Three-Dimensional Bone Response to Commercially Pure Titanium, Hydroxyapatite, and Calcium-Ion-Mixing Titanium in Rabbits

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Three-dimensional bone response to 3 biomaterials—commercially pure titanium (Ti), hydroxyapatite (HA), and calcium-ion-mixing titanium (Ca-Ti)—embedded in the tibiae of rabbits was examined chronologically. The rabbits were sacrificed at 2, 4, and 8 weeks after implantation, and the percent bone volume around each implant was calculated from the implant surface to each of 4 measurements: 36 μm, 0.25 mm, 0.5 mm, and 1.0 mm in 2 regions (cortical bone and bone marrow regions). Percent bone volume in the cortical bone was consistent, whereas in the bone marrow region, the percent bone volume varied according to implant material, implantation period, and distance from the implant surface. With Ti implants the percent increased gradually up to 8 weeks at each distance, whereas in HA and Ca-Ti implants the percent was largest at 4 weeks and increased closer to the surface. The percent with Ti implants was largest at 36 μm to 0.25 mm. Aspect of bone response to Ca-Ti was its position intermediate between those of HA and Ti. The decrease of the percent at 8 weeks was smaller than HA. (Int J Oral Maxillofac Implants 2000;15:231–238)

Key words: *calcium-ion-mixing titanium, hydroxyapatite, percent bone volume, 3-dimensional bone response, titanium*

Commercially pure titanium is widely used as a biocompatible implant material because of the biomechanical bond between the material surface and bone through so-called "osseointegration."[1] Where low bone density exists, osseointegration is often not possible when primary fixation of the implant is not obtained during the bone healing period.[2,3] It has been reported that the bone healing period following implantation of hydroxyapatite (HA) is shorter than that following titanium implant placement and that HA can be applicable in low-bone-density situations because of high bone affinity.[4–7] However, HA is subject to soluble dissolution and cellular degradation. Subsequent opportunistic microbiologic colonization on unstable surfaces and the mechanical weakness of HA itself may result in unfavorable clinical response.[8–11] Hydroxyapatite plasma-sprayed (HPS) titanium is a hybrid material that combines both high bone conductivity and mechanical strength.[12,13] However, HPS titanium remains problematic because of the adhesive fracture between HA and titanium and cohesive fracture and dissolution with HA.[14,15]

Recently, it has been reported that titanium implanted by calcium ions (Ca^{2+}) with an acceleration energy of 18 keV has useful properties as a biomaterial.[16–23] Calcium phosphate precipitation on titanium in an electrolyte is accelerated tremendously by Ca^{2+} implantation.[16] In addition, osteogenic cells on titanium are activated and the formation of osteoid tissue by the cells is accelerated when calcium ions are implanted in titanium.[17] Furthermore, it has been shown that a larger amount of new bone was formed on Ca^{2+}-implanted titanium than on unimplanted titanium.[18] The results indicated that Ca^{2+}-implanted titanium is superior to titanium alone for bone conduction. Therefore, Ca^{2+}-implanted titanium may be a superior biomaterial. This favorable property of

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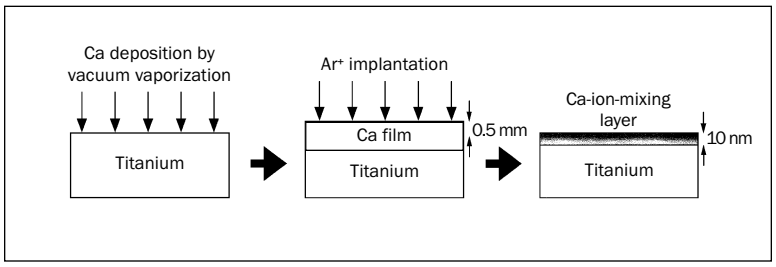

Fig 1 Diagram of calcium-ion-mixing process.

