

High Bone Bonding Ability and Affinity of New Low-rigidity β -type Ti-29Nb-13Ta-4.6Zr Alloy as a Dental Implant

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Abstract

Ti-29Nb-13Ta-4.6Zr alloy (TNTZ) has a low modulus of elasticity and is composed of non-toxic and non-allergenic elements. This study investigated the bone bonding ability and bone affinity of TNTZ for its application to dental implants. TNTZ subjected to solution treatment (TNTZ_{ST}), TNTZ subjected to aging treatment after solution treatment (TNTZ_{AT}), commercially pure titanium (CP-Ti), and Ti-6Al-4V ELI alloy (Ti64) were implanted into canine mandibular implant beds. The bone bonding ability and bone affinity of all metal rods were compared 3 months (3M) and 6 months (6M) after implantation. Biomechanical analysis showed that the failure loads of TNTZ_{AT} and TNTZ_{ST} at 3M and 6M were higher than those of CP-Ti and Ti64. Histological analysis indicated that TNTZ_{AT} and TNTZ_{ST} were surrounded by thick newly formed bone and their direct contact areas with newly formed bone were markedly high. Histomorphometric analysis also indicated significant differences in newly formed bone in the cancellous bone area between TNTZ_{AT} and/or TNTZ_{ST} and the other two groups. These findings suggest that TNTZ could be a useful dental implant material because it has higher bone bonding ability than CP-Ti and Ti64, and would be expected to maintain osseointegration with high bone affinity in the long term.

Keywords: Dental implant, Ti-29Nb-13Ta-4.6Zr alloy, Bone bonding ability, Bone affinity, Canine

Introduction

Titanium (Ti) and its alloys are commonly used metallic biomaterial as dental implants and bone fixation materials [1,2] because they are lightweight, strong, and have excellent corrosion resistance and high bone affinity. Ti alloys are classified into three types according to their crystal structures: α -type represented by CP-Ti (commercially pure titanium JIS grade 2), ($\alpha + \beta$)-type represented by the Ti-6Al-4V ELI alloy (referred to as Ti64), and β -type.

Although CP-Ti and Ti64 are mainly used as dental implants, fractures of CP-Ti implants have occasionally been reported by the insufficiency of the strength [3]. Therefore, Ti64 has been used generally for dental implants because of their superior strength. However, aluminum (Al) and vanadium (V) are included in Ti64, and it was reported that Al is associated with neurotoxicity and Alzheimer's disease, and V is suspected of cytotoxicity. Therefore, the elution of these metal ions could affect vital tissue [4,5]. Since the elastic modulus of CP-Ti and Ti64 (around 110 GPa) is markedly higher than that of jaw bone (around 30 GPa), stress shielding [6] has occurred between dental implant and jaw bone. This stress shielding would elicit bone resorption around the dental implant, bone atrophy, and restriction of bone remodeling. To resolve these problems, many new β -type titanium alloys have been developed for biomedical applications [7,8]. They are composed of non-toxic and non-allergenic elements and exhibit low moduli of elasticity.

In this study, β -type Ti-29Nb-13Ta-4.6Zr alloy (referred to as TNTZ) designed using the theory of the d-electrons alloy method [9] and developed at the Institute for Materials Research, Tohoku University was used. TNTZ is composed of niobium (Nb), tantalum (Ta), and zirconium (Zr), which are also non-toxic and non-allergenic elements. Nb and Ta are β -phase-stabilizing elements, which also improve corrosion resistance. Zr, which adjusts the strength, is distributed in both β phase and α phase. After solution treatment, TNTZ has shown a low modulus of elasticity, about 60 GPa, after solution treatment, which is relatively close to the elastic modulus of jaw bone.

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Moreover, the tensile strength of TNTZ after aging treatment is equal to or greater than that of Ti64. TNTZ could replace Ti64 and SUS316L stainless steel as fracture fixation materials because intramedullary nailing and plate fixation by TNTZ enhanced bone remodeling and achieved rapid bone healing [1,6] in a rabbit tibia fracture model. However, these experiments did not evaluate neither the bone bonding ability nor bone affinity with TNTZ, and the surrounding bone. In this study, bone bonding ability and bone affinity of TNTZ were evaluated in order to emphasize its potential in dental implants by using a canine mandibular model.

