Original paper

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Surface Properties and Biocompatibility of Nitrided Titanium for Abrasion Resistant Implant Materials

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Corrosion, other related properties and biocompatibility of surface nitrided titanium were investigated to examine its possible use as an abrasion resistant implant material. The nitrided layer about 2μm thick composed of TiN and Ti_2N was formed on titanium by a gas nitriding method. The dissolved amount of titanium ion in SBF was as low as the detection limit of ICP, and that in the 1% lactic acid showed no significant difference from titanium. The tissue reaction of the cylindrical implant in soft tissue of rats showed no inflammation, and fine particles of 1μm induced phagocytosis, which was similar to titanium. The implantation in the femor showed the new bone formed in direct contact with implants. All the results suggested that the wettability, corrosion resistance, *S.mutans* adhesion and biocompatibility were nearly equivalent to those of titanium. The surface of nitrided titanium was promising, with biocompatibility comparable with titanium, as an implant material such as for an abutment part of a dental implant, which requires high abrasion resistance.

Key words : Titanium nitride, Implant, Biomaterial

INTRODUCTION

In the increasingly aged society, implants as a method for recovering the function of lost teeth will become more and more important. Titanium is used most as the implant material at present with several variations including apatite coated titanium implants and functionally graded implants[1−9]. Corrosion resistance is one of the most important factors to affect the biocompatibility of implant materials[10−14]. The titanium surface has a passive film formed by a thin and stable oxide, which aids biocompatibility under the severe environments found *in vivo*. Titanium has, however, also negative points. One of them is low abrasion resistance[15]. Minute titanium abrasion powders may cause an inflammatory reaction[16,17]. Therefore, it is desirable to develop implant materials with both biocompatibility and abrasion resistance. The surface nitriding method would be one of the surface treatments of metallic material to solve this problem.

Titanium nitride is known for its high surface hardness and mechanical

strength[18,19]. It was also reported that the dissolution of Ti ion is very low[19,20]. From these findings, the surface nitriding method appears to be useful. However, research to evaluate the surface properties and biocompatibility of nitrided titanium for application as an implant has rarely been carried out.

As for dental implants it is comprised various components. The implant abutment part (the mucosa penetration part) is exposed in the oral cavity and so plaque and dental calculus easily adhere on it. Removal of the plaque and dental calculus is necessary to obtain a good prognosis throughout the long term maintenance of the implant. Therefore, it is desirable for the abutment part to have abrasion resistance against the scaling treatment.

In a previous study on the mechanical properties of nitrided titanium, the present authors reported that a nitrided layer on titanium with a thickness of 2μm showed a Vickers hardness of about 1,300, approximately 10 times that of pure titanium, and was strongly bonded with the pure titanium base metal. In both the Martens scratch test and abrasion resistance test with a dental ultrasonic scaler, nitrided titanium showed very high abrasion resistance[21].

The purpose of the present study was to evaluate corrosion resistance, wettability of the surface, quality of *S.mutans* adhesion and biocompatibility of the surface nitrided titanium and to examine its possible use, as an abrasion resistant implant material, especially for the abutment part of dental implants.

Pure titanium and surface nitrided titanium are abbrevicted as Ti and Ti(N), respectively in the following figures.

MATERIALS AND METHODS

Specimen preparation

A 1 mmϕ ×7 mm length 99.9% titanium wire (NILACO Ltd., Tokyo, Japan) and JIS type 1 pure titanium plate (10×10×0.5 mm: KOBE STEEL Ltd., Kobe, Japan) were used as the cylindrical and plate specimens, respectively. Both specimens were polished with #2000 waterproof paper (the #2000 polishing specimen). Part of the plate specimens were also polished with 6μm diamond emulsion (BUEHLER, Illinois, USA)(the 6μm polishing specimen). These specimens were treated with 0.1% hydrofluoric acid solution for 10 sec to clean the surface. They were then ultrasonically cleaned with distilled water, 100% ethanol and 99.5% acetone for 15 min each. Nitriding was performed under the conditions of a N_2 atmosphere of 1 atm at 850 ℃ for 7 hours. (N_2 flow rate: 50 L/min, Furnace volume: 0.574 m^3)

Evaluation of surface quality

1) Surface observation

Surface structure was observed by atomic force microscopy (AFM) (TMX-2000 Explorer, TopoMetric, Santa Clara, USA).

2) Confirmation of the nitrided layer

The cross-section of the surface nitrided titanium was observed by SEM (S-4000N,

HITACHI, Tokyo, Japan) after the specimen was cut and polished. X-ray diffraction of the specimen surface was performed using X-ray diffraction equipment (JDX-3500, JEOL, Tokyo, Japan).

3) Roughness measurement

On the #2000 polishing specimen, AFM was used for a scaning area $50 \times 50 \mu m$ and with a scanning rate $5 \mu m/min$. Measurements were performed 5 times for each specimen and the average height deviation from the mean plane (Ra) was obtained.

Corrosion resistance test

The dissolution test of titanium was carried out using Simulated body Fluid (SBF) and 1% lactic acid in order to evaluate corrosion resistance of nitrided titanium. A plate specimen with a 220 mm^2 surface area (the #2000 polished specimen in the SBF and the $6 \mu m$ polished specimen in the 1% lactic acid) was used as a test piece. They were ultrasonically cleaned and then sterilized with ethylene oxide gas. These specimens were soaked in SBF and in 1% lactic acid 5 ml for 10 and 30 days. The test was performed 5 times for each specimen. The vessel of the blank condition where the specimen was not soaked was prepared as a control population. The composition of SBF followed the methods of Kokubo *et al.*[22]. The SBF was prepared by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$ and $CaCl_2$ into deionization distilled water so that ion concentrations of the fluid were almost equal to those in human blood plasma. The ion concentrations are shown in Table 1. The lactic acid used was high-pure lactic acid (reagent prime class, Wako PURE CHEMICAL INDUSTRIES, Ltd., Osaka, Japan) with 90% purity and was diluted with deionization distilled water at 1.0%. The sealing polypropylene vessel was used for the dipping test. The vessel was set at 37℃ under sealed conditions for the whole period of the dipping. After dipping, the titanium concentration (ppm=mg/L) was analyzed using the ICP emission analysis equipment (P-4010 type, HITACHI, Tokyo, Japan), and the amount (ng/mm^2) of Ti dissolution per unit area was deduced.

