Bone Response to Endosseous Titanium Implants Surface-Modified by Blasting and Chemical Treatment: A Histomorphometric Study in the Rabbit Femur

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Abstract: This study evaluated the effects of the addition of oxide structure with submicron-scale porous morphology on the periimplant bone response around titanium (Ti) implants with microroughened surfaces. Hydroxyapatite-blasted Ti implants with (experimental) and without (control) a porous oxide structure produced by chemical treatment were investigated in a rabbit femur model. Surface characterizations and *in vivo* bone response at 4 and 8 weeks after implantation were compared. The experimental implants had submicron-scale porous surface structure consisted of anatase and rutile phase, and the original R_a values produced by blasting were preserved. The histomorphometric evaluation demonstrated statistically significantly increased bone-to-implant contact (BIC) for experimental implants, both in the three best consecutive threads ($p < 0.01$) and all threads ($p < 0.05$) at 4 weeks. There was no remarkable difference in the BIC% or bone area percentage between the two groups at 8 weeks. The porous Ti oxide surface enhanced periimplant bone formation around the Ti implants with microroughened surfaces at the early healing stage. Based on the results of this study, the addition of crystalline Ti oxide surface with submicron-sized porous morphology produced by chemical treatment may be an effective approach for enhancing the osseointegration of Ti implants with microroughened surfaces by increasing early bone-implant contact. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 84B: 400–407, 2008

Keywords: titanium implants; osseointegration; surface modification; surface topography; histomorphometry

INTRODUCTION

Titanium (Ti) and its alloys are the most frequently used materials for endosseous implants in dentistry and orthopedics due to a high degree of biocompatibility and good mechanical properties. The long-term success of Ti implants is greatly affected by local bone conditions, such as bone quality and quantity. Relatively high implant failure rates have been reported in areas of poor quality bone, such as in the posterior maxilla.[1]

Ti is generally considered to be bioinert and not likely to form direct bonds with bone; it must be fixed clinically to bone by mechanical interlocking. However, mechanically fixed implants can loosen over long periods of use and numerous surface modifications have been studied to improve clinical results and shorten healing periods. It has been suggested that microroughness, produced by blasting and/or acid etching, gives an increase in the implant surface area and enhances biomechanical bonding by optimizing the biological response of the bone and micromechanical interlocking.[2–4]

Many approaches have focused on activating the implant surfaces to improve osseointegration by increasing the reactivity of the metallic Ti surface through chemical modification, including ion implantation,[5] alkali treatment,[6] and coating with bioactive materials.[7] Of these, hydroxyapatite (HA) plasma spraying is the most frequently used coating method to produce potentially bioactive implant surfaces, but several problems, such as delamination and unpredictable biodegradation, have been reported.[8–10]

It has been suggested that the reactivity of Ti implants can also be enhanced by a TiO_2 layer, because of the capability to induce calcium phosphate formation *in vitro* and

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in vivo.[11] Sul et al.[12] reported that surface porosity and changed oxide crystallinity seem to be the most probable reasons for the finding of a greater bone response to oxidized implants in animal models. To improve the bioactivity of Ti implants by using a TiO_2 layer, several methods such as anodic oxidation,[12] alkali treatment,[6] and sol–gel coating[13] have been studied. Several studies indicated that the oxide structure and porous morphology of the Ti surface are responsible for apatite deposition.[14–16]

Recently, Uchida et al.[17] reported that the *in vitro* apatite-forming ability of Ti induced by NaOH and heat treatment could be enhanced by the use of water and subsequent heat treatment, and they suggested that higher *in vivo* bone bonding ability could be obtained in the same way. The *in vitro* apatite-forming ability of Ti surfaces in simulated body fluid was reported to be consistent with the *in vivo* bone bonding behavior of the implants.[18,19]

We expected that this porous oxide structure of Ti implants might have synergic effects in periimplant bone formation when combined with the micron-scale surface properties of implants related to biomechanical interlocking. The purpose of this study was to evaluate the effects of the addition of oxide structure with submicron-sized porous morphology on the periimplant bone response around Ti implants with microroughened surfaces.

For this purpose, the porous oxide structure on the surface of Ti implants was prepared by water and heat treatment after alkali treatment. The surface characteristics of the Ti implants were observed before and after surface treatment. Periimplant bone responses were evaluated by histological and histomorphometric evaluation after 4 and 8 weeks of implantation in rabbit femurs.

