Effects of Ion Beam–Assisted Deposition of Hydroxyapatite on the Osseointegration of Endosseous Implants in Rabbit Tibiae

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The aim of this study was to evaluate the effects of coating implants with hydroxyapatite (HA) by an ion beam–assisted deposition (IBAD) method and to compare them with implants prepared with sandblasted and machined surfaces. Examination of osteoblast cultures displayed no difference in the secretion of alkaline phosphatase (ALP) between the various surfaces, but the IBAD-HA specimen showed low ALP secretion ($P < .05$). Removal torque tests showed that implants coated with HA by the IBAD method had values similar to the implants with a sandblasted surface, but values for the machined-surface implants differed. Implants placed in a group of ovariectomized rabbits showed lower mechanical test values than implants placed in sham-operated rabbits ($P < .05$). Implants coated with HA by the IBAD method demonstrated the highest mean bone-to-metal contact ratio on all threads and on the 3 best consecutive threads, followed by the implants with a sandblasted surface and implants with a machined surface ($P < .05$). Hydroxyapatite-coated implants showed a slightly higher bone-to-implant contact ratio than sandblasted implants, but no statistically significant difference was seen between the 2 materials. The implants placed in ovariectomized rabbits showed lower amounts of bone-to-metal contact than the implants placed in sham-operated rabbits, but no statistically significant difference was seen between the 2 groups. Evaluation of bone volume on all threads and the 3 best consecutive threads showed no statistically significant difference among the different surface treatment groups, but lower bone volume was seen in the ovariectomized rabbits than in the sham-operated animals ($P < .05$). (Int J Oral Maxillofac Implants 2001;16:809–818)

Key words: *alkaline phosphatase, endosseous dental implants, histomorphometry, hydroxyapatites, materials testing, osteoblasts, ovariectomy*

Albrektsson and coworkers[1] have suggested 6 factors that are important to successful osseointegration: material, design, implant surface characteristics, bone condition, surgical technique, and loading conditions. To date, metals such as pure titanium, tantalum, niobium, zirconium, cobalt-chromium alloy, titanium-6aluminum-4vanadium alloy, and ceramic materials such as aluminum oxide, hydroxyapatite (HA), or β-tricalcium phosphate have been used as materials for oral implants. The most widely used oral implant material, commercially pure titanium (cpTi), has been used with a passive oxide coating on a machined surface.[2] Different surface treatments such as titanium plasma coating or calcium phosphate coating have been developed. When metals are used alone for the implant material, their biocompatibility may be lower than that of bioceramic materials, and if these metals are in vivo for a long period, dissolution of metal ions and the formation of inorganic material have been reported.[3]

Hydroxyapatite is composed of the same inorganic materials as bone, chemically and structurally, and also displays some bioactivity for chemical adhesion to surrounding bone. However, the solid form of HA has a greater hardness than bone but

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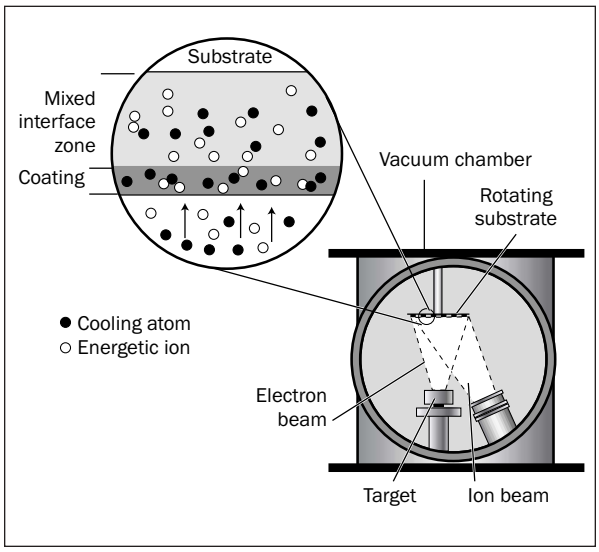


Fig 1 Schematic representation of IBAD system.

low tensile strength. Therefore, its application has been limited to areas of minimal loading, such as auricular bone.[4] The deposition of HA on metal implants has been actively developed, and a coating can be applied by electrophoretic deposition,[5] dipping,[6] hot isostatic pressing,[6] flame spraying,[7] plasma spraying,[8,9] or pulsed laser deposition.[10]

Currently, a widely used plasma spraying method has solved to some extent the problem of physiologic, immunologic, and chemical stability that is brought about by the use of a metal surface implant. However, the problems of chemical nonuniformity of this coating, degradation or absorption in the human body, low dynamic characteristics, and low adhesion strength between the metal and HA coating remain. These render HA unacceptable in fields such as orthopedics or dental applications, which require a strong and intimate HA coating.[11,12]

