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Original Article

Resorption of Apatite-wollastonite Containing Glass-ceramic and β-tricalcium Phosphate *in vivo*

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Apatite-wollastonite containing glass ceramic is considered to be difficult to resorb, but we experienced the disappearance of the porous type of Apatite-wollastonite glass ceramic particles *in vivo*. In this study, the resorption of porous apatite-wollastonite glass-ceramic implanted in the femurs of rabbits was investigated, and the process was compared with β-tricalcium phosphate, a resorbable ceramics. Porous apatite-wollastonite glass-ceramic (70, 80, and 90% porosity) and β-tricalcium phosphate (75% porosity) were implanted in the femurs of Japanese white rabbits. Samples were harvested and examined 0, 4, 8, 12, 24 and 36 weeks after implantation. Quantitative analysis of the radiographic and histologic findings was performed with NIH Image software. Radiographic examination demonstrated that the radiopacity and size of the porous apatite-wollastonite glass-ceramic cylinders decreased gradually after implantation. Histologic examination revealed that the surface area of the apatite-wollastonite glass-ceramic cylinders decreased continuously, and approached 20% of the original area 36 weeks after implantation. However, the resorption rate of porous apatite-wollastonite glass-ceramic was slower than that of β-tricalcium phosphate. Toluidine blue staining showed abundant new bone formation on the surface of the apatite-wollastonite glass-ceramic matrix. Considering its mechanical strength, gradual resorption characteristics, and good osteochonductive activity, porous apatite-wollastonite glass-ceramic appears to be a suitable artificial bone substitutes.

Key words: apatite-wollastonite containing glass-ceramic (A-W GC), resorption, porous, β-tricalcium phosphate (β-TCP)

Bioactive ceramics, have a variety of uses, including use as artificial vertebrae or bone defect supplementation material. These ceramics are classified into 2 types: surface-bioactive ceramics or resorbable ceramics [1]. Apatite-wollastonite containing glass ceramic (A-W GC) is classified as a surface bioactive ceramic it is considered to be stable *in vivo,* and difficult to be resolved when implanted in bone [1–3]. When dense A-W GC (0.7% porosity) is used as a bone substitute, no significant change is observed, even after a long implantation period. However, Fujita *et al.* reported subtotal resorption, within 24 months, of porous A-W GC intramedullary plugs implanted in femurs of dogs [4]. We also observed disappearance of

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the porous A-W GC particles that was implanted in the bone defects after the curettage of benign bone tumors was also observed in our department (unpublished paper). Therefore, A-W GC could be a resorbable bioactive material when used as a porous condition. However, there are few reports documenting the resorption of porous A-W GC that was implanted in bones [4, 5]. To our knowledge, the histologic and radiographic findings of the resorption of porous A-W GC implanted *in vivo* have not been investigated. In the current study, the resorption process of porous A-W GC that was implanted in the femur of rabbit was examined quantitatively and compared with that of β-tricalcium phosphate (β-TCP), a resorbable bioactive ceramics.

Materials and Methods

Implants. Cylinders of porous A-W GC (4.4 mm diameter $\times$ 9 mm length, mean pore size 200 μm) were prepared and provided by Nippon Electric Glass, Co., Ltd. (Ohtsu, Japan). The chemical composition of A-W GC was 4.6% MgO, 44.9% CaO, 34.2% SiO_2, 16.3% P_2O_5, 0.5% CaF_2, and the crystallised glass-ceramic consisted of 28% glass, 38% apatite [$Ca_{10}(PO_4)_6(O, F_2)$], and 34% β-wollastonite ($SiO_2 \cdot CaO$). Three different porosities (70%, 80%, and 90%) of A-W GC were used. Their compressive strengths (mