Ultrastructure of the Interface Between Titanium and Surrounding Tissue in Rat Tibiae – A Comparison Study on Titanium-Coated and -Uncoated Plastic Implants

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ABSTRACT

Purposes: The purposes of this study were to prepare experimental titanium-coated plastic implants suitable for electron microscopy examination of the titanium-bone interface and the response of tissue surrounding titanium, and to histologically compare surrounding tissue responses in coated and uncoated implants.

Materials and Methods: Experimental plastic implants were prepared from a plastic rod coated with a thin film of titanium. Plastic implants without coatings were used as controls. The implants were placed into tibiae of 10-week-old male rats. The specimens with implants were harvested 4 weeks after placement and observed under a light microscope, a transmission electron microscope, and a scanning electron microscope.

Results: In the transmission electron microscopy, the titanium layer of the experimental implant was a uniform layer that was approximately 150- to 250-nm wide. The new bone formation was observed around both titanium-coated implants and plastic implants. However, there was no direct bone contact with the plastic implant.

Discussion: The responses of tissue surrounding the experimental implants varied. Under an electron microscope, the following areas were observed: (1) an area with a direct contact between the titanium and bone, (2) an area at the interface where an amorphous layer was observed, (3) an area with progressing calcification in the surrounding tissue where the cells were adjacent to the titanium surface, and (4) an area in which bone resorption and apposition were observed and remodeling was thought to be occurring.

Conclusion: The experimental titanium was homogenous and was considered to be highly useful in observing the responses of the surrounding tissue to the titanium surface.

KEY WORDS: osseointegration, plastic implant, rat, tissue-implant interface, titanium coating, ultrastructure

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Today, titanium implants have become mainstream. The essential condition of their success is the occurrence of a direct contact between bone and titanium implant surface, osseointegration.[1–4] Osseointegration has been evaluated by observing the bone interface at the level of light microscopy (LM) and comparing the bone–implant surface contact rate. It has also been evaluated by comparing the removal torque or force necessary for pushing out implants placed in animals.[5–17] These studies made evident that implants with rough surfaces were better in achieving osseointegration than implants with machined surfaces.[12,13] Electron microscopic studies have been conducted in order to investigate the ultrastructure of osseointegration. In many reports, because implants were removed in the course of

preparing specimens for electron microscopy, completely intact interfaces were not observed. A method of coating a thin film of titanium on plastic has been used in order to examine an intact titanium-bone interface. However, there have been only a few such reports in the past 20 years.[18–23] In addition, opinions in these reports have not necessarily been consistent regarding the existence of an amorphous layer at the titanium-bone interface and the response of surrounding tissue. One cause of such inconsistencies in opinions is the relative difficulty of achieving a homogenous titanium coating on plastic. Also, a few reports lack detailed explanations of the titanium coating method and its characteristics.

In recent years, indications for implant treatment have broadened because of its combination with bone regeneration therapy. To evaluate these treatment methods, it is necessary not only to take a molecular biological approach but also to evaluate calcification kinetics and morphology of cells related to calcification. Establishment of a standard experimental system for titanium-coated implants should make a large contribution to the progress of future studies.

The first objective of this study was to prepare experimental titanium-coated plastic implants suitable for electron microscopy examination of the titanium-bone interface and the response of tissue surrounding titanium. The second objective was to place the experimental implants into the tibiae of rats and to histologically compare the titanium-bone interfaces and surrounding tissue responses 4 weeks after placement in coated and uncoated implants. In doing so, specific responses of bone tissue against titanium could be made evident.

MATERIALS AND METHODS

An experimental implant was prepared from a plastic rod (acrylic resin) of 1.6 mm in diameter and 7 mm in length. Its axial surface was coated with a thin film of titanium. A DC magnetron sputtering apparatus (L332S-FHS, ANELVA, Tokyo, Japan) was used for titanium coating.[24] A 99.9% pure titanium target was used in sputtering, which was performed under the conditions of 300 W DC power, 20 minutes in 2.2×10^{-1} Pa argon (Table 1 and Figure 1). After the titanium coating, the thin film of the surface of an experimental implant was analyzed using an electron probe micro-analyzer (EPMA) (JXA-8900 L, JEOL, Japan). Plastic implants without coatings were used as controls.

TABLE 1 DC Sputtering Apparatus Condition

- Target: 99.9% Ti
- DC power: 300 W
- Sputtering gas: Ar
- Ar pressure: 2.2×10^{-1} Pa
- Deposition time: 1200 seconds
- Deposition temperature: room temperature
- Distance between the Ti target and plastic implant: 90 mm

Specimens were obtained 4 weeks after implant placement to obtain detailed observation of the titanium-bone interface after achieving osseointegration, because several investigators reported the successful osseointegration 4 to 12 weeks after implant placement.[18–23,25,26]

Twenty 10-week-old male Sprague Dawley rats were used in the experiment. After ether inhalation anesthesia, an intraperitoneal injection of pentobarbital sodium (0.1 mg/100 g) was given to each rat. When each rat became unconscious, its fur surrounding the bilateral knee joints was shaved. An incision of approximately 15 mm was made from the knee joint along the anterior border of the tibia. Then, the bone surface was exposed. An implant site was placed 10 mm inferiorly from the apical region of the knee joint, and the socket was prepared so that it penetrated from the tibial medial to lateral side with 1.6 mm in diameter. The experimental implant was placed in the implant site of one tibia and the control implant was placed in the implant site of the other tibia (Figure 2). The experimental implants were carefully placed to keep the titanium coating intact. After the placement of implants, the periosteal flaps

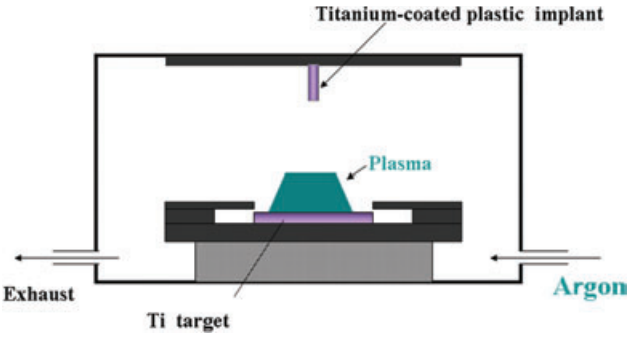

Figure 1 The schema of the DC magnetron sputtering apparatus. A 99.9% pure titanium target was used. The apparatus was filled with argon. The generated argon plasma and target were used to produce titanium ions, and the plastic implant surface was coated.

were sutured into appropriate positions, and the surgical treatment was then completed. This study was carried out under the control of the committee in accordance with "The Guidelines on Animal Experiments" at Fukuoka Dental College (approval number 03005).