To resolve these kinds of problems, coating methods using ion application (ion-sputtering,[11,13] ion plating,[14] and ion implantation,[12] all of which are used in the semiconductor industry) are being developed. Adherence of metal and an HA coating is 8 to 45 MPa with sputtering,[15] 53 MPa with radiofrequency magnetron sputtering,[16] and 59 to 65 MPa with ion beam dynamic mixing.[12] These are higher values than the 7 MPa commonly obtained for plasma-sprayed coatings.[17] Unfortunately, one problem with ion sputtering[15] is the high dissolution rate of the coating, which brings about separation of the coating and a reduction of adherence. This is owing to the change in the calcium/phosphorus (Ca/P) ratio that occurs during the deposition process; this can be corrected by heat processing, but cracking of the coating may occur, and the reduction of adhesion has been reported.[13,18]

Implantation of Ca^{2+} ions and control of the Ca/P ratio have been developed recently.[12] One of the factors in the success and failure of implants is the quality and quantity of bone.[19] Thus, interest in factors that can affect the quality and quantity of bone has increased.

The purpose of this study was to investigate the biocompatibility of HA-coated root-form endosseous implants, comparing alkaline phosphatase (ALP) concentrations after osteoblast culturing, and to compare biomechanically and histomorphologically the effect of 3 different surface treatment methods: a machined surface, an aluminum oxide (Al_2O_3) particle–blasted surface, and a surface coated with HA using the ion beam–assisted deposition (IBAD) method. Implants placed in osteoporotic rabbits (induced by ovariectomy) and control (sham-operated) rabbits were compared.

MATERIALS AND METHODS

Hydroxyapatite Coating

Preparation of Evaporant. Evaporants used for coating were made with a 17.5% mass ratio of CaO powder (Cerac, Milwaukee, WI) to HA powder (Alfa Aesar Company, Ward Hill, MA), and ball-milled in ethyl alcohol for 24 hours with Al_2O_3 balls as media. The powder mixtures were then sintered in air at 1,200°C for 24 hours to make evaporants. Pure HA powder was sintered for the control group samples.

The IBAD System. Figure 1 shows schematically the IBAD system employed in this study. Roughing evacuation was done using a mechanical rotary pump to acquire 5×10^{-2} mmHg and maintained down to 10^{-7} mmHg using a cryopump (Helix Technology, Mansfield, MA). Before deposition, the surface of the implants was cleaned for better adhesion with an ion beam (120 V, 2 A) extracted from an end-hall–type ion gun (Mark II, Commonwealth Scientific, Alexandria, VA). For the evaporation, voltage of the electron beam (Telemark, Fremont, CA) was 8.5 kV, and the current was initially 0.06 to 0.08 A and then increased to 0.15 A. The substrate holder was rotated at a speed of 8 rpm during the deposition for uniformity of the coating layer. The thickness of the coating layer was 1 μm, as measured by a surface profiler (Model P-10, Tencor, Santa Clara, CA).

Heat Processing. Heat treatment was utilized for better conversion of the amorphous into a crystalline phase in the normal specimens that were

used in the osteoblast culture test. The specimens were placed in the furnace at 3 mmHg vacuum, and the temperature was increased at a rate of 5°C/min up to 630°C and maintained for 1 hour. The specimens were then cooled to room temperature.

Osteoblast Culturing

Specimen Preparation. Titanium was cut and machined into disks with a thickness of 2 mm and a diameter of 25 mm. The surfaces of the specimens were ground with 200-grit, 320-grit, 500-grit, 1,000-grit, and 1,200-grit silicon carbide paper and subsequently polished with 1-μm and 6-μm diamond spray (Struers, Copenhagen, Denmark). Then they were ultrasonically cleaned for 5 minutes and divided into 3 groups (HA17.5CaO-deposited samples, HA17.5CaO-deposited and heat-treated samples, and pure HA-deposited samples). As controls, 2 additional groups of samples were prepared; 1 group was composed of pure Ti disks that were ground by 600-grit silicon carbide paper to a roughness according to the surface roughness parameter (R_a) that was measured. The second group was blasted with Al_2O_3 particles of a mean particle size of 50 μm at a pressure of between 4 and 5 kg. For each group, 3 samples were tested; for each sample, 3 tests were done. Mean values of surface roughness parameters (R_a) for each sample were as follows: 1.10 μm for HA17.5CaO-deposited samples, 1.07 μm for HA17.5CaO-deposited and heat-treated samples, 0.98 μm for pure HA-deposited samples, 1.12 μm for machined samples, and 5.7 μm for blasted samples.

Sample Preparation. Three of the prepared disks from each group were placed on a dish with a diameter of 30 mm. Osteoblasts that were derived from the calvaria of newborn Institute for Cancer Research (ICR) mice were multilayer-cultured for 3 days in α minimum essential medium (MEM) (commercially available medium including 10% fetal bovine serum) at 37°C in a 5% CO_2 incubator and treated with trypsin-EDTA. The cell layer was derived from the sample and centrifuged at 1,500 rpm for 8 minutes to release the cells. After mixing with 20 μL of the MEM, a 10-μL suspension solution was made and reacted with NaOH buffer (pH 10.4) in 10-μL 0.1% Triton X-100/saline and 10 μL double distilled water (DDW) at 37°C for 30 minutes and then with lysed cells.

