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Original

Comparison of Bone Mineral Density and Area of Newly Formed Bone Around Ti-15%Zr-4%Nb-4%Ta Alloy and Ti-6%Al-4%V Alloy Implants

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Abstract:A newly developed titanium alloy, Ti-15%Zr-4%Nb-4%Ta (Ti15Zr4Nb4Ta), has been shown to have excellent mechanical characteristics, corrosion fatigue strength, anticorrosion, cytocompatibility, and biocompatibility. The purpose of this study was to compare the bone mineral density (BMD) and area of newly formed bone around Ti15Zr4Nb4Ta implants to that around Ti-6%Al-4%V alloy (Ti6Al4V) implants. We inserted 2 types of implants (surface treat of the machine and the blast implant) in New Zealand white rabbit femurs under general anesthesia. The rabbit was done perfusion fixation of 4, 8, 16, 24, and 48 weeks later, and the femurs with the implants were collected and we observed the surrounding bone by an implant drawing test. We measured the BMD and the area of the newly formed bone surrounding the implant inserted part using image analysis software Image-Pro PLUS Ver. 4.0® which it developed newly in micro focus computed tomography (micro CT). The results showed the machine and the blast implant together, which the BMD and area around the Ti15Zr4Nb4Ta implants were comparable to or greater than those around the Ti6Al4V implants. These results suggested that the new alloy, Ti15Zr4Nb4Ta, might be useful for orthopedic or dental implants with the added advantage of superior biologic safety and mechanical properties than the current alloy, Ti6Al4V.

Key words: Implant, Surface treatment, Bone area, Bone mineral density, Micro CT

Introduction

Surface treatment of implants also plays an important role in their stability. Plasma-sprayed HA has been used for coating orthopedic and dental implants, thus combining the mechanical strength of Ti alloy (e.g., Ti-6%Al-4%V: Ti6Al4V) and the bioactivity of calcium phosphate (specifically, hydroxyapatite, HA)[1]. HA-coated implants were shown to accelerate junction with the bone and provide a stronger bone-implant interface[1-3]. However, analysis of HA-coated orthopedic and dental implants showed that the coating consisted of HA and amorphous calcium phosphate, ACP[4] and that the ACP/HA ratio may affect the stability of the coating (e.g., high ACP/HA ratio may cause premature resorption and delamination of the coating)[5]. This would in turn affect the stability of the implant. Surface treatment by grit-blasting [6, 7] (e.g., Astra Tech® [8] and Ankylos® [9, 10]) were also shown to improve osseointegration.

Okazaki et al developed a Ti alloy containing 15%-Zr, 4%-Niobium (Nb), and 4%-Tantalm (Ta) to provide a Ti alloy (Ti15Zr4Nb4Ta) which has been shown to have excellent mechanical characteristics, corrosion fatigue strength, anticorrosion, cytocompatibility, and biocompatibility and to promote bone formation when implanted in tibia of rats and femurs of rabbits[11-19].

Newly formed bone around implants is usually evaluated two-dimensionally by histological analysis of thin sections[7, 15, 18]. In this study, we used micro focus computed tomography (micro CT) to observe the three-dimensional organization of the trabecular and determine bone mineral density (BMD) and area of newly formed bone around implants. There were no studies that measured

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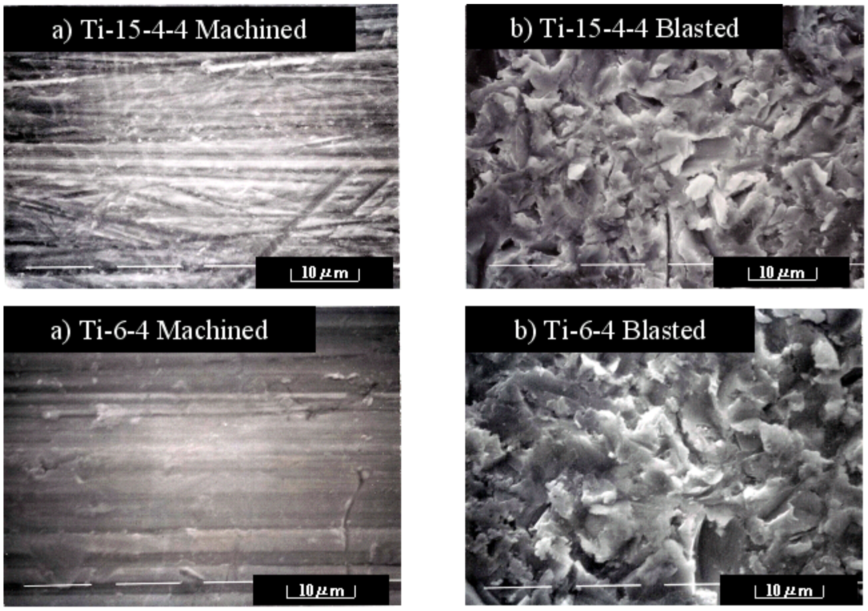
Table I Chemical composition (mass %) of the two types of Ti alloy used in the study.