Materials and Methods

Preparation of materials

A hot-forged bar of TNTZ (Nb: 29.0, Ta: 13.2, Zr: 4.66, O: 0.12, N: 0.011, Ti: bal, mass %) with a diameter of 24 mm was used in this study. Then, it was subjected to solution treatment in a vacuum at 1063 K, which was about 50 K higher than the β transus temperature, for 3.6 ks, followed by water quenching (referred to as TNTZ_{ST}). Moreover, TNTZ_{ST} was aged in a vacuum at 723 K for 259.2 ks, followed by water quenching (referred to as TNTZ_{AT}). Bars of CP-Ti and Ti64 with a diameter of 24 mm were employed as reference materials. They were subjected to annealing treatment in a vacuum at 933 K for 10.8 ks and 1023 K for 3.6 ks, respectively, followed by air cooling. Each experimental metal rod of TNTZ_{ST}, TNTZ_{AT}, CP-Ti, and Ti64 was cut by mechanical processing, and was adjusted to 1.7 mm in diameter and 8.0 mm in length. After the metal rods were polished using waterproof emery papers up to 1500 grid, they were buffed using a suspension of silicon dioxide to obtain a mirror-like surface (Figure 1a ~ d). Table 1 shows the mechanical properties of each metal rod and the mandible [6,10,11].

Experimental animals and implantation procedures

Eighteen-month-old, male beagle dogs ($n = 12$; NARC Co., Chiba, Japan) were used. The principles of laboratory animal care were followed, as were nation laws, and all procedures were approved by the Animal Research Committee of Tohoku University (Figure 1).

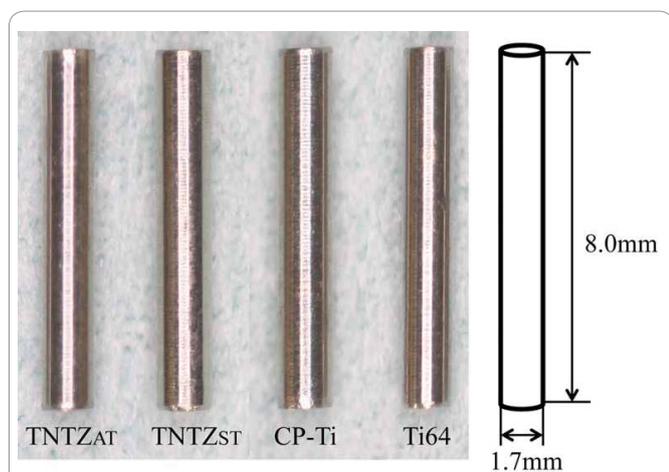


Figure 1. Experimental metal rods

Each experimental metal rod of TNTZ_{ST}, TNTZ_{AT}, CP-Ti, and Ti64 is cut by mechanical processing, and is adjusted to 1.7 mm in diameter and 8.0 mm in length. The metal rods are polished to a mirror-like surface.

	Young's modulus (GPa)	Tensile strength (MPa)	Fatigue strength (MPa)
TNTZAT	80	900	685
TNTZST	58	500	320
CP-Ti	110	345	180 -200
Ti64	110	850	570
Mandible	10 -30	100 -200	-

Table 1. Mechanical properties of the metal samples and the mandible [6,10,11].

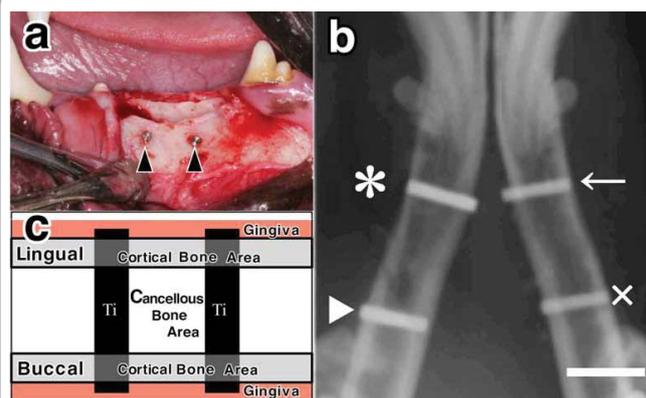


Figure 2. Implantation procedures and schematic diagram

(a) The metal rods are positioned into the implant beds to penetrate buccal and lingual cortical bone. \blacktriangle : The metal rods. (b) The occlusal radiographs of immediately after implantation indicate all metal rods penetrate through buccal to lingual cortical bone without dropout. \blacktriangle : TNTZ_{AT}, $*$: TNTZ_{ST}, \leftarrow : CP-Ti, \times : Ti64, bars: 10.0 mm, (c) The implanted site of the metal rod is designated as cortical bone area and cancellous bone area. Ti: The metal rods.

