Analysis of Bone Mineral Density and Area of Newly Formed Bone Around Implants Using Micro-CT After Pull-Out Tests

Hiroshi Nakada,^{1,9} Taketoshi Suwa,² Yasuko Numata,² Yoshimitsu Okazaki,³ Toshiro Sakae,^{4,9} Tohru Hayakawa,^{5,9} Masataka Kato,⁶ Takashi Kaneda,^{6,9} Racquel Z. LeGeros,⁷ Takao Kato,^{8,9} Atsuko Gunji,^{1,9} Katsuhisa Kuwahara,^{1,9} and Kihei Kobayashi^{1,9}

Departments of ¹Gnatho-Oral Prosthetic Rehabilitation, ⁴Histology, Cytology and Developmental Anatomy, ⁵Dental Biomaterials, ⁶Radiology, ⁸Oral and Maxillofacial Implantology, and ⁹Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

²Nihon University Graduate School of Dentistry at Matsudo, Complete Denture Prosthodontics, Matsudo, Chiba 271-8587, Japan

³Institute for Human Science and Biomedical Engineering, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8564, Japan

⁷Department of Biomaterials and Biomimetics, New York University College of Dentistry, 345 East 24th Street, New York, NY 10010, USA

Correspondence to :

Hiroshi Nakada

E-mail : nakada.hiroshi@nihon-u.ac.jp

Abstract

New bone formation around implants has been evaluated two-dimensionally by the histopathological examination of sections. However, micro-computed tomography (micro-CT) allows the three-dimensional observation of bone structure. The purpose of this study is to compare bone mineral density and area of newly formed bone around implants with two types of surface treatment. We evaluated the influence of differences in surface treatment using micro-CT. The Ti-6Al-4V alloy implants (3.1 mm in diameter and 30.0 mm in length) underwent mechanical polishing ('machined') as a control or shot-blast surface treatment ('blasted') and their average surface roughness was ≤ 0.70 . The implants were inserted into the femurs of New Zealand White rabbits (16 weeks old). After 4, 8, 12, 24, and 48 weeks, pull-out tests were performed and the rabbit femurs were studied after the tests. The bone mineral density of newly formed bone was slightly higher for the blasted implants than for the machined implants from 4 to 48 weeks. Blasted implants showed a significantly greater area of newly formed bone around them after each observation period than did machined implants. These results suggested that surface treatment of implants is a factor affecting the bone mineral density and bone area. These micro-CT measurement results revealed that blasted surface was a factor for increasing the bone mineral density and bone area with time, in contrast to machined surface. Furthermore, micro-CT is a valuable analytical tool for assessing the implant-bone interface and the effect of implant surfaces on the quality and structure of newly formed bone.

Keywords :

implant, surface treatment, bone area, bone mineral density, micro-CT

Introduction

The most important factor in implant treatment is that biomaterial properties, host tissue, and surgical techniques are harmonized with the body. In particular, implant surface characteristics are dependent on

the chemical, physical, and mechanical properties of the surface. Larsson et al (1). reported that these different properties act mutually and that the functional activity of cells close to the implant surface is influenced by implant surface properties. Implant

surface roughness and irregularity greatly influence the surface energy, oxidized membrane thickness, and chemical composition.

In general, surface treatment methods include sand-blasting or grit-blasting with abrasives (2-5), chemical treatments (acid or alkali) (6-8), and deposition of a calcium phosphate coating. The coating deposition method used for commercial orthopedic and dental-coated implants is the plasma-spray method using hydroxyapatite (HA) (9-11). Other coating methods include electrochemical deposition (12, 13), physical vapor deposition (14), plasma sputtering (15, 16), and precipitation (17, 18). It has been reported that implants with a rough surface require a higher removal torque force than smooth surface implants, showing improvement in the interfacial strength (4, 19-21). Bowers et al (22). reported greater cell adhesion to an irregular rough surface obtained by sand-blasting. Morphological evaluation of the relationship between implant surface roughness and an increase in bone contact (23) revealed that a sand-blasted surface showed an increased rate of new bone formation on the implant surface (20). Cochran et al (24). reported that bone resorption around the implant was significantly lower in implants after acid etching, due to the bone conductivity of the acid-etched implant surface.

