Enhanced Cell Integration to Titanium Alloy by Surface Treatment with Microarc Oxidation

A Pilot Study

Young Wook Lim MD, Soon Yong Kwon MD,
Doo Hoon Sun MD, Hyoun Ee Kim PhD,
Yong Sik Kim MD

Published online: 12 May 2009

© The Association of Bone and Joint Surgeons® 2009

Abstract Microarc oxidation (MAO) is a surface treatment that provides nanoporous pits, and thick oxide layers, and incorporates calcium and phosphorus into the coating layer of titanium alloy. We presumed such modification on the surface of titanium alloy by MAO would improve the ability of cementless stems to osseointegrate. We therefore compared the *in vitro* ability of cells to adhere to MAOed titanium alloy to that of two different types of surface modifications: machined and grit-blasted. We performed energy-dispersive x-ray spectroscopy and scanned electron microscopy investigations to assess the structure and morphology of the surfaces. Biologic and morphologic responses to osteoblast cell lines (SaOS-2) were then examined by measuring cell proliferation, cell differentiation (alkaline phosphatase activity), and $\alpha v \beta 3$ integrin. The

cell proliferation rate, alkaline phosphatase activity, and cell adhesion in the MAO group increased in comparison to those in the machined and grit-blasted groups. The osteoblast cell lines of the MAO group were also homogeneously spread on the surface, strongly adhered, and well differentiated when compared to the other groups. This method could be a reasonable option for treating the surfaces of titanium alloy for better osseointegration.

Introduction

Various surface treatments, including grit blasting and hydroxyapatite (HA) coating, have been developed for promoting bone ongrowth of cementless femoral stems. These two methods for bone ongrowth are widely used and their long-term clinical survival rates are 66% to 100% at about a 10-year followup [8, 19, 27, 28]. However, these methods have some disadvantages. In the case of grit blasting, alumina (Al_2O_3) is frequently used as the blasting material. The blasting material is often embedded into the implant and remains even after ultrasonic cleaning, acid passivation, and sterilization [3]. In some cases, these particles have been released into the surrounding tissues and have interfered with the osseointegration of the implants; moreover, the chemical heterogeneity of this implant surface may decrease the excellent corrosion resistance of titanium in a physiological environment [3]. With HA coatings, it is hard to maintain the intended surface roughness with the added coating [20], and coating delamination from the surface of the titanium implant and failure at the implant-coating interface despite the fact that the coating is well attached to the bone tissue, thus causing the clinical failure of implants [9, 34].

Each author certifies that he has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

This work was performed at Seoul St. Mary's Hospital and St. Mary's Hospital, Seoul, Korea.

Y. W. Lim, Y. S. Kim (✉)

Department of Orthopaedic Surgery, Seoul St Mary's Hospital,
505 Banpo-dong, Seocho-gu, Seoul, South Korea
e-mail: yongsik@korea.com

S. Y. Kwon

Department of Orthopaedic Surgery, St Mary's Hospital, Seoul,
South Korea

D. H. Sun

Department of Orthopaedic Surgery, Sun Hospital, Daejeon,
South Korea

H. E. Kim

Department of Materials Science and Engineering, Seoul
National University, Seoul, South Korea

Porous coated implants for bone ingrowth, whether they are proximally or extensively coated, have a long term survival rate of 79% to 94.7% at about a 15 year followup [18, 36]. However, these implants are sometimes associated with delamination of coating layer, osteolysis, and stress shielding due to excessive bone ingrowth in the diaphyseal area, especially in extensively porous coated implants [6, 24, 25].

In the dental field, an anodized surface modification method has recently been developed to enhance osseointegration. Microarc oxidation (MAO) provides nanoporous pits, thick oxide layers, and the incorporation of calcium and phosphorus into the coating layer which results in improved osteoblast cell responses, such as an enhanced rate of actin, vinculin cytoskeletal reorganization, and the formation of integrins mediating osteoblast adhesion [7, 14, 23, 37, 38]. The average roughness of a Ti-alloy surface, treated by the MAO process, is about 0.3 to 0.5 μm [22]. Smooth surfaces are sufficient for dental implants for early fixation and long-term mechanical stability possibly owing to the screw fixation and their small size. Orthopaedic implants such as cementless femoral stems seemingly require rough surfaces for early fixation and long-term mechanical stability because these are more or less cylindrical and large in size [7]. The average roughness of Ti-alloy surfaces treated by the MAO process is too low to apply to the cementless femoral stem. Therefore, we modified the surface of the titanium alloy by grit-blasting, then MAO to enhance the surface roughness in order to utilize the advantages of MAO.

