapy should be performed carefully in osteoporotic patients.

CONCLUSIONS

Newly developed experimental titanium-coated plastic implants were prepared for mice tibias. These implants were effective for micro-QCT because artifacts were undetectable. The results of this study using a mouse osteoporosis model showed lower BMD in the bone marrow region. This suggests that senile osteoporosis might be a risk factor for implant therapy. In contrast, the experimental group (osteoporosis model) and the control group had no difference in peri-implant BMD in the cortical bone region suggesting that risk might be avoided by careful implant placement that effectively uses cortical bone. However, further investigations are needed to clarify the relevance between our results and the clinical situation.

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