Ca^{2+}-implanted titanium is the result of implanted calcium ions, ie, the surface-modified layer formed by ion implantation. The surface-modified layer consists of calcium titanate when ions are implanted with 10^{16} and 10^{17} ions/cm^2, and both calcium oxide (hydroxide) and calcium titanate when implanted with 10^{18} ions/cm^2.[19] The mechanism of improvement of hard tissue compatibility of Ca^{2+}-implanted titanium is as follows.[20] The Ca^{2+}-implanted titanium surface is more positively charged by dissociation of hydroxyl radicals than the titanium surface. In addition, the number of charging sites of Ca^{2+}-implanted titanium is greater than that of non–Ca^{2+}-implanted titanium. Adsorption of phosphate ions in bioliquid is greater on the Ca^{2+}-implanted titanium surface than on the plain titanium surface because of the attractive force of the electric charge. Greater adsorption of phosphate ions causes greater attraction of calcium ions by the surface, and more calcium phosphate precipitates. Simultaneously, calcium ions dissolve from the surface-modified layer of Ca^{2+}-implanted titanium, as revealed in previous studies.[21,22] This causes supersaturation for calcium phosphate precipitation in the bioliquid near the surface, and acceleration of calcium phosphate precipitation. Recently, Ca^{2+}-mixing titanium (Ca-Ti), whose surface-modified layer was the same as that of Ca^{2+}-implanted titanium, was developed as a successional material to Ca^{2+}-implanted titanium.[23] The Ca-Ti has the same properties as Ca^{2+}-implanted titanium and is useful as a biomaterial, having both the mechanical strength of titanium and the bone conductivity of HA.

In the evaluation of the biomaterial, especially as a dental implant, the results of in vitro experiments do not always compare with those of in vivo experiments. Albrektsson et al emphasized the importance of evaluating materials by in vivo experiments.[24] A new system for 3-dimensional examination of the bone structure around implants in vivo has been reported.[25] This system provides details of 3-dimensional bone structure after the implantation of biomaterials. In the present study, the 3-dimensional bone structure after implantation of 3 biomaterials (commercially pure [cp] titanium, HA, and Ca^{2+}-implanted titanium in rabbits) was examined chronologically using this system. The differences in bone response to the biomaterials were evaluated, and influence on the clinical response was discussed.

MATERIALS AND METHODS

Specimen Preparation

Commercially pure titanium (Ti) grade 2 and dense HA implants were custom-made (Pentax, Tokyo, Japan) for this experiment. The surface condition of the implants was the same as that in the report of Wigianto et al.[26] Each was prepared as a cylinder 4.0 mm in diameter and 5.0 mm in length with a smooth surface. In the manufacturing of Ca-Ti, calcium was deposited on the Ti implant specimen with a thickness under 0.5 mm using the vacuum vapor deposition method. Thereafter, argon ions (Ar^+) were implanted with an amount of 10^{17} ions/cm^2 into the surface to mix the deposited calcium and titanium as the substrate using an ion implantation instrument (original machine, Ishikawajima-Harima Heavy Industries Co, Ltd, Tokyo, Japan) (Fig 1). The acceleration energy of Ar^+ implantation was 25 keV. The color of the Ca-Ti was pale gold. The Ca^{2+}-mixing titanium samples were ultrasonically rinsed in acetone and ethanol for 900 seconds each and dried with a stream of high-purity nitrogen.

Throughout the present study, the guidelines for animal experimentation at Tokushima University, Japan, were followed. Before embedding, the implants were sterilized in ethylene oxide gas. Each implant material was embedded in the tibiae of 18 adult male (weight 3.0 to 3.5 kg) New Zealand white rabbits. After the rabbits were sedated using Nembutal (Dairabot Co Ltd, Tokyo, Japan) administered intravenously and enhanced by lidocaine at the operation site, implant placement was carried out. To prevent postoperative infection, antibiotic topically (Terra-Cortril, Pfizer, Tokyo, Japan) was