Wettability of the specimen surface

To evaluate the wettability of the specimen surface, measurement of contact angle was carried out by the dropping test. First, $2 \mu l$ of distilled water was dropped on the specimen surface, and the droplet was photographed from the horizontal direction. The contact angle was defined as the double of angle ∠CAB (Fig. 1). Measurements were performed 5 times on each specimen, and the mean value was

Table 1 Ion concentrations (mM) in the simulated body fluid (SBF) and human plasma

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0
Human plasma	142.0	5.0	1.5	2.5	103.0	13.5	1.0

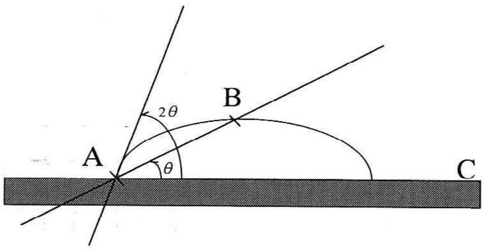

Fig. 1 Measuring method of the contact angle.
A: Intersection point of the droplet surface and specimen surface
B: Top of the droplet. C: specimen surface

obtained.

S.mutans adhesion

To compare the adhesion of *S.mutans* on titanium and nitrided titanium, the *S.mutans* attachment test was carried out. The 6μm polished specimen was used as a test piece. They were ultrasonically cleaned and then sterilized with ethylene oxide gas.

1) Bacteria and culture

Streptococcus mutans JC 2 which was divided from the Department of Oral Pathobiological Science Hokkaido University was used. *S.mutans* was inoculated in Brain Heart Infusion Broth (EIKEN CHEMICAL Co., Ltd, Tokyo, Japan) with 5% sucrose added (5% sucrose BHI culture medium). Precultivation was carried out at 37℃ for 24 hours. This fungus liquid of 60μl was added to 5% sucrose BHI culture medium of 3 ml. These specimens were suspended with 5-0 nylon thread for oral surgery, and cultivation was carried out at 37℃ for 24, 48, and 72 hours. Measurements were performed 5 times on each specimen, and the mean value was obtained.

2) Measurement of adhered bacteria

After the culture, specimens were washed with 10 ml distilled water. These specimens were invested in 2 ml 1N sodium hydroxide solution, elimination and solubilization of the fungus were by ultrasonic cleaning for 60 seconds. The absorbence was measured at 550 nm using the spectrophotometer (U-1100, HITACHI, Tokyo, Japan), and the mean value was regarded as adhesion of *S.mutans*.

Biocompatibility

To compare the biocompatibility of titanium and nitrided titanium, these materials were implantation.

1) Implantation in subcutaneous tissue

(1) Animal experiment

Wistar strain rats aged 14 weeks (weight, 350-380 g) were used. After anesthesia

with diethyl ether (Wako Pure Chemical Industries, Osaka, Japan), pentobarbital sodium (30 mg/kg; NEMBUTAL INJECTION, Dainabot, Osaka, Japan) was injected into the abdominal cavity of the rats, and the cylindrical implants (1 mm ϕ ×7 mm length) were inserted in the subcutaneous connective tissue in the abdominal region. The wound was then sutured. Four specimens were implanted for each mateial. These rats were sacrificed at 4 and 8 weeks after surgery, and tissue block involved specimens were harvested.

(2) Histological evaluation

Implants inserted in the subcutaneous tissue were carefully removed from the tissue blocks after fixation in 10% neutral buffered formalin, and the tissue blocks were then embedded in paraffin by a conventional method. The tissue blocks were sectioned and stained with Hematoxylin-Eosin. These specimens were histopathologically observed with an optical microscope (Axioskop, ZEISS, Germany).

2) Implantation test of fine particles

(1) Specimens

Titanium fine particles (1~3μm) (SOEKAWA CHEMICALS Co., Ltd, Tokyo, Japan), nitrided titanium fine particles (1~1.5μm) (KISHIDA CHEMICAL Co., Ltd, Osaka, Japan) were used as experimental specimens. Each specimen was sterilized by ethylene oxide gas after it was washed in 100% ethanol, and then it was used for the experiment.

(2) Animal experiment

Wistar strain rats aged 14 weeks (weight, 350-380 g) were anesthetized by the methods similar to those for the subcutaneous implant, and fine particles were inserted in the subcutaneous connective tissue in the abdominal region. The wound was then sutured. Four specimens were implanted for each material. These rats were sacrificed at 1, 4, and 8 weeks after surgery, and tissue block specimens were harvested.

(3) Histological evaluation

After fixation in 10% neutral buffered formalin, and the tissue blocks were then embedded in paraffin by a conventional method. The tissue blocks were sectioned and stained with Hematoxylin-Eosin. These specimens were histopathologically observed with an optical microscope.

3) Implantation in the hard tissue

(1) Animal experiments

Wistar strain rats aged 14 weeks (weight, 350-380 g) were anesthetized by the methods similar to the subcutaneous implant. A hole was carefully made in the lateral surface of the diaphysis of the femur using a dental round bur (ϕ 1 mm), with a physiological saline external coolant, and the cylindrical implants (1 mm ϕ ×7 mm length) were inserted in the bone marrow. The wound was then sutured. Four specimens were implanted for each material. These rats were sacrificed at 4 and 8 weeks after surgery, and tissue block specimens were harvested.