For transmission electron microscopy (TEM) and LM, an intraperitoneal anesthesia was administered to the 20 rats 4 weeks after implant placement, similar to implant placements. The tibiae were fixed by vascular perfusion with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4) through the ascending aorta for 5 minutes. After perfusion, the tibiae with implants were cut, and the specimens were immersed in the same fixative for 24 hours at 4°C. Then, they were immersed in 10% EDTA for 3 weeks for decalcification. Post-fixation was performed in 2% osmium tetroxide for 2 hours at 4°C, and then block staining was performed with 0.25% uranyl acetate overnight at 4°C. After dehydration through a graded series of ethanol and then propylene, they were embedded in Quetol 651 (Nisshin EM, Kyoto, Japan). Semi-thin sections (0.5 µm) and thin sections (70 nm) were stained with toluidine blue and double stained with uranyl acetate and lead citrate, respectively. The observations were performed using a light microscope (BX51-DP 12, OLYNPUS, Tokyo, Japan) and a transmission electron microscope (1200-EX, JEOL) at 80 kV.

For scanning electron microscopy (SEM), the specimens were fixed using the same methods as the above-mentioned. Then, a microtome (BS 3000, EXAKT, Norderstedt, Germany) was used to prepare a specimen approximately 3- to 4-mm thick, which was sectioned in the central plane parallel to the long axis of the implant body. Postfixation in osmium tetroxide and block staining were performed. After dehydration in a graded series of ethanol and isoamyl acetate, they were critical-point dried with liquid CO_2 and coated with platinum. The central plane of the implant was examined with an electron microscope (JSM-6330 F, JEOL) at 5 kV.

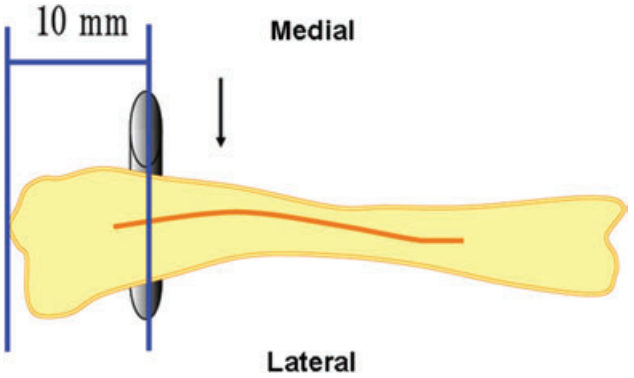

Figure 2 Implant placement in rat tibia. An experimental implant was placed in the rat tibia approximately 10 mm from the knee joint and in the direction from the medial to the lateral side. The arrow shows the direction of implant placement.

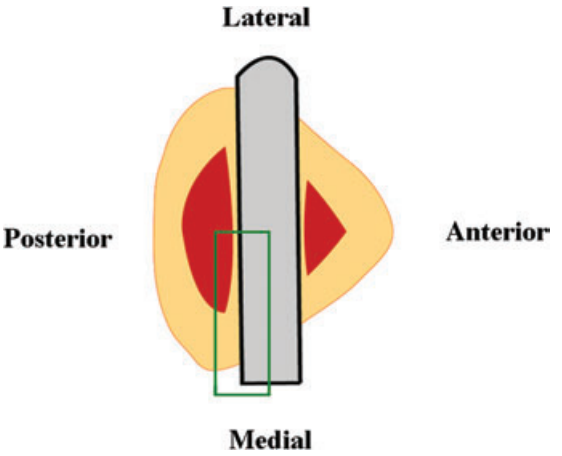

Figure 3 Cross section of rat tibia with the experimental implant. The mechanical effect of muscles at the observed region was taken into consideration in determining the medial posterior direction. The region that was microscopically examined is framed in square.

The observed region was in the medial posterior area of the tibia to avoid the effects of stress caused by muscular activities (Figure 3). This area has no muscle attachments and is less likely to experience any effect of external force. Therefore, this area was considered well suited for observing responses of the tissue surrounding an implant. We decided cell types by morphological characteristics, ultrastructure in cells, and the locations.

RESULTS

Surface Properties of an Experimental Implant

The surface of an experimental implant had a metallic luster and macroscopically appeared smooth with a mirrorlike surface. By SEM examination, striations that were axially and parallel to the surface were observed. These striations were thought to have been created when plastic was molded (Figure 4). As a result of EPMA, a titanium (Ti) peak was found other than the peak from carbon (C) from the plastic. Therefore, we were able to confirm that a thin titanium film was coated on the experimental implant surface without the incorporation of impurities (Figure 5).[27]

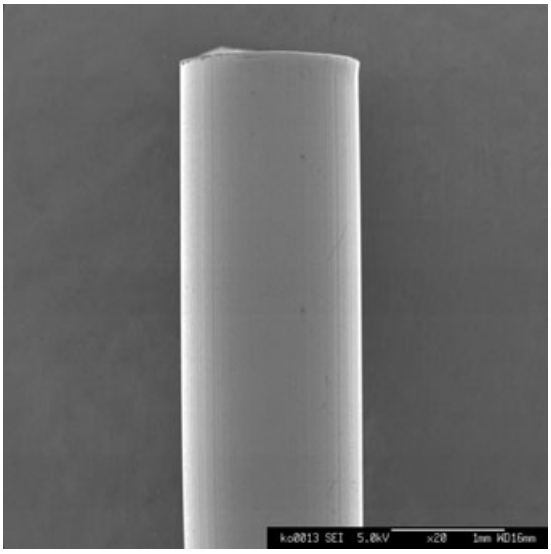

Figure 4 Scanning electron microscopy image of the experimental implant. The surface was mostly smooth, and the surface morphology of the plastic prior to coating was mostly preserved.