General anesthesia was applied by administering intravenous sodium pentobarbital (0.5 ml/kg), and local anesthesia (2% lidocaine with 1/80,000 epinephrine) was applied by injection. First, the left lower second and third mandibular premolars were extracted, and the buccal and lingual mucoperiosteal flap from the first to the fourth premolar was ablated. Then, the alveolar septum of the second and third mandibular premolars, and the circumferential cancellous bones were removed using a surgical bar under saline irrigation. In order to prepare implant beds for the metal rods, two sites of the ablated mandible were perforated through the buccal to the lingual side using a twist drill 1.5 mm in diameter. Then, the metal rods were positioned in the implant beds to penetrate buccal and lingual cortical bone (Figure 2a). Finally, the operative wound was closed in a watertight manner. As the implant surgery was performed in the same way on the right side, four metal rods made of TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 were implanted in bilateral mandibles of dogs (Figure 2b). The implanted (observation) site of the metal rod was designated as the cortical bone area and the cancellous bone area (Figure 2c). To prevent infection, flomoxef sodium was used during the operation, and cefcapene pivoxil hydrochloride hydrate was administered orally for 3 days postsurgically. A soft diet was provided to rest the operative wounds for 2 weeks postsurgically.

At 3 months ($n = 6$; referred to as 3M) and 6 months ($n = 6$; referred to as 6M) after implant surgery, the experimental animals were euthanized by intravenous injection of an overdose of sodium pentobarbital. The mandible and surrounding tissues

were resected and fixed with 10% formalin neutral buffer solution, pH 7.4.

Biomechanical Analysis

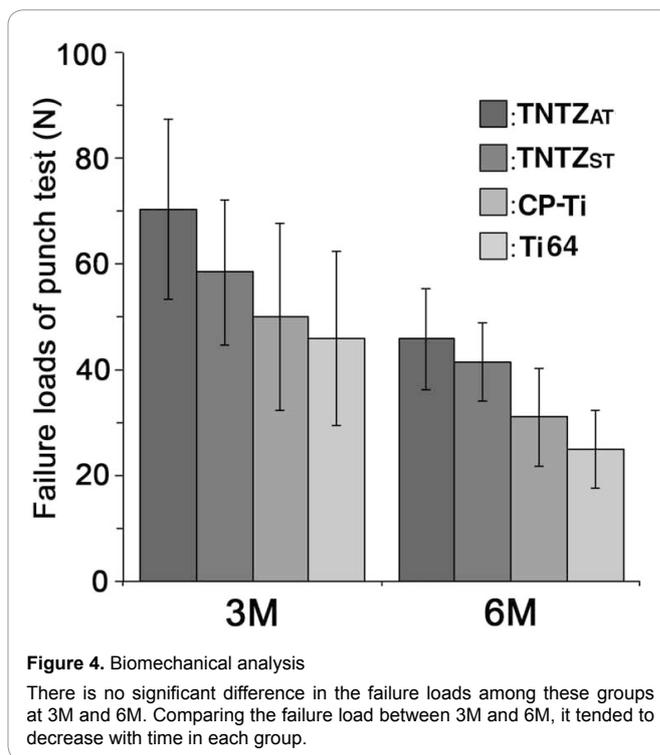
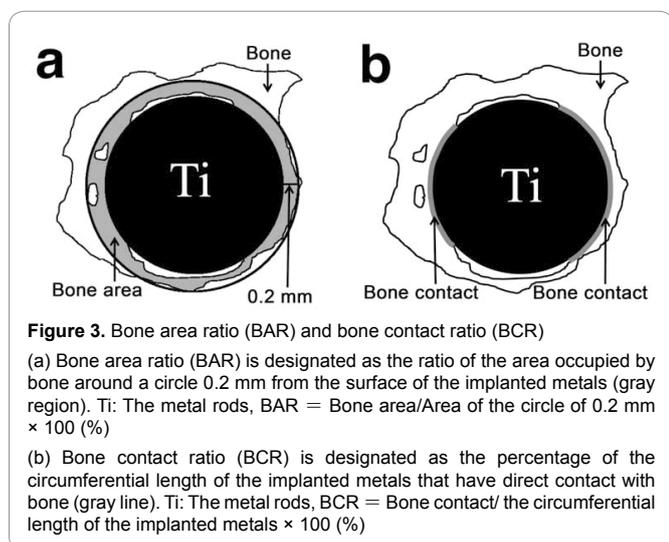
A small punch test was applied to evaluate bone bonding to each metal rod using a punch test device of AUTOGRAPH® AGS-5kNG (Shimadzu Co., Kyoto, Japan) with a capacity of 100 N. A cross head speed of 1 mm/min was employed for punch tests. The metal rods were exposed completely by removing the surrounding soft tissue. The punch tests were performed on three samples for each experimental period (3 and 6M) and group (TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64).