(2) Histological evaluation

The tissue blocks from the rat femurs were fixed in 10% neutral buffered formalin, washed, stained with Villanueva bone stain, and then embedded in PMMA. The

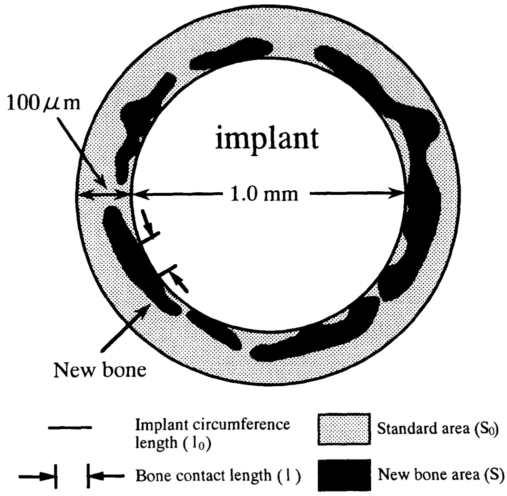

Fig. 2 Measurement methods of the area ratio of new bone formation and the ratio in contact with a specimen.

tissue blocks were then sectioned at 400 μm with a precision sawing machine (ISOMET 2000, BUEHLER, Illinois, USA) and thinner sections of about 100μm thickness were prepared by mechanical polishing. These specimens were histopathologically observed with an optical microscope. In addition, histomorphometric investigation by image processing was carried out according to the method of Matsuno *et al.*[4]. As shown in Fig. 2, new bone (S) that formed in the area of the region within 100μm from the specimen surface (standard area: S_0) was measured, and the area ratio of new bone formation (A) was obtained as $A=S/S_0$. The proportion of bone contact length (l) for each specimen circumference length (l_0) (The ratio of bone in contact with the specimen: C) was obtained as $C=l/l_0$. Eight polishing specimens were used per implantation period for histomorphometric analysis.

In each test, a significance test was carried out on the statistical analysis using the Mann-Whitney U test (Stat View 5.0, HULINKS Ltd., Tokyo, Japan).

RESULTS

Evaluation of surface quality

1) Surface observation

The specimen surface after nitriding showed a gold color. Fig. 3 shows the typical surface structure of pure titanium (a) and surface nitrided titanium (b) observed by AFM. In titanium, the ruggedness formed by mechanical polishing was observed nearly parallel in a unidirection. The surface nitrided titanium showed a ruggedness with an irregular form. In the roughness measurement, the Ra of the #2000 polishing specimen was 0.13μm (Standard deviation: 0.015) in the titanium, and 0.32μm

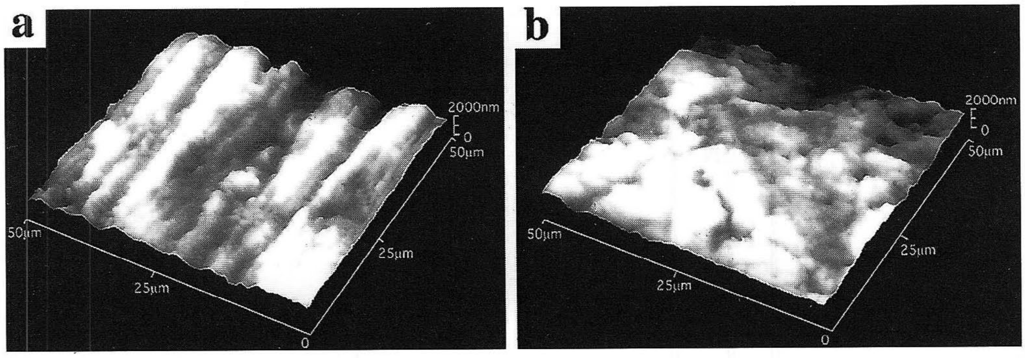

Fig. 3 Surface structure of titanium observed by AFM (#2000 polished specimen).
a: before nitriding, b: after nitriding

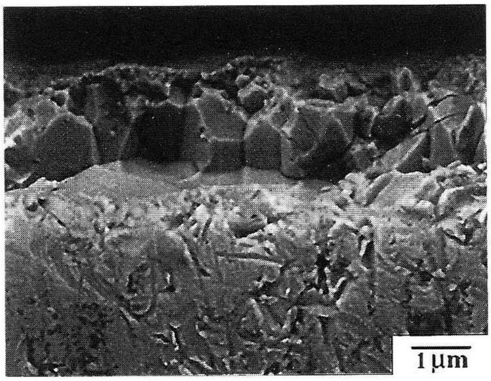

Fig. 4 Cross-section of nitrided layer observed by SEM.

(0.020) in the nitrided titanium. The Ra of the 6μm polished specimen was 0.04μm (0.001) in the titanium, and 0.08μm (0.002) in the nitrided titanium. The surface of nitrided titanium was rougher than that of titanium.

2) Confirmation of the nitrided layer

Fig. 4 shows the cross-section of the nitrided layer observed by SEM. The nitrided layer was approximately 2μm thick on surface of the pure titanium. Fig. 5 shows the X-ray diffraction of nitrided titanium. The composition of the nitrided layer was a mixture of TiN and Ti_2N. The Ti peaks were very few. It was confirmed that the surface was almost completely nitrided.