We asked whether the modified MAO process would improve in vitro cell responses to the titanium alloy as reflected by (1) cell morphology; (2) confocal microscopy of $\alpha\text{v}\beta 3$ integrin; (3) proliferation rate; and (4) alkaline phosphatase activity compared to two different types of surface modification (machined and grit blasted).

Materials and Methods

We studied three types of titanium alloy surfaces (MAO processed, machined without any surface treatment, grit-blasted) in vitro to compare cell morphology, confocal microscopy of $\alpha\text{v}\beta 3$ integrin, proliferation rate, and alkaline phosphatase activity. We then performed energy-dispersive x-ray spectroscopy (EDS) and scanning electron microscopy (SEM) investigations to assess the structure and morphology. We finally examined biologic and morphologic responses to osteoblast cell lines (SaOS-2) by measuring cell proliferation, cell differentiation (alkaline phosphatase activity), $\alpha\text{v}\beta 3$ integrin expression, and by cytoskeleton staining (rhodamine phalloidin).

We used 100 Ti6Al4 V discs, measuring 12 mm in diameter and 10 mm in thickness to create the three different types of specimens: machined—which do not process the surface of titanium alloy ($n = 32$); grit-blasted (see below) ($n = 32$); and MAO ($n = 32$). Grit blasting was achieved with Al_2O_3 particles with a diameter of 200 to 500 μm moving in a high-velocity air stream (KSSA-

Fig. 1A–D SEM images of the surfaces of (A) Ti-alloy ($\times 2000$), (B) grit-blasted ($\times 2000$), and (C) MAO specimens ($\times 2000$) show different surface characteristics. (B) The grit-blasted specimen shows a finely textured surface. (C) This MAO specimen ($\times 2000$) shows a multilayered porosity of 1 to 5 μm . (D) This MAO specimen ($\times 3000$) shows micropores ranging in size from 0.4 to 7.4 μm .

5FD; Kumkang Tech, Seoul, Korea). The roughness of grit-blasted specimens was in the range of approximately 5 to 7 μm . For MAO, $\text{Ti}_6\text{Al}_4\text{V}$ specimens were used as anodes, and stainless steel plates were used as cathodes in an electrolytic bath. The $\text{Ti}_6\text{Al}_4\text{V}$ plates were ground with abrasive papers, ultrasonically washed with acetone and distilled water, and dried at 40°C. A fresh electrolyte prepared by dissolving reagent-grade chemicals of Ca (CH_3COO) $_2 \cdot \text{H}_2\text{O}$ (6.3 g L^{-1}), $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (13.2 g L^{-1}), EDTA-2Na (15 g L^{-1}), and NaOH (15 g L^{-1}) into deionized water was used for the MAO process. The applied voltage, frequency, duty cycle, and oxidizing time were 230 V, 600 Hz, 8%, and 5 minutes, respectively. The temperature of the electrolyte was kept at 40°C by a cooling system. The highest surface roughness (R_a values) was that of the MAO group (mean $\pm$ standard deviation, $6.5 \pm 0.13 \mu\text{m}$), followed by grit blasted ($5.0 \pm 0.24 \mu\text{m}$), and machined ($1.8 \pm 0.13 \mu\text{m}$). SEM findings showed different surface characteristics (Fig. 1A). The grit-blasted surface was finely textured (Fig. 1B). The MAO surface showed a multilayered porosity of 0.7 to 7.4 μm (average, 2.9 μm) (Figs. 1C–D at two magnifications).

Energy-dispersive x-ray spectroscopy (JEOL JSM-6700F; JEOL Ltd, Tokyo, Japan) was performed to evaluate the chemical composition of the titanium surfaces of the three groups. The morphologic comparison on the titanium surfaces of the three groups was performed using an SEM (JEOL JSM-6700F; JEOL Ltd, Tokyo, Japan) after the test specimens had been coated with platinum. MAO was able to cover the contaminated alumina particles impacted by the grit-blasted surfaces. An equivalent observation of the Al_2O_3 was confirmed by the EDS spectrum results of the surface chemical characterization (Fig. 2A–C). The EDS for the MAO group showed high peaks of calcium and phosphate compared to the machined and grit-blasted groups.

