Mechanical and Histologic Examination of Titanium Alloy Material Treated by Sandblasting and Anodic Oxidization

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To evaluate the biocompatibility and the bone-bonding strength of new titanium alloy materials treated by sandblasting and anodic oxidization, 3 cylindric test pieces having different surface roughnesses were manufactured and implanted into the diaphyses of the femurs of New Zealand white rabbits. Six weeks later, shear loading tests and histologic examination were carried out. Strong interfacial bonding strength and active new bone formation were confirmed in the peripheral area of the test pieces having a surface roughness (Ra = 2.7 μm and Ra = 4.7 μm). Judging from stable fixation to shear loading in bone tissue, it was concluded that group C (Ra = 2.7 μm) had the best surface condition of the 2 groups. Further detailed examination is required to demonstrate that the surface treatment used for group C (a micro rough surface on a macro rough surface structure) can enhance active bone formation and stable fixation in bone tissue. Int J Oral Maxillofac Implants 2005;20:48–53

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Since 1975, the authors have developed and clinically applied various screw-type dental implants made of alumina single crystals (sapphire).[1,2] It has been shown that an alumina porous implant could support a dental prosthesis independently.[3,4] However, a 2-stage-type implant could not be produced for clinical use because of the brittle property of the sapphire. Pure titanium or titanium alloys have been clinically used as 2-stage-type implant materials, but the surfaces are smooth and coated only by a thin passive oxide layer with a thickness of 1 to 2 nm.[5,6] Therefore, the decrease of interfacial bonding strength in a jaw after superstructure placement and possible local foreign body reactions caused by titanium ion exudation[4] has been a cause for concern. Subsequently, the authors prepared a sand-blasted rough surface on titanium alloy (Ti-6Al-4V, ELI specification) with a thick oxide layer (140 nm) by a direct-current anodizing treatment and investigated the interfacial bonding strength and histologic reaction in the rabbit femur.

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MATERIALS AND METHODS

Preparation of Test Pieces

A total of 32 titanium alloy cylinders (Ti-6Al-4V, ELI specification), 3.1 mm in diameter and 8 mm long, were prepared by machine grinding. Eight of these cylinders were set aside to be used in group A. An

anodic oxidation layer with a thickness of 135 to 140 nm and an uneven macrostructure was formed on the surface of the remaining test pieces in 0.1% phosphoric acid solution at 150 to 200 V for a few minutes to produce 3 groups of 8 (groups B, C, and D), each with a different condition (Fig 1). The cylinders in groups B, C, and D were then sandblasted. Different levels of roughness were achieved by sandblasting with particles of varying sizes.

Observation of Surface Microstructure

The surface of 1 test piece from each group was sputter-coated with gold and observed by the scanning electron microscope (SEM) (JSM-5410LV, JEOL, Kyoto, Japan) at 15 kV.

Surface Roughness Measurement

Surface roughness (Ra) in 5 different places on the same test piece selected for SEM observation was measured by a surface profilometer (Suricom, Tokyo Seimitsu, Tokyo, Japan) under the following conditions: sampling length = 0.8 mm, measurement speed = 0.3 mm/s, cut-off value = 0.8 mm, stylus; r = 5 μm. The standard deviation of Ra values in these 5 different places was in the 5% range in all groups.

Animal Experiment

Six test pieces from each group were used in an animal experiment. All test pieces were sterilized in an autoclave. Eight 16-week-old male New Zealand white rabbits weighing approximately 3 kg each were used in the animal experiment. All animals were handled in accordance with the Guidelines for Animal Experimentation at Osaka Dental University. After general anesthesia was achieved with a pentobarbital sodium injection (Nembutal, Abbott Laboratories, North Chicago, IL), the diaphysis of each femur was exposed and 3 holes were created at 10-mm intervals using a dental drill with a diameter of 3 mm. The test pieces were then placed firmly into the holes (Fig 2). After receiving an antibiotic (Viccillin, Meiji Seika, Tokyo, Japan), the rabbits were returned to their cages. Six weeks later, the rabbits were injected with a lethal dose of pentobarbital sodium, and femoral bone blocks containing the test pieces were harvested.

Interfacial Bonding Strength Measurement

Harvested femoral bone blocks were fixed on the original stand using resin (GC-Ostron II, GC Corporation, Tokyo, Japan). Femoral bone blocks were adjusted to be perpendicular to the bottom of the original stand. The original stand with the femoral bone block was set in a universal testing machine (model no. 1123, Instron, Canton, MA), and the shear loading test was carried out at a crosshead speed of 0.5 mm/min (Fig 3). The shear loading was stopped at the first peak of load value after starting the test, and that value was designated the interfacial bonding strength. Bone blocks were continuously moistened with saline solution to avoid drying.