Corrosion resistance test

1) Simulated body fluid immersion test

Fig. 6 shows the dissolved amounts of titanium in the SBF at 10 and 30 days. In both specimens, dissolution was almost equal to the blank condition. This

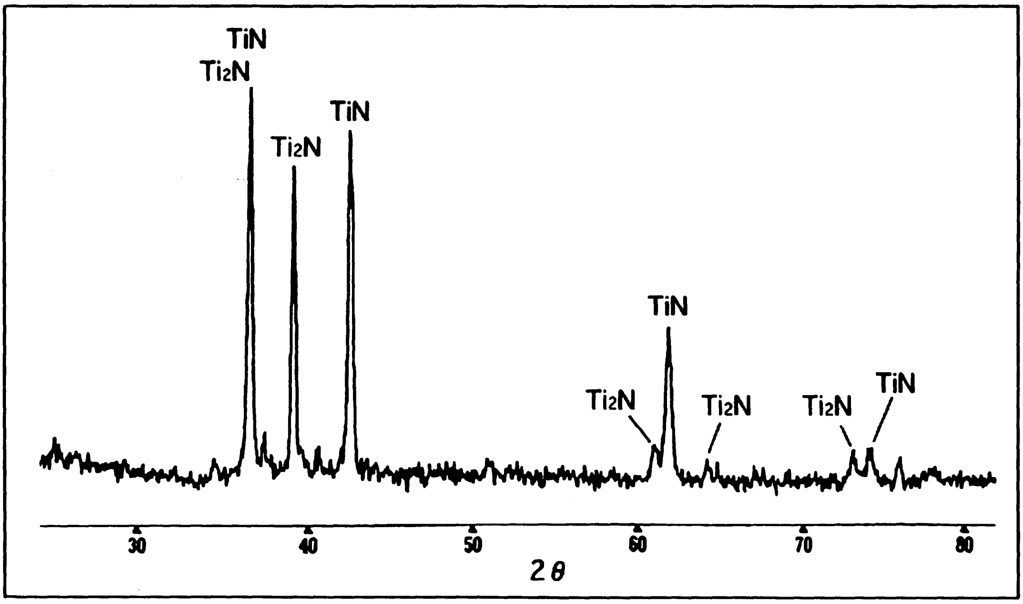

Fig. 5 X-ray diffraction of the surface nitrided titanium.

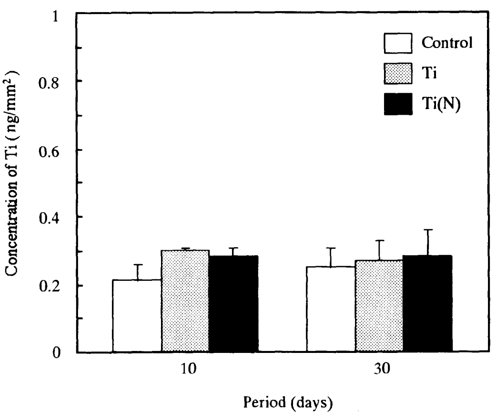

Fig. 6 Titanium dissolution in simulated body fluid from titanium and nitrided titanium.

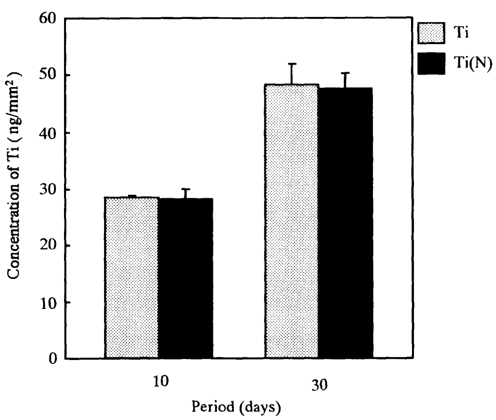

Fig. 7 Titanium dissolution in 1% lactic acid from titanium and nitrided titanium.

background level was the detection limit of ICP.

2) 1% lactic acid immersion test

Fig. 7 shows the dissolved amount of titanium in the 1% lactic acid at 10 and 30 days. In the dipping for 10 days, the dissolved titanium was 28.3 ng/mm^2 in the titanium, 28.1 ng/mm^2 in the nitrided titanium, and after 30 days, 48.8 ng/mm^2 in the titanium, 47.2 ng/mm^2 in the nitrided titanium. No significant difference was found

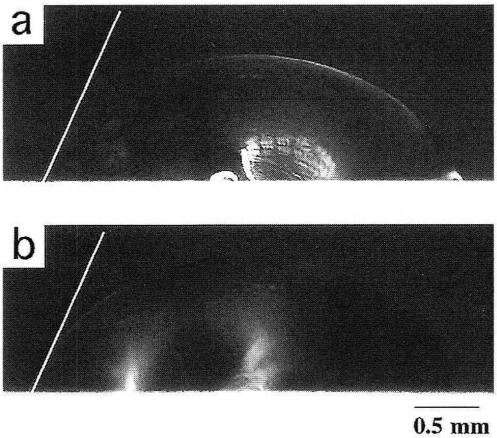

Fig. 8 Image of water droplet and contact angle on a specimen's surface.
a: titanium, b: nitrided titanium

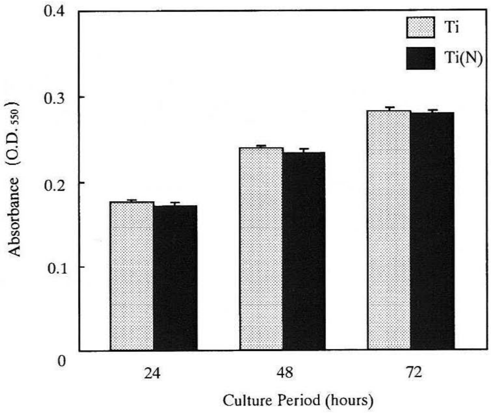

Fig. 9 Quantity evaluation of *S.mutans* adhesion by absorptiometry on titanium and nitrided titanuim.

between titanium and nitrided titanium for either 10 or 30 days.

Wettability evaluation of the specimen surface

Fig. 8 shows an image of a water droplet and the contact angle on the specimen surface. The mean value of the contact angle was 59.0° in the titanium, 60.6° in the nitrided titanium. There was no significant difference between titanium and nitrided titanium.

S.mutans adhesion

Fig. 9 shows the quantity of *S.mutans* adhered on titanium and nitrided titanium by absorptiometry. The average absorbance after the 24, 48, and 72 hours was 0.170, 0.226, 0.278 in the titanium, and 0.175, 0.238, 0.282 in the nitrided titanium, respectively. There was no significant difference between titanium and nitrided titanium.