Titanium alloy	Zr	Nb	Ta	Pd	Fe	O	N	H	C	Al	V	Ti
Ti-15Zr-4Nb-4Ta	15.24	3.90	3.92	0.22	0.022	0.162	0.048	0.011	0.002	-	-	Bal.**
Ti-6Al-4V ELI*	-	-	-	-	0.198	0.101	0.003	0.005	0.011	6.24	4.19	Bal.**

* ELI. : Extra Low Interstitial ** Bal. : Balance


a) Ti-15-4-4 Machined

b) Ti-15-4-4 Blasted

a) Ti-6-4 Machined

b) Ti-6-4 Blasted

Fig. 1 Scanning electron micrographs (SEM) of the machined and blasted surfaces (*2000).

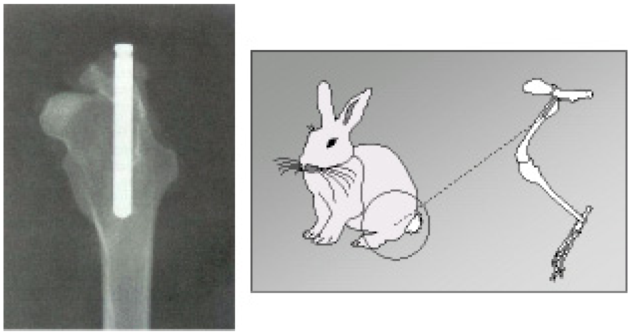


Fig. 2 Radiographic appearance of Ti15Zr4Nb4Ta implants 24 weeks after implantation in the rabbit femur (Left side). Location of implant probe in the mesial femur of New Zealand White rabbit (Right side).

the BMD surrounding the Ti15Zr4Nb4Ta implants by the implant drawing test and the distribution domain of newly formed bone for three dimensions till now.

The purpose of this study was to compare the BMD and area of newly formed bone around two types of Ti alloy implants (Ti6Al4V and Ti15Zr4Nb4Ta) that had received two kinds of surface treatments (machined and blasted) using micro CT.

Materials and Methods

Implant materials

We showed the chemical composition of Ti15Zr4Nb4Ta and Ti6Al4V used in this study to Table I. The implants used a cylindrical (a diameter of 3.1mm and 30.0mm in length) type. The implants were surface treated by either mechanical polishing (machined) or grit-blasting with alumina abrasive (blasted). Both

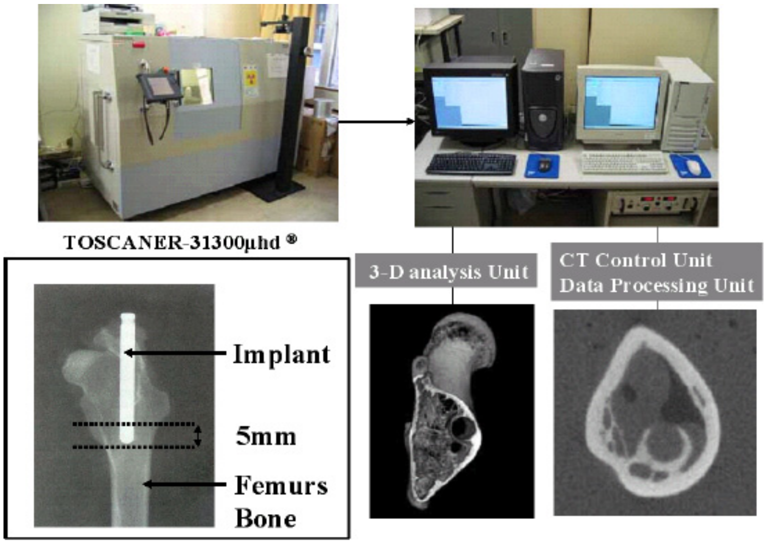


Fig. 3 Photograph of Micro CT systems and measurement site in a rabbit femur.