MATERIALS AND METHODS

Titanium Implants and Chemical Treatment

Screw-type implants ($n = 42$) with an external diameter of 3.75 mm and a length of 7 mm were turned from commercially pure Ti rods (ASTM Grade 2) and the surfaces were roughened by HA blasting (MegaGen, Kyungsan, Korea). The implants were then cleaned in nitric acid to remove the remaining HA sandblasted particles (blasted implants). To prepare a submicron-scale porous anatase structure on the surface of the Ti implants, blasted implants were chemically treated as described elsewhere.[17] Briefly, blasted implants were treated in 5*M* NaOH at 60°C for 24 h. Then, implants were thoroughly rinsed and immersed in deionized water (Milli-Q Ultra-Pure water) at 80°C for 24 h. The implants were then dried at 40°C for 24 h and subjected to heat treatment at 600°C for 1 h. The heat treatment was performed in an electric furnace heated at a rate of 5°C/min and then allowed to cool to room temperature (blasted/AWH implants). In this study, two groups of implants were used: blasted implants as the control, and blasted/AWH implants as the experimental group. All implants were sterilized by gamma irradiation before use. To evaluate the crystalline structure and composition of the Ti oxides after surface treatments, rectangular Ti samples ($20 \times 10 \times 1$ mm^3) were treated in the manner described above.

Surface Characterization

The crystalline phase of the surface TiO_2 layer was evaluated by thin-film X-ray diffractometry (XRD, X'Pert-APD, Philips, Netherlands) using rectangular Ti samples. One experimental and one control implant were observed by scanning electron microscope (SEM, S-4200; Hitachi, Tokyo, Japan) to evaluate their surface morphology. The chemical composition of the Ti surfaces was analyzed by X-ray photoelectron spectroscopy (XPS, MT 500/1; VG Microtech, UK). Implant surface roughness measurement was performed by stylus profilometry (Form Talysurf Series 2; Taylor Hobson, London, UK). For this purpose, two implants from each group were measured, and three measurements of the amplitude parameters were performed on each implant. All measurements were performed on the lateral flat surface of the apical part of the implants.

Animals and Surgical Procedure

Eighteen adult male New Zealand white rabbits weighing 3–3.5 kg were used in this study. This experiment was approved by the Institutional Animal Care and Use Committee of Kyungpook National University Hospital, Daegu, Korea.

General anesthesia was induced by intramuscular injection of a combination of 1.3 mL of ketamine (100 mg/mL; Ketara, Yuhan, Korea) and 0.2 mL of xylazine (7 mg/kg body weight; Rompun, Bayer Korea, Korea). The medial flat surfaces of femoral condyle were used as the surgical sites. The surgical areas were shaved and the skin was washed with a mixture of iodine and 70% ethanol prior to surgical draping. Local anesthesia with 1.0 mL of 2% lidocaine (1:100,000 epinephrine; Yuhan, Korea) was performed to control bleeding and to provide additional anesthesia. The surgical sites were exposed with an incision through the skin, fascia, and periosteum at the medial surface of the distal femur using sterile surgical techniques.

The implant site osteotomies were prepared in the usual manner. A final drill diameter of 3 mm was used. All drilling procedures were carried out under profuse sterile saline irrigation. One femur received one implant. Blasted/AWH experimental implants ($n = 18$) and blasted control implants ($n = 18$) were placed in the right and left femur according to a random scheme. Implants were placed by self-tapping and all implants penetrated the first bone cortex only.

After surgery, surgical sites were closed in layers and sutured using Vicryl (Ethicon, Somerville, USA). Postoperatively, antibiotics (Baytril, Bayer Korea, Korea) and analgesics (Nobin, Bayer Korea, Korea) were injected intramuscularly for 3 days to prevent postsurgical infection and to control pain. The animals were divided into two groups for histomorphometric evaluation. After 4 weeks of healing, nine animals were killed by intravenous injection of air. The remaining nine animals were killed after 8 weeks of healing.