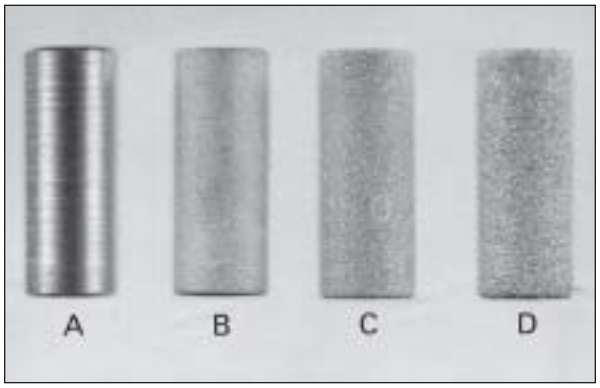


Fig 1 Test pieces used in this study. The cylinders in group A were machine-surfaced. Those in groups B, C, and D were sandblasted after anodic oxidation processing. Ra was 1.2 μm for group B, 2.7 μm for group C, and 4.7 μm for group D.

Histologic Examination

After the shear loading test, femoral bone blocks were cut in half and test pieces were carefully removed. Bone blocks were dipped in 10% neutral buffered formalin solution for 2 days, then they were decalcified in 10% EDTA solution for 1 week and embedded with paraffin. The paraffin-embedded block was set into a rotary microtome (HM340E, Apogent/Microm International, Walldorf, Germany). Tissue sections were cut and hematoxylin-eosin staining was performed. The boundary area between the surface of the test piece and the newly formed tissue was observed using an optical microscope (ECLIPSE E600; Nikon, Tokyo, Japan).

RESULTS

Observation of Test Piece Surfaces

The surface of group A exhibited flaws perpendicular to the long axis of the cylinder at approximately 100-μm intervals caused by mechanical grinding (Fig 4a). The surface of group B was a rough microstructure (Fig 4b). The surface of group C was classified as a rough macrostructure, but it included rough microstructure, as seen in group B (Fig 4c). The surface of group D was the coarsest macrostructure, without evidence of any rough microstructure such as that seen in group C (Fig 4d).

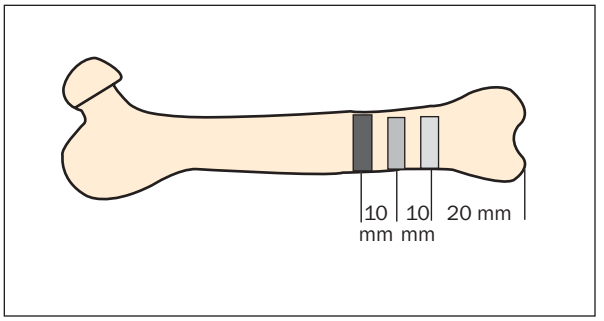


Fig 2 *(Above)* Test pieces were implanted in the metaphysis of the distal femur at 10-mm intervals.