New bone formation around implants has been evaluated two-dimensionally by the histopathological examination of sections (8, 20, 25). However, micro-computed tomography (micro-CT) allows the three-dimensional observation of bone structure. There have been no studies in which the bone mineral density (BMD) and area of newly formed bone around Ti-6Al-4V ELI alloy (Ti-6-4) implants were measured in bone after removal of the implant seated for a long period.

The purpose of this study is to compare the BMD and area of newly formed bone around implants with two types of surface treatment. We evaluated the influence of differences in surface treatment on the newly formed bone around the implant using micro-CT.

Materials and Methods

Test materials

The chemical composition of Ti-6-4 used in this study is shown in Table 1. Ti-6-4 is widely used at present in clinical practice, mainly in the departments of dentistry and orthopedic surgery. The implants were columnar (3.1 mm in diameter and 30.0 mm in length), considering the size of the rabbit femur. A small hole (1.0 mm in diameter) was made at the upper end of the implant for pull-out tests. The implants underwent mechanical polishing ('machined') as a control or shot-blast surface treatment ('blasted') (Fig. 1), and their average surface roughness (R_a) was ≤ 0.70 . The surface of each implant was observed using a scanning electron microscope (SEM), (JSM-T200, JEOL Ltd.) at an accelerating voltage of 20 kv (Fig. 2).

Table 1. Chemical composition (mass %) of materials used.

Titanium alloy	Al	V	Fe	O	C	H	N	Ti
Ti-6Al-4V ELI*	6.24	4.19	0.198	0.101	0.011	0.005	0.003	Bal.**

*ELI: Extra Low Interstitial

**Bal.: Balance

Fig. 1. Photograph of each implant. a) Machined, b) Blasted.

Fig. 2. Scanning electron micrographs (SEM) of implant surfaces. a) Machined, b) Blasted (magnifications: 2000).

Surgery

The animal experimental protocol in this study was approved by the Ethics Committee for Animal Experiments in our department (approval No. ECA-03-0003). Forty male New Zealand White rabbits (body weight, 2.93 ± 0.13 kg; Sankyo Labo Service Corporation) were maintained for two weeks and used at the age of 16 weeks. General anesthesia was performed by injection of pentobarbital sodium (Nembutal®, Dainippon Pharmaceutical Co., Ltd.) via the auricular vein at a rate of 25 mg/kg. Subsequently, an incision (about 30 mm) was made in the apophysis on the central side of the femur and the bone surface was exposed. An implantation pit was made parallel to the long axis of the femur using an engine for implants (Implanter-S®, Kyocera Co., Ltd.) at a low rotation rate (gear ratio, 1:16) and implants were inserted into each of the bilateral femurs (Fig. 3). The rabbits were sacrificed under general anesthesia 4, 8, 16, 24, and 48 weeks after implantation and the femurs were resected. The specimens ($n=2$ /each week) were rabbit femurs after the implants were removed (the pull-out tests were performed as reported by Machida (26)). Specimens were selected after confirming the absence of trabecular attachments on the surface of Ti-6-4.

Fig. 3. Radiographic appearance of a blasted Ti-6-4 implant 24 weeks after implantation in the rabbit femur (Left). Location of the implant probe in the mesial femur of a New Zealand White rabbit (Right).

Measurement of BMD and bone area by micro-CT

1) Site of measurement of BMD and bone area

First, the measurements of BMD and bone area

were acquired from three-dimensional images of whole femurs by a high-resolution micro-CT scanner (Toscaner-31300 μ hd®, Toshiba Co., Ltd.) (Fig. 4). Next, the extraction of the newly formed bone site was based on the detection of radio-opaque bone within a circular region of interest around the medullary cavity in the transverse plane of the three-dimensional image (Fig. 5). Then, BMD and area of newly formed bone around the implants were measured using the micro-CT scanner by the cone beam scan method with the non-helical mode at a tube voltage of 74 kv, a tube electric current of 110 μ A, a slice thickness of 0.2 mm, and a slice pitch of 0.1 mm. The measurement site was 5 mm superior to the implant tip and 100 μ m below the newly formed bone around the implants in the long axis direction (Fig. 4).

2) Measurement of BMD

BMD was measured by the simultaneous imaging of five BMD quantification phantoms as standards (hydroxyapatite contents: 0, 50, 100, 150, and 200 mg/cm³; Fig. 6) and specimens. Obtained images were stored in the CT Control Unit Data Processing Unit. Five slice images were randomly selected and new bone that was not continuous with cortical bone was selected as a measurement site. To obtain three-dimensional images, slice images were transferred to the 3-D Analysis Unit and three-dimensional reconstruction was performed using 3-D trabecular structure measurement software (3D BON®, RATOC System Engineering) (Fig. 4).