Histological Analysis

The specimens were then fixed in 70% ethanol, stained with Villanueva bone stain, dehydrated in graded ethanol, and embedded in methyl methacrylate. They were cut as sagittal sections using a low-speed saw (Isomet 5000; Buehler, Lake Bluff, IL, USA) with a diamond-wafering blade. The sections were mounted on plastic slides and were ground and polished until they were 20-30 μ m thick. The specimens were repeatedly stained with Villanueva bone stain and then observed with a photomicroscope (Leica DFC290HD; Leica Microsystems Japan, Tokyo, Japan) in the cortical and cancellous bone areas.

Histomorphometric Analysis

The sections of each experimental group stained with Villanueva bone stain were used for histomorphometric measurement (Figure 3). The bone area ratio (BAR) was designated as the ratio of the area occupied by bone around a circle of 0.2 mm from the surface of the implanted metal rods (Figure 3a). The bone contact ratio (BCR) was designated by the percentage of the circumferential length of the implanted metal rods that directly contacted bone (Figure 3b). The BAR and BCR were measured using Image J (National Institutes of Health, Bethesda, MD, USA) on digital images of the sections. The mean values of BAR and BCR obtained from four different sites of each specimen were defined as the BAR and BCR of each specimen. In addition, BAR and BCR were assessed at the cortical bone area and the cancellous bone area. Histomorphometric analysis was



performed on three samples for each experimental period (3 and 6M) and group (TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64).

Statistical Analysis

Statistical analysis was performed on the punch test results, BAR, and BCR using Excel ver. X (Microsoft Co., Redmond, WA, USA). One-way analysis of variance (ANOVA) was used to compare the means among groups. If significant differences in the mean values were detected, Tukey-Kramer multiple comparison analysis was used as a post hoc test. All values are reported as the means \pm standard error (SE). Statistical significance was accepted at $p < 0.05$.

Results

Biomechanical analysis

The failure loads of TNTZ_{AT} ($n = 3$), TNTZ_{ST} ($n = 3$), CP-Ti ($n = 3$), and Ti64 ($n = 3$) at 3M were 70.4 ± 17.1 N, 58.4 ± 13.7 N, 50.0 ± 17.7 N, and 46.0 ± 16.5 N, respectively. There were no significant differences in mean values of the failure loads at 3M among these groups. The failure loads of TNTZ_{AT} ($n = 3$), TNTZ_{ST} ($n = 3$), CP-Ti ($n = 3$), and Ti64 ($n = 3$) at 6M were 45.8 ± 9.54 N, 41.5 ± 7.38 N, 31.1 ± 9.32 N, and 25.0 ± 7.40 N, respectively. There were no significant differences in mean values of failure loads at 6M among these groups. The failure load at 6M was smaller than that at 3M in each group (Figure 4).