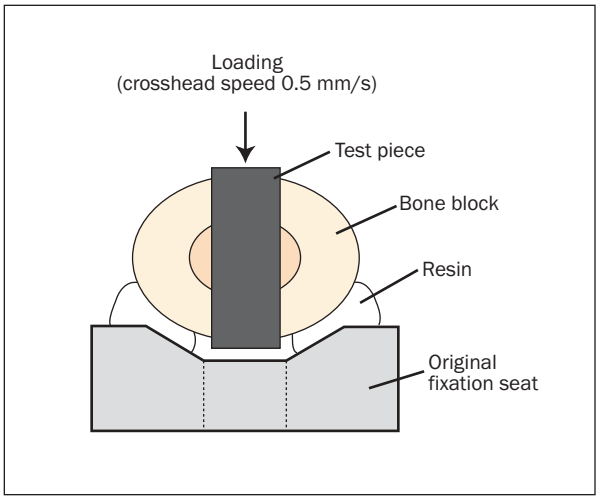
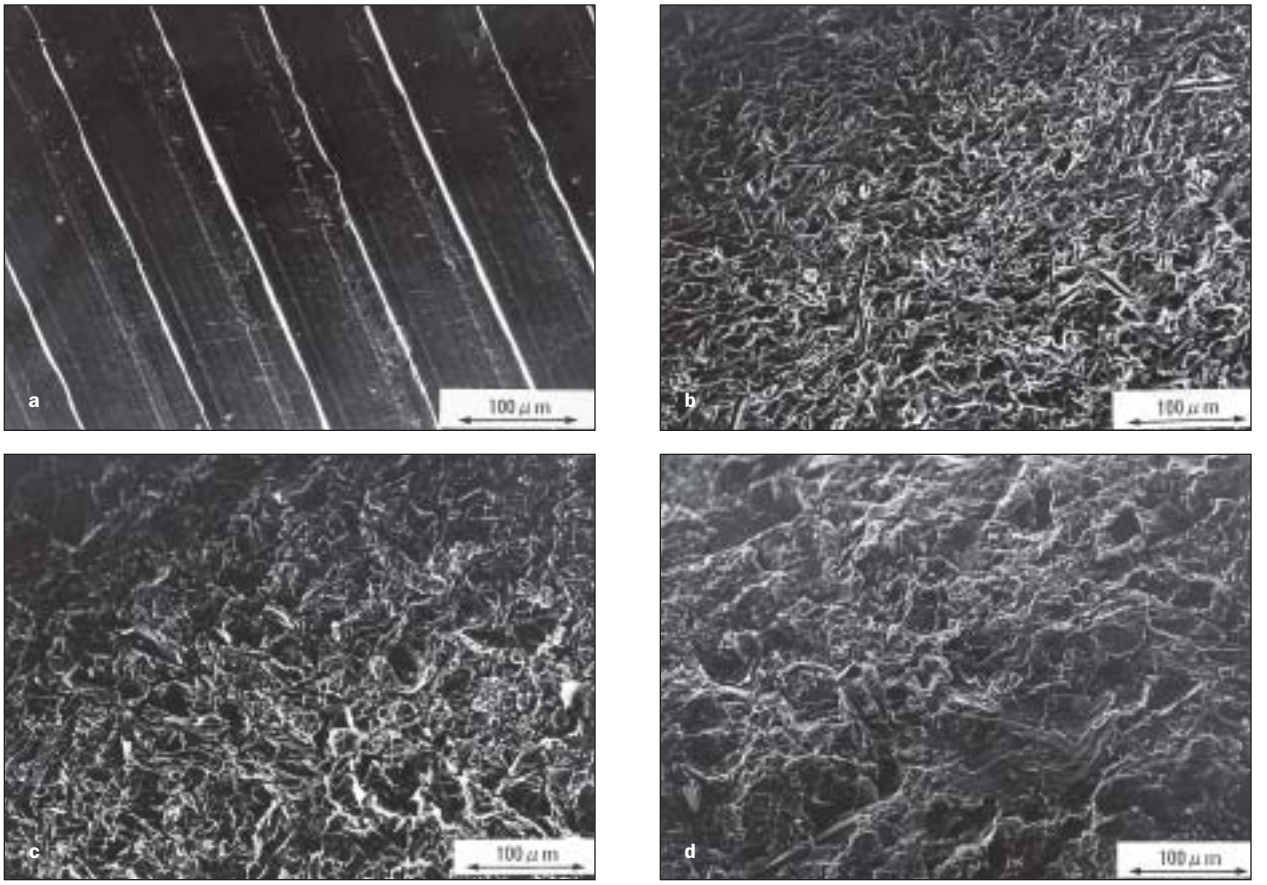


Fig 3 *(Right)* Shear loading test. After the bone block was fixed on the original fixation seat with resin, the bone block containing the test piece was loaded perpendicularly by the pushpin at the speed of 0.5 mm/s.



Figs 4a to 4d SEM images of the microsurface structures of test pieces from *(a)* group A, *(b)* group B, *(c)* group C, and *(d)* group D.

Surface Roughness Measurement

The arithmetic mean Ra values were 1.1 μm for group A, 1.2 μm for group B, 2.7 μm for group C, and 4.7 μm for group D (Fig 5).

Shear Loading Test

The average shear strength value of the test piece was 4.5 ± 0.9 kg for group A, 8.8 ± 5.0 kg for group B, 17.7 ± 5.7 kg for group C, and 21.7 ± 10.7 kg for group D (n = 6 for each group) (Fig 6). As a result of the statistical analysis, it was found that there were significant differences ($P < .01$) between group A (machine-ground surface) and groups C and D, which had been sandblasted. Significant differences ($P < .05$) also were found between group B and groups C and D. However, there were no significant

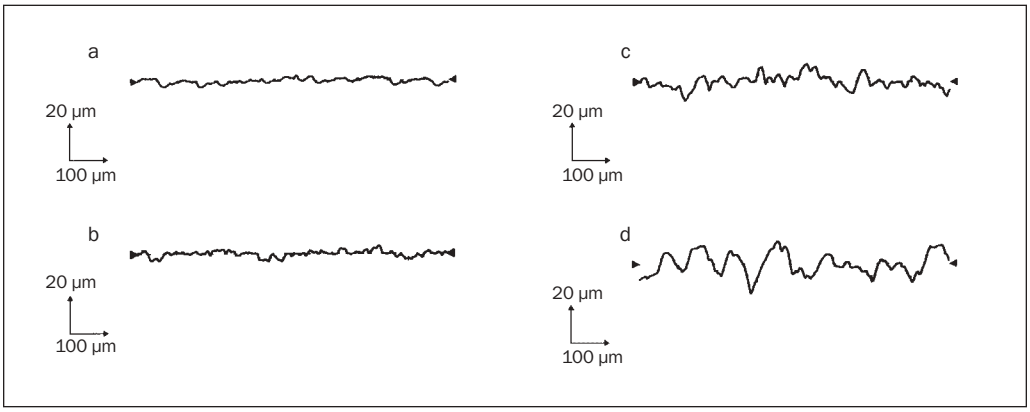


Fig 5 The degree of Ra according for *(a)* group A (Ra = 1.1 μm), *(b)* group B (Ra = 1.2 μm), *(c)* group C (Ra = 2.7 μm), and *(d)* group D (Ra = 4.7 μm).

differences between groups A and B or between groups C and D.

Histologic Examination

The peripheral tissue area in contact with the test pieces from each group was observed by optical microscope. In group A, newly formed bone tissue directly contacted the surface of test pieces in the pre-existing bone area, but the contact was weak, and some fibrous connective tissue was found in the bone marrow area. Fractured bone sites were not seen in the implant-bone interfacial area after the shear loading test (Fig 7a). In group B, newly formed bone tissue was seen along the surface of the test piece in both the pre-existing bone area and the bone marrow area. Bone fracture sites were not seen in the implant-bone interfacial area after the shear loading test (Fig 7b). In group C, newly formed bone thicker than that seen in group B was observed in the bone marrow area. Bone fracture was detected in the implant-bone interfacial area after the shear loading test (Fig 7c). In group D, newly formed bone of equal or greater thickness compared with group C was observed in the bone marrow area. Bone fracture was detected in the implant-bone interfacial area after the shear loading test (Fig 7d).

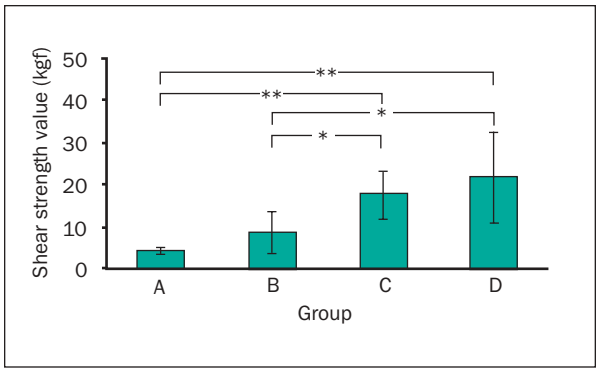
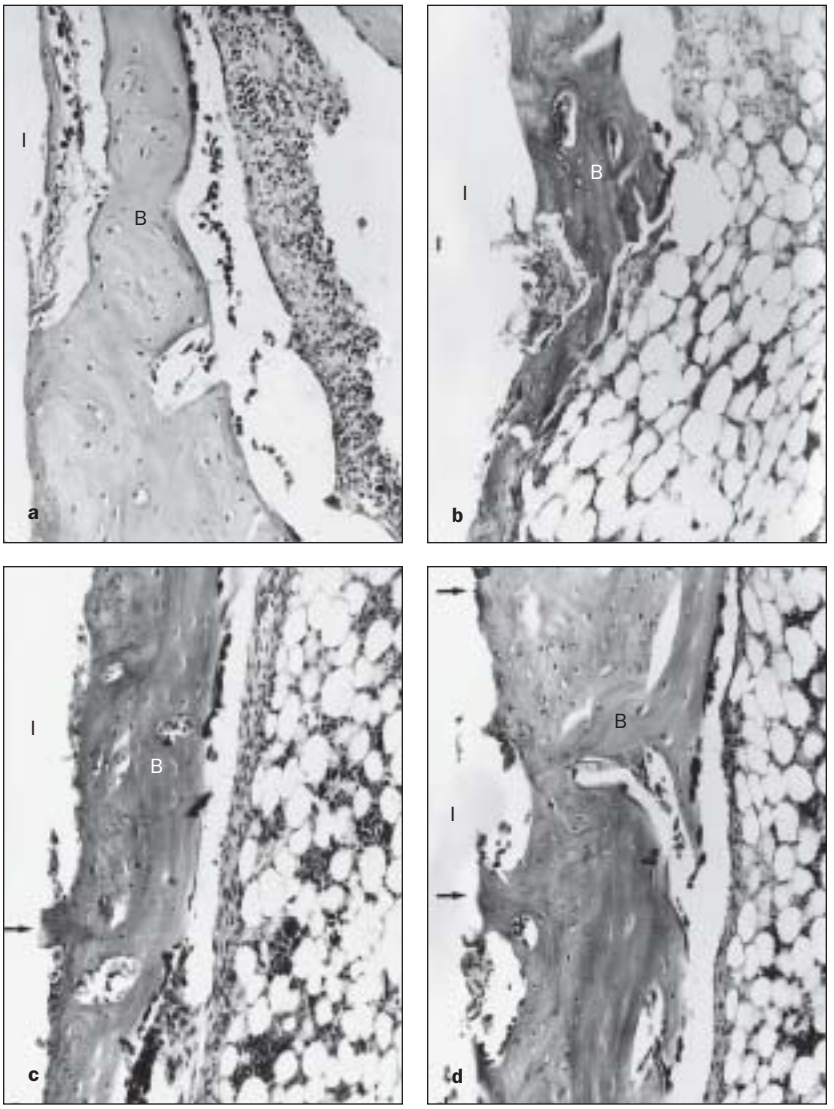