To prepare the specimens for SEM observation, 0.5 mL SaOS-2 cells (1×10^5 cells/mL) were seeded on each of the 12 titanium samples, and after 8 hours of incubation, the medium was then removed. Phosphate-buffered saline (PBS) was added and the cells were irrigated with PBS two to three times. After adding the 2% glutaraldehyde-PBS solution, the cells were left for 2 hours to stabilize. The fixative solution was removed after 2 hours and the cells were irrigated with distilled water three times. At 30 minute intervals, 50%, 70%, 90%, and 100% concentrations of the ethanol acceptor solution were added in sequence and dehydrated. Lastly, the ethanol was removed and the cells were left at room temperature to vaporize the ethanol completely.

Fig. 2A–C EDS spectrum images of (A) Ti-alloy as machined, (B) grit-blasted, and (C) MAO specimens are shown. (C) The MAO specimen shows high peaks of calcium and phosphate compared to other groups. The peaks of calcium and phosphate of A–B were results of being contaminated.

We visualized the $\alpha v \beta 3$ integrins using confocal microscopy. For $\alpha v \beta 3$ integrin, 0.5 mL SaOS-2 cells (5×10^4 cells/mL) were seeded on each of the six titanium samples and incubated for 6 hours. The cells were irrigated with PBS three times and stabilized with 4% paraformaldehyde for 10 minutes. After the stabilized cells were irrigated with PBS three times and treated with 0.1% Triton® X-100 for 10 minutes, they were again irrigated with PBS. After blocking with 5% serum, mouse anti-human $\alpha v \beta 3$ monoclonal antibody (MAB 1976; Chemicon, Temecula, CA) was left to react for 16 hours at 4°C. The following day, the cells were reacted with Alexa Fluor® 488 goat anti-mouse IgG (Molecular Probes Inc) and irrigated with PBS; the distribution of the cell adhesion agent, integrin, was subjectively assessed using a confocal and multiphoton microscope system (Bio-Rad Laboratories, Mississauga, ON, Canada).

For the cell proliferation assays, 0.5 mL SaOS-2 cells (5×10^4 cells/mL) were seeded on each of the 18 titanium samples and incubated (37°C, 5% CO₂, 95% humidity) at intervals of 4, 24, 48, 72 hours, and 1 week. We exchanged the medium with fresh medium before measuring the proliferation using the CellTiter 96® non-radioactive cell proliferation assay (Promega Corp, Madison, WI) as described by the manufacturer. This assay is a colorimetric method for determining the number of viable cells in proliferation assays. The amount of formazan formed can be measured by its absorbance at 450 nm using a plate reader and is directly proportional to the number of viable cells in culture. Using this kit, 15 μ L dye solution was placed on the titanium sample and the cells were incubated in 5% CO₂ at 37°C for 4 hours. After 100 μ L of stop solution was added, each 100 μ L of pipetted cells was transferred onto a 96-well plate (Nunc A/S, Roskilde, Denmark). We measured cell proliferation at 450 nm using a spectrophotometer (EL 312e; BioTek Instruments, Inc, Winooski, VT).

The alkaline phosphatase activity was measured by seeding 0.5 mL SaOS-2 cells (5×10^4 cells/mL) on each of the 18 titanium samples and incubated for 21 days. The medium was then removed and the cells were irrigated with PBS three times to remove as much of the serum in the culture fluid as possible. We placed 1 milliliter of 0.02% Triton® X-100 on the titanium sample to dissolve the cells. Cytolytic solution was transferred into a 1.5-mL tube, and the cells were crushed by sonication. The tube was then centrifuged at 14,000 rpm at 4°C for 15 minutes, and the supernatant was transferred to a new 1.5-mL tube. We added 100 microliters 1 mol/L Tris-HCl, 20 μ L 5 mmol/L MgCl₂, and 20 μ L 5 mmol/L p-nitrophenyl phosphate to the supernatant. It was left to react at 37°C for 30 minutes, and 50 μ L 1 N NaOH was then added to stop the reaction. Using p-nitrophenol as a standard, we

measured the optical density at 410 nm with a spectrophotometer. The measured alkaline phosphatase activity was expressed as the value of p-nitrophenol production quantity divided by the reaction time and the protein synthesis quantity, as measured by the Bio-Rad Protein assay kit (Bio-Rad Laboratories, San Jose, CA).