Specimen Preparation and Histomorphometric Evaluation

The distal femurs containing the implants were removed *en bloc*, fixed in 4% neutral buffered formaldehyde, dehydrated using an ascending series of alcohol, and embedded in methyl methacrylate for undecalcified sectioning. Undecalcified cut-and-ground sections, which were prepared in a plane parallel to the long axis of each implant, containing the central part of the implants were produced at a final thickness of 20 μm using a Macro cutting and grinding system (Exakt 310 CP series; Exakt Apparatebau, Norderstedt, Germany). The sections were stained with Villanueva stain, and histomorphometric analysis was carried out using a light microscope (Axioplan 2, Carl Zeiss, Germany) with an image analysis system (i-Solution, iMTechnology, Korea) under 50× magnification. Images were captured using a digital camera (AxioCam MRc 5, Carl Zeiss, Germany) attached to the microscope and displayed on a computer monitor. The percentage of bone-to-implant contact (BIC%) for the best three consecutive threads and percentage of bone area within the same threads were measured. The percentage of BIC was measured as the percentage of the length of mineralized bone in direct contact with the implant surface. In addition, the percentage of BIC for all threads was measured.

Statistical Analysis

The histomorphometric data were processed using the SAS statistical system. After having checked the normality of the data the significance of the differences between the two groups was analyzed using Student's paired *t* test. The significance of the differences between the two healing periods in the same group was also analyzed. Values of p less than 0.05 were considered statistically significant.

RESULTS

Surface Characteristics

SEM observations showed typical irregular indentations produced by blasting on the surfaces of both groups of implants at a magnification of 1000× [Figure 1(a,c)]. Submicron-sized porous structure (~200 nm in size) was observed on the surfaces of the blasted/AWH implants at a magnification of 10,000× [Figure 1(d)]. Based on profilometry, the average surface roughness (R_a) of both groups of implants were almost identical (Table I). Blasted/AWH surfaces exhibited a set of peaks of reflection from anatase and rutile in the XRD analysis (Figure 2). All surfaces of the blasted and blasted/AWH implants consisted primarily of Ti and O. Carbon (C) was detected as a surface contaminant in the XPS survey spectra; no traces of Ca in the surfaces of blasted samples or Na in the blasted/AWH samples were detected (Figure 3).

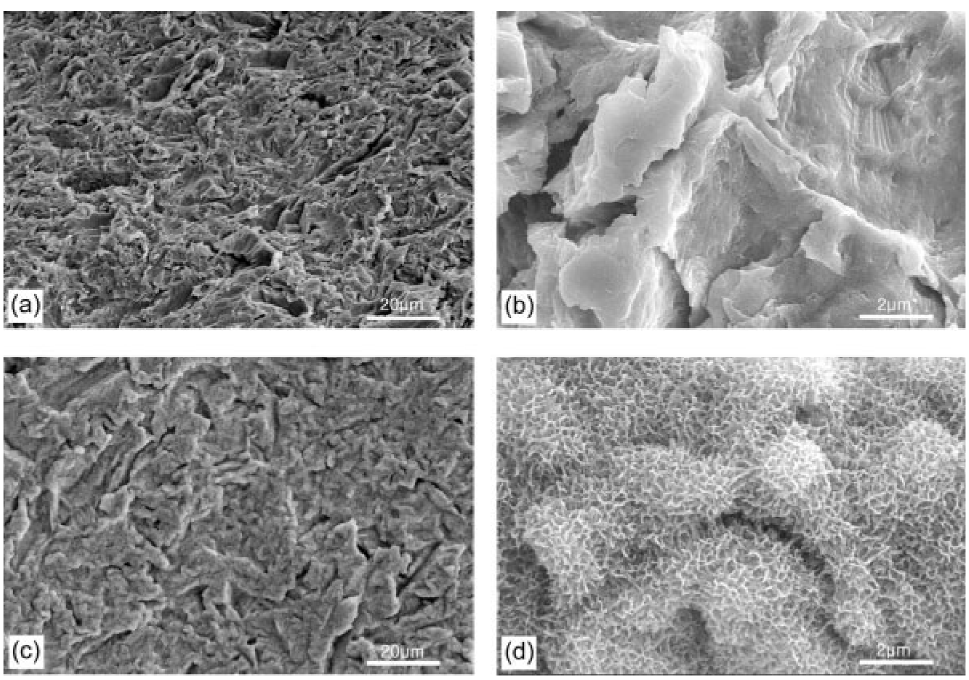

Figure 1. SEM images of blasted (a,b) and blasted/AWH (c,d) implants at magnifications of ×1000 (a,c) and ×10,000 (b,d).