Histological Evaluation of a Control Implant

As controls, plastic implants without titanium coatings were used. Under a light microscope, a new bone formation surrounding the plastic implants was observed, but a direct contact between the plastic implant surface and bone was not observed. A new bone was formed starting from the cortical bone region and extended toward the side of the marrow, covering the entire surroundings of an implant with almost the same width. On the marrow side, there were very small areas, which in part had no bone. A layer of soft tissue with many flat cells and blood vessels existed between the implant and bone. This layer was observed almost all around the implant. Between the cortical bone area and the marrow area, there was no morphological difference in the interface of the implant and the surrounding tissue (Figure 6).

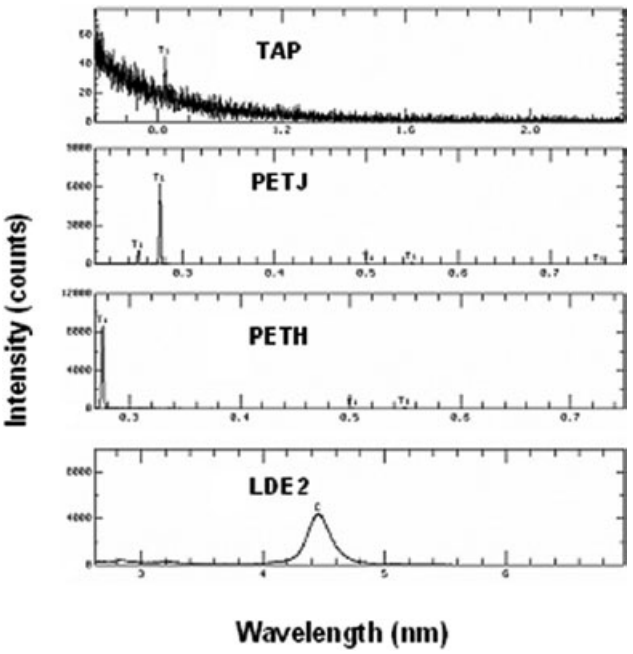

Figure 5 Electron probe micro-analyzer data of the experimental implant. The analyzing crystals for EPMA qualitative analysis were thallium acid phthalate (TAP), pentaerythritol(J) (PETJ), pentaerythritol(H) (PETH), and layered dispersion element 2 (LDE2). Titanium (Ti) was detected in addition to carbon (C) from the plastic, and the presence of titanium on the surface was confirmed. Other impurities were not detected.

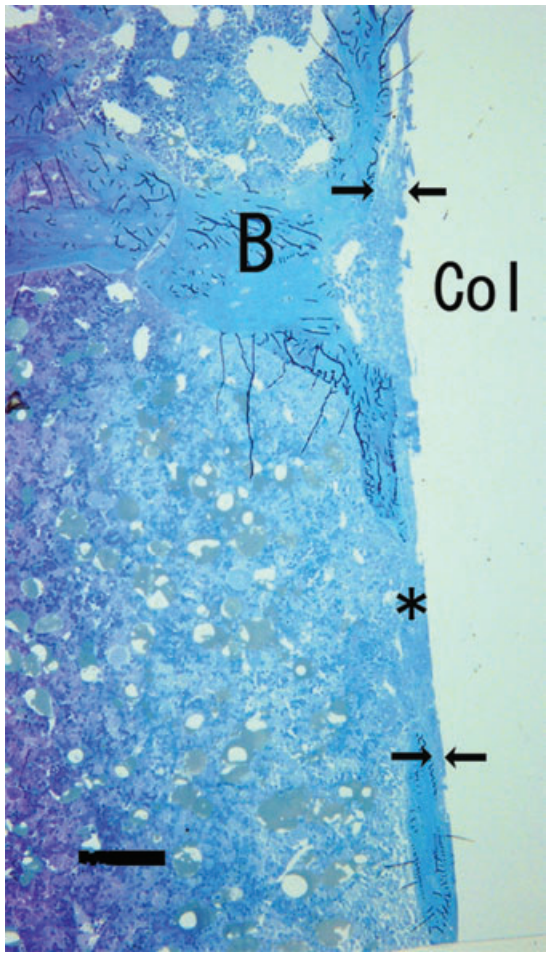

Figure 6 Light micrograph of the tissue surrounding the control implant. A new bone (B) formation was observed in the surroundings of the control implant. A few layers of cells were seen between the control implant (CoI) and new bone (shown between the arrows). In a narrow area on the marrow side, there was a region in which no bone formation was observed in the implant surroundings (*). Bar = 50 μm.

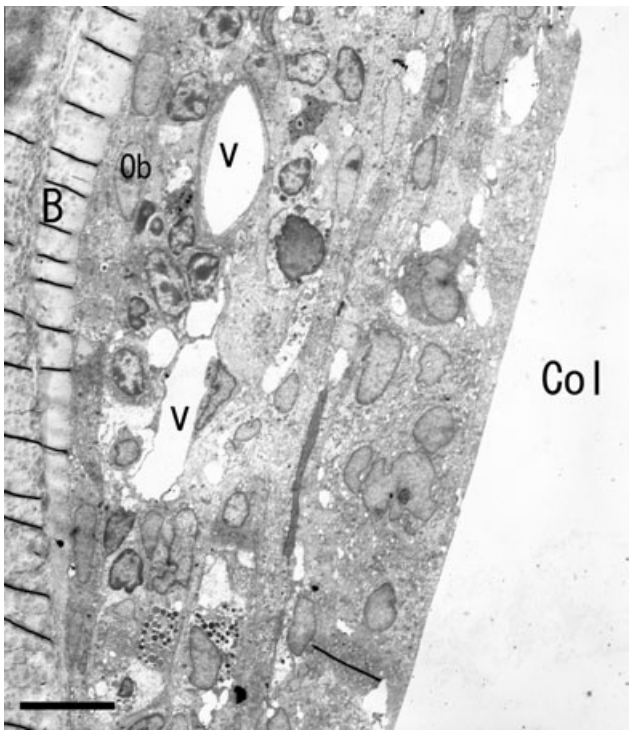

Figure 7 Electron micrograph of the control implant. A few layers of cells were observed between the control implant (CoI) surface and the bone (B) surface. Osteoblast-like cells (ob) were observed on the bone surface. Blood vessels (V) were observed between the control implant surface and bone surface. Bar = 10 μm.