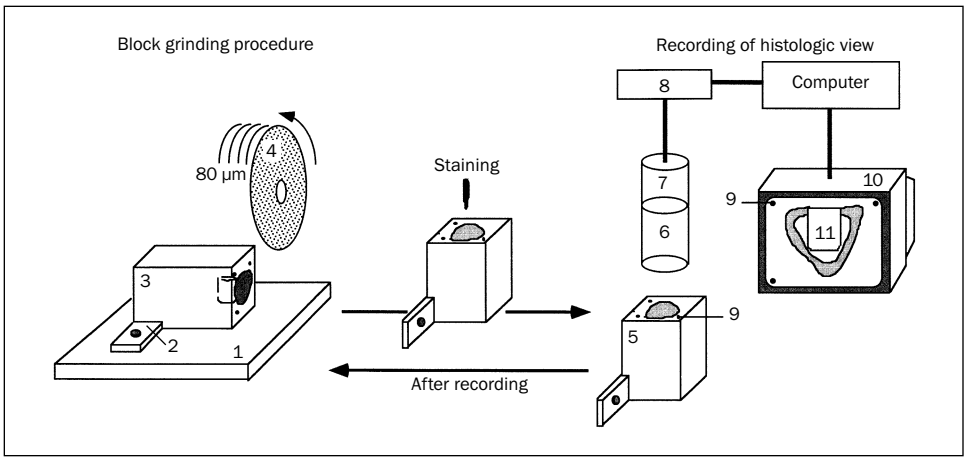

Fig 2 Schematic block diagram for 3-dimensional bone examination, including the block grinding procedure and recording of the stained block's surface. 1 = ceramic plate; 2 = screw holder; 3 = block specimen; 4 = grinding disk; 5 = stained block specimen; 6 = light microscope; 7 = CCD camera; 8 = camera control unit; 9 = marker of fine copper wires; 10 = computer monitor; 11 = image of block surface.

applied to the sutured area. The rabbits were sacrificed 2, 4, and 8 weeks after implantation by overdose of Nembutal. The bone column including the implant was placed immediately into 10% buffered formalin solution. Following a non-decalcifying histologic procedure, the specimens were embedded in polyester resin (Maruto Co, Tokyo, Japan). Four implants of each implant type and time were used under the same conditions.

Three-Dimensional Modeling and Percent Bone Volume

Three-dimensional bone structure around the implant was examined following the procedure of Wigianto et al (Fig 2).[25] The embedded specimen was ground at intervals of 80 μm along the long axis of the implant. The block was removed from the grinding machine and the block surface was then stained with Alizarin red S. The stained ground block surface was examined under a light microscope, and the histologic view was recorded with a CCD camera (KP-C251, Hitachi, Tokyo, Japan) and a computer with a resolution of 36 μm/pixel. Block specimen grinding and image recordings were repeated, and serial 2-dimensional images were constructed into a 3-dimensional model using image software.

Percent bone volume was calculated using image-analyzing software (IP Lab Spectrum 3.1, Signal Analytics Co, Fairfax, VA). The percent bone volume was determined for 2 regions: cortical bone and bone marrow. In the region from the implant surface to each measurement—36 μm, 0.25 mm, 0.5 mm, and 1.0 mm (Fig 3)—each percent bone volume was determined using the following equation:

$$\text{Percent bone volume} = \frac{\text{Bone volume (boxes)}}{\text{Volume to be examined (boxes)}} \times 100$$

Therefore, percent bone volume in the region from the implant surface to a 36-μm distance was equal to the bone-implant contact rate.

RESULTS

Three-dimensional models in Fig 4 show the chronologic changes of the bone structure around 3 biomaterials. The quality of bone healing depended on the implant materials and implantation period. More bone was observed around the HA implants in the early period, but then decreased at 8 weeks, whereas around the cp Ti implants, bone increased gradually up to 8 weeks. The chronologic aspect of bone remodeling of Ca-Ti was similar to that of HA. The bone volume of Ca-Ti appeared to be larger than that of HA.