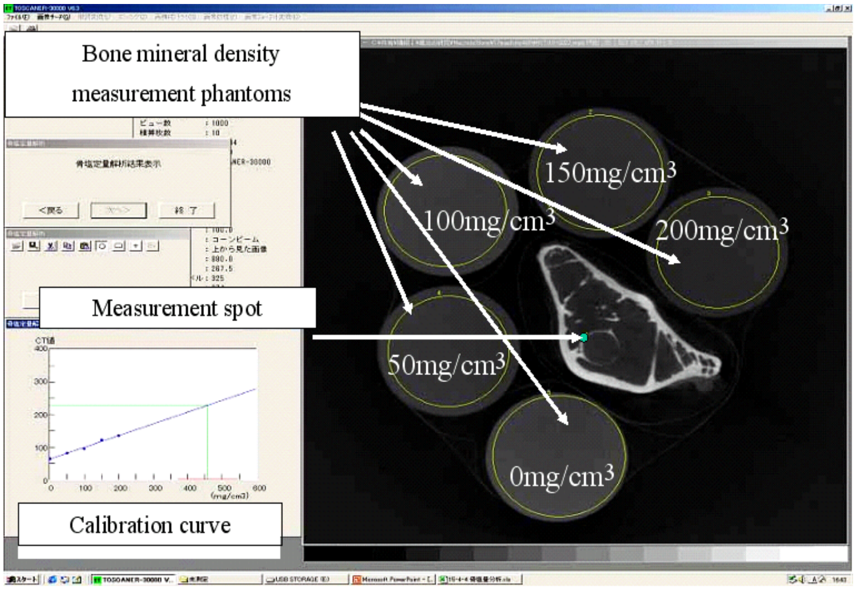


Fig. 4 Measurement method of bone mineral density using Micro CT (HA content of standards: 0, 50, 100, 150, 200 mg/cm^3).

implants (machined and blasted) underwent ultrasonic cleaning twice. The absence of residual alumina particles on the surface of each implant was confirmed using a scanning electron microscope (JSM-T200, JEOL Ltd., Japan) at an accelerating voltage of 20 kV (Fig. 1). The average surface roughness of the implants was £ 0.70 Ra as measured using a profilometer.

Experimental animals

Twenty male New Zealand White rabbits (Sankyo Lab, Japan) (average body weight: 2.93 ± 0.13 kg) were taken care of for two weeks and used at the age of 16 weeks. The Ethical Committee for Animal Experiments of our university approved the experimental protocol of this animal study (ECA-No.03-0003).

Surgery and an implant drawing test

We did dosage by intravenous injection of 74mg from auricular veins at a rate of sodium pentobarbital (Nembutal®, Dainippon Pharmaceutical) 25mg/kg for general anesthesia. An incision (about 30 mm) was made in the apophysis along the central side of the femur, and the bone surface was exposed. The implant bed was prepared parallel to the long axis of the femur using a device for implants (Implanter-S®, Kyocera Co., Japan) and an implant

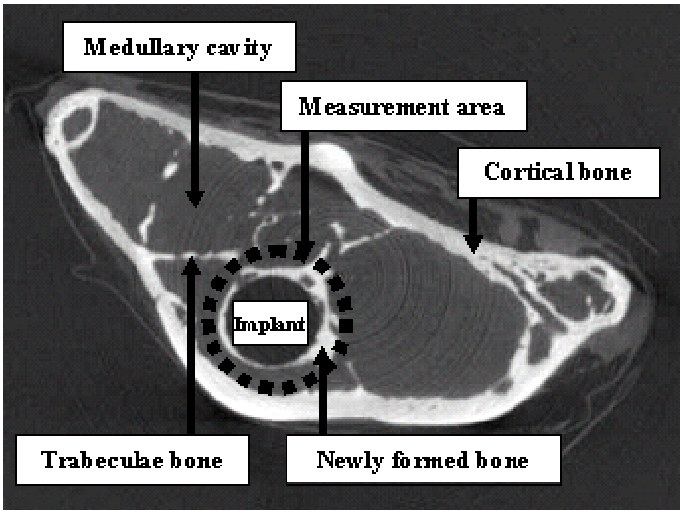


Fig. 5 Illustration of area (a dotted line) of newly formed bone around implants.