We compared the means of the proliferation rate and alkaline phosphatase activity of the three different surfaces using Student's t-test. Statistical analysis was performed using SPSS® 11.5 software (SPSS, Inc, Chicago, IL).

Results

After 6 hours of incubation the grit-blasted surface was covered with small, slender osteoblasts (Fig. 3A–B), whereas the MAO surface was largely covered with lamellipodia from the osteoblast cells (Fig. 3C). In addition, the lamellipodia largely covered and the filopodia extended into the micropores of the MAO surface. The thin cytoplasmic processes branched out from the filopodia to enter the micropores (Fig. 3D).

Cell adhesion appeared more intense in the MAO group. These findings were reflected in the expression and distribution of the integrin using the mouse anti-human $\alpha v \beta 3$ monoclonal antibody; the intensity was less in the Ti alloy (Fig. 4A) and gritblasted surface (Fig. 4B) than in the MAO surface (Fig. 4C).

Cell proliferation was similar in all three groups: machined (100.43 ± 13.44 OD), grit blasted (124.18 ± 16.13 OD), MAO group (141.68 ± 14.67 OD) (Fig. 5). Alkaline phosphatase activity was also similar in all three groups: machined (93.17 ± 9.76 nmol/min/ug), grit blasted (107.09 ± 8.15 nmol/min/ug), MAO group (117.81 ± 7.89 nmol/min/ug) (Fig. 6).

Discussion

To enhance the potential for titanium alloys to osseointegrate, we explored the MAO method, which had been successfully applied in the dental field, though not in the orthopaedic field. Because of the typical low surface roughnesses of orthopaedic implants we developed a modified MAO process of grit-blasting followed by MAO. We then asked whether the modified MAO process would improve the cell responses to the titanium alloy in vitro as reflected by (1) cell morphology, (2) confocal microscopy of $\alpha v \beta 3$ integrin, (3) proliferation rate, and (4) alkaline phosphatase activity, when compared to the two different types of surface modification (machined and grit blasted).

We note several limitations to our study. First, this was a study on the in vitro nature of cell morphology,

Fig. 3A–D SEM images show SaOS-2 cells after 6 hours of incubation on (A) Ti alloy ($\times 700$), (B) grit-blasted ($\times 700$), and (C, D) MAO surfaces ($\times 700$). (B) The grit-blasted surface is weakly covered with small, slender osteoblasts. (C) The MAO surface is largely and strongly covered with healthy lamellipodia of the osteoblast cells. (D) A thin cytoplasmic process branches out from the filopodia to enter the inside of a micropore.

proliferation rate, and alkaline phosphatase activity. Future studies are needed to determine whether MAO would improve the osteointegration, as it has yet to be studied in vivo. Second, we used SaOS-2 cells, an osteosarcoma cell line, which could possibly distort the action of osteoblasts, especially when drawing conclusions regarding osseointegration. We also did not compare our experiments with porous or HA coated implants. In the grit blasted and MAOed implants, the osseointegration was performed by bone ongrowth. In contrast, with the porous-coated implants, the osseointegration was performed by bone ingrowth. Because of this difference, we chose not to compare the MAO method with porous coated implants. In the case of HA coating, the surface roughness could not be manually controlled to different levels when grit blasting and MAO. Therefore, we did not compare with the HA coating.

Recently, a MAO method was successfully applied to form a rough TiO_2 layer on titanium surfaces in the dental field. This method, which increases surface roughness, forms TiO_2 on the surface through the oxidation process, and incorporates calcium or phosphorus ions into the surface layer, and is beneficial to osteoblastic cell activity [1, 21, 22, 38]. MAO forms a relatively thick TiO_2 layer by applying a positive voltage above a certain point to the titanium [22]. In this process, the repeated breakdown and

regeneration of TiO_2 results in porous and rough surfaces [22, 33]. Components of the surface commonly consist of calcium and phosphate and contain a porous structure with pores approximately the size of a micron or submicron, which facilitates osseointegration [14, 15, 21, 23, 26]. Moreover, MAO is a simple, controllable, and cost-effective method [22], the latter even more so than HA coatings. The properties of the oxide layer, such as its thickness, microstructure, roughness, and concentrations of Ca and P are easily controllable by adjusting the voltage, current, processing time, and the concentration of the electrolyte during the MAO process [14, 31, 32]. Despite these structural characteristics and chemical advantages, MAO has not yet been introduced and is currently being used for the surface treatment of cementless stems.