Figure 5 shows the percent bone volume at each distance from the implant surface of the cortical bone and the bone marrow regions. Table 1 shows

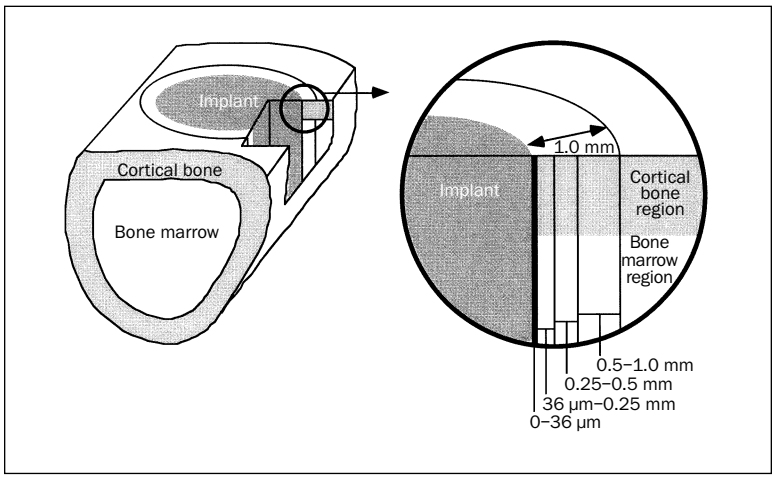

Fig 3 Schematic figure of medial-superior view of the implant in tibia bone. The percent bone volume was examined up to 1 mm from the implant surface and categorized into 0 to 36 μm, 36 μm to 0.25 mm, 0.25 to 0.5 mm, and 0.5 to 1.0 mm. The percent bone volume of each was also divided into 2 regions, cortical bone and bone marrow.