Test Protocol. For measurement of ALP activity, a commercially available assay kit (Procedure No. ALP-10, Sigma, St Louis, MO) was used. Approximately 1 mL ALP in the assay kit was heated up to 30°C and mixed with the samples prepared above. The mixture was left to react for 30 minutes and the absorbance at 405 nm was measured (set as initial value). After 2 more minutes' reaction, the procedure was repeated (set as final value). According to these 2 values, the ALP concentration was calculated using the following formula: ALP concentration = (final value – initial value) × 2,764.

Histomorphometric Analysis and Measurement of Removal Torque

Experimental Animals. Thirty-six female adult New Zealand white rabbits (average age 15 months, average 3 kg in weight) were used. Throughout the study the animals were kept in separate double cages and fed with standard food. Of these, 18 underwent an ovariectomy to induce artificial osteoporosis, and the remaining 18 underwent a sham operation.

Implants. One hundred forty-four cpTi screw-type implants 10 mm in length and 3.8 mm in diameter (Dong Myeong Company, Seoul, Korea) were used (n = 4 implants per rabbit). They were divided into 3 groups: Group 1 implants were as-delivered, machined surface; group 2 implants were blasted with Al_2O_3 particles (mean particle size of 50 μm) at a pressure of between 4 and 5 kg; and group 3 implants received HA deposition using the IBAD technique with HA17.5CaO (n = 48 implants per group) (Fig 2). Mean values for surface roughness parameters (R_a) were as follows: 1.13 μm for machined surfaces, 5.8 μm for blasted surfaces, and 1.04 μm for IBAD surfaces.

Methods. Distribution of the Experimental Group. Thirty-six rabbits were divided into 2 groups (18 rabbits per group) depending on whether or not an ovariectomy had been done. Within each group, the rabbits were subdivided according to the implant surfaces mentioned above; ie, group 1 = machined-surface implants, group 2 = Al_2O_3-blasted implants, and group 3 = IBAD-treated implants (n = 6 rabbits per group). Two kinds of implants were selected randomly and placed in each tibia of each rabbit (n = 12 legs/24 implants for each group). Twelve weeks after implant placement, the rabbits were sacrificed and histomorphometric analysis and removal torque tests were performed.

Ovariectomy. In each rabbit, an intramuscular injection was made and general anesthesia induced with 50 mg/mL ketamine HCl (Ketalar, Yuhan Company, Seoul, Korea) 2 mL/kg. A 2-cm incision was made at the center of the abdomen and an ovariectomy was done. The rest of the organs were repositioned, and the wound was closed with layered sutures. Daily intramuscular injections of Cefazolin (Yuhan Company; 250 mg) were given for 1 week. The sham operation was performed in the same way, except for removal of the ovaries.

Implant Placement. Two months after ovariectomy or the sham operation, 50 mg/mL ketamine HCl (Ketalar, Yuhan Company) 2 mL/kg was injected intramuscularly for general anesthesia, and 2% lidocaine HCl (Yuhan Company) with epinephrine (1:100,000) was administered under aseptic conditions at the surgical site.

Rabbits were randomly selected from both the ovariecomized and sham-operated groups. A skin incision was made on the right and left sides of each rabbit tibia and a flap was created to expose the tibia. On the inner-anterior side of the tibia, 2 kinds of experimental implants were selected randomly and placed in the rabbit. These implants were randomly selected from 3 groups of prepared implants. Thus, a total of 4 randomly selected implants were placed in each randomly selected rabbit. The implants were placed 1 cm apart, and drilling and placement were done under copious saline irrigation. Tapping was limited to the cortical bone and initial fixation. The distal specimen was used to measure removal torque, and the medial specimens were used for histomorphometric analyis. After implant placement, the periosteum and fascia were sutured with resorbable suture material (polyglactin 910) and the skin was closed with silk sutures. To prevent infection, daily intramuscular injections of Cefazolin (Yuhan Company; 250 mg) were given for 1 week.

Removal Torque Measurement. Nine rabbits (5 rabbits in the sham-operated group and 4 ovariectomized rabbits) died at various intervals postoperatively for reasons unrelated to the surgical procedures. The remaining 27 rabbits were sacrificed after a 12-week period by intravenous injection of air into the rabbits' ears. The fascia and periosteum were removed, and the distal implant was exposed. The specimen was connected to a mount and torque strain gauge (Tohnichi, Tokyo, Japan) capable of measuring 6 kg/cm (58.8 Ncm). The sample numbers were as follows: The sham-operated group included 8 HA17.5CaO-deposited specimens (group 3), 8 particle-blasted samples (group 2), and 10 machined samples (group 1); the ovariectomized group included 8 HA17.5CaO-deposited specimens (group 3), 10 particle-blasted samples (group 2), and 10 machined samples (group 1).