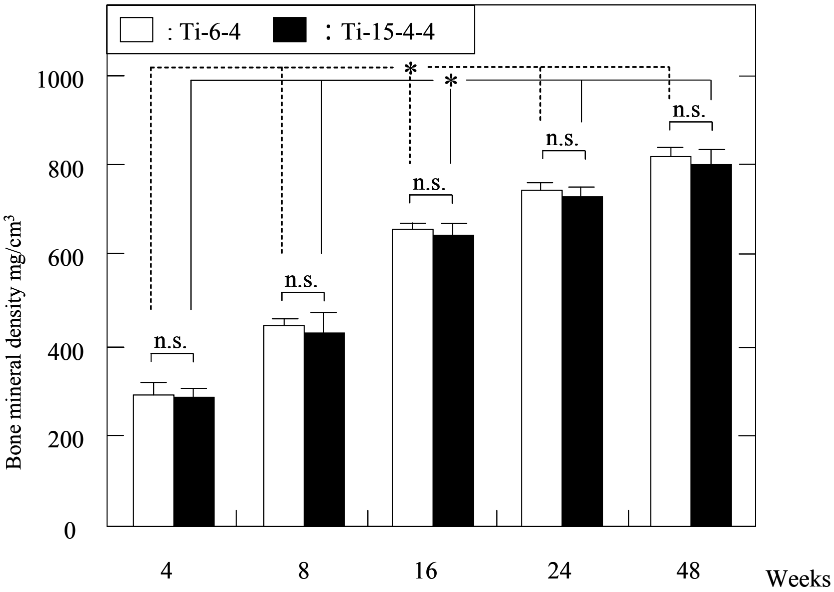


Fig. 6 Bone mineral density of newly formed bone around machined Ti15Zr4Nb4Ta and Ti6Al4V implants. Difference was analyzed by one-way ANOVA and Bonferroni's method (*: $P<0.05$, n.s.: Non significant).

was inserted into each of the bilateral femurs (Fig. 2). The rabbits were done perfusion fixation of 4, 8, 16, 24, and 48 weeks later by general anesthesia since implants inserted. And the femurs were removed surgically. The specimens examined were rabbit femurs after the implants were removed[18]. Specimens were selected after confirming the absence of trabecular attachments on the surface of both Ti alloy implants.

Measurement of bone mineral density (BMD) and bone area by micro CT

BMD and bone area were measured using a high resolution micro CT scanner (Toscaner-31300 mhd®, Toshiba Corp., Japan) (Fig. 3) by the cone beam scan method in the non-helical mode at a tube voltage of 74 kV, and tube electric current of 110 mA with a slice thickness of 0.2 mm and slice pitch of 0.1 mm. In the measurement site, as for the newly formed bone of the implant surrounding, 100µm of long axes were in 5.0mm than an implant tip[20].

BMD was measured by simultaneous imaging of the specimens and 5 BMD quantification phantoms, which served as standards (hydroxyapatite contents: 0, 50, 100, 150, and 200 mg/cm^3: Fig. 4). Five slice images were randomly selected, and newly formed bone that was not continuous with cortical bone was selected as the