TABLE I. Surface Roughness Parameters of the Implants (mean ± SD)

Implant	R_a (μm)	R_q (μm)	R_t (μm)	R_{zDIN} (μm)
Blasted	1.3214 ± 0.0681	1.7119 ± 0.0725	10.0344 ± 1.6138	7.5741 ± 0.4363
Blasted/AWH	1.3327 ± 0.0897	1.6795 ± 0.0867	8.3275 ± 0.2047	6.7940 ± 0.4402

R_a = The arithmetic average of the absolute height values of all points of the profile.
R_q = The root mean square of the values of all points of the profile.
R_t = The maximum peak-to-valley height of the entire measurement trace.
R_{zDIN} = The arithmetic average of the maximum peak-to-valley height of the roughness values of five consecutive sampling sections over the filtered profile.

Histological and Histomorphometrical Evaluation

At 4 and 8 weeks after implantation, all implants in the control and experimental groups were histologically in direct contact with the surrounding bone, with no signs of inflammation at the bone-implant interface (Figures 4 and 5). Four weeks after implantation, the blasted/AWH implants showed greater bone-implant contact than the blasted implants (Figure 4), the mean BIC% over the total implant length was 66.23% ± 10.28% for the blasted/AWH implants and 50.13% ± 15.35% for the blasted implants. In the three best consecutive threads, the mean BIC% was 80.44% ± 9.95% for the blasted/AWH implants and 60.41% ± 9.72% for the blasted implants. The blasted/AWH implants showed a statistically significantly higher percentage of BIC than the blasted implants both in the total implant length ($p = 0.019$; Figure 6) and three best consecutive threads ($p = 0.001$; Figure 7). Within the same three consecutive threads, the mean bone area percentage was 71.94% ± 10.65% for the blasted/AWH implants, and 63.21% ± 11.87% for the blasted implants. There was no statistical difference between the two groups ($p > 0.05$; Figure 8).

Eight weeks after implantation, all implants, both blasted and blasted/AWH, were surrounded by mineralized bone, the bone filled large areas within all threads of the implants of both groups (Figure 5). There was no statistical difference in the BIC% or bone area percentage between the control and experimental groups ($p > 0.05$; Figures 6–8). The mean BIC% over the total implant length was 69.71% ± 10.23% for the blasted/AWH implants and 66.54% ± 10.51% for the blasted implants. In the three best consecutive threads, the mean BIC% was 75.35% ± 10.92% for the blasted/AWH implants and 73.88% ± 9.06% for the blasted implants. The mean bone area percentage within the same three consecutive threads was 82.9% ± 4.39% for the blasted/AWH implants, and 78.88% ± 12.17% for the blasted implants.

In the blasted/AWH implants, the mean values of BIC% at 4 and 8 weeks after implantation were similar except the mean bone area percentage in the three consecutive threads ($p = 0.016$). In the blasted implants, the mean values of the histomorphometric parameters at 8 weeks were significantly higher compared with those at 4 weeks ($p = 0.008$, 0.018, and 0.014, respectively for BIC% in three best consecutive threads, BIC% in all threads and bone area percentage; Figures 6–8).

DISCUSSION

Numerous surface modifications have been tried in order to optimize cell and tissue interactions and thereby obtain rapid bone response in endosseous implant therapy. Several studies have suggested the effectiveness of specific Ti oxide structure on Ti implants for improvement of osseointegration, reporting that an oxidized Ti surface can enhance the periimplant bone healing response, thereby providing successful osseointegration.[12,20,21]

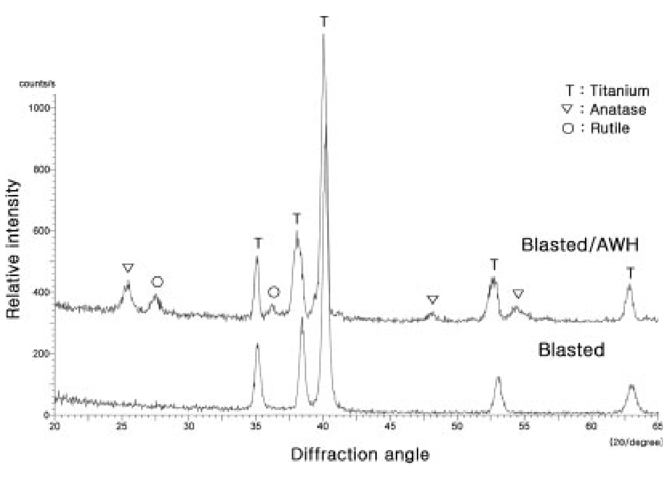

Figure 2. XRD patterns of the blasted and blasted/AWH Ti samples.