Preparation and Observation of Tissue Specimens. For preparation of the tissue specimens, the rabbit tibiae were removed and fixed with 70% alcohol to minimize deformation of the tissue specimens, and cut along the long axis of the implant. Then they were preserved and dyed in a villanueva bone stain solution for 1 week. They were then dehydrated in 70%, 90%, and 95% alcohols (once each) and 100% alcohol (4 times) for 12 hours each and then embedded in methylmethacrylate resin and left in a thermoregulated vacuum at 37°C for 40 days. After the slices had hardened, they were cut along the long axis of the implant successively to a thickness of 200 μm by means of a low-speed diamond wheel saw (Maruto Company, Tokyo, Japan), then ground to a thickness of 30 μm with the Hard Tissue Grinding System (Maruto Company).

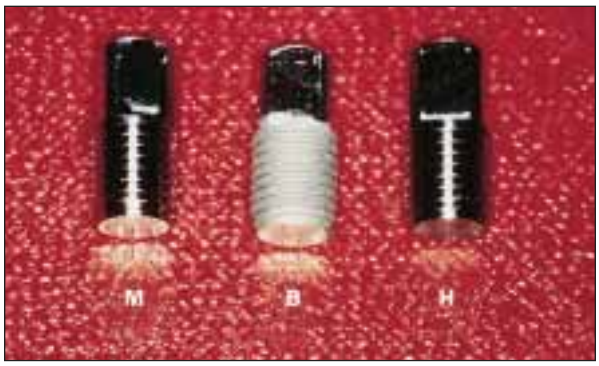


Fig 2 Implants used in this experiment. M = machined surface; B = blasted surface; H = surface deposited with HA by IBAD method.

Histomorphometric Analysis. Computer-based histomorphometric analysis was carried out under a light microscope (Olympus BX50, Olympus, Tokyo, Japan) equipped with a charge-coupled distributor camera (Samsung Aerospace, Seoul, Korea), connected to a computer. This system enabled the observer to perform histomorphometric quantifications directly in the eyepiece of the microscope using a 10× objective and a zoom of 2.5×. The histomorphometric investigations included measurement of bone-to-metal contact and bone volume at every thread (both on the anterior and posterior side of the implant in the tibia; ie, all threads were measured on the ground sections, along with the 3 best consecutive threads in the cortical region). In brief, bony contact measurements involved first outlining the entire thread length and then outlining the non–bone-contacting lengths. Percentages of bone-to-metal contact were obtained by dividing the latter by the former. Bone volume was measured by outlining the total area bounded by the threads, measuring the total area occupied by bone within this region, and dividing the latter area by the former area to express it as the percentage of bone volume in the thread.

Statistical Analysis

Statistical analysis was performed using the SAS program (version 6.12, SAS Institute, Cary, NC). The means and standard deviations of all measured

Table 1 Alkaline Phosphatase Secretion

Surface treatment	ALP (U/L) (mean ± SD)
Machined	47.7 ± 2.4
Blasted	45.7 ± 1.9
IBAD with HA17.5CaO	40.2 ± 2.1
IBAD with HA17.5CaO + heat treatment	50.1 ± 3.2
IBAD with pure HA	6.4 ± 0.9*

*Statistically significant difference from other samples (Tukey test; $P < .05$).

Table 2 Removal Torque Measurements (in NcM, mean ± SD)

Rabbit group	HA-coated implants	Blasted implants	Machined implants
Sham-operated	48.5 ± 5.4[a]	47.3 ± 5.8[a]	32.3 ± 2.91[b]
Ovariectomized	35.6 ± 3.6*,[a]	34.4 ± 3.9*,[a]	23.4 ± 4*,[b]

[a,b] Statistically significant difference between columns ($P < .05$).
*Statistically significant difference between rows ($P < .05$).

categories were calculated, and comparison analysis between each group at the significance level of 95% was performed with the Kruskal-Wallis analysis of variance and the Tukey test.

RESULTS

Osteoblast Culture Test

Alkaline phosphatase concentrations of osteoblasts were measured after culturing them on the following 5 surfaces: machined surface, blasted surface, surface with HA17.5CaO deposition, heat-treated surface with HA17.5CaO deposition, and surface deposited with pure HA. The results showed that the osteoblast concentration was significantly lower than that seen on the other surfaces ($P < .05$) only on the surface treated with pure HA deposition, leading to the assumption that the dissolution product resulting from the high dissolution rate of this surface had an adverse effect on the growth and differentiation of osteoblasts. Other surfaces showed similar results (Table 1).