In the observation of control implants under TEM, bone formation was observed around the implant, and osteoblasts in contact with the osteoid-like tissues were seen. There was a distance of approximately 1 to 50 μm between the implant surface and bone, and multinuclear and mononuclear cells which were relatively flat were layered in between the implant and bone. The layer contained blood vessels and white blood cells. On the new bone surface formed surrounding the implant, cell processes extending from the osteoblasts toward the bone were observed. The osteoblasts had well-developed Golgi complexes, and numerous mitochondria and rough endoplasmic reticula. In addition, multinuclear and mononuclear cells, which were relatively flat with few organelles, were observed contacting the implant (Figure 7).

Experimental Implants

Observation using a light microscope or a scanning electron microscope revealed that the titanium coating of the experimental implant was a uniform thin layer. A new bone formation was observed surrounding the implant (Figures 8 and 9). Under the scanning electron microscope and light microscope, we observed that much of the new bone was in direct contact with the titanium layer. There was no morphological difference between the cortical bone side and marrow side of the titanium-bone interface.

In the TEM, the titanium layer of the experimental implant was a uniform layer that was approximately 150- to 250-nm wide. The titanium layer and bone were adjacent to each other for almost the entire implant surface. There were areas in which a mature bone was in contact with titanium and areas in which a layer with a relatively low calcification level existed between the bone and the titanium. In areas where a mature bone was in contact with the titanium, we observed on the titanium surface a structurally indistinct layer, approximately 30- to 60-nm wide, with a low electron density. Adjacent to this layer, we observed another structurally indistinct layer, approximately 100- to 200-nm wide, with a high electron density (Figure 10). At the interface where layers with different densities were not observed clearly, collagen fibers were adjacent to the titanium surface

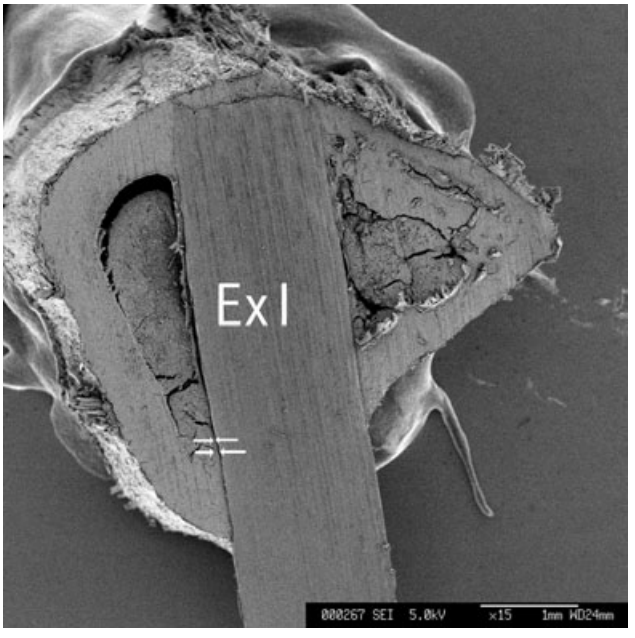

Figure 8 Scanning electron micrograph of the experimental titanium-coated implant. A cross section of rat tibia with the experimental implant (ExI). A new bone (shown between the white arrows) was observed along the experimental implant surface. Bar = 1 mm.

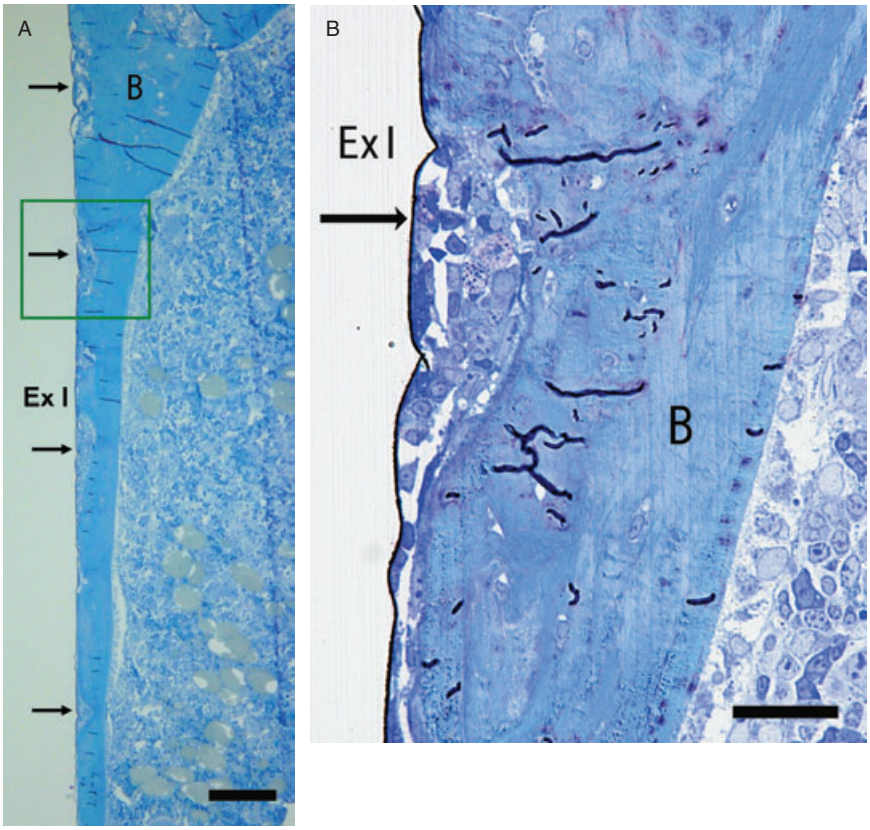

Figure 9 *A,* Light micrograph of the experimental titanium-coated implant. A new bone (B) was observed along the experimental implant (ExI), and much of the new bone was in direct contact with the implant. Parts of the implant surface were in contact with clusters of cells (arrows). Bar = 100 µm. *B,* Enlargement of the green frame in *A*. Bar = 20 µm.

(Figure 11). The course of collagen fibers was clearly observed in the bone or osteoid, which was thought to have a low calcification level. The course of collagen was not clearly observed in the existing mature bone (see Figure 11). These fibers ran parallel to the titanium surface near the implant surface.

From the osteocytes found near the titanium layer, cytoplasmic processes extending toward the titanium layer were observed. The osteocytes resided in lacunae within the calcified interstitial substance and had slender processes in the canaliculi. These processes reached the titanium layer and extended along the titanium layer (Figure 12).

A few types of cells, which contacted the titanium layer on the cortical bone side and marrow side, were observed. In particular, many flat, multinuclear cells in contact with the titanium surface were observed (Figures 9 and 13).