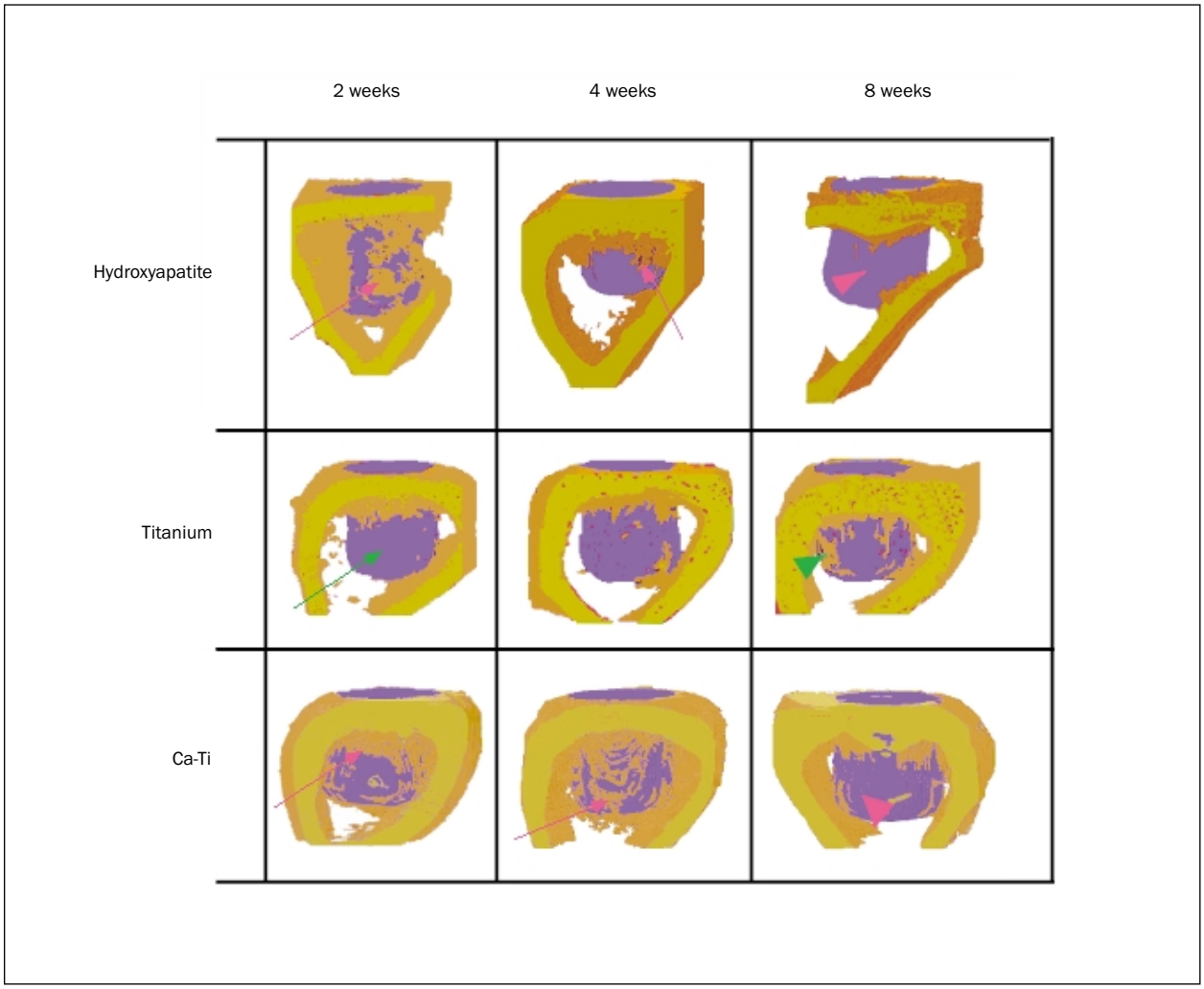

Fig 4 Three-dimensional graphics were used to visualize chronologic changes in bone structure around the 3 implants. Bone in the marrow cavity was observed more around HA and Ca-Ti *(red arrows)* implants than around Ti implants in the early period (up to 4 weeks) *(green arrow)*. The bone around the HA and Ca-Ti implants *(red arrowheads)* then decreased, whereas bone around the Ti implant increased *(green arrowhead)*.