Histological analysis

Cortical bone area: In 3M and 6M for each group, the surfaces of the implanted metal rods directly contacted the surrounding cortical bone, and atypical bone resorption was not observed. The osteocytes around the implanted metal rods concentrically assembled on their surfaces, whereas those

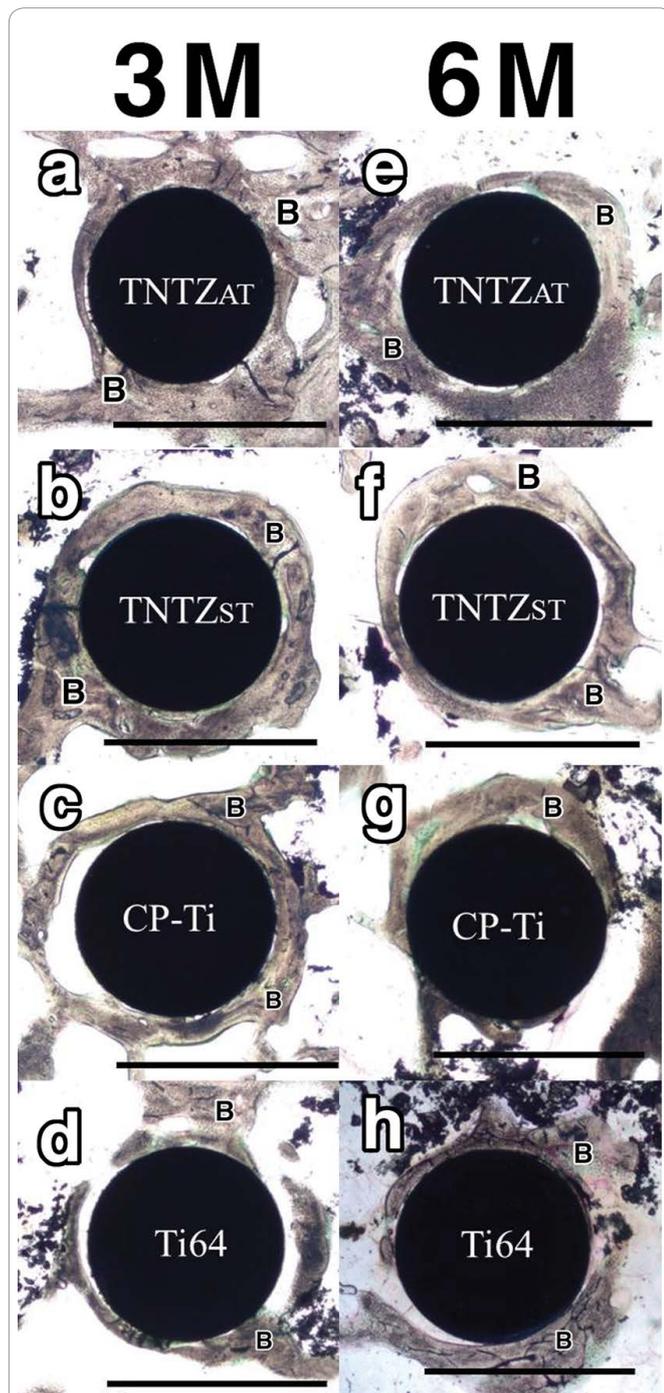


Figure 5. Histology of the cancellous bone area at 3M and 6M
 Histological findings of TNTZ_{AT} (a), TNTZ_{ST} (b), CP-Ti (c), and Ti64 (d) at 3M, and TNTZ_{AT} (e), TNTZ_{ST} (f), CP-Ti (g), and Ti64 (h) at 6M. B: Bone, Bars: 2.00 mm.

in the pre-existing cortical bone were arranged horizontally (data not shown).

Cancellous bone area: In the TNTZ_{AT} at 3M, the rods were circumferentially enclosed by thick newly formed bone with a lamellar structure. The surfaces of the rods exhibited considerable direct contact with the surrounding bone without

intervention of connective tissue (Figure 5a). In TNTZ_{ST} and CP-Ti at 3M, the rods were surrounded by considerably thick newly formed bone with a lamellar structure. Although most of the rod surface directly contacted with the surrounding bone, partial gaps between bone and rod were observed (Figure 5b and 5c). In the Ti64, at 3M a thin layer of newly formed bone was observed near the surface of the rod. The gaps between bone and rod were filled with intervening connective tissue (Figure 5d).

In the TNTZ_{AT}, the rods were circumferentially enclosed by thick newly formed bone with a lamellar structure at 6M. But the gaps between the bone and the rod were observed partially (Figure 5e). In TNTZ_{ST} at 6M, the rods are mostly surrounded by thick newly formed bone, and partial gaps between bone and rod were observed (Figure 5f). In the CP-Ti and Ti64 at 6M, there were thin layers of newly formed bone near the surfaces of the rods, and the surfaces of the rods extensively contacted connective tissue (Figure 5g and 5h). The thickness of the newly formed bone and direct bone contact with the rod surface seemed to be reduced from 3M to 6M.