The surface roughness of titanium implants affects the rate of osseointegration and biomechanical fixation [10, 20]. Several in vitro studies have reported the effects of surface roughness on the adhesion, proliferation, and differentiation of osteoblastic cells. Osteoblastic cells attach, spread, and proliferate more rapidly on smooth surfaces than on rough surfaces while their differentiation is enhanced by rough surfaces [2, 4, 17]. Both the early fixation and long-term mechanical stability of the prosthesis can be improved by a higher surface roughness. However, too much surface roughness may cause an increase in peri-

Fig. 4A–C Confocal microscopy images show the expression and distribution of integrin using the mouse anti-human $\alpha v \beta 3$ monoclonal antibody in (A) Ti alloy ($\times 400$), (B) grit-blasted ($\times 400$), and (C) MAO specimens ($\times 400$). (C) Note the greater intensity of staining of integrin around the cytoplasm in the MAO specimen.

Fig. 5 A graph shows the results of SaOS-2 cell proliferation assays, expressed as relative proliferation rates, for the machined, grit-blasted, and MAO specimens. Cell proliferation was similar in the three groups.

Fig. 6 A graph shows the alkaline phosphatase activity of SaOs-2 cells cultured for 21 days on machined, grit-blasted, and MAO surfaces. Secretion of alkaline phosphatase was similar in the three groups.

implant inflammation and in ionic leakage [5]. Therefore, the optimal average roughness of cementless stems is reportedly 5 to 7 μm [13]. The average roughness of grit-blasted cementless stems, which have been used commercially, is 5 to 7 μm [11, 29]. In the present study, the average surface roughness of MAOed titanium was at a controlled 6.5 μm . When grit-blasting, the blasting material would have been embedded. MAO however is not a mechanical bond, rather a chemical one. Therefore, the binding strength is stronger than a mechanical bond, so although the blasting material would have been embedded, it could not have been released.

The cell morphology and proliferation assay indicated all surfaces were cytocompatible. This is in agreement with one study suggesting titanium is a biocompatible substrate for cell culturing [30]. We found similar cell proliferation and actin filaments on the various surfaces. However, compared to the machined and grit-blasted titanium surfaces, osteoblastic cells in MAOed titanium spread and formed lamellipodia on mirror-polished titanium surfaces. Moreover, we observed increased cell adhesion on the confocal images of the $\alpha\text{v}\beta 3$ integrin. This suggests that the osteoblastic cells were rapidly attached and differentiated on the microporous structure of MAOed titanium.

Alkaline phosphatase activity is an indicator of osteogenic differentiation, bone formation, and matrix mineralization [20]. Several studies have demonstrated the influence of surface roughness on ALP activity [12, 22, 35]. We could not, however, corroborate the finding of ALP activity increasing with the roughness parameters, as reported by Kim et al. [16], though this discrepancy may be attributed to the short duration (21 days) of our experiments.

To increase the surface roughness, we modified the surface of the titanium alloy by grit-blasting followed by MAO to enhance surface roughness and utilize the advantages of MAO. This modification should overcome the disadvantages of grit blasting and HA coating. In this method, the blasting material is also embedded, as with grit blasting. MAO is not a mechanical bond, but a chemical bond to the surface of Ti-alloy, in which the stable oxide layer surrounds the embedded particles (Fig. 1C). Therefore, the blasting material would have been embedded, but could not have been released. The oxide layer formed by MAO could not be delaminated, like HA, and because the oxide layer formed by MAO was stable, it could not be delaminated or degraded like HA [22, 37].