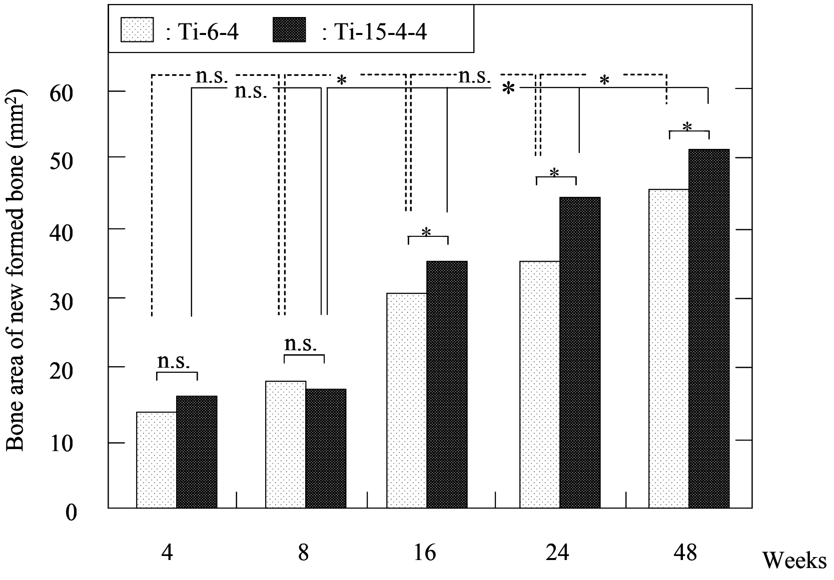


Fig. 7 Bone mineral density of newly formed bone around blasted Ti15Zr4Nb4Ta and Ti6Al4V implants. Difference was analyzed by one-way ANOVA and Bonferroni's method (*: P<0.05, n.s.: Non significant).

measurement site.

To measure the area of newly formed bone around implants, 10 images were randomly selected from 128 slice images obtained by micro CT, and measurement was performed using image analysis software Image-Pro PLUS Ver. 4.0®(Media Cybernetics Nippon Roper Co., Ltd.).

Statistical analysis

For statistical analysis, the means and standard deviations of BMD and area of newly formed bone around implants made of both alloys with two types of surface treatment were calculated, and compared by one-way analysis of variance (one-way AVOVA) and multiple comparison (Bonferroni's method). SPSS (SPSS, Chicago, IL, USA) was used for statistical analysis.

Results

BMD around machined Ti15Zr4Nb4Ta and Ti6Al4V implants

The BMD values of newly formed bone around machined Ti15Zr4Nb4Ta implants increased significantly after each period (Fig. 6): 240.6 ± 22.1 mg/cm^3 at 4 weeks, 440.4 ± 33.5 mg/cm^3 at 8 weeks, 638.7 ± 41.0 mg/cm^3 at 16 weeks, 731.2 ± 25.9 mg/cm^3 at 24 weeks and 802.7 ± 38.3 mg/cm^3 at 48 weeks. The BMD values of newly formed bone around machined Ti6Al4V implants also showed a significant increase after each period (Fig. 6): 260.9 ± 12.7 mg/cm^3 at 4 weeks, 437.7 ± 22.0 mg/cm^3 at 8 weeks, 660.0 ± 18.1 mg/cm^3 at 16 weeks, 740.5 ± 26.0 mg/cm^3 at 24 weeks and 821.3 ± 23.1 mg/cm^3 at 48 weeks. The BMD of newly formed bone around both Ti alloy implants increased linearly from 4 to 16 weeks after implantation, but the increase was gradual from 16 to 48 weeks, with no significant difference between the two types of alloys after each period.

BMD around blasted Ti15Zr4Nb4Ta and Ti6Al4V implants

The BMD values of newly formed bone around the blasted Ti15Zr4Nb4Ta implants also increased significantly after each period (Fig. 7): 285.4 ± 41.0 mg/cm^3 at 4 weeks, 445.4 ± 26.6 mg/cm^3 at 8 weeks, 708.8 ± 25.1 mg/cm^3 at 16 weeks, 756.2 ± 23.0 mg/cm^3 at 24 weeks and 849.3 ± 51.6 mg/cm^3 at 48 weeks after implantation. The BMD values of newly formed bone around the blasted Ti6Al4V implants also showed a significant increase after each period (Fig. 7): 296.8 ± 25.6 mg/cm^3 at 4 weeks, 525.0 ± 72.4 mg/cm^3 at 8 weeks, 691.2 ± 26.0 mg/cm^3 at 16 weeks, 776.9 ± 27.7 mg/cm^3 at 24 weeks and 845.2 ± 23.1 mg/cm^3 at 48 weeks after implantation. The BMD of newly formed bone around both Ti alloy implants increased linearly from 4 to 16 weeks, but the increase was gradual from 16 to 48 weeks. Ti6Al4V showed significantly higher values after 8 weeks, but there was no significant difference between the two types of alloys after any other period.