Histomorphometric Analysis

BAR (bone area ratio) of the cortical bone area: The values of BAR in TNTZ_{AT} ($n = 3$), TNTZ_{ST} ($n = 3$), CP-Ti ($n = 3$), and Ti64 ($n = 3$) at 3M were $98.0 \pm 0.72\%$, $94.2 \pm 4.46\%$, $91.8 \pm 3.62\%$, and $90.5 \pm 6.46\%$, respectively. No significant differences in mean values among the groups were recognized. The values of BAR in TNTZ_{AT} ($n = 3$), TNTZ_{ST} ($n = 3$), CP-Ti ($n = 3$), and Ti64 ($n = 3$) at 6M were $92.3 \pm 2.38\%$, $91.4 \pm 3.15\%$, $91.8 \pm 1.99\%$, and $88.1 \pm 6.58\%$, respectively. No significant differences in mean values among the groups were recognized.

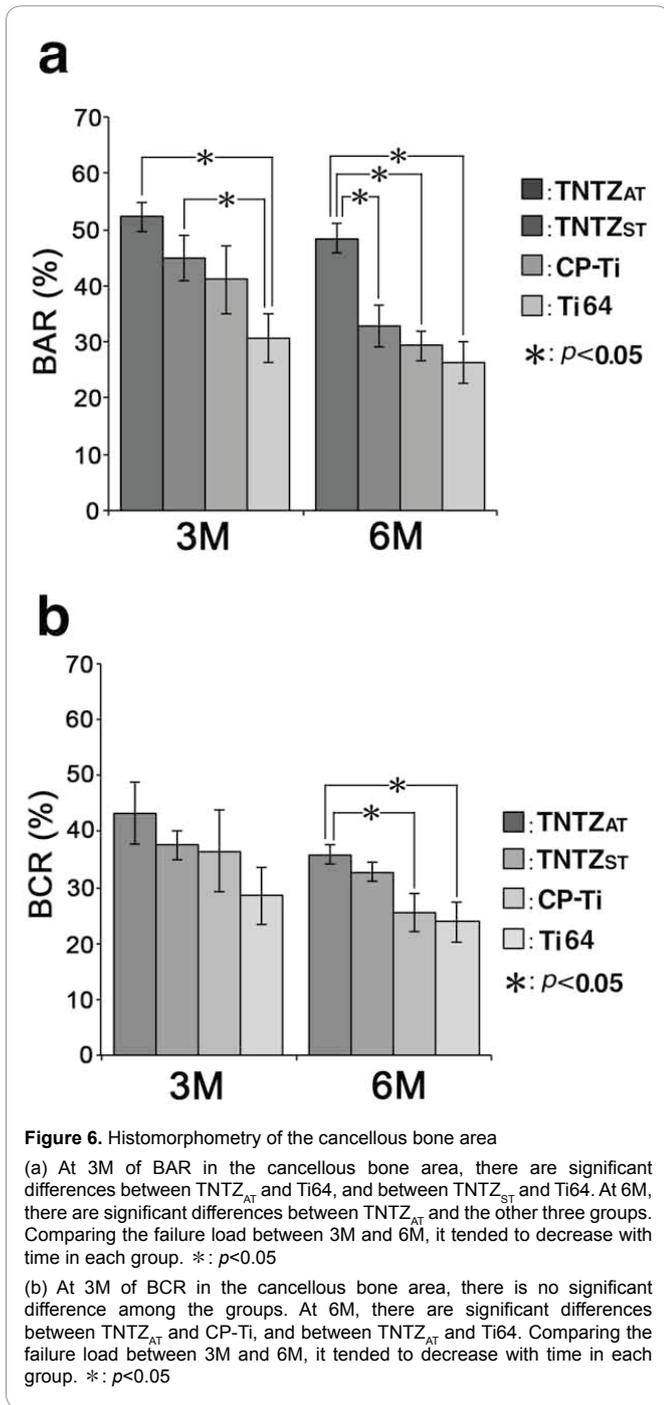
BCR (bone contact ratio) of the cortical bone area: The values of BCR in TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 at 3M were $86.8 \pm 2.00\%$, $83.3 \pm 4.59\%$, $81.9 \pm 1.40\%$, and $81.7 \pm 2.26\%$, respectively. No significant differences in mean values among the groups were recognized. The values of BCR in TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 at 6M were $84.7 \pm 1.46\%$, $80.6 \pm 2.17\%$, $79.4 \pm 2.10\%$, and $73.0 \pm 9.92\%$, respectively. No significant differences in mean values among the groups were recognized.