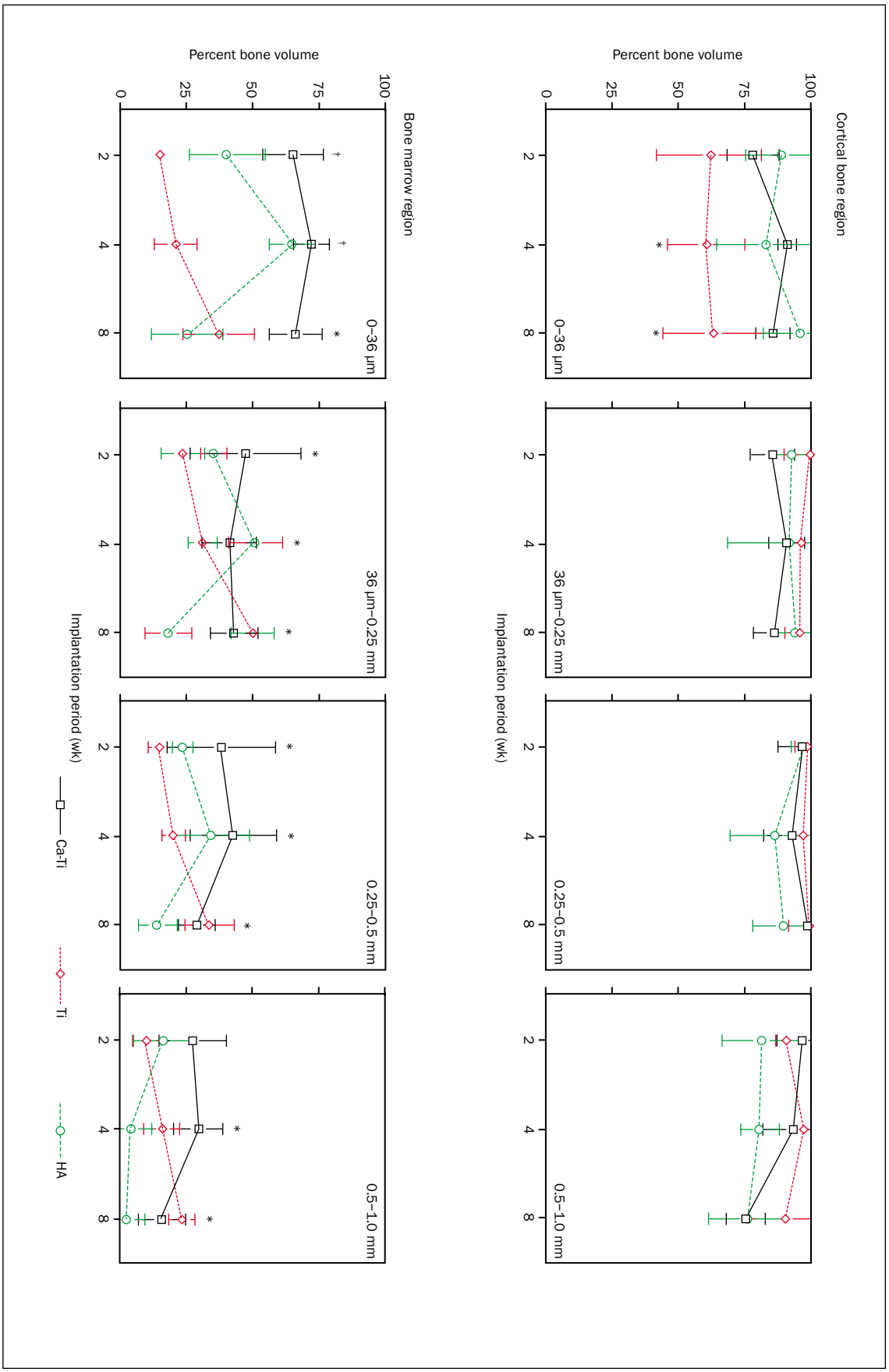

Fig 5 Chronologic changes of percent bone volume. Symbols and error bars represent mean and standard deviation (n = 4). Bone volume in the cortical region was relatively stable at each distance around both the HA and the Ti implants. In bone marrow regions, bone volume varied among implant types, implantation period, and distance from the implant surface (3-way ANOVA, $P < .05$). * = Significant difference (ANOVA, $P < .05$); † = Significant difference (ANOVA, $P < .0001$).

Table 1 Summary of 3-Way ANOVA

Region	df	Sum of squares	Mean square	F value	*P* value
Cortical bone region					
Types (T)	2	.007	.003	.248	.7808
Periods (P)	2	.006	.003	.234	.7919
Range (R)	3	.578	.193	13.928	< .0001
T vs P	4	.037	.009	.675	.6109
T vs R	6	.717	.120	8.647	< .0001
P vs R	6	.101	.017	1.220	.3015
T vs P vs R	12	.088	.007	.532	.8897
Total	108	1.493	.014		
Bone marrow region					
Types	2	.956	.478	51.847	< .0001
Periods	2	.113	.056	6.108	.0031
Range	3	1.759	.586	63.607	< .0001
T vs P	4	.743	.186	20.144	< .0001
T vs R	6	.557	.093	10.060	< .0001
P vs R	6	.059	.011	1.061	.3906
T vs P vs R	12	.221	.018	1.995	.0314
Total	108	.996	.009		

Types = Ti, HA, and Ca-Ti; periods = at 2, 4, and 8 weeks; range = 0 to 36 μm, 36 μm to 0.25 mm, 0.25 to 0.5 mm, and 0.5 to 1.0 mm.

the results of a 3-way analysis of variance (ANOVA). Percent bone volume in cortical bone was consistent regardless of implant materials, implantation period, and distance from the implant surface, whereas in the bone marrow region, percent bone volume varied among implant material, implantation period, and distance from the implant surface. With cpTi implants, the percent increased gradually up to 8 weeks at each distance, whereas with HA implants the percent was largest at 4 weeks. With HA implants, the percent closer to the surface was largest, and the percent for cpTi implants was largest at 36 μm to 0.25 mm. Bone surrounding the HA implant was thinner than that around the cp Ti implant, and the healing field of HA implants was narrower. In total, up to the 1.0 mm distance, percent bone volume of HA implants at 2 and 4 weeks after implantation was higher than for cpTi implants but showed a decline at 8 weeks.

Calcium-ion-mixing titanium had a characteristic that positioned it intermediate to that of HA and cpTi. The percent bone volume was largest at 4 weeks. The percent bone volume closer to the surface was largest, and the bone decreased relative to the distance from the surface in the same way as HA. Percent bone volume was higher than HA, and the decrease at 8 weeks was lower.