Fig 6 The shear loading test was carried out to measure the bone-implant interfacial bonding strength. The shear strength value increased as the degree of Ra increased. *$P < .05$; **$P < .01$.

DISCUSSION

A number of researchers have attempted to increase the interfacial bonding strength in bone tissue by roughening the surface of material.[7–11] It is commonly accepted that increased Ra strengthens interfacial bonding in the bone tissue. The Ra values of the machine ground surface (group A) and the blasted and anodized surface (group B) were similar, but the shear strength and response to new bone formation were excellent for the latter. Previous studies have confirmed that the oxide layer formed by anodic oxidation in water inhibits metal ion elution to the peripheral tissue in phosphoric acid solution, and the authors hypothesize that the oxide layer formed by blasting and anodizing enhanced active new bone formation in the group B (unpublished data, 1984). In addition, the tendency of the quantity of newly formed bone to increase as the surface roughness increases was confirmed. This finding is concurrent with a report[12–14] that bone apposition is enhanced by making the material surface rougher. It is speculated that surface roughening increases the bone contact area of the material surface and thus promotes the initial adhesion of bone marrow cells. Then cell differentiation to osteoblasts and the generation of bone matrix leads to active bone formation for a short period.

In the shear loading test, it was found that interfacial bonding strength increased as a function of increased surface roughness. No bone tissue damage was seen at the interfacial area in groups A and



Figs 7a to 7d Histologic images of the interface between the test pieces *(I)* and the newly formed bone *(B)* for *(a)* group A, *(b)* group B, *(c)* group C, and *(d)* group D. In groups C and D, bone fracture *(arrows)* was seen in the interface area (hematoxylin-eosin, original magnification ×40).

B, while bone tissue damage at the interfacial area was found in groups C and D, where the surface roughness was relatively high. This result showed that firm fixation resulted from new bone tissue on anodized titanium alloy surface with a surface roughness of at least 2.7 μm (the mean Ra of group C). In terms of the average shear strength value, group D, which had the greatest surface roughness, might have the best interfacial bonding strength. However, bonding strength varied widely within group D, and a number of samples showed the same shear strength value as group B. At the same time, data dispersion within group C was small, and the shear strength values were more uniform. A small difference between the surface structure of samples from groups C and D was seen. The surface of the group C test piece had a rough microsurface structure within a rough macrosurface structure. On the other hand, group D had a rough macrosurface but hardly demonstrated any microstructure surface roughness.

CONCLUSION

In this study, it was found that titanium alloy treated by sandblasting and anodic oxidation can promote osseointegration at an early stage. The treated test pieces demonstrated stronger fixation in bone tissue than machine-ground titanium alloy. Judging from the stable fixation in bone tissue during shear loading, it was concluded that group C (Ra = 2.7 μm) had the best surface condition of the 4 groups. However, further detailed examination is required to demonstrate that the surface structure used in group C, a rough microsurface in a macrosurface structure, can enhance active bone formation and stable fixation in bone tissue.

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