Biocompatibility

1) Implantation in subcutaneous tissue

Fig. 10 shows tissue responses to titanium and nitrided titanium implanted in subcutaneous tissue of rats at 4 and 8 weeks after surgery. The blank, upper part of each photograph is the position where the specimen was implanted. In both titanium and nitrided titanium at 4 weeks, the specimens were encapsulated with fibrous connective tissue in which fibroblasts were observed, and no inflammatory response was observed. At 8 weeks, the fibrous connective tissue became much thinner than at 4 weeks. No inflammatory change was observed around the specimens. There was significant difference between titanium and nitrided titanium in the tissue response.

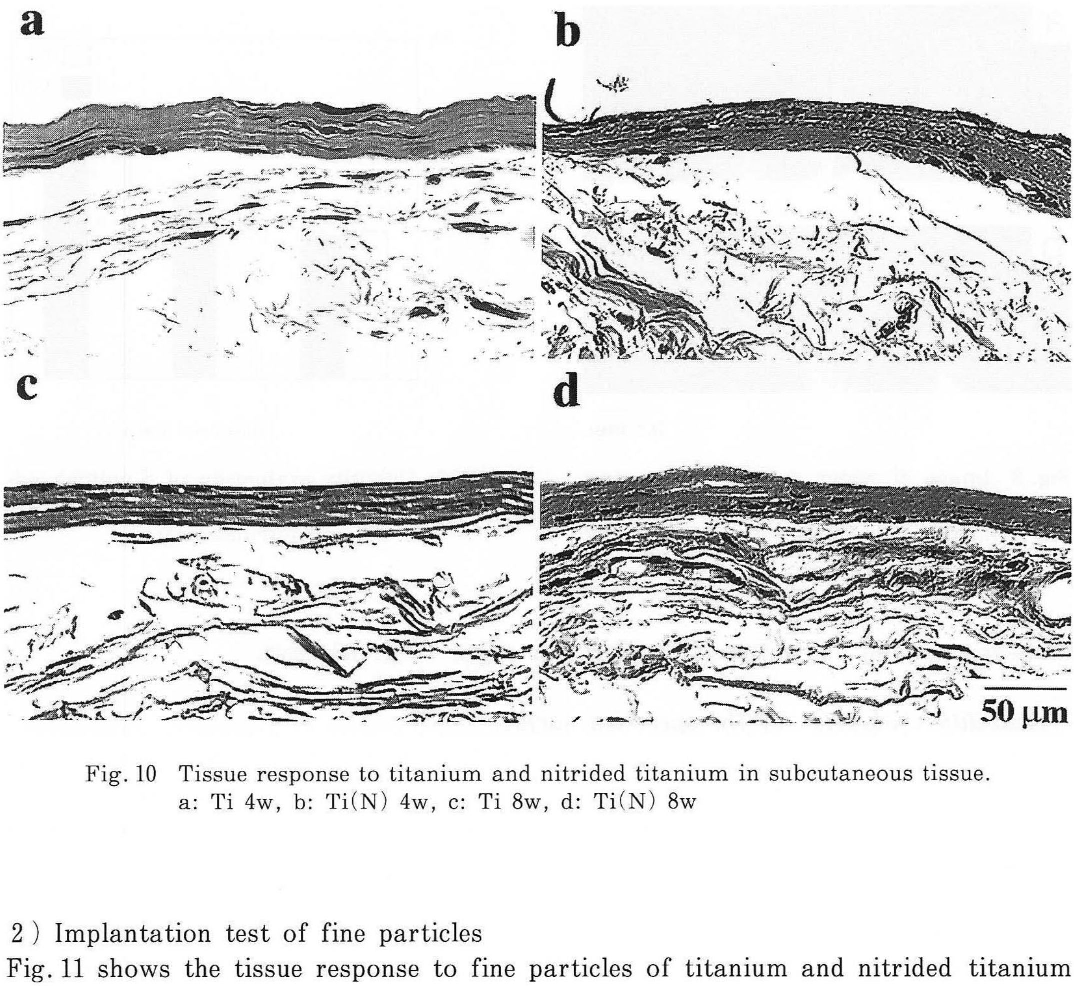

Fig. 10 Tissue response to titanium and nitrided titanium in subcutaneous tissue.
a: Ti 4w, b: Ti(N) 4w, c: Ti 8w, d: Ti(N) 8w

2) Implantation test of fine particles

Fig. 11 shows the tissue response to fine particles of titanium and nitrided titanium in subcutaneous tissue at 1, 4 and 8 weeks. In both titanium and nitrided titanium, numerous inflammatory cells were observed at 1 week. The distribution of the fine particles was widely extended. The high magnification shows that fine particles were phagocytized into the cytoplasm by macrophages. At 4 week, numerous inflammatory cells were still observed, and fine particles were phagocytized by macrophages as at 1 week. The distribution region of fine particles was reduced a little in comparison with at 1 week. At 8 week, the inflammatory response became slightly. Fine particles were arranged with a high density in the distributed region in comparison with at 4 weeks. The difference between titanium nitride and titanium could not be recognized during the experimental period in the degree of inflammatory response and the phagocytosis by the macrophages.