DISCUSSION

Bone response after implantation is influenced by composition and the surface condition of the biomaterial. It has been reported that the rate of contact between cpTi and bone is dependent more on the surface condition than material composition.[27] Although numerous studies on the bone-biomaterial interface have been carried out, bone structure around the biomaterial must be fully and objectively understood. Microfracture of bone around the implant is often observed with dental implants,[28] and adequate bone thickness surrounding the implants is required to act as support over the long term. There is little information on the thickness of surrounding bone, despite a large number of reports on bone-implant contact rate.[29–31] Therefore, the percent bone volume as proposed herein could be an important factor with which to evaluate the bone-implant interface. In the present unloaded situation, the percent bone volume was calculated within 1-mm distances, because Brunski reported that bone remodeling after implantation expanded within 1 mm from the implant surface in the loaded case.[29]

Results of the present study showed that the chronologic change in 3-dimensional bone structure in the bone marrow area differed among the 3

biomaterials tested. In the cortical bone area, the percent bone volume was comparatively consistent for 3 implants throughout the observation period. The dissolution of ions from the biomaterials might be an important factor in explaining the differences in bone response after implantation. There have been numerous reports on the dissolution and cellular resorption of HA. Hanawa et al also observed the dissolution of calcium ions from Ca-Ti.[21] Differences in the biomechanical environment attributed to the biomaterial-bone interface conceivably represent another reason. The Ti implants are mechanically attached to the bone; therefore, placement of a Ti implant into a tibia is equal to form the defect region in the tibia. The stress is transmitted to the tibia by the movement of the rabbit, and the biomechanical stimulation promotes bone repair in the region around the Ti implant. Hydroxyapatite binds the bone chemically, and the implant is recognized as bone. The stress is not transmitted to the cancellous region, and the bone formed primarily in the cancellous region may decrease. In contrast, no finding indicating that the cpTi binds to the bone has been obtained to date. Therefore, the region is discontinuous, even if so-called osseointegration occurs around the implant, and bone formation will be continued gradually until the implant is surrounded with adequate bone thickness, including the cancellous bone region.

Bone response to Ca-Ti was similar to that of HA implants. Rapid osteogenesis, as in the MC3T3-E1 cell culture,[3] and rapid calcium complex extraction in pseudo-body fluid have been shown on Ca-Ti. Results of the present study also supported the fact that Ca-Ti has the same bone conductivity as HPS and HA in vivo. The decrease in bone volume around Ca-Ti at 4 weeks was less than that with HA. Bone conductivity of Ca-Ti is induced by calcium-related materials on the Ca-Ti surface, eg, calcium oxide and calcium titanium oxide. For HA, an apatite lattice on the material side is connected to an apatite lattice on the bone side epitaxially. The interface between Ca-Ti and bone may differ from that of HA. The binding strength of Ca-Ti against the bone may be less than HA. Some stress will be transferred to the cancellous region through Ca-Ti.

The aforementioned biomechanical hypothesis may confirm a well-known clinical fact, that HA implants have a high success rate in early periods and a high failure rate in the long term compared with Ti implants.[32] If bone-HA integration around the crestal region of the implant is destroyed because of overstressing and infection, only thin supporting bone remains in the apical region. Therefore, support against occlusal stress will be low, and the supporting bone will be destroyed rapidly. However, the high bone affinity of HA is desirable in situations of low bone density. Ca^{2+}-implanted titanium has the characteristics both of bone conductivity, as in HA, and the more chronologic stability of bone volume in the cancellous region than HA. No adhesive fracture was observed with Ca-Ti, whereas this is often the case with titanium plasma-spray or HA plasma-spray. In addition, when the surface of Ca-Ti is exposed in the mouth, periodontal management may be as easy as for machined-surface implants.

CONCLUSION

The bone response to cpTi, HA, and Ca-Ti in rabbits was examined. Chronologic changes of bone structure around the 3 biomaterials were different. This research was based on interaction between the material and bony tissue without occlusal stress, so that the long-term response to the biomaterials in practical, clinical terms must be examined.

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