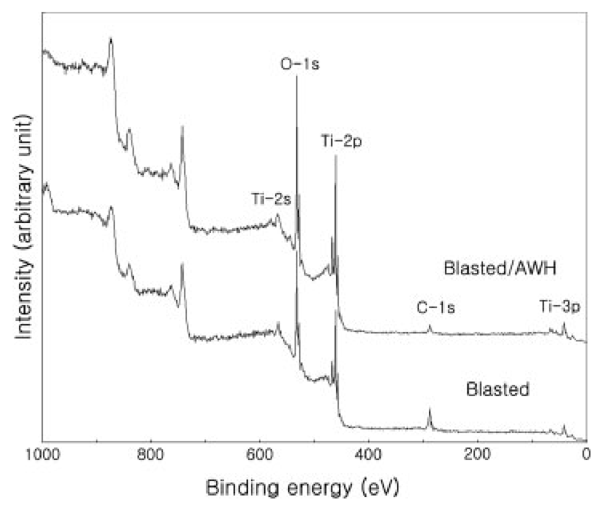

Figure 3. XPS survey spectra from the surfaces of blasted and blasted/AWH implants at binding energies up to 1000 eV.

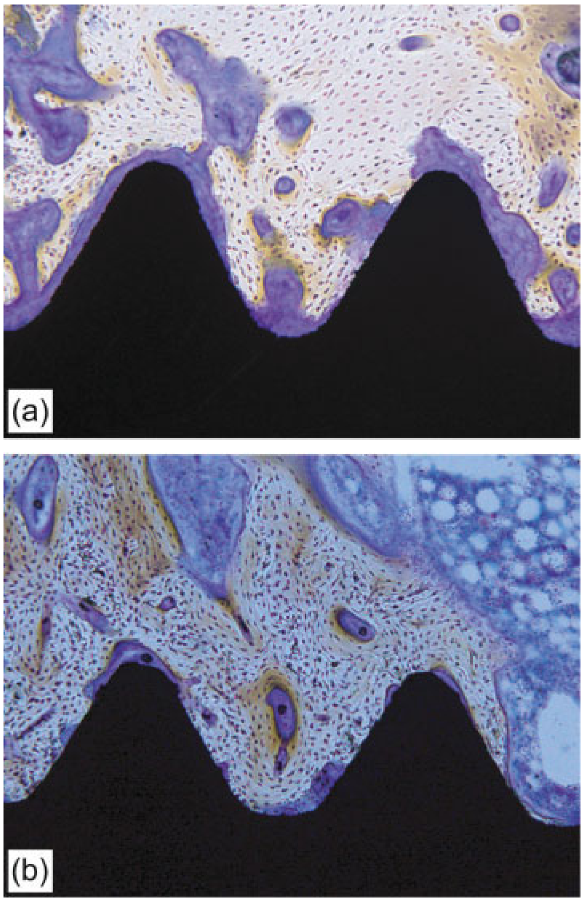

Figure 4. Histological sections of blasted (a) and blasted/AWH (b) implants 4 weeks after implantation in rabbit femurs. The blasted/AWH implant shows a higher degree of bone-implant contact compared with the blasted implant. The distance between the threads is 600 μm (stained with Villanueva stain). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In this study, we evaluated the surface characteristics and bone responses to microroughened Ti implants with porous Ti oxide surface in rabbit femurs. The porous crystalline Ti oxide structure produced by chemical treatment preserved the underlying micron-scale surface properties produced by HA blasting of the Ti implants. There were no remarkable differences in the mean values of the surface roughness parameters when comparing the blasted/AWH and blasted surfaces (Table I). Although the optimal surface roughness to guarantee implant success has not been clearly defined yet, many experimental studies have shown positive correlations between an increased surface roughness and bone fixation.[22–24] Wennerberg et al.[25–27] suggested an optimal surface roughness in terms of height deviation, in the range of 1–1.5 μm, for enhancing periimplant bone formation in grit-blasted implants.