3) Implantation in the hard tissue

(1) Observation by optical microscopy

Fig. 12 shows the new bone formation around titanium and nitrided titanium in femoral bone marrow at 4 and 8 weeks. At 4 weeks, a 30 μm thick layer of newly

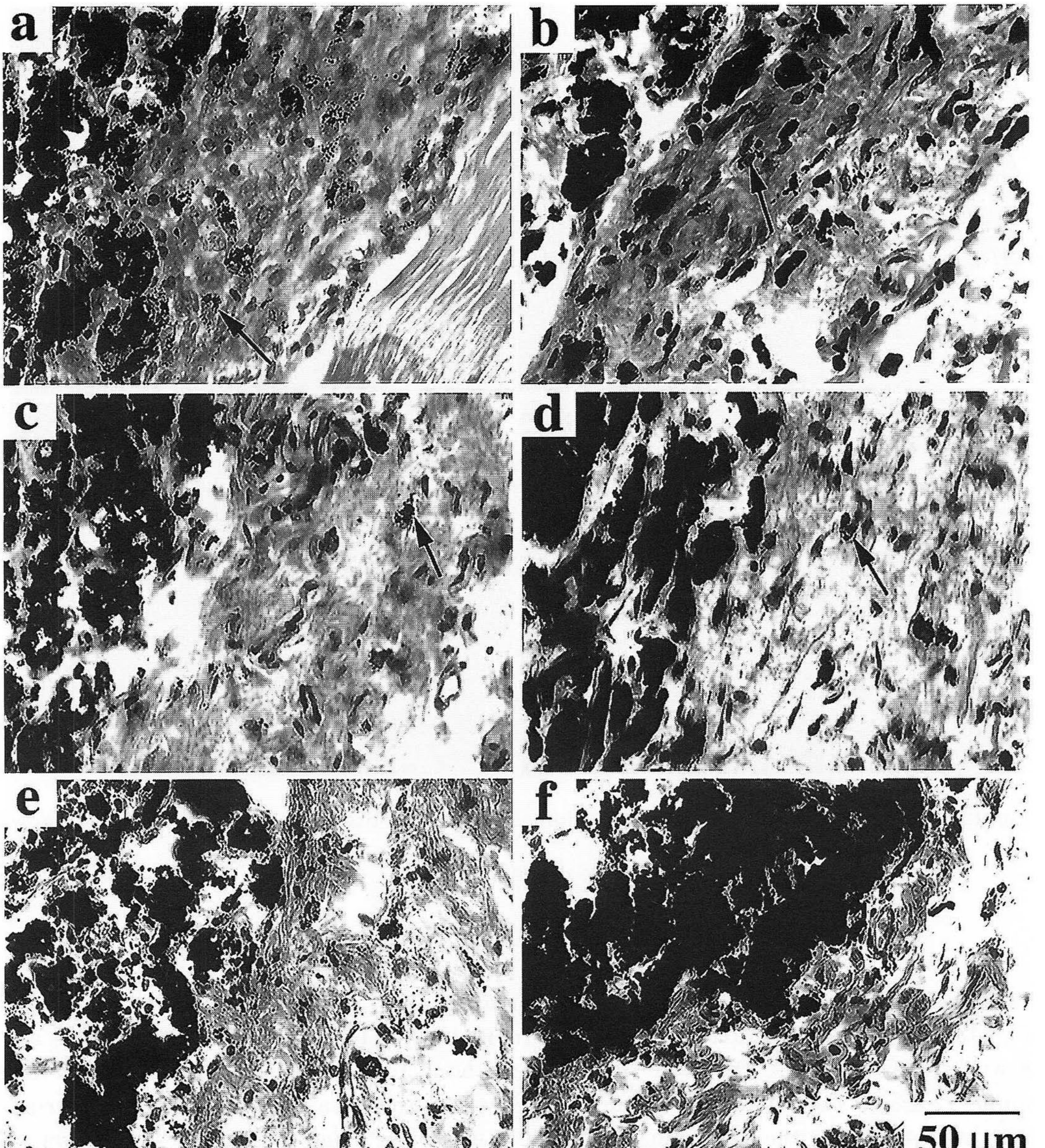

Fig. 11 Tissue response to fine particles of titanium and nitrided titanium in subcutaneous tissue. (Arrow: Phagocytosis by a macrophage)
a: Ti 1w, b: Ti(N) 1w, c: Ti 4w, d: Ti(N) 4w, e: T1 8w, f: Ti(N) 8w

formed bone was in direct contact with some parts of the surface of the metal implants. The area of direct contact at the bone-metal interface appeared to have increased between 4 and 8 weeks in both specimens.

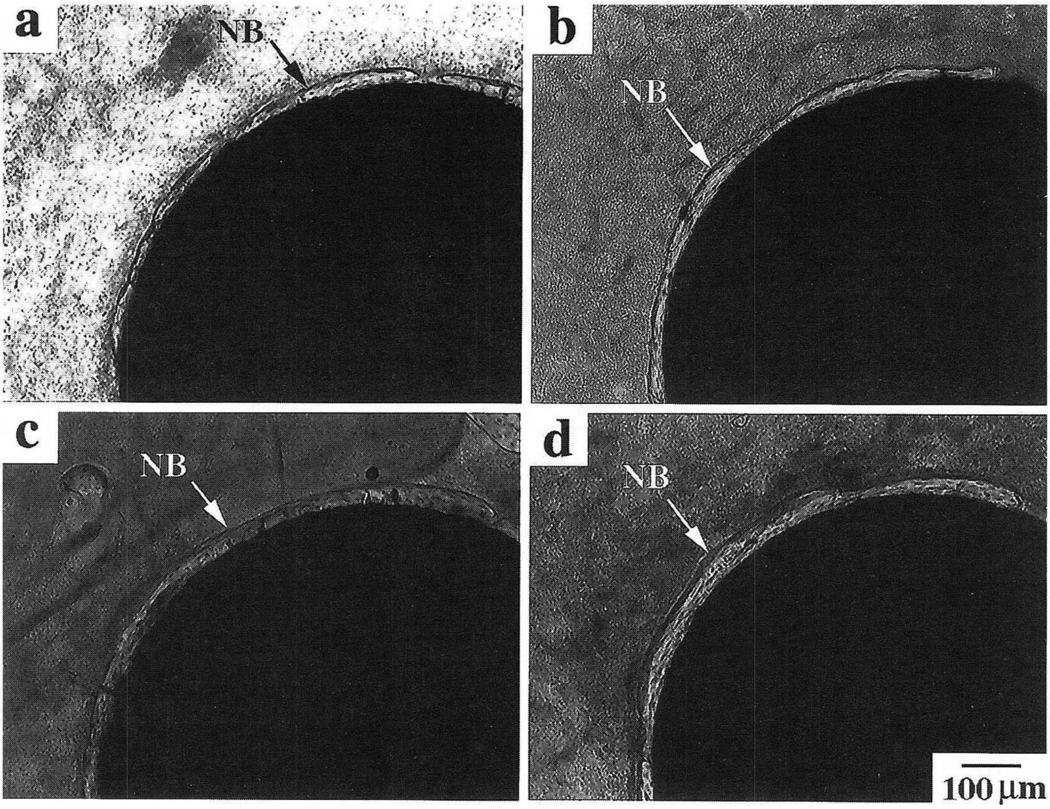

Fig. 12 New bone formation around titanium and nitrided titanium in femoral bone marrow.
NB: New bone
a: Ti 4w, b: Ti(N) 4w, c: Ti 8w, d: Ti(N) 8w