In the area where the osteoblasts were in contact with the titanium layer, we observed their cell processes extending toward the osteocytes in the surrounding bone. In the surroundings of these cells, we clearly observed the course of collagen fibers (Figure 14).

DISCUSSION

To make possible the observation of the implant-bone interface and its relationship with the surrounding tissue using an electron microscope, experimental plastic implants coated with titanium were prepared. It has become evident that implant surface properties affect the response of the surrounding bone, and the coating quality is an important factor in examining the responses of the surrounding bone tissue.

In this study, we used a DC magnetron sputtering machine with the titanium target below a holder of the specimen, and prepared the experimental implants without the adhesion of impurities to the surface of the specimens. As a result, we were able to coat the implant with a titanium layer with homogeneity, a width of approximately 150 to 250 nm, and low amounts of

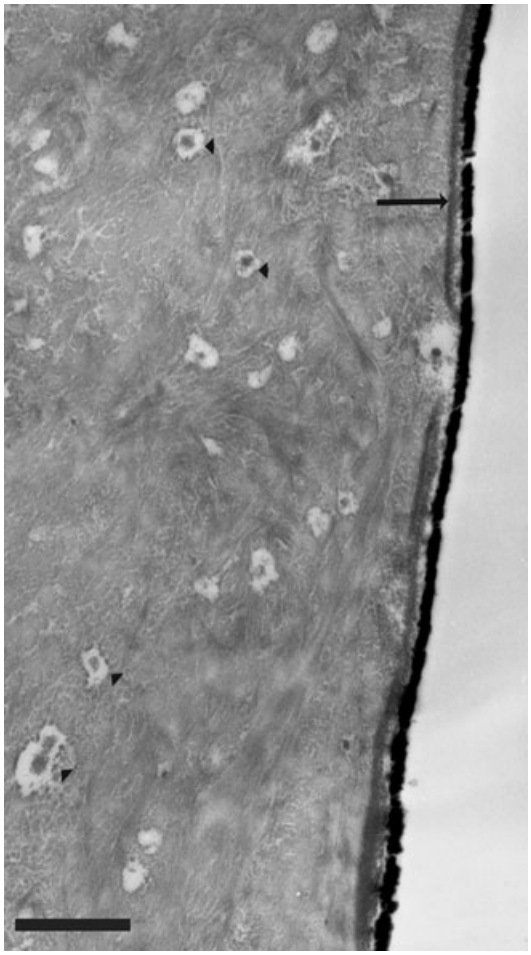

Figure 10 Electron micrograph of the experimental titanium-coated implant. The titanium layer was in contact with relatively mature bone tissue. Two layers with different electron densities were observed adjacent to the titanium layer. One layer had a low electron density and the other had a high electron density (arrow). A cross section of bone canaliculi was observed in the bone tissue (arrowheads). Bar = 1 μm.

impurities. The implant-bone interface was observed using a transmission electron microscope. As a result, we confirmed the osseointegration of the experimental titanium-coated implants. There were some parts of the specimens in which the titanium separated from the surrounding tissue during the process of preparing the specimens. However, no detachment of the titanium coating occurred in vivo, and no migration of the titanium coating occurred into the tissue.

A new bone formation was observed around both titanium-coated implants and plastic implants, and cell attachment on both implant surfaces was also observed. However, there was no direct bone contact with the plastic implant. Therefore, the bone formations in the nearest region to the implant surface, within 1 to 50 μm, depended on the implant material, titanium or plastic. Zakiah and colleagues[28] compared osteoblast proliferation and gene expression on different materials. They concluded that all the titanium-surface studies supported cellar growth and the temporal expression of an array of bone-related genes and transcription factors.[28–32] However, their results show no significant differences in osteoblast gene expression between plastic and titanium surfaces. Other unknown factors may participate in the osteogenesis on an implant surface in a living bone.

In this study, a 30- to 60-nm-wide layer with a low electron density was observed contacting the titanium surface. Contacting this layer, we observed a layer with a high electron density and it was as wide as or wider than the low-electron density layer. The structures of these layers are indistinct, and the layers differed in structures from the bone which included the surrounding collagen fibers. However, these layers were not clear in the area that was considered to have a high level of bone maturity, and the existence of an amorphous layer

Figure 11 Electron micrograph of the experimental titanium-coated implant. A layer with high electron density, which contacted the titanium layer, was not observed at a site where mature bone tissue was in contact with the titanium layer. Collagen fibers were distinct near the titanium layer, but collagen fibers were indistinct far from the titanium layer. Bar = 400 nm.