In contrast, other studies[4,22–24] indicated that the roughness window of 1–1.5 μm could not be applied for all kinds of microroughened surfaces, which found that the strength of biomechanical attachment of bone tissue to Ti implants increases with increasing the degree of surface roughness beyond the roughness range suggested by Wennerberg et al.[25–27]

In this study, the surface treatment method for producing the porous Ti oxide surface did not affect the original roughness values produced by blasting. This chemical treatment may be considered an effective method for providing bioactivity to bioinert Ti implants with intermediately rough surfaces.

The histomorphometric analysis showed a greater degree of bone contact with the blasted/AWH implants at the early healing stage. Recently reported *in vivo* studies have indicated a strong correlation between the degree of BIC and surface properties of implants.[28,29] The finding of increased bone-implant contact may be correlated with the high apatite-forming ability of porous crystalline Ti oxide surface. Several studies have suggested that apatite formation on Ti surfaces increases with increases in anatase.[17,30]

The histomorphometric results indicate that a porous Ti oxide surface increases the osteoconductivity of microroughened implants, thereby increasing BIC% at the early healing stage. These results coincide with the findings of

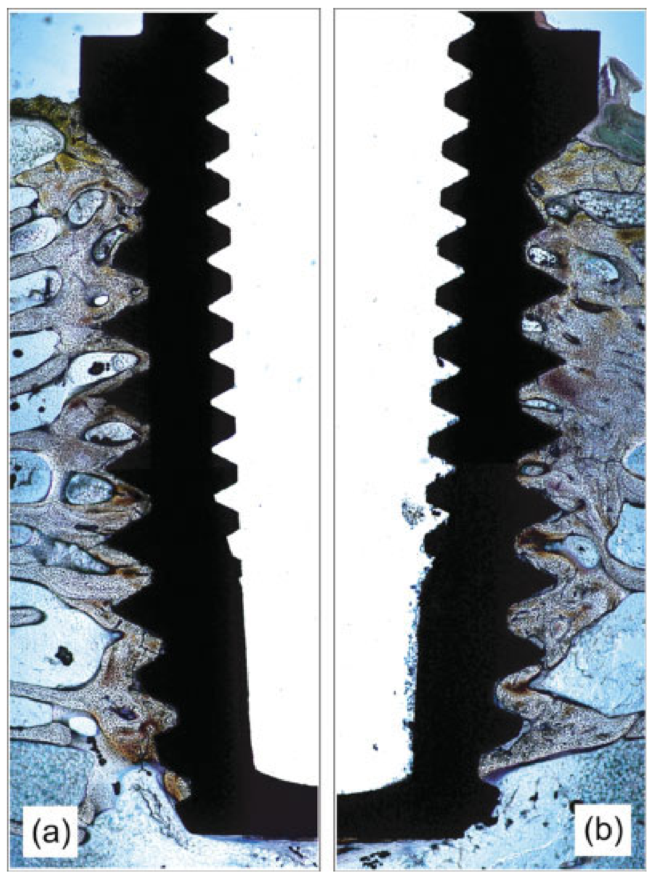

Figure 5. Histological sections of blasted (a) and blasted/AWH (b) implants 8 weeks after implantation. Direct bone-implant contact is observed in all threads of both groups of implants and the bone fills almost the entire area within the threads. The distance between the threads is 600 μm (stained with Villanueva stain).

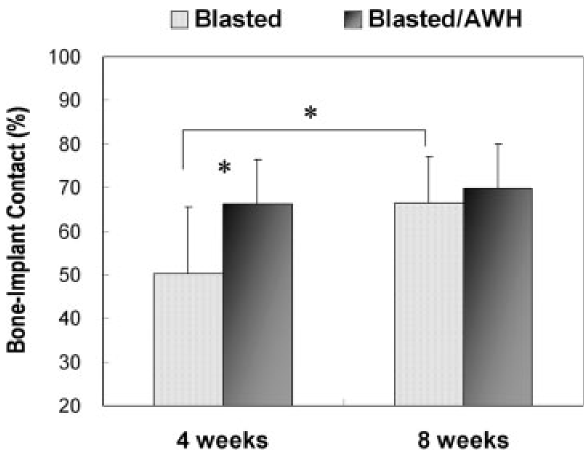

Figure 6. Mean percentage of bone-to-implant contact (BIC%) in all threads. The BIC% was significantly different between the two groups at 4 weeks (*$p < 0.05$). The blasted implants show significant increases in BIC% at 8 weeks compared with 4 weeks (*$p < 0.05$).