Area of newly formed bone around machined Ti15Zr4Nb4Ta and Ti6Al4V implants

The area of newly formed bone around the machined Ti15Zr4Nb4Ta implants was 16.2 ± 0.1 mm^2 at 4 weeks, 16.5 ± 0.1 mm^2 at 8 weeks, 35.1 ± 0.1 mm^2 at 16 weeks, 44.8 ± 0.1 mm^2 at 24 weeks and 51.1 ± 0.2 mm^2 at 48 weeks after implantation (Fig. 8). The area of newly formed bone around the machined Ti6Al4V implants was 13.6 ± 0.3 mm^2 at 4 weeks, 17.3 ± 0.2 mm^2 at 8 weeks, 30.7 ± 0.4 mm^2 at 16 weeks, 34.7 ± 0.2 mm^2 at 24 weeks and 46.0 ± 0.7 mm^2 at 48 weeks after implantation (Fig.

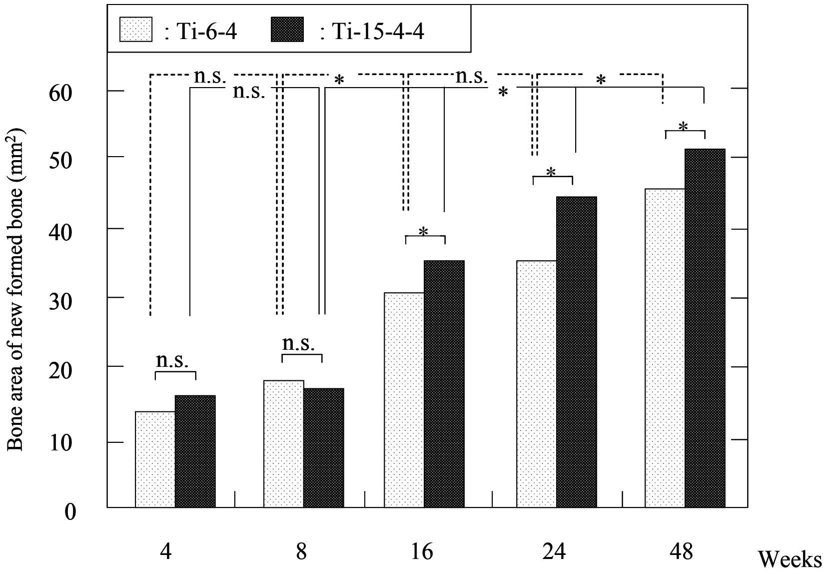


Fig. 8 Area of newly formed bone around machined Ti15Zr4Nb4Ta and Ti6Al4V implants. Difference was analyzed by one-way ANOVA and Bonferroni's method (* : $P<0.05$, n.s.: Non significant).

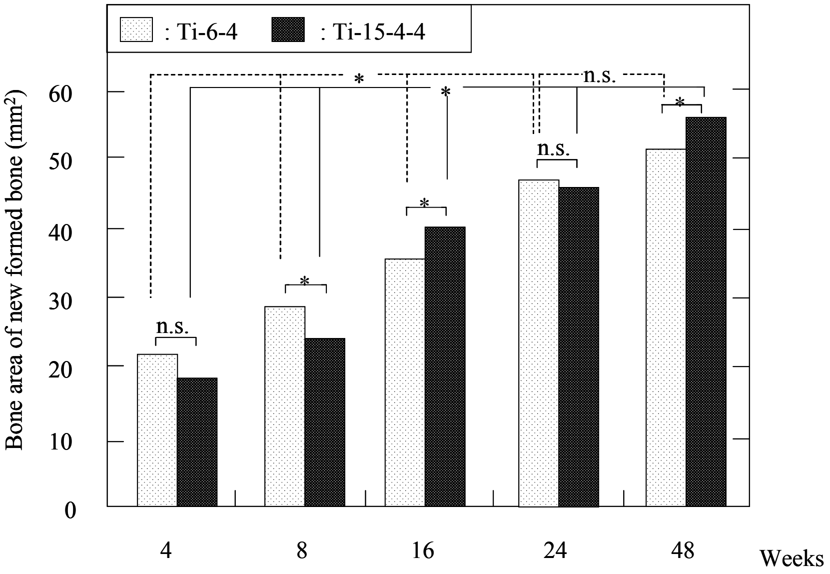


Fig. 9 Area of newly formed bone around blasted Ti15Zr4Nb4Ta and Ti6Al4V implants. Difference was analyzed by one-way ANOVA and Bonferroni's method (* : $P<0.05$, n.s.: Non significant).