High long-term survival (66%~100%) has been reported with currently used cementless artificial joints and dental implants and there are no major problems with stability. However, when the bone quality is poor, such as in revision surgery or severe osteoporosis, surface treatment that can achieve or induce strong initial stability is

essential. In such situations MAO with enhanced cell responses compared to the conventional surface treatment methods for bone ongrowth may provide a new approach in creating the titanium nanoporous oxide layer with appropriate surface roughness.

Acknowledgments We thank William J. Maloney, Seung Soo Kim, and Shang Joong Lee for providing editorial assistance.

References

1. Anselme K. Osteoblast adhesion on biomaterials. *Biomaterials*. 2000;21:667–681.
2. Anselme K, Biggerelle M. Topography effects of pure titanium substrates on human osteoblast long-term adhesion. *Acta Biomater*. 2005;1:211–222.
3. Aparicio C, Gil FJ, Fonseca C, Barbosa M, Planell JA. Corrosion behaviour of commercially pure titanium shot blasted with different materials and sizes of shot particles for dental implant applications. *Biomaterials*. 2003;24:263–273.
4. Bächle M, Kohal RJ. A systematic review of the influence of different titanium surfaces on proliferation, differentiation and protein synthesis of osteoblast-like MG63 cells. *Clin Oral Implants Res*. 2004;15:683–692.
5. Becker W, Becker BE, Ricci A, Bahat O, Rosenberg E, Rose LF, Handelsman M, Israelson H. A prospective multicenter clinical trial comparing one- and two-stage titanium screw-shaped fixtures with one-stage plasma-sprayed solid-screw fixtures. *Clin Implant Dent Relat Res*. 2000;2:159–165.
6. Bourne RB, Rorabeck CH, Ghazal ME, Lee MH. Pain in the thigh following total hip replacement with a porous-coated anatomic prosthesis for osteoarthritis: a five year follow-up study. *J Bone Joint Surg Am*. 1994;76:1464–1470.
7. 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*. 1991;25:889–902.
8. Capello WN, D'Antonio JA, Feinberg JR, Manley MT. Ten-year results with hydroxyapatite-coated total hip femoral components in patients less than fifty years old: a concise follow-up of a previous report. *J Bone Joint Surg Am*. 2003;85:885–889.
9. Chang YL, Lew D, Park JB, Keller JC. Biomechanical and morphometric analysis of hydroxyapatite-coated implants with varying crystallinity. *J Oral Maxillofac Surg*. 1999;57:1096–1108.
10. 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*. 1998;40:1–11.
11. Delaunay C, Bonnomet F, North J, Jobard D, Cazeau C, Kempf JF. Grit-blasted titanium femoral stem in cementless primary total hip arthroplasty: a 5- to 10-year multicenter study. *J Arthroplasty*. 2001;16:47–54.
12. Giordano C, Sandrini E, Busini V, Chiesa R, Fumagalli G, Giavaresi G, Fini M, Giardino R, Cigada A. A new chemical etching process to improve endosseous implant osseointegration: in vitro evaluation on human osteoblast-like cells. *Int J Artif Organs*. 2006;29:772–780.
13. Hacking SA, Bobyn JD, Tanzer M, Krygier JJ. The osseous response to corundum blasted implant surfaces in a canine hip model. *Clin Orthop Relat Res*. 1999;364:240–253.
14. Ishizawa H, Ogino M. Formation and characterization of anodic titanium oxide films containing Ca and P. *J Biomed Mater Res*. 1995;29:65–72.