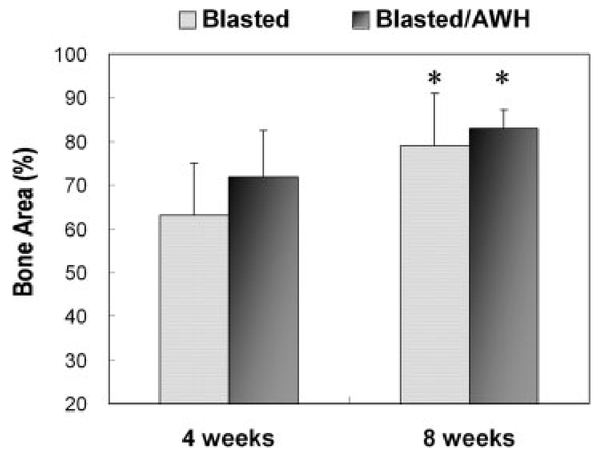

Figure 8. Mean percentage of the bone area in three best consecutive threads of implants. The bone area percentage was not significantly different between the two groups at 4 and 8 weeks ($p > 0.05$). Both the control and experimental implants show significantly increased bone area percentage at 8 weeks compared with 4 weeks (*$p < 0.05$).

other studies, which reported that sodium-free alkali- and heat-treated Ti surfaces showed faster bone-bonding ability.[31,32]

The faster bone-bonding ability can be explained by the mechanism described by Kokubo et al.,[30] who indicated that among different Ti—OH groups, the Ti—OH groups in the anatase structure are the most effective for apatite nucleation. However, it also has been shown that both anatase and rutile structure of Ti oxide surfaces induce apatite formation on their surfaces *in vitro*,[14–16,33,34] Li et al.[14] and Wang et al.[16] have suggested that the surface porosity is more critical for inducing apatite deposition than is crystal structure of Ti oxide layer. Furthermore, the submicron-sized porous surface structure of implants increases the total surface area and thus exposes a larger area of bioactive structure, which subsequently increases nucleation sites

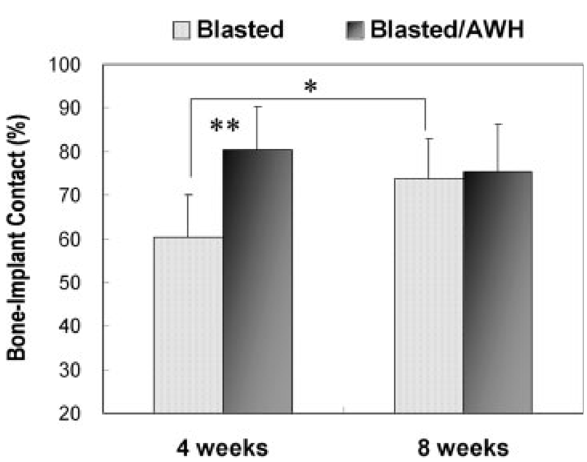

Figure 7. Mean percentage of bone-to-implant contact (BIC%) in three best consecutive threads. The BIC% of the blasted/AWH implants is significantly greater than that of the blasted implants at 4 weeks (**$p < 0.01$). The blasted implants show significant increases in BIC% at 8 weeks compared with 4 weeks (*$p < 0.05$).

for calcified tissue nuclei in the biological environment. It is believed that apatite formation on implant surfaces is the first step of osseointegration.[18,35] It has also been suggested that the *in vitro* apatite-forming ability of Ti surfaces in simulated body fluid is consistent with the *in vivo* bone-bonding behavior of the implants.[18,19]