3) Measurement of bone area

For the measurement of the area of newly formed bone around implants, 10 images were randomly selected from 128 slice images obtained by micro-CT and measurement was performed using the image analysis software Image-Pro PLUS Version 4.0® (Media Cybernetics). Newly formed bone only at sites not affected or only negligibly affected by preexisting cortical bone was used and the bone circumference was visualized. Measurement was performed using the software and the mean value at 10 sites was defined as the bone area (Fig. 5).

Fig. 4. Photograph of the micro-CT system and measurement site in a rabbit femur.

Fig. 5. Illustration of the bone area (dotted line) of newly formed bone around an implant.

Statistical analysis

For statistical analysis, the mean BMD, mean bone area, and standard deviation were calculated in newly formed bone around implants of Ti-6-4 with

two types of surface treatment and compared by one-way analysis of variance (one-way ANOVA) and multiple comparison (Bonferroni's method). Statistical analyses were conducted using SPSS software (SPSS Inc.).

Results

The results of BMD and bone area measurement in newly formed bone around implants of Ti-6-4 with two types of surface treatment are as follows.

Measurement of BMD around Ti-6-4 implants

The results of BMD measurement around the Ti-6-4 implants are shown in Fig. 7. The BMD of the machined implants was 260.9 ± 12.7 mg/cm³ 4 weeks after implantation, 437.7 ± 22.0 mg/cm³ after 8 weeks, 660.0 ± 18.1 mg/cm³ after 16 weeks, 740.5 ± 26.0 mg/cm³ after 24 weeks, and 821.3 ± 23.1 mg/cm³ after 48 weeks, showing a significant increase after each period. The BMD of the blasted implants was 296.8 ± 25.6 mg/cm³ 4 weeks after implantation, 525.0 ± 72.4 mg/cm³ after 8 weeks, 691.2 ± 26.0 mg/cm³ after 16 weeks, 776.9 ± 27.7 mg/cm³ after 24 weeks, and 845.2 ± 23.1 mg/cm³ after 48 weeks, also showing a significant increase after each period. Comparison between the machined and blasted implants showed a higher BMD of new bone for the blasted implants after 4, 8, and 24 weeks but no significant difference after 16 or 48 weeks. The BMD of new bone was slightly higher for the blasted implants than for the machined implants from 4 to 48 weeks. The BMD of newly formed bone increased linearly from 4 to 16 weeks but increased more slowly from 16 to 48 weeks.

Fig. 7. Bone mineral density of newly formed bone around Ti-6-4 implants with machined or blasted surface treatments. Significance of differences was examined by one-way ANOVA and Bonferroni's method (**: $P < 0.01$, *: $P < 0.05$, n.s.: Not significant).

Measurement of the new bone area around Ti-6-4 implants

The results of measurement of the newly formed bone area around the Ti-6-4 implants are shown in Fig. 8. The bone area around the machined implants was 13.59 ± 0.24 mm² 4 weeks after implantation, 17.25 ± 0.16 mm² after 8 weeks, 30.74 ± 0.41 mm² after 16 weeks, 34.74 ± 0.14 mm² after 24 weeks, and 46.02 ± 0.65 mm² after 48 weeks. A significant increase was observed from 8 to 16 weeks and from 24 to 48 weeks after implantation. The bone area around the blasted implants was 22.35 ± 0.09 mm² 4 weeks after implantation, 28.92 ± 0.4 mm² after 8 weeks, 35.38 ± 0.06 mm² after 16 weeks, 47.2 ± 0.06 mm² after 24 weeks, and 51.59 ± 0.15 mm² after 48 weeks. A significant increase was observed from 4 to 24 weeks after implantation and a slight increase was seen from 24 to 48 weeks after implantation. Comparison between the machined and blasted implants showed a significantly greater area around the blasted implants after each observation period. The newly formed bone area gradually increased from 4 to 48 weeks after implantation.

Discussion

As implant materials in the dental field, titanium implants have been selected due to their high biocompatibility and safety, which is based on bone

Fig. 8. Bone area of newly formed bone around Ti-6-4 implants with machined or blasted surface treatments. Significance of differences was examined by one-way ANOVA and Bonferroni's method (**: $P < 0.01$, *: $P < 0.05$, n.s.: Not significant).