Removal Torque Measurements

Removal torque measurements were made at the distal surfaces of the implants. The measurements of the sham-operated group were as follows: 32.30 Ncm for machined surfaces, 47.25 Ncm for blasted surfaces, and 48.50 Ncm for the surfaces with HA17.5CaO deposition. In the ovariectomized group, the measurements were 23.00 Ncm for machined surfaces, 34.40 Ncm for blasted surfaces, and 34.63 Ncm for the surface with HA17.5CaO deposition. According to the statistical analysis, the removal torque measurements obtained from the ovariectomized rabbits were significantly lower than those of the control rabbits. Implants with a blasted surface and HA17.5CaO deposition showed similar measurements, obtaining significantly higher figures ($P < .05$) in comparison to implants with a machined surface (Table 2).

Histomorphometric Analysis

Histomorphometric analysis was performed on each sample of all categories (Figs 3 and 4). The total bone contact ratio of the different surfaces in the sham-operated group was as follows: 33.8% bone contact for machined surfaces, 48.5% for blasted surfaces, and 52.4% for surfaces with HA17.5CaO deposition. The contact ratios in the ovariectomized group were as follows: 30.0% for machined surfaces, 40.9% for blasted surfaces, and 48.4% for surfaces with HA17.5CaO deposition.

A significant difference was shown among all implant surfaces in both groups. Also, bone-to-metal contact measurements were generally lower in the ovariectomized group, while blasted-surface implants showed a statistically significant difference ($P < .05$) among the 2 groups (Table 3).

For the 3 best consecutive threads, the bone-to-metal contact ratios were as follows. In the sham-operated group, the ratio on HA17.5CaO-deposited implant surfaces was 62.5%, that of blasted-surface implants was 54.2%, and that of machined-surface implants was 38.2%. In the ovariectomized group, the ratio on HA17.5CaO-deposited implant surfaces was 59.9%, that of blasted-surface implants was 47.3%, and that of machined-surface implants was 35.5%. A significant difference was shown among all the surfaces in both groups. Bone-to-metal contact was generally lower in the ovariectomized rabbits, and a significant difference ($P < .05$) was detectable in blasted-surface implants between the 2 groups (Table 4).

The total bone volume measurements in both groups were as follows: in the sham-operated group, 76.4% for HA17.5CaO-deposited implant surfaces, 72.2% for blasted-surface implants, and 74.8% for machined-surface implants; in the ovariectomized group, 63.2% for HA17.5CaO-deposited implant surfaces, 63.8% for blasted-surface implants, and 61.7% for machined-surface implants. No significant difference was found among the different types of surfaces in both groups, and the ovariectomized

Figs 3a to 3f The view of undecalcified specimens in each group, examined under light microscopy.

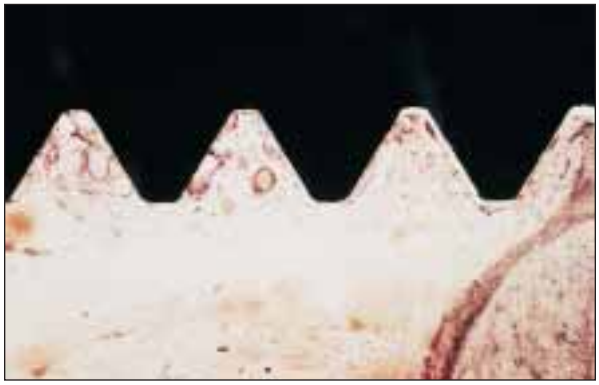


Fig 3a Surface deposited with HA by IBAD method in sham-operated group. The degree of bone contact was quite satisfactory.

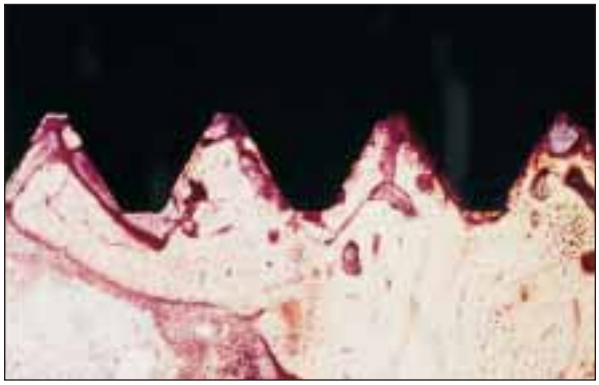


Fig 3b Aluminum oxide–blasted surface in sham-operated group. The degree of bone contact was good but inferior to HA deposited surface by IBAD method.