15. Johansson C, Albrektsson T. Integration of screw implants in the rabbit: a 1-year follow-up of removal torque of titanium implants. *Int J Oral Maxillofac Implants*. 1987;2:69–75.
16. Kim MJ, Choi MU, Kim CW. Activation of phospholipase D1 by surface roughness of titanium in MG63 osteoblast-like cell. *Biomaterials*. 2006;27:5502–5511.
17. Kim MJ, Kim CW, Lim YJ, Heo SJ. Microrough titanium surface affects biologic response in MG63 osteoblast-like cells. *J Biomed Mater Res A*. 2006;79:1023–1032.
18. Kim YH. Long-term results of the cementless porous-coated anatomic total hip prosthesis. *J Bone Joint Surg Br*. 2005;87:623–627.
19. Kim YH, Kim JS, Oh SH, Kim JM. Comparison of porous-coated titanium femoral stems with and without hydroxyapatite coating. *J Bone Joint Surg Am*. 2003;85:1682–1688.
20. Le Guehennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater*. 2007;23:844–854.
21. Lee IS, Whang CN, Kim HE, Park JC, Song JH, Kim SR. Various Ca/P ratios of thin calcium phosphate films. *Mater Sci Eng C*. 2002;22:15–20.
22. Li LH, Kong YM, Kim HW, Kim YW, Kim HE, Heo SJ, Koak JY. Improved biological performance of Ti implants due to surface modification by micro-arc oxidation. *Biomaterials*. 2004;25:2867–2875.
23. Liu F, Song Y, Wang F, Shimizu T, Igarashi K, Zhao L. Formation characterization of hydroxyapatite on titanium by microarc oxidation and hydrothermal treatment. *J Biosci Bioeng*. 2005;100:100–104.
24. Maloney WJ, Harris WH. Comparison of a hybrid with an uncemented total hip replacement: a retrospective matched-pair study. *J Bone Joint Surg Am*. 1990;72:1349–1352.
25. Maric Z, Karpman RR. Early failure of noncemented porous coated anatomic total hip arthroplasty. *Clin Orthop*. 1992;278:116–120.
26. Nie X, Leyland A, Matthews A. Deposition of layered bioceramic hydroxyapatite/TiO₂ coatings on titanium alloys using a hybrid technique of micro-arc oxidation and electrophoresis. *Surface Coatings Technol*. 2000;125:407–414.
27. Parvizi J, Sharkey PF, Hozack WJ, Orzoco F, Bissett GA, Rothman RH. Prospective matched-pair analysis of hydroxyapatite-coated and uncoated femoral stems in total hip arthroplasty: a concise follow-up of a previous report. *J Bone Joint Surg Am*. 2004;86:783–786.
28. Reikerås O, Gunderson RB. Excellent results of HA coating on a grit-blasted stem: 245 patients followed for 8–12 years. *Acta Orthop Scand*. 2003;74:140–145.
29. Schreiner U, Scheller G, Herbig J, Jani L. Mid-term results of the cementless CLS stem: a 7- to 11-year follow-up study. *Arch Orthop Trauma Surg*. 2001;121:321–324.
30. Schuler M, Trentin D, Textor M, Tosatti SG. Biomedical interfaces: titanium surface technology for implants and cell carriers. *Nanomed*. 2006;1:449–463.
31. Sul YT, Johansson CB, Jeong Y, Albrektsson T. The electrochemical oxide growth behavior on titanium in acid and alkaline electrolytes. *Med Eng Phys*. 2001;23:329–346.
32. Sul YT, Johansson CB, Petronis S, Krozer A, Jeong Y, Wennerberg A, et al. Characteristics of the surface oxides on turned and electrochemically oxidized pure titanium implants up to dielectric breakdown: the oxide thickness, micropore configurations, surface roughness, crystal structure and chemical composition. *Biomaterials*. 2002;23:491–501.
33. Wei D, Zhou Y, Jia D, Wang Y. Characteristic and in vitro bioactivity of a microarc-oxidized TiO₂-based coating after chemical treatment. *Acta Biomater*. 2007;3:817–827.
34. Wheeler SL. Eight-year clinical retrospective study of titanium plasma-sprayed and hydroxyapatite-coated cylinder implants. *Int J Oral Maxillofac Implants*. 1996;11:340–350.
35. Xavier SP, Carvalho PS, Beloti MM, Rosa AL. Response of rat bone marrow cells to commercially pure titanium submitted to different surface treatments. *J Dent*. 2003;31:173–180.
36. Yoon TR, Rowe SM, Kim MS, Cho SG, Seon JK. Fifteen- to 20-year results of uncemented tapered fully porous-coated cobalt-chrome stems. *Int Orthop*. 2008;32:317–323.
37. Zhu X, Chen J, Scheideler L, Althebaumer T, Geis-Gerstorf J, Kern D. Cellular reactions of osteoblasts to micron- and submicron-scale porous structures of titanium surfaces. *Cells Tissues Organs*. 2004;178:13–22.
38. Zhu X, Kim KH, Jeong Y. Anodic oxide films containing Ca and P of titanium biomaterial. *Biomaterials*. 2001;22:2199–2206.