-titanium binding, so-called osseointegration. Albrektsson et al (27). reported the following factors associated with good osseointegration: (a) implant material, (b) implant design, (c) implant finish (implant surface), (d) status of the bone, (e) surgical technique, and (f) implant loading conditions. Among these factors, implant material, implant design, and implant finish (implant surface) are implant-associated factors. In this study, attention was paid to implant surface.

For structural morphometry of bone tissue, many scholars have produced thin tissue specimens and observed them by microscopy (28-30). However, this method requires pretreatment, such as embedding, and specialized measurement skills. Micro-CT used in this study does not require pretreatment or specific skills, allows noninvasive measurement (31), and is particularly useful for the ultrastructural analysis of bone structure (32, 33). In particular, micro-CT scanners have a high resolution and allow the visualization of the trabecular structure itself and also the measurement of bone volume, bone surface area, and cancellous bone thickness (34). Therefore, this scanner is frequently used for the study of trabecular structure and hard tissue ultrastructure and has been applied to studies in various medical fields (35, 36). The resolution of micro-CT scanners in the early period was about 50 μm (37), but has improved to about 14 μm (38) in recent years, also allowing the accurate evaluation of the trabecular structure of small animals. Because the resolution of the Toshiba micro-CT scanner used in this study is 5-8 μm , very clear scans could be obtained. In addition, micro-CT using synchrotron radiation has a maximum voxel size of 2 μm and allows the observation of fractures and irregularities on the trabecular surface and the distribution of bone cells (39). Although the focus size of the routinely used X-ray structure for dental use is about 1 $\times$ 1 mm, the changeover between large/small foci (small focus, 5-7 μm ; large focus, 45 μm) is possible by the electric power value (tube voltage $\times$ tube current) in micro-CT. In general, imaging for a long period is necessary to obtain high-resolution images by micro

-CT. However, prolongation of the imaging time does not improve image quality. Therefore, the optimal imaging conditions according to the characteristics of the scanner and the object for imaging should be evaluated.

In experiments involving implants in animals, observation by micro-CT is not reliable because the measurement of areas close to the implant is difficult due to the influences of artifacts. In this study, the measurement site was chosen to be 100 μm below the bone surface in contact with the implant based on information that occlusal force associated with mastication requires at least a 100- μm maintenance area and buffering ability of peri-implant bone and also a risk of destruction of new bone after the implant is pulled out. The measurement of specimens after pulling-out allowed the acquisition of more accurate raw data. BMD was measured by the simultaneous imaging of specimens and BMD measurement phantoms. Because HA has a lower radiation transmittance than other tissues, its amount can be measured by calculating the radiation absorption rate. Five BMD measurement phantoms and specimens may be simultaneously measured and the BMD corresponding to the CT value of the specimen may be calculated based on a calibration curve. Therefore, measurement does not require a special device and the BMD per unit volume could be readily obtained.

In this study, BMD and the bone area were measured in newly formed bone around implants with two types of surface treatment. All measurements showed a higher BMD and greater area of newly formed bone around the blasted implants than around the machined implants. In general, for bone/implant binding, blasted or HA implants are considered to be more advantageous than machined implants (9). Blasted implants have been reported to show improved reactivity on the titanium surface due to the removal of contaminants on the surface layer and production of fine irregular unevenness on the surface by blasting with titanium oxide or aluminum oxide (21). Another study suggested that the bone/implant contact area is larger in blasted

Fig. 9. Micro-CT appearance of each implant from 4 to 48 weeks after implantation in the rabbit femur.

implants than machined implants ; therefore, cells on the rough surface can exist and proliferate for a long period (40).

Micro-CT showed the formation of numerous trabeculae and newly formed bone around the area of the pulled-out implant in all specimens from 4 to 48 weeks after implantation (Fig. 9). Around the machined implants, the thickness of newly formed bone increased with time and the number and thickness of trabeculae bone extending to the cortical bone, supporting the newly formed bone, also increased. The amount of bone formation around the blasted implant was marked and the increases with time in the thickness of newly formed bone and the number and thickness of trabeculae bone were also marked, resulting in firm support for the implant. These micro-CT findings supported the increases in the BMD and bone area.