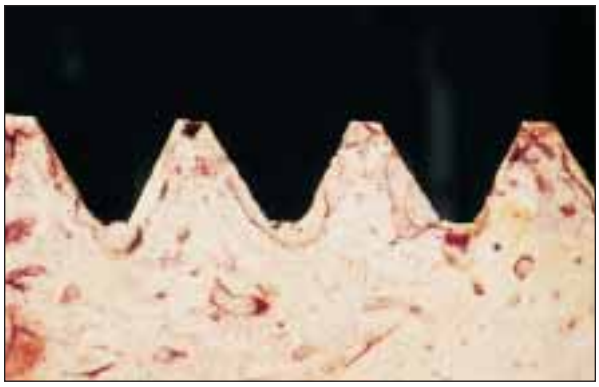


Fig 3c Machined surface in sham-operated group. The degree of bone contact was less than that seen for the other 2 types of implants.

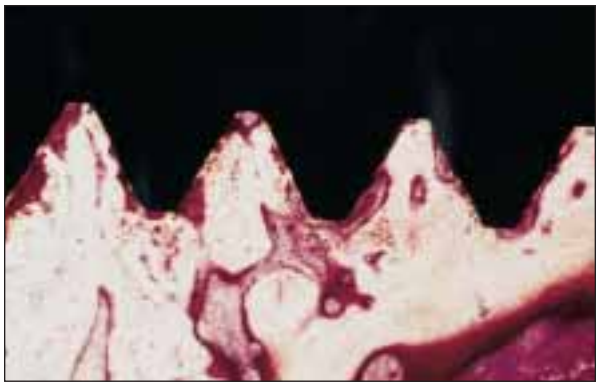


Fig 3d Surface deposited by IBAD method in ovariectomized group. There was no significant difference compared to the sham-operated group, but the bone volume was less than the normal group.

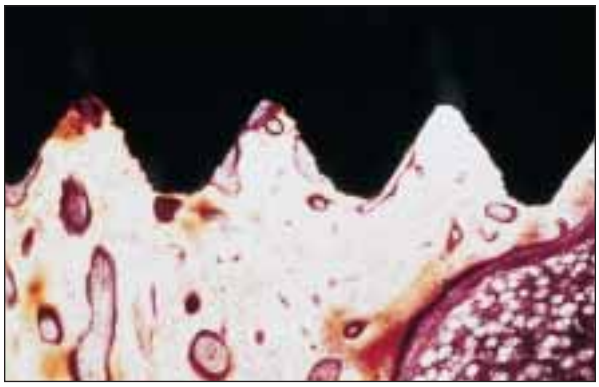


Fig 3e Aluminum oxide–blasted surface in ovariectomized group. The degree of bone contact was less than the sham-operated group, but an increase in bone volume was found.

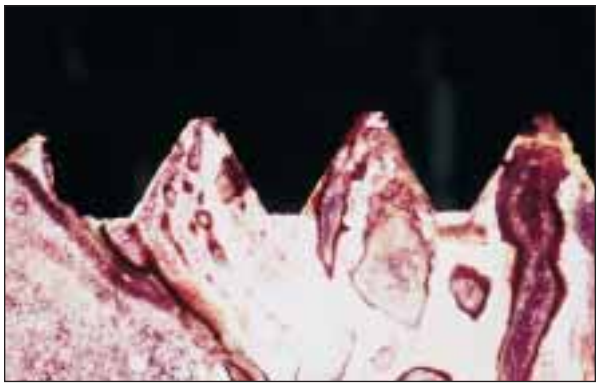
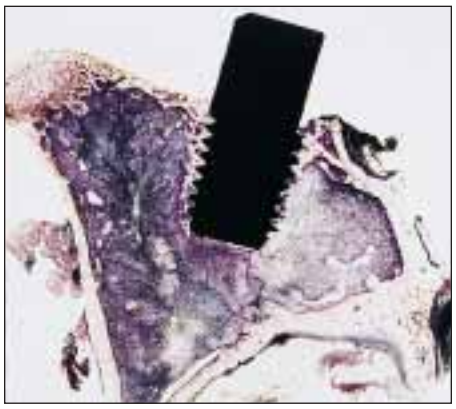


Fig 3f Machined surface in ovariectomized group. There was no significant difference compared to the sham-operated group, but a decrease in bone volume was seen.



Figs 4a and 4b Undecalcified specimens in *(left)* the sham-operated group and *(right)* the ovariectomized group. Downward growth of cortical bone around 2 implants was found. In the ovariectomized group, the thickness of cortical bone was reduced and the bone quality was inferior.

Table 3 Bone-to-Implant Contact (%) in All Threads (mean ± SD)

Rabbit group	HA-coated implants	Blasted implants	Machined implants
Sham-operated	52.4 ± 6.3[a]	48.5 ± 3.8[a]	33.8 ± 4.7[b]
Ovariectomized	48.4 ± 4.5[a]	40.9 ± 5.0*[,b]	30.0 ± 3.4[c]

[a,b,c] Statistically significant difference between columns ($P < .05$).
*Statistically significant difference between rows ($P < .05$).