(2) Quantitative analysis of new bone formation

Fig. 13 shows the area ratio of new bone formation in femoral bone marrow at 4 and 8 weeks. At 4 weeks, the area ratio of new bone formation was 22.4% in the titanium, 22.0% in the nitrided titanium. At 8 weeks, the area ratio of new bone formation was 42.6% in the titanium, and 40.8% in the nitrided titanium. There was a significant increase between 4 and 8 weeks. There were no significant differences between titanium and nitrided titanium for each period. Fig. 14 shows the ratio of new bone in direct contact with the implant surface at 4 and 8 weeks. At 4 and 8 weeks, the ratio was 51.5% and 67.8% in the titanium, and 46.6% and 65.8% in the nitrided titanium. There was a significant increase between 4 and 8 weeks. There were no significant differences between titanium and nitrided titanium for each period.

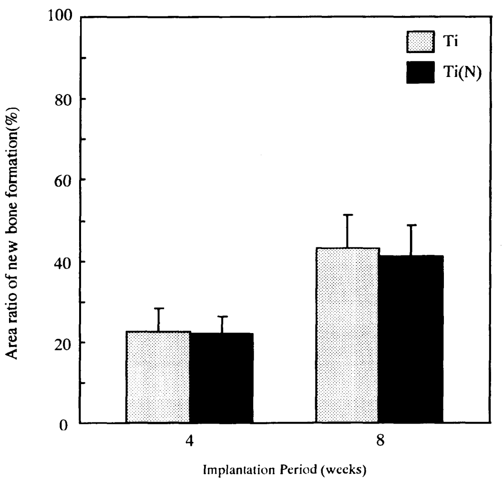

Fig. 13 The area ratio of new bone formation at 4 and 8 weeks.

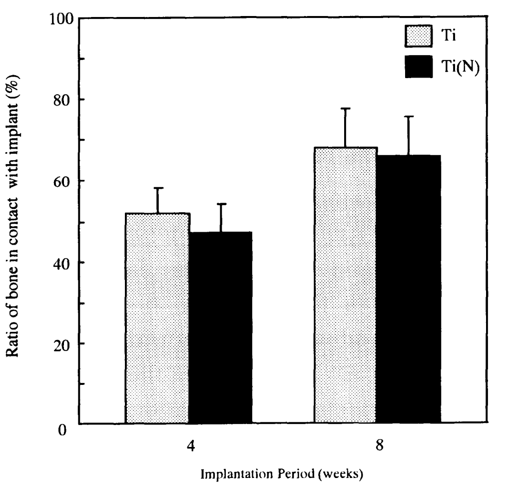

Fig. 14 The ratio of bone in contact with specimens at 4 and 8 weeks.

DISCUSSION

Evaluation of surface quality

Macroscopically, the nitrided layer appeared like surface film with uniform thickness. However, the surface roughness of titanium nitride was greater than that of titanium for both the #2000 and 6μm polished specimens. Ttitanium nitride is formed through the process of nucleation and growth, and is expanded to the full surface from the insular distribution at the beginning. Therefore, as observed in the cross-section of Fig. 5, which consists of polycrystals, the surface layer has some ruggedness and porosity, which differs, depending on the conditions of the nitriding temperatures, N_2 partial pressure and processing time. The surface roughness of nitrided layer is thus coarsened compared with before nitriding treatment.

The composition of the nitrided layer was a mixture of TiN and Ti_2N as revealed from X-ray diffraction. It was confirmed that the surface was sufficiently nitrided, and the thickness of the nitrided layer was about 2μm.

Watari reported the formation of oxide, carbide, and a nitrided layer on the surface of titanium by controlling the gas partial pressure in a vacuum, and showed that the layer thickness grew in proportion to the square root of treatment time and Ti_2N was formed by nitriding[23,24].

Takamura[25] reported that the minute chemical compound layer, mainly consisting of TiN, is formed on the surface, followed in the inside by a hard layer with solid solution hardening of the nitrogen to α-Ti. The thickness of the chemical compound layer and the thickness of the hard layer changed almost proportional to the square root of the nitriding time at a constant temperature. When nitriding was carried out at 850℃ for 16 hours, the thickness of the chemical compound layer was

about 3μm, and that of the hard layer was about 50μm. In the present study, the thickness of the nitrided layer was about 2μm, which is slightly smaller due to the difference in nitriding time. The composition of the nitrided layer was a mixture of TiN and Ti_2N as revealed from X-ray diffraction. In the Ti-N phase diagram[26], TiN and Ti_2N intermingle at a temperature of 850℃. In a previous study on the mechanical properties of nitrided titanium, the present authors reported that Vickers hardness of nitrided titanium was about 1,300, approximately 10 times that of pure titanium and was strongly bonded with pure titanium base metal. In both the Martens scratch test and abrasion resistance test with a dental ultrasonic scaler, nitrided titanium showed very high abrasion resistance and it was estimated that there would be almost no damage on the surface under the clinically used conditions[21].