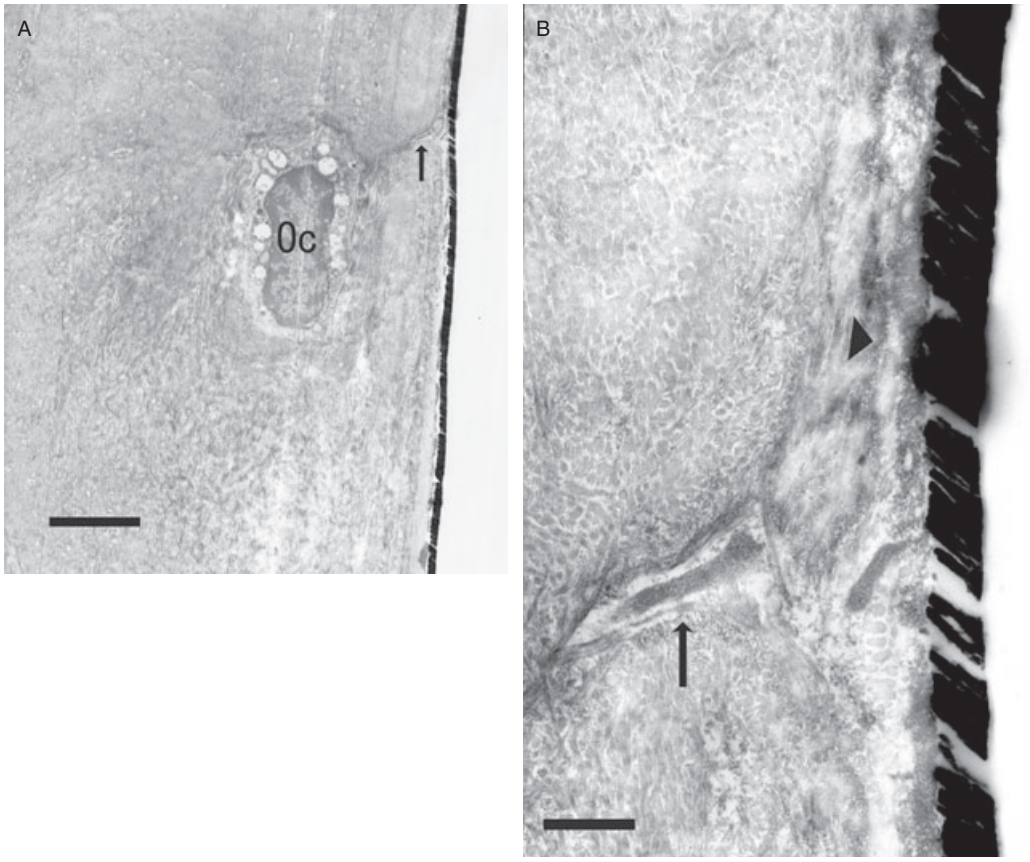

Figure 12 *A,* Electron micrograph of the experimental titanium-coated implant. Osteocytes (Oc) were observed in the bone tissue near the titanium layer. The cell processes of the osteocytes (arrow) were extended toward the titanium layer and were adjacent to the titanium layer. Bar = 4 µm. *B,* Enlargement of *A*. Cell processes extending toward the titanium layer (arrow) were spreading along the titanium layer (arrowhead). Bar = 500 nm.

might have some relationship with the maturity of the bone. Bone canaliculi were sometimes observed in the electron dense layer. These canaliculi were believed to be the cell processes extending from the osteocytes toward the titanium layer. Sennerby and colleagues[33] reported that the lamina limitans-like structure adjacent to the amorphous layer was continuous with the canaliculi of the osteocyte surroundings. Their findings were considered to be similar to those in our study.

Among the studies of the titanium-bone interface using a transmission electron microscope, a few studies reported that an amorphous layer existed 20 to 500 nm from the implant surface.[3,4,9,34–37] An abundance of proteoglycans was observed in this amorphous layer using immunostaining and ruthenium red staining.[14,22,38] Proteoglycans on the implant surface are thought to play some roles in the achievement of bony union. Because structures and compositions of the cement line in the bone and the bone-implant interface are similar, Nanci and colleagues[35] stated that body response to a biomaterial could be analogous to the bone formation process occurring at the bone interface in the body. However, the existence of an amorphous layer has been disputed in some studies using similar TEM.[20,39] Budd and colleagues[40] removed titanium screw implants by a fracture technique and made observations using TEM. As a result, they stated that the electron dense layer observed at the bone-implant interface was a residual oxidized titanium layer of the implant body. Depending on the interpretation of the researcher, the term *amorphous layer* itself can indicate different areas of an indistinct structure in the implant surroundings.

In our study, a few types of cells contacting the titanium layer were observed to be surrounded by bone or osteoid-like tissue. These cells included not only bone-forming cells but also multinuclear cells, indicating possible bone remodeling. Cell processes of osteocytes near the implant surface were also observed extending along

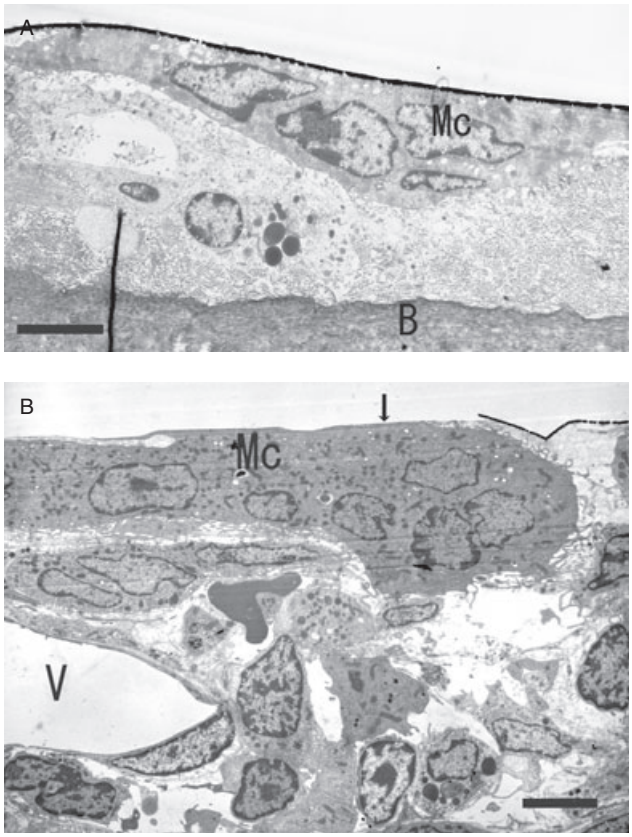

Figure 13 *A,* Electron micrograph of the experimental titanium-coated implant. Multinuclear cells (Mc) were observed contacting the surface of the titanium layer. In the surroundings, other cells and collagen fibers were observed clearly. The course of collagen fibers was not clearly observed in the existing bone (B) far from the titanium layer. Bar = 4 μm. *B,* Further enlargement of multinuclear cells on the titanium surface. Many cell processes were observed on the cellular surface and many mitochondria were observed intracellularly. A portion of the titanium (arrow) may detach during preparation of the specimen. Bar = 5 μm.

the titanium surface toward the implant surface. This state indicated the possibility that information at the implant surface was transmitted to the osteocytes. Furthermore, cell processes of multinuclear cells were observed extending parallel to the implants, and these processes were adjacent to the processes of the osteoblast-like cells. This state indicated the possibility that information was being communicated among the cells near the implant surface.

Collagen fibers in the bone were in contact with the titanium layer in some areas in our observations. We observed that the distance from the titanium layer did not affect the size of the collagen fibers. The collagen fibers were indistinct in the existing bone far from the titanium layer, but collagen in the newly formed bone was distinct. Therefore, the characteristics of the collagen fibers were thought to be affected by the difference in bone calcification levels. Listgarten and colleagues[21] reported that the density of collagen near the titanium layer was low, and the collagen diameter was small. These findings suggested that the presence of the implant body affected the properties of the surrounding bone. In our study, similar findings were made in areas thought to have relatively low calcification. However, as mentioned previously, there was no difference in structure between the areas near and far from the implant in the mature bone.