BAR of the cancellous bone area: The values of BAR in TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 at 3M were $52.3 \pm 2.67\%$, $44.9 \pm 4.16\%$, $41.1 \pm 6.09\%$ and $30.7 \pm 4.36\%$, respectively. There were significant differences in mean values between TNTZ_{AT} and Ti64, and between TNTZ_{ST} and Ti64. The values of TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 at 6M were $48.4 \pm 2.56\%$, $32.8 \pm 3.73\%$, $29.3 \pm 2.66\%$, and $26.4 \pm 3.75\%$, respectively. There were significant differences between TNTZ_{AT} and the other three groups (Figure 6a).

BCR of the cancellous bone area: The values of BCR in TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 at 3M were $43.3 \pm 6.21\%$, $37.5 \pm 2.55\%$, $36.4 \pm 7.32\%$, and $28.5 \pm 5.13\%$, respectively. No significant differences in mean values among the groups were recognized. The values of BCR in TNTZ_{AT}, TNTZ_{ST}, CP-Ti, and Ti64 at 6M were $35.9 \pm 1.78\%$, $32.8 \pm 1.65\%$, $25.5 \pm 3.40\%$, and $23.8 \pm 3.59\%$, respectively. There were significant differences between TNTZ_{AT} and CP-Ti and between TNTZ_{AT} and Ti64 (Figure 6b).

Discussion

The results of these punch tests indicated that the failure loads at 3M and 6M showed no significant differences among



these four groups, although the largest mean value was obtained in TNTZ_{AT}, followed in order by TNTZ_{ST}, CP-Ti, and Ti64. It has been reported that no significant differences in the failure loads obtained from pull-out tests among CP-Ti, Ti-15Zr-4Nb-4Ta, and TNTZ when these alloys were implanted into tibiae of rabbits [12]. The previous report also indicated that the failure load obtained from pull-out tests for Ti-15Zr-4Nb-4Ta alloy was higher than that for Ti64 if implanted into femora of rabbits [13]. These results suggest that the bone bonding ability of TNTZ is similar to those of CP-Ti and Ti-15Zr-4Nb-4Ta alloy, and higher than that of Ti64, despite the different methods of biomechanical

testing being applied. Consequently, TNTZ_{AT} and TNTZ_{ST} should be appropriate as dental implant materials, with bone bonding ability equal to or greater than those of CP-Ti and Ti64.

In the cortical bone area, the newly formed bone around the implant would be involved in bone remodeling because the osteocytes around the implanted metals were concentrically assembled on the surfaces of the metals. In terms of BAR (bone area ratio) and BCR (bone contact ratio) values of the cortical bone areas at 3M and 6M, there were no significant differences among the four groups. These results suggest that these four metals have no difference in bone affinity in the cortical bone area and would exhibit equivalent osseointegration.

In the cancellous bone area, BAR and BCR values of TNTZ_{AT} and TNTZ_{ST} tended to be higher than those of CP-Ti and Ti64 at 3M and 6M. Histological sections revealed that most of the implant surface in the TNTZ_{AT} and TNTZ_{ST} directly contacted the surrounding bone, without the intervention of connective tissue. It has been reported that the amount of new bone formation and the percentage of bone contact in Ti, Nb, and Ta were significantly higher than those of hafnium and rhenium, when these metals were implanted into the femora of rats. [14]. Also, the adhesion ratio of the proliferation of osteoblast-like MG63 cells on TNTZ was significantly higher than that on CP-Ti, and tended to be superior to that of Ti64 [15]. Furthermore, osteoclast formation [16] and the production of PGE₂, which activates the expression of RANKL (receptor activator of NF- κ B ligand) [17], are much more stimulated by CP-Ti than by TNTZ and Ti64. These reports suggest that the bone affinity of TNTZ would be higher than those of CP-Ti and Ti64.