Butterfield et al (41). measured BMD after maxillary sinus lift using autologous bone and reported a BMD of 349 mg/cm³ after 4 weeks. A direct comparison between their results and those in this study is difficult because of the differences in the implantation material and site, but their results were similar to those for blasted Ti-6-4 implants in this study. Comparison of the results of the bone area is also difficult because of the differences in the implanta-

tion site and measurement conditions, but our finding of an increase with time in BMD, similar to that in the bone area, is in agreement with their results.

Concerning trabeculae formation around implants, a previous study showed active bone formation in the early stage from 2 to 6 weeks after implantation but trabeculae resorption after 24 weeks or more (42). In this study, a linear increase in BMD was observed from 4 to 16 weeks after implantation and a gradual increase thereafter until 48 weeks after implantation for both types of surface treatment. The area of newly formed bone also increased from 4 to 48 weeks after implantation. Newly formed bone around the implant should be maintained despite loads such as the occlusion force applied to bone. Newly formed trabeculae are not adequately mature and the crystallinity of trabeculae should be gradually improved by stimulating bone precursor cells, increasing alkaline phosphatase activity, and enhancing the incorporation of newly formed collagen into the bone matrix. In addition, to maintain BMD, modeling-remodeling is indispensable and the bone area should be further increased for the establishment of bone as a basis for osseointegration.

These micro-CT measurement results revealed that the blasted implant was a factor for increasing the BMD and bone area with time, in contrast to the

machined implant. Micro-CT is a non-destructive analytical method for investigating bone structure. This method, unlike the conventional light microscope method, does not need tedious specimen preparation and specialized skills in microscopy. However, imaging techniques such as stereology, back-scattered imaging, and scanning electron microscopy are necessary for further details. In this study, micro-CT is a valuable analytical tool for assessing the implant-bone interface and the effect of implant surfaces on the quality and structure of newly formed bone. For this purpose, further research is necessary to analyze the crystallinity, i.e., the quality of bone formed around Ti-6-4 implants.

Conclusion

In this study, using micro-CT of rabbit femur specimens after implant pull-out tests, the bone mineral density of new bone 100 μm below the surface in contact with implants and the area of newly formed bone around implants were measured. We evaluated the influence of differences in surface treatment on the newly formed bone around implants and the usefulness of micro-CT. The following conclusions were obtained.

Bone mineral density measurement

It was revealed that the newly formed bone around implants showed a significantly higher bone mineral density 4 to 48 weeks after implantation in both the surface treatment groups. The serial bone mineral density was higher with blasted treatment than with machined treatment.

Bone area measurement

It was revealed that the area of newly formed bone around implants significantly increased 4 to 48 weeks after implantation in both surface treatment groups. The bone area increase was higher with blasted treatment than with machined treatment.

These micro-CT measurement results revealed that blasted treatment was a factor for increasing the bone mineral density and area with time, in contrast to machined treatment. Micro-CT is a non-

destructive analytical method for investigating bone structure. This method, unlike the conventional light microscope method, does not need tedious specimen preparation and specialized skills in microscopy. However, imaging techniques such as stereology, back-scattered imaging, and scanning electron microscopy are necessary for further details. The present study demonstrated that micro-CT is a valuable analytical tool for assessing the implant-bone interface and the effect of implant surfaces on the quality and structure of newly formed bone.

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References

1. Larsson C, Thomsen P, Lausmaa J, Rodahl M, Kasemo B, Ericson LE: Bone response to surface modified titanium implants: studies on electropolished implants with different oxide thicknesses and morphology. *Biomaterials*, 15: 1062-1074, 1994.
2. Nakashima Y, Hayashi K, Inadome T, Uenoyama K, Hara T, Kanemaru T, Sugioka Y, Noda I: Hydroxypapatite-coating on titanium arc sprayed titanium implants. *J Biomed Mater Res*, 35: 287-298, 1997.
3. Li D, Liu B, Han Y, Xu K: Effects of a modified sandblasting surface treatment on topographic and chemical properties of titanium surface. *Implant Dent*, 10: 59-64, 2001.
4. Wennerberg A, Albrektsson T, Lausmaa J: Torque and histomorphometric evaluation of c.p. titanium screws blasted with 25- and 75-microns-sized particles of Al_2O_3 . *J Biomed Mater Res*, 30: 251-260, 1996.
5. Abbron A, Hopfensperger M, Thompson J, Cooper LF: Evaluation of a predictive model for implant surface topography effects on early osseointegration in the rat tibia model. *J Prosthet Dent*, 85: 40-46, 2001.
6. Cochran DL, Schenk RK, Lussi A, Higginbottom FL, Buser D: Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: a histometric study in the canine mandible. *J Biomed Mater Res*, 40: 1-11, 1998.
7. MacDonald DE, Betts F, Doty SB, Boskey AL: A methodological study for the analysis of apatite-coated dental implants retrieved from humans. *Ann Periodontol*; 5: 175-184, 2000.