In our study, osteoblasts were observed adjacent to the implant surface. The osteoblasts were thought to contribute to the calcification occurring in between the existing bone and implant surface. A layer of approximately 5 μm with low calcification level existed between the existing bone and osteoblasts, and collagen fibers

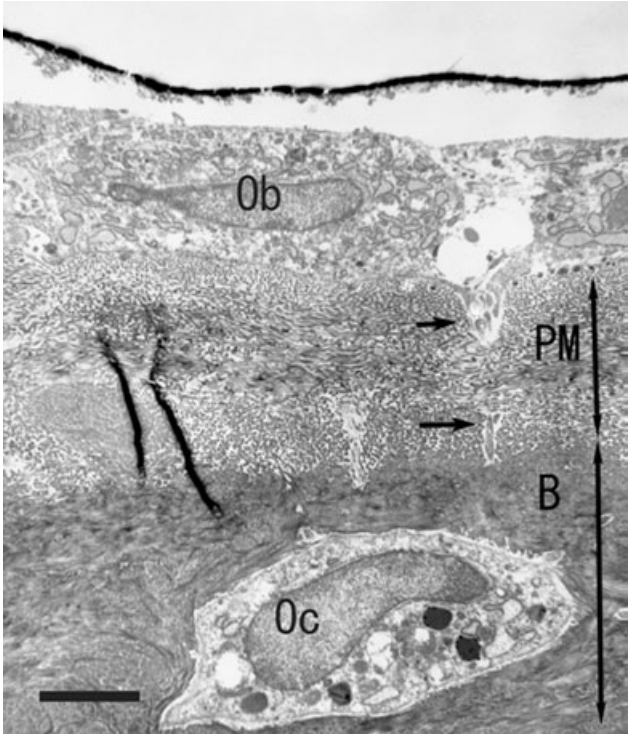

Figure 14 Osteoblasts on the titanium surface. In the process of preparing the specimen, the titanium layer became detached from the surface, but osteoblasts (Ob) adjacent to the implant surface were observed with their cell processes (arrow) extending toward the bone. A 5-μm-wide layer with low calcification level (PM) was observed between the existing bone and osteoblasts. Collagen fibers were observed clearly in this layer. Osteocytes (Oc) were in the bone, and their cell processes were observed extending toward the titanium layer. Bar = 2 μm.

were observed to be distinct. The course of collagen fibers was irregular, and the fibers included structures believed to be cytoplasmic processes of osteoblasts. Granules with a high electron density were dispersed in the collagen fibers, and this layer was speculated to be in the process of calcification. Structures thought to be cytoplasmic processes from osteocytes in the existing bone extended toward the osteoblasts, and these structures could have contributed to the intercellular communication of information. This type of phenomenon could be one of the causes resulting in the difference between the responses of tissues surrounding the control implants and the titanium-coated implants. A new bone was observed to form from the side of the existing bone in the gap between the titanium surface and the existing bone. There was no evidence that the new bone formed from the titanium surface.

In our model, the interface between the titanium and the surrounding tissue could be observed without damages. In this study, only smooth surfaces were investigated. However, other studies have reported excellent results from the clinical use of various types of rough surfaces prepared by treatments such as anodic oxidation, acid etching, blasting, and etching and blasting. Therefore, rough surfaces should be used in our model, and more investigation is necessary to decide the ultrastructure of tissue surrounding a rough surface implant.

CONCLUSION

1. We confirmed the osseointegration with the experimental titanium-coated implants.
2. The experimental titanium coating prepared in this study was homogenous and was considered to be highly useful in observing the responses of the surrounding tissue to the titanium surface.
3. The responses of tissue surrounding the implants varied 4 weeks after placement. Under an electron microscope, we observed the following areas: (1) an area with direct contact between the titanium and bone, (2) an area at the interface where an amorphous layer was observed, (3) an area with progressing calcification in the surrounding tissue where the cells were adjacent to the titanium surface, and (4) an area in which bone resorption and apposition were observed and remodeling was thought to be occurring.
4. All around the control implants, a new bone formation was observed with a cellular layer approximately 1- to 50-μm wide in between the bone and the implant.

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REFERENCES

1. Albrektsson TO, Johansson C, Sennerby L. Biological aspects of implant dentistry: osseointegration. Periodontol 2000 1994; 4:58–73.
2. Brånemark PI. Osseointegration: biotechnological perspective and clinical modality. In: Brånemark PI, Rydevik BL, Skalak R, eds. Osseointegration in skeletal reconstruction and joint replacement. Chicago, IL: Quintessence, 1997: 1–24.
3. Albrektsson T, Brånemark PI, Hansson HA, Lindström J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand 1981; 52:155–170.
4. Albrektsson T, Jacobsson M. Bone-metal interface in osseointegration. J Prosthet Dent 1987; 57:597–607.
5. Cook SD, Kay JF, Thomas KA, Jarcho M. Interface mechanics and histology of titanium and hydroxyapatite-coated titanium for dental implant applications. Int J Oral Maxillofac Implants 1987; 2:15–22.
6. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: a 1-yr follow-up of removal torque of titanium implants. Int J Oral Maxillofac Implants 1987; 2:69–75.
7. Carlsson L, Röstlund T, Albrektsson B, Albreksson T. Removal torques for polished and rough titanium implants. Int J Oral Maxillofac Implants 1988; 3:21–24.
8. Weinlaender M, Kenney EB, Lekovic V, Beumer J, Moy PK, Lewis S. Histomorphometry of bone apposition around three types of endosseous dental implants. Int J Oral Maxillofac Implants 1992; 7:491–496.
9. Martinez-Gonzalez JM, Garcia-Saban F, Ferrandiz-Bernal J, Gonzalo-Lafuente JC, Cano-Sanchez J, Braona-Dorado C. Removal torque and physico-chemical characteristics of dental implants etched with hydrofluoric and nitric acid. An experimental study in beagle dogs. Med Oral Patol Oral Cir Bucal 2006; 11:E281–E285.