8). A slight increase was observed from 4 weeks to 8 weeks after implantation. Both the Ti alloy implants showed a significant increase after 16 weeks or longer. A significant increase was observed in Ti15Zr4Nb4Ta implants from 8 weeks or longer. In Ti6Al4V implants, a significant increase was observed from 8 weeks to 16 weeks and from 24 weeks to 48 weeks after implantation. A significantly greater area of newly formed bone was observed around the Ti15Zr4Nb4Ta implants when compared to that around the Ti6Al4V implants from 16 weeks to 48 weeks after implantation. There was no significant difference between the two types of alloys after 4 weeks or 8 weeks, but a greater area was observed around the Ti15Zr4Nb4Ta after each period, except at 8 weeks, than around the Ti6Al4V implants.

Area of newly formed bone around blasted Ti15Zr4Nb4Ta and Ti6Al4V implants

The area of newly formed bone around the blasted Ti15Zr4Nb4Ta implants was 18.7 ± 0.2 mm^2 at 4 weeks, 24.1 ± 0.1 mm^2 at 8 weeks, 40.2 ± 0.1 mm^2 at 16 weeks, 46.4 ± 0.1 mm^2 at 24 weeks and 56.2 ± 0.2 mm^2 at 48 weeks after implantation (Fig. 9), showing a significant increase after each observation period. The area of newly formed bone around the blasted Ti6Al4V implants was 22.4 ± 0.1 mm^2 at 4 weeks, 28.9 ± 0.4 mm^2 at 8 weeks, 35.4 ± 0.1 mm^2 at 16 weeks, 47.2 ± 0.1 mm^2 at 24 weeks and 51.6 ± 0.2 mm^2 at 48 weeks after implantation (Fig.9). A significant increase was observed from 4 weeks to 24 weeks after implantation and there was a slight increase from 24 weeks to 48 weeks after implantation. The area of newly formed bone around the blasted Ti15Zr4Nb4Ta implants was significantly greater when compared to that around the Ti6Al4V implants after 16 weeks and 48 weeks. A slightly greater area was observed around the Ti-6-4 implants after 4 and 24 weeks, and the area around the Ti6Al4V implants were significantly greater after 8 weeks.

Discussion

Commercially pure Ti and Ti alloy (Ti6Al4V) are the metals of choice for dental implants due to their biocompatibility, safety and ability to osseointegrate. Albrektsson et al[21)]. associated the following factors with good osseointegration: 1) implant material, 2) implant surface treatment, 3) implant morphology, 4) bone state at the implantation site, 5) surgical technique, and 6) degree of the load. Among these, implant morphology, and material and surface morphology are implant-associated factors. Morphology enters these, and the materials and the surface morphology are factors related to the implant. Then, in this research paid attention to the implant material and the surface morphology.

Johansson et al[22)]. observed no difference between pure Ti and Ti6Al4V implanted into the rabbit tibia based on histologic and quantitative histomorphometric analyses. However, the two metals have different physical characteristics: pure Ti is inferior to Ti6Al4V in strength. In recent years, dental implants have been used not only for support of single teeth[23)] but also for support of partial dentures,[24)] support for reconstruction of the entire dental arch,[25)] support of complete dentures,[26)] as structures penetrating the mucosa for support for the reconstruction of maxillofacial defects,[27, 28)] and for treatment allowing oral, cranial, and facial reconstruction. To avoid clinical fracture of pure titanium implants,[29, 30)] mechanical properties appropriate for implantation are required for long-term functioning of the implant materials *in vivo*. Ti15Zr4Nb4Ta was developed with improved properties. The annealing strength of Ti15Zr4Nb4Ta is slightly higher than the standard value for Ti6Al4V established by the International Organization for Standardization (ISO) and the physical characteristics are similar to those of Ti-6Al-7Nb alloys.[12] Thus, they show no problems in mechanical properties, such as fracture.

Micro CT does not require pretreatment (e.g., embedding, sectioning) or any specific skills, allows noninvasive measurement,[31)] and is particularly useful for ultrastructural analysis of bone tissue[32, 33)]. In particular, micro CT scanners have high resolution, and allow visualization of the trabecular structure itself and the measurement of bone volume, bone surface area, and cancellous bone thickness[34)]. Therefore, micro CT is frequently used for the study of trabecular structure and hard tissue ultrastructure, and has been applied to studies in various medical fields[35, 36)]. In animal experiments involving implants, observation by micro CT may not be reliable because measurement of areas close to the implant is difficult due to the artifacts[37, 38)]. Therefore, in this experiment, rabbit femurs were used after the implants had been removed. Specimens were selected after confirming that trabecular attachments were absent on the surface of both Ti alloy implants. The optimal imaging conditions should be determined according to the characteristics of the scanner and the object of imaging.