8. Nishiguchi S, Kato H, Fujita H, Oka M, Kim HM, Kokubo T, Nakamura T: Titanium metals form direct bonding to bone after alkali and heat treatments. *Biomaterials*, 22 : 2525-2533, 2001.
9. Gotfredsen K, Wennerberg A, Johansson C, Skovgaard LT, Hjorting-Hansen E: Anchorage of TiO₂-blasted, HA-coated, and machined implants: an experimental study with rabbits. *J Biomed Mater Res*, 29 : 1223-1231, 1995.
10. Lee TM, Yang CY, Chang E, Tsai RS: Comparison of plasma-sprayed hydroxyapatite coatings and zirconia-reinforced hydroxyapatite composite coatings: in vivo study. *J Biomed Mater Res A*, 71 : 652-660, 2004.
11. Wang H, Eliaz N, Xiang Z, Hsu HP, Spector M, Hobbs LW: Early bone apposition in vivo on plasma-sprayed and electrochemically deposited hydroxyapatite coatings on titanium alloy. *Biomaterials*, 27 : 4192-4203, 2006.
12. Rossler S, Sewing A, Stolzel M, Born R, Scharnweber D, Dard M, Worch H: Electrochemically assisted deposition of thin calcium phosphate coatings at near-physiological pH and temperature. *J Biomed Mater Res A*, 64 : 655-663, 2003.
13. Lin S, LeGeros RZ, LeGeros JP: Adherent octacalciumphosphate coating on titanium alloy using modulated electrochemical deposition method. *J Biomed Mater Res A*, 66 : 819-828, 2003.
14. Antonov EN, Bagratashvili VN, Popov VK, Ball MD, Grant DM, Howdle SM, Scotchford CA: Properties of calcium phosphate coatings deposited and modified with lasers. *J Mater Sci Mater Med*, 14 : 151-155, 2003.
15. Piattelli A, Scarano A, Corigliano M, Piattelli M: Effects of alkaline phosphatase on bone healing around plasma-sprayed titanium implants: a pilot study in rabbits. *Biomaterials*, 17 : 1443-1449, 1996.
16. Yang Y, Kim KH, Agrawal CM, Ong JL: Influence of post-deposition heating time and the presence of water vapor on sputter-coated calcium phosphate crystallinity. *J Dent Res*, 82 : 833-837, 2003.
17. Wen HB, de Wijn JR, Cui FZ, de Groot K: Preparation of calcium phosphate coatings on titanium implant materials by simple chemistry. *J Biomed Mater Res*, 41 : 227-236, 1998.
18. LeGeros RZ, Lin S, Rohanizadeh R, Mijares D, LeGeros JP: Biphasic calcium phosphate bioceramics: preparation, properties, and applications. *J Mater Sci Mater Med*, 14 : 201-209, 2003.
19. Carlsson L, Rostlund T, Albrektsson B, Albrektsson T: Removal torques for polished and rough titanium implants. *Int J Oral Maxillofac Implants*, 3 : 21-24, 1988.
20. Feighan JE, Goldberg VM, Davy D, Parr JA, Stevenson S: The influence of surface-blasting on the incorporation of titanium-alloy implants in a rabbit intramedullary model. *J Bone Joint Surg Am*, 77 : 1380-1395, 1995.
21. Wennerberg A, Albrektsson T, Johansson C, Andersson B: Experimental study of turned and grit-blasted screw-shaped implants with special emphasis on effects of blasting material and surface topography. *Biomaterials*, 17 : 15-22, 1996.
22. Bowers KT, Keller JC, Randolph BA, Wick DG, Michaels CM: Optimization of surface micromorphology for enhanced osteoblast responses in vitro. *Int J Oral Maxillofac Implants*, 7 : 302-310, 1992.
23. 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 Mater Res*, 25 : 889-902, 1991.
24. Cochran DL, Nummikoski PV, Higginbottom FL, Hermann JS, Makins SR, Buser D: Evaluation of an endosseous titanium implant with a sandblasted and acid-etched surface in the canine mandible: radiographic results. *Clin Oral Implants Res*, 7 : 240-252, 1996.
25. Okazaki Y, Nishimura E, Nakada H, Kobayashi K: Surface analysis of Ti-15Zr-4Nb-4Ta alloy after implantation in rat tibia. *Biomaterials*, 22 : 599-607, 2001.
26. Machida T: A study of osseointegration of titanium alloy implants: the investigation on effect of different surface treatment (Japanese). *Nihon Univ J Oral Sci*, 30 : 245-257, 2004.
27. Albrektsson T, Brånemark PI, Hansson HA, Lindstrom J: Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. *Acta Orthop Scand*, 52 : 155-170, 1981.
28. 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*, 7 : 62-71, 1992.
29. Ericsson I, Johansson CB, Bystedt H, Norton MR: A histomorphometric evaluation of bone-to-implant contact on machine-prepared and roughened titanium dental implants: A pilot study in the dog. *Clin Oral Implants Res*, 5 : 202-206, 1994.
30. Evans GH, Mendez AJ, Caudill RF: Loaded and non-loaded titanium versus hydroxyapatite-coated threaded implants in the canine mandible. *Int J Oral Maxillofac Implants*, 11 : 360-371, 1996.
31. Beck TJ, Looker AC, Ruff CB, Sievanen H, Wahner HW: Structural trends in the aging femoral neck and proximal shaft: analysis of the Third National Health and Nutrition Examination Survey dual-energy X-ray absorptiometry data. *J Bone Miner Res*, 15 : 2297-2304, 2000.
32. Ito M, Nishida A, Nakamura T, Uetani M, Hayashi