10. Shalabi MM, Wolke JGC, Jansen JA. The effects of implant surface roughness and surgical technique on implant fixation in an in vitro model. Clin Oral Implants Res 2006; 17:172–178.
11. Büchter A, Joos U, Wiesmann HP, Seper L, Meyer U. Biological and biomechanical evaluation of interface reaction at conical screw-type implants. Head & Face Medicine 2006; 2:5. http://www.head-face-med.com/content/pdf/1746-160X-2-5.pdf.
12. Sennerby L, Thomsen P, Ericson LE. A morphometric and biomechanic comparison of titanium implants inserted in rabbit cortical and cancellous bone. Int J Oral Maxillofac Implants 1992; 7:62–71.
13. Brånemark R, Öhrnell LO, Nilsson P, Thomsen P. Biomechanical characterization of osseointegration during healing: an experimental in vivo study in the rat. Biomaterials 1997; 18:969–978.
14. Buser D, Schenk RK, Steinemann S, Fiorellini JP, Fox CH, Stich H. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. J Biomed Mat Res 1991; 25:889–902.
15. Larsson C, Thomsen P, Aronsson BO, et al. Bone response to surface-modified titanium implants: studies on the early tissue response to machined and electropolished implants with different oxide thicknesses. Biomaterials 1996; 17:605–616.
16. Gottlander M, Albrektsson T. Histomorphometric studies of hydroxyapatite-coated and uncoated CP titanium threaded implants in bone. Int J Oral Maxillofac Implants 1991; 6:399–404.
17. Kido H, Saha S. Effect of HA coating on the long-term survival of dental implant: a review of the literature. J Long Term Eff Med Implants 1996; 6:119–133.
18. Albrektsson T, Brånemark PI, Hansson HA, Ivarsson B, Jönsson U. Ultrastructural analysis of the interface zone of titanium and gold implants. Clinical Applications of Biomaterials 1982; 4:167–177.
19. Linder L, Albrektsson T, Brånemark PI, et al. Electron microscopic analysis of the bone-titanium interface. Acta Orthop Scand 1983; 54:45–52.
20. Albrektsson T, Hansson HA. An ultrastructural characterization of the interface between bone and sputtered titanium or stainless surfaces. Biomaterials 1986; 7:201–205.
21. Listgarten MA, Buser D, Steinemann SG, Donath K, Lang NP, Weber HP. Light and transmission electron microscopy of the intact interfaces between non-submerged titanium-coated epoxy resin implants and bone or gingiva. J Dent Res 1992; 71:364–371.
22. Ayukawa Y, Takeshita F, Inoue T, et al. An ultrastructural study of the bone-titanium interface using pure titanium-coated plastic and pure titanium rod implants. Acta Histochem Cytochem 1996; 29:243–254.
23. Ayukawa Y, Takeshita F, Yoshinari M, et al. An immunocytochemical study for lysosomal cathepsins B and D related to the intracellular degradation of titanium at the bone-titanium interface. J Periodontol 1998; 69:62–68.
24. Watazu A, Teraoka K, Kido H, et al. An inverted observation technique for interactions between titanium oxide and cells on commercially pure titanium. Key Eng Mater 2006; 309:363–366.
25. Futami T, Fujii N, Ohnishi H, et al. Tissue response to titanium implants in the rat maxilla: ultrastructural and histochemical observations of the bone-titanium interface. J Periodontol 2000; 71:287–298.
26. Masuda T, Salvi GE, Offenbacher S, Felton DA, Cooper LF. Cell and matrix reactions at titanium implants in surgically prepared rat tibiae. Int J Oral Maxillofac Implants 1997; 12:472–485.
27. Watazu A, Teraoka K, Kido H, et al. Formation and properties of transparent titanium thin films by DC magnetron sputtering for observing interactions between titanium and cells. Trans Mater Res Soc Jpn 2006; 31: 677–680.
28. Zakiah MI, Galen BS, Rebecca Z, Denise S, Clark MS. Effects of fluoride-modified titanium surfaces on osteoblast proliferation and gene expression. Int J Oral Maxillofac Implants 2006; 21:203–211.
29. Schneider GB, Zaharias R, Seabold D, Keller J, Stanford C. Differentiation of preosteoblasts is affected by implant surface microtopographies. J Biomed Mater Res 2004; 69A:462–468.
30. Ogawa T, Sukotjo C, Nisimura I. Modulated bone matrix-related gene expression is associated with differences in interfacial strength of different implant surface roughness. Int J Prosthodont 2002; 11:241–247.
31. Schneider GB, Perinpanayagam H, Clegg M, et al. Implant surface roughness affects osteoblast gene expression. J Dent Res 2003; 82:372–376.
32. Knabe C, Howlett CR, Klar F, Zreiqat H. The effect of different titanium and hydroxyapatite-coated dental implant surfaces on phenotypic expression of human bone-derived cell. J Biomed Mater Res 2004; 71A:98–107.
33. Sennerby L, Thomsen P, Ericson LE. Early tissue response to titanium implants inserted in rabbit cortical bone. Part II: ultrastructural observations. J Mater Sci Mater Med 1993; 4:494–502.
34. Sennerby L, Thomsen P, Ericson LE. Ultrastructure of the bone-titanium interface in rabbits. J Mater Sci Mater Med 1992; 3:262–271.
35. Nanci A, McCarthy GF, Zalzal S, Clokie CML, Warshawsky H, McKee MD. Tissue response to titanium implants in the rat tibia: ultrastructural, immunocytochemical and lectin-cytochemical characterization of the bone-titanium interface. Cells and Materials 1994; 4:1–30.
36. Murai K, Takeshita F, Ayukawa Y, Kiyoshima T, Suetugu T, Tanak T. Light and microscopic studies of bone-titanium interface in the tibiae of young and mature rats. J Biomed Mater Res 1996; 30:523–533.

37. Steflik DE, Sisk AL, Perr GA, Lake FT, Hanes PJ. Experimental studies of the implant-tissue interface. J Oral Implantol 1993; 19:90–94.
38. Klinger MM, Rahemtulla F, Prince CW, Lucas LC, Lemons JE. Proteoglycans at the bone-implant interface. Crit Rev Oral Biol Med 1998; 9:449–463.
39. Chehroudi B, Ratkay J, Brunette DM. The role of implant surface geometry on mineralization in vivo and in vitro: a transmission and scanning electron microscopic study. Cell and Materials 1992; 2:89–104.
40. Budd TW, Nagahara K, Bielat KL, Meenaghan MA, Schaaf NG. Visualization and initial characterization of the titanium boundary of the bone-implant interface of osseintegrated implants. Int J Oral Maxillofac Implants 1992; 7:151–160.

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