Table 4 Bone-to-Metal Contact (%) in 3 Best Threads (mean ± SD)

Rabbit group	HA-coated implants	Blasted implants	Machined implants
Sham-operated	62.5 ± 5.2[a]	54.2 ± 5.4[b]	38.2 ± 3.5[c]
Ovariectomized	59.9 ± 6.4[a]	47.3 ± 5.7*[,b]	35.0 ± 3.8[c]

[a,b,c] Statistically significant difference between columns ($P < .05$).
*Statistically significant difference between rows ($P < .05$).

Table 5 Bone Volume (%) in All Threads (mean ± SD)

Rabbit group	HA-coated implants	Blasted implants	Machined implants
Sham-operated	76.4 ± 4.8	72.2 ± 5.9	74.8 ± 4.9
Ovariectomized	63.2 ± 8.5*	63.8 ± 5.3*	61.7 ± 3.5*

*Statistically significant difference between rows ($P < .05$).

Table 6 Bone Volume (%) in 3 Best Threads (mean ± SD)

Rabbit group	HA-coated implants	Blasted implants	Machined implants
Sham-operated	82.4 ± 5.6	78.9 ± 5.2	79.0 ± 6.1
Ovariectomized	71.6 ± 7.7*	73.98 ± 7.8*	68.5 ± 5.1*

*Statistically significant difference between rows ($P < .05$).

group showed significantly lower bone volume measurements compared to the sham-operated group ($P < .05$; Table 5).

For bone-to-metal contact ratio, the findings were as follows: in the sham-operated group, 82.4% for HA17.5CaO-deposited implant surfaces, 78.9% for blasted-surface implants, and 79.0% for machined-surface implants; in the ovariectomized group, 71.6% for HA17.5CaO-deposited implant surfaces, 73.9% for blasted-surface implants, and 68.5% for machined-surface implants. There was no significant difference among the surfaces in both groups. When the results of the ovariectomized group were compared to those of the sham-operated group, the ovariectomized group showed a significantly lower bone-to-metal contact ratio ($P < .05$; Table 6).

DISCUSSION

Fibroblasts[20] and fetal bovine bone cells[21] have been used for cell culture tests in previous studies. However, in the present study, calvaria-derived cells taken from newborn rats were used following the methods of Bellows and colleagues.[22] It is known through immunocytochemistry studies that ALP, bone sialoprotein, osteocalcin, and osteoblast-specific matrix proteins are important factors in achieving osteoblast differentiation and bone formation.[23,24] The serum factor and materials added to the medium have been known to influence cultured osteoblasts.[21,23] In previous mineralizing osteoblast culture studies comparing HA surfaces with pure titanium and pure titanium[25] with sprayed surfaces,[26] no difference was shown in ALP, bone sialoprotein,

and osteocalcin. Also, HA surfaces and pure titanium surfaces showed similar results after 14 and 21 days.[25] In studies of osteosarcoma cell line or rat calvaria–derived osteoblasts, an increase in surface roughness has been shown to lead to an increase in osteoblast growth and ALP secretion.[27,28]

In a study that used an ion beam dynamic mixing method similar to the present method, fibroblasts were able to mature on both HA and titanium surfaces, proving that growth and development of these cells are not affected by dissoluted product.[20] In the present study, the analysis of ALP manifestation of calvaria-derived osteoblasts on different implant surface types has shown that there was no significant difference among HA17.5CaO-deposited implant surfaces, blasted-surface implants, and machined-surface implants. However, pure HA-deposited surfaces showed significantly lower concentrations. In the case of HA, circular defects were visible around the surface because of the inactive growth of osteoblasts. Considering the results obtained, it can be presumed that the dissolution rate of the coating layer has an influence on osteoblast culture and ALP concentration, and achievement of the correct Ca/P ratio is essential.

Many studies have been conducted on the quantity and quality of bone in osteoporotic patients,[5,29] and many methods for inducing osteoporosis in vivo have been introduced. Animal models that have been artificially ovariectomized have been used.[30] Roberts and associates[31] and Dao and coworkers[32] have stated that osteoporosis is not a contraindication for implant placement. However, Mori and colleagues[33] reported a delay in bone remodeling and healing time in a study on osseointegration in rabbit bone with low mineral density. Motohashi and associates[34] reported that the presence of ovaries in mice had no relationship to the degree of bone contact, and the minimal production of bone was a result of osteoclast activation resulting from lack of estrogen.

In the present study, removal torque measurements were significantly lower in ovariectomized groups for all surfaces used ($P < .05$). Also, in the histomorphometric analysis, the bone-to-metal contact ratio was lower in the ovariectomized rabbits (not significant) and the amount of cortical bone was significantly lower. Under light microscopy, the cortical bone was thinner in the ovariectomized group (Fig 4), and characteristic absorbed fossae with rough boundaries were frequently identified.