- K: Differences of three-dimensional trabecular microstructure in osteopenic rat models caused by ovariectomy and neurectomy. *Bone*, 30 : 594-598, 2002.
33. Shiraishi A, Higashi S, Masaki T, Saito M, Ito M, Ikeda S, Nakamura T: A comparison of alfacalcidol and menatetrenone for the treatment of bone loss in an ovariectomized rat model of osteoporosis. *Calcif Tissue Int*, 71 : 69-79, 2002.
 34. Sone T, Tamada T, Jo Y, Miyoshi H, Fukunaga M: Analysis of three-dimensional microarchitecture and degree of mineralization in bone metastases from prostate cancer using synchrotron microcomputed tomography. *Bone*, 35 : 432-438, 2004.
 35. Hara T, Takizawa M, Sato T, Ide Y: Mechanical properties of buccal compact bone of the mandibular ramus in human adults and children: relationship of the elastic modulus to the direction of the osteon and the porosity ratio. *Bull Tokyo Dent Coll*, 39 : 47-55, 1998.
 36. Balto K, Muller R, Carrington DC, Dobeck J, Stashenko P: Quantification of periapical bone destruction in mice by micro-computed tomography. *J Dent Res*, 79 : 35-40, 2000.
 37. Feldkamp LA, Goldstein SA, Parfitt AM, Jesion G, Kleerekoper M: The direct examination of three-dimensional bone architecture in vitro by computed tomography. *J Bone Miner Res*, 4 : 3-11, 1989.
 38. Ruegsegger P, Koller B, Muller R: A microtomographic system for the nondestructive evaluation of bone architecture. *Calcif Tissue Int*, 58 : 24-29, 1996.
 39. Sharp JC, Copps JC, Liu Q, Ryner LN, Sebastian RA, Zeng GQ, Smith S, Niere JO, Tomanek B, Sato M: Analysis of ovariectomy and estrogen effects on body composition in rats by X-ray and magnetic resonance imaging techniques. *J Bone Miner Res*, 15 : 138-146, 2000.
 40. Piattelli M, Scarano A, Paolantonio M, Iezzi G, Petrone G, Piattelli A: Bone response to machined and resorbable blast material titanium implants: An experimental study in rabbits. *J Oral Implantol*, 28 : 2-8, 2002.
 41. Butterfield KJ, Bennett J, Gronowicz G, Adams D: Effect of platelet-rich plasma with autogenous bone graft for maxillary sinus augmentation in a rabbit model. *J Oral Maxillofac Surg*, 63 : 370-376, 2005.
 42. Kawamura H, Ito A, Muramatsu T, Miyakawa S, Ochiai N, Tateishi T: Long-term implantation of zinc-releasing calcium phosphate ceramics in rabbit femora. *J Biomed Mater Res A*, 65 : 468-474, 2003.