In this study, the measurement site was 100 mm inwards from the bone surface in contact with the implant because occlusal force associated with mastication requires at least 100 mm for maintenance and buffering action of the peri-implant bone, and there is also a risk of damage to newly formed bone after drawing tests[20)]. The measurement of specimens after removed out allowed acquisition of more accurate findings from raw data. BMD was measured by simultaneous imaging of specimens and BMD measurement phantoms. Since hydroxyapatite has lower radiation transmittance than other tissues, its amount can be measured by calculating the radiation absorption rate. Five BMD measurement phantoms and specimens were simultaneously measured, and BMD corresponding to the CT value of the specimen was calculated based on a calibration curve. Therefore, BMD measurement did not require any special device, and the BMD per unit volume could be readily obtained.

In this study, BMD and area were measured in the newly formed bone around implants with two types of surface treatment. The grit-blasted implants showed a greater BMD and area of newly formed bone compared to the machined implants. In general, for bone/implant bonding, grit-blasted or plasma-sprayed HA coated implants are considered to be more advantageous than machined implants[39)]. Grit-blasted implants have been reported to exhibit improved reactivity on the titanium surface due to removal of contaminants on the surface layer and production of fine irregular unevenness on the surface by blasting with titanium oxide or alumina oxide[40)]. Another study suggested that the bone/implant contact area is larger in grit-blasted implants than machined implants, and therefore, cells on the rough surface can exist and proliferate for a longer period[41)].

Butterfield et al[42)]. measured BMD after maxillary sinus lift using autologous bone and reported a BMD of 349 mg/cm^3 after 4 weeks. Direct comparison between the above results and those of the present study is difficult due to differences in the implantation material and site, but their results were similar to those of blasted Ti6Al4V implants in the present study. Comparison of the results of the bone area is also difficult because of the differences in the implantation site and measurement conditions, but our findings of an increase in both BMD and bone area with time is in agreement with their results.

Concerning trabecular formation around implants, a previous study showed active bone formation in the early stage from 2 weeks to 6 weeks after implantation, but trabecular resorption occurred after 24 weeks or more.[43] In the present study, a linear increase in BMD was observed from 4 weeks to 16 weeks after implantation, and a gradual increase thereafter until 48 weeks after implantation for both types of surface treatment on both alloys. The area of newly formed bone also increased from 4 weeks to 48 weeks after implantation.

Newly formed bone around the implant should be maintained despite loads such as occlusion force applied on bone. Newly formed trabeculae are not mature enough, and the crystallinity of trabeculae should be gradually improved by stimulating bone precursor cells, increasing alkaline phosphatase activity, and enhancing the incorporation of newly formed collagen into bone matrix. In addition, to maintain BMD, modeling-remodeling is indispensable, and the bone area should be further increased for the establishment of the bone basis for osseointegration.

These results suggest the usefulness of Ti15Zr4Nb4Ta as an implant material for biological applications. Though many titanium alloys have been developed as implant materials, artificial materials that do not induce negative responses during their constant functioning in vivo should be selected. Hence, further research is necessary to analyze the crystallinity, i.e., quality of bone formed around Ti15Zr4Nb4Ta implants.

Conclusion

We investigated the characteristics of bone on the machined or blasted surface of Ti15Zr4Nb4Ta, a potential titanium alloy for biological use, and Ti6Al4V, a control that was conventionally used. The BMD and bone area were measured by micro CT in newly formed bone 100 mm from the bone surface around implants of these alloys with two types of surface treatment.

The BMD and bone area around the Ti15Zr4Nb4Ta implants were comparable to or greater than those around the Ti6Al4V implants. The Ti15Zr4Nb4Ta contains ions that are biologically safer. These results suggested that the new alloy, Ti15Zr4Nb4Ta might be useful for orthopedic or dental implants with the added advantage of superior biologic safety and mechanical properties than the current alloy, Ti6Al4V. Further studies are warranted on the new alloy to evaluate its potential as an implant material for orthopedic and dental applications.

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