In the cancellous bone area, the values of BAR and BCR were lower in Ti64 than in the other three groups at 3M and 6M. Histologically, the gaps between the surface of Ti64 and bone were filled with intervening connective tissue. Previous studies have shown that the rate of bone contact with the intervening connective tissues is lower in Ti64 than in CP-Ti, and this phenomenon would be caused by the elution of metal ions from Ti64 [18]. Likewise Al ions, which are eluted from Ti64, would affect compete with calcium ions, which are involved in bone formation [19]. Furthermore, several studies in vitro have found that Al ions eluted from Ti64 restrain bone growth by inhibiting osteogenic cells [20,21], and Al and V ions prevent the mineralization of bone by interfering with the formation of hydroxyapatite [22].

In this study, the values of BAR and BCR for TNTZ_{AT} were higher than those for TNTZ_{ST}. This would depend upon the difference in corrosion resistance between them: the corrosion resistance of TNTZ_{AT} is lower than that of TNTZ_{ST} [23], and TNTZ_{AT} is inclined to elute metal ions (Ti, Nb, Ta) [14]. It should be clearly elucidated whether the difference in bone affinity between TNTZ_{AT} and TNTZ_{ST} is related to the difference in corrosion resistance. Although previous studies have shown that the composition of titanium alloy influences the proliferation and differentiation of cells, protein synthesis, and the production of local factors [24,25], the influence of the composition of titanium alloy for bone affinity should be investigated further. This study revealed that TNTZ_{AT} and TNTZ_{ST} would be expected to acquire and maintain osseointegration, because they formed mature

lamellar bone around the surface of the implants due to their high bone affinity for a long period.

The failure loads of Ti-15Zr-4Nb-4Ta alloy and Ti64 increased over time up to 24 weeks, and then slightly decreased [13]. When Ti-6Al-7Nb alloy and Ti64 were implanted into femora of rabbits, new bone matured until 16 weeks and bone trabecular decreased after 24 weeks [26]. Another study has shown that trabecular bone surrounding implant formed vigorously in the early stage at 2 to 8 weeks, but its resorption occurred after 24 weeks, when ceramic-coated implants were inserted in femora of rabbits [27]. In the present study, the values of the failure loads, BAR, and BCR at 6M were decreased compared with those at 3M in all experimental groups. Although it was considered that appropriate bone remodeling was essential for the long-term stability of osseointegration [28], the appropriate load to the metal implants would not be achieved in this experimental model to maintain appropriate bone remodeling. Excessive or insufficient load to the implants elicits bone resorption due to the microdamage of bone and disuse bone atrophy [29]. In this study, the time-dependent decreases of the values of failure loads, BAR, and BCR were approximately the same for all experimental groups, and it would be difficult with this experimental method to provide an appropriate continuous load to the implants. Considering this background, the stable failure loads, BAR, and BCR to the implants would be obtained after 6 months in this experimental model. In this experimental model, the metal implants were applied to penetrate buccal and lingual cortical bone, and this model would not achieve an appropriate continuous load to the metal implants. Previous studies introduced a dental implant model that could achieve an appropriate continuous load to metal implants because the external force to the implants was directed to the tooth axis like occlusal pressure [30]. It might be more suitable to apply this dental implant model to evaluate the differences in bone reaction caused by differences in the elastic modulus between bone and the metal rods. However, this study did not adopt the dental implant model for the reason that it was complicated to ensure equal load distribution to the implanted metals. Moreover, there is a considerable risk of infection around the implant with this model because it is difficult to keep the intra-oral wound clean after implant surgery. Consequently, the most appropriate experimental model for application of TNTZ as a dental implant and for the establishment of its usefulness as a low-elastic-modulus metal should be evaluated further.

TNTZ is a low-elastic-modulus metal of β -type titanium alloy composed of non-toxic and non-allergenic elements. In this study, the efficacy of TNTZ as a dental implant was investigated to evaluate its bone bonding ability and bone affinity in comparison with CP-Ti and Ti64, when these metals were implanted into a canine mandibular model. The obtained results suggest that the bone bonding ability of TNTZ are equal to or greater than those of CP-Ti and Ti64, and bone affinity of TNTZ is greater than those of CP-Ti and Ti64. Since the low elastic modulus of TNTZ is expected to limit stress shielding, it might be possible to apply TNTZ as a dental implant.

Acknowledgments

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