Corrosion resistance test

Titanium surface has a passive film formed by stable oxide, and this aids biocompatibility under the severe environment at conditions found *in vivo*. Therefore, titanium is used mostly as implant material at present. However, titanium has a few negative points. It was suggested that the dissolution of metal ions can be generated where the oxide film is exfoliated by the abrasion and in implants with a porous structure[27-29]. Surface nitriding may be one of the methods to solve these problems. In the present study, SBF and 1.0% lactic acid were chosen from the general solvent for the dissolution test of biocompatible metals, and the corrosion resistance of titanium and nitrided titanium was compared. The dissolution depends on the material and also surface roughness. The effect of surface roughness would be more influenced in acid etching. Therefore, fine mechanical finishing was performed for lactic acid dipping to compare the dissolution properties of the materials.

The dissolved amount of titanium in SBF was the background level, as low as the detection limit of ICP (Fig. 6). The dissolved amount of titanium in titanium and nitrided titanium in the 1.0% lactic acid showed no significant difference after 10 and 30 days immersion (Fig. 7). The corrosion resistance of titanium nitride was excellent and similar to titanium. The surface of nitrided titanium formed a minute chemical compound layer, therefore, it is suggested that the corrosion resistance was improved in comparison with the titanium[15]. In the present study, no significant difference could be found between titanium and nitrided titanium. This may be due to the roughened Ra after nitriding, and minute cracks induced during the cooling process after heating at 850℃ through which titanium might be dissolved out.

Wettability and S.mutans adhesion

In general the adhesion mechanism of bacteria to a solid surface is affected by nonspecial factors such as the physiochemical binding power of the hydrophobic interaction, the electrostatic interaction and the peculiar interaction by glycoproteins of the bacterial surface layer[30,31]. In the oral cavity environment, the surface material is covered with saliva and serum, and the force to detach bacteria from the surface material by caloric intake and tongue motion always works. Therefore, it is

difficult to examine the adhesive properties of bacteria to surface materials *in vivo*. In the present study, wettability of the surface material was examined by contact angle and adhesion of bacteria *in vitro*.

The contact angle was about 60° and there was no significant difference between titanium and nitrided titanium in wettability (Fig. 8). The quantity of *S.mutans* adhesion, evaluated by absorptiometry, showed no significant difference between titanium and nitrided titanium (Fig. 9). The surface free energy of a solid surface such as a tooth and various restorative materials is low, when the contact angle is large[32]. The quantity of plaque adhesion to a solid surface *in vivo* correlates with the solid surface free energy, and the formation of plaque is small on a surface with small surface free energy[33-35]. These findings suggest that based on the nearly equivalent surface free energy, the wettability of titanium and nitrided titanium were similar.

Biocompatibility

1) Biocompatibility in soft tissue

In the examination of the bioreaction in soft tissue (subcutaneous tissue) of titanium and nitrided titanium, the biocompatibility of both cylindrical specimens and fine particles were investigated. Although titanium specimens of macro size such as the cylindrical implants showed good biocompatibility, fine particles as small as a cell induced cytotoxicity, as reported previously[16,17]. The abrasion resistance of nitrided titanium was reported to be significantly much higher than titanium. The simulated experiments showed that there would be almost no damage on the surface material under general clinical conditions, when nitrided titanium is used as an abutment of dental implants[21]. By the using an ultrasonic scaler or metallic scaler, however, some of the nitrided layer may be detached from the surface and enter the gingival tissue. If the toxicity of abraded powders is high, it may bring about harmful results even if the abrasion resistance is high. Therefore, the biocompatibility of fine particles was also examined in addition to that of cylindrical specimens in this study.

In the case of cylindrical specimens, titanium and nitrided titanium were encapsulated with fibrous connective tissue in which fibroblasts were observed, and no inflammatory response was observed (Fig. 10). In both specimens, the fibrous connective tissue at 8 weeks became thinner than at 4 weeks (Fig. 10). In the tissue response to fine particles, both titanium and nitrided titanium induced phagocytosis by macrophages and inflammation continuously, but no significant differences beyween titanium and nitrided titanium could be recognized (Fig. 11). These results suggest that the tissue reaction to both macroscopic size and fine particles of nitrided titanium were nearly equivalent to those of titanium.

2) Biocompatibility in hard tissue

The abutment of implants has been connected with fixtures of implants, and it is close to the bone which supports the fixture. Therefore, it is necessary to use material without harmful effects on the bone for the abutment. Pure titanium is used for the abutment at present. To use nitrided titanium as the abutment part, it is neces-

sary to confirm its biocompatibility for hard tissue. In this study, biocompatibility in hard tissue was evaluated by comparing the quantity of new bone formation and the ratio of bone in direct contact with the implant surface.

In observations by optical microscopy, no inflammatory reactions could be recognized in either specimens. New bone was in contact in both specimen surfaces, and the thickness of new bone was also almost equivalent. In the quantitative measurement by image analysis, the area ratio of new bone formation and the ratio of bone in contact with the specimens, were increased significantly between 4 and 8 weeks. There were no significant differences between both specimens for each period.

The chemically very stable properties of titanium nitride, especially the excellent corrosion resistance which may be better than titanium, would contribute to its biocompatibility in both soft and hard tissue.

Therefore, the application of nitrided titanium was suggested from the view point of osteocompatibility for the parts which require both biocompatibility and abrasion resistance such as an abutment of dental implants and the sliding part of artificial joints.

CONCLUSIONS

To examine its possible use as an implant material, especially for the abutment, corrosion resistance, wettability of the surface, quality of *S.mutans* adhesion and biocompatibility, tests of nitrided titanium were carried out and the following findings were obtained.

1. The corrosion resistance of titanium nitride was excellent and similar to those of titanium.
2. The wettability of the surface and quality of *S.mutans* adhesion showed no significant difference between titanium and nitrided titanium.
3. The biocompatibility of nitrided titanium in soft and hard tissue suggested that tissue reactions in soft tissue and the formation of new bone in hard tissue were excellent, and equivalent to those of titanium.

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