Hydroxyapatite plasma-sprayed implant surfaces have been reported to have qualities similar to those of the HA17.5CaO-deposited implant surface, blasted-surface implants, and machined-surface implants, in comparison to Ti implants.[18,35] Other clinical and animal studies argue that HA ceramic, because of its weak mechanical properties, is inadequate for use in areas of implant loading. They reported that even though bone contact of HA is sound in the early stages, HA coatings delaminate after 48 weeks.[36]

The long-term results of HA-coated implants, demonstrating less bone in comparison to uncoated cpTi implants,[37] could have several explanations. First, the high bone contact ratio of HA may serve as a cause of bone loss.[38] Second, the abundance of osteoblasts around HA-coated areas promotes bone absorption.[39] Third, disintegrated HA particles serve as activators that promote secretion of interleukin-1 or prostaglandin E_2, which in turn activates osteoclasts.[40]

In the present study, it was possible to analyze the HA-deposited implant surface that was used for removal torque measurements with an electrodiagnostic system. It was found that none of the HA particles were left. Thus, it was anticipated that the advantages of the HA-deposited implants in the early healing phase would be apparent, while the disadvantages, such as separation or fracture of the coating layer, would be prevented. However, another problem, ie, the control of resorption, needs to be further investigated.

The HA-deposited surface used in this study maintains a 1-μm thickness and appears to be an improvement over the plasma-spray method. An electronic beam in a high vacuum environment of 10^{-7} mmHg is blasted from an electronic beam gun and collides with evaporating materials after changing direction under the magnetic field. The electronic beam technique has become prevalent for use in many industries because of its excellent physical and chemical characteristics.[41] The bonding force of metal to HA coatings deposited by electronic beams was measured. The forces were 8 to 45 MPa for ion sputtering,[42] 53 MPa for radiofrequency magnetron sputtering,[16] 59 to 65 MPa for beam dynamic mixing,[12] and 7 MPa for plasma spraying.[17] Ion beam–assisted deposition, which was used in this study, showed a bonding force of 35 to 70 MPa.[43]

Stabilization of implants is greatly influenced by dissolution, which causes breakdown in the coating layer and decreases the bonding forces, thus leading to separation of the implants and tissue. This apparently is not the case when the dissolution rate is controlled (not rapid); time may be provided for the bone to replace the dissolved areas.[13] Many methods have been introduced to control the dissolution rate. One is to decrease the degree of resolution by heating the coating layer,[44] but this method introduces problems such as cracking of the coating layer during the

heating process. An alternative is to control the Ca/P ratio.[12,45] The Ca/P ratio of HA is 1.67 because of differences in the evaporation rate and ionic energy between Ca and PO_4, and it is difficult to obtain a coating layer that is similar to evaporant in its contents.[14] In this study, the dissolution rate was controlled by adding CaO to the evaporant. In instances in which pure HA was used as the evaporant, the Ca/P ratio of the specimen reached 1.1 to 1.2, which was minimal, and β-tricalcium phosphate was created as a result. In cases where the Ca/P ratio was controlled to a level of 1.67 by changing the amount of CaO, an HA-type phase was formed. Other studies have mentioned a Ca/P ratio of 1.67.[12,45]

CONCLUSION

The effect of the IBAD deposition method on the osseointegration of root-form endosseous implants was assessed in rabbits. To compare the biocompatibility of each different surface, ALP measurements were also made in vitro on these surfaces.

1. Alkaline phosphatase concentration was measured after culturing osteoblasts on 5 different surfaces: a machined surface, a blasted surface, a machined surface with HA17.5CaO deposition without treatment, a machined surface with HA17.5CaO deposition with heat treatment, and a machined surface with pure HA deposition. Significantly, low concentrations were only seen on pure HA deposited surfaces.
2. Removal torque tests showed implants coated with HA by the IBAD method to be similar (ie, showed higher values) to implants with aluminum oxide–blasted surfaces compared to the implants with a machined surface. An ovariectomized group of rabbits showed lower mechanical test values than a sham-operated group ($P < .05$).
3. Histomorphometric comparisons were made on undecalcified ground sections. Implants coated with HA by the IBAD method demonstrated the highest mean bone-to-metal contact ratio on all threads and the 3 best consecutive threads; this was followed by implants with a blasted surface and those with a machined surface ($P < .05$). Hydroxyapatite-coated implants showed a slightly higher bone-to-metal contact ratio than the blasted implants, but no statistically significant difference was seen between the 2 materials. Implants that had been placed in ovariectomized rabbits showed less bone-to-metal contact than those placed in sham-operated rabbits, but no statistically significant difference was seen between the 2 groups.
4. Evaluation of bone volume on all threads and the 3 best consecutive threads showed no statistically significant difference among the different surface treatment groups, but lower bone volume was shown in implants placed in ovariectomized rabbits versus sham-operated rabbits ($P < .05$).

According to these results, implants coated with a thin film of HA showed high bone contact ratio, bone volume, and removal torque strength in the short term, but long-term observation is needed.

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