Additionally, the submicron-scale porous structure may influence bone healing by enhancing osteoblast adhesion and subsequent cellular function on the surfaces of Ti implants.[36,37] The blasted/AWH implants had a submicron-scale surface structure ~200 nm in size. In this study, increased bone formation at the early healing stage may be explained by the effects of the bioactivity of porous Ti oxide structure, with increased osteoblast adhesion and deposition of proteins including bone sialoprotein and osteopontin on the surfaces of the Ti implants.[38] Basically, contaminant-free, micron-scale surface properties produced by HA blasting might be the most important reason for favorable bone formation on Ti implant surfaces by enhancing biomechanical anchorage through optimizing the biological response of the bone and micromechanical interlocking.[2–4,39]

In contrast to the results at 4 weeks, there were no remarkable differences in the bone-implant contact and bone area percentage when comparing the blasted/AWH surfaces and blasted surfaces at 8 weeks. For the blasted/AWH implants, there were no differences in BIC% between the two healing times. However, blasted implants showed statistically significantly increased bone-implant contact with increased healing time (73.88% vs. 60.41% for the three best consecutive threads, 66.54% vs. 50.13% over the total implant length; $p < 0.05$). It may be postulated that in unloaded condition, coating of a porous Ti oxide structure onto microroughened Ti surfaces accelerates early-bone formation, but not in more prolonged healing periods, where it does not override the biocompatible, microroughened surfaces free of surface contaminants such as alumina grit produced by HA-blasting.

This is in agreement with the findings of other studies, which indicated that bone-implant contact remains steady or slightly increased when a longer healing time is allowed, especially when the implants already achieved relatively high degree of BIC at early healing phase.[40,41]

Based on the histomorphometric results at 4 weeks, the coating of crystalline Ti oxide structure with submicron-scale porous morphology onto Ti implant surfaces may be considered an effective method for enhancing early bone formation, thereby shortening healing periods for osseointegration. The BIC% of blasted/AWH implants at 4 weeks showed similar or higher values of BIC, both in the three best consecutive threads (80.44% vs. 73.88%) and all threads (66.23% vs. 66.54%), to those of blasted implants at 8 weeks.

It was reported that the long-term stability of implants depends on bone anchoring to micron-scale surface structures.[42] The combined use of submicron-scaled porous Ti oxide structure and a microroughened surface may have advantages in improving osseointegration of endosseous Ti implants. However, sodium-free porous Ti oxide structure may result in relatively weak mechanical properties of the implant surfaces, as described by Fujibayashi et al.[32] The generation of Ti particles arising from Ti implant surfaces due to frictional force during implant insertion, especially in thick and dense cortical bone regions, may result in particulate-induced osteolysis.[43,44] It has been reported that phagocytosis of submicron- to micron-sized particles of any metallic species stimulates macrophages, thereby releasing bone-resorbing intermediates including IL-1α, IL-1β, and IL-6.[45–48] In this study, no histological findings of osteolysis related with the generation of Ti particles were noted at either 4 or 8 weeks. In dentistry, in contrast to orthopedic applications, particulate-induced implant loosening has not been reported as a major finding, but more definitive studies are needed.[49,50] The grit-blasting produces isotropic microstructures composed of peak, valley, and undercut, on the surface of Ti implant. Unlike the peak area, the undercut and valley area are less likely affected by frictional force, which will be generated during implant insertion. So it can be postulated that undamaged porous structure in those area plays a role in enhancing osteoconductivity of blasted implants after implant installation.

The results of this animal study suggest that the addition of porous crystalline Ti oxide surface to the Ti implants with microroughened surfaces may be an effective way to optimize the bone healing response and shorten the healing period for early clinical loading.

CONCLUSION

In this study, it was found that the coating of crystalline Ti oxide structure with submicron-sized porous morphology onto Ti surfaces could promote the periimplant bone healing response around endosseous Ti implants with microroughened surface at the early healing stage. In conclusion, we suggest that a porous Ti oxide surface on Ti implants prepared by water and heat treatment after alkali treatment may be an effective approach to increase the osteoconductivity of microroughened Ti implants, thereby enhancing early bone responses and shortening the healing period. A synergistic effect on osseointegration, related to biomechanical interlocking and bioactive Ti oxide surface can be expected when combined with micron-scale surface properties of implants. However, further detailed studies are required to investigate the long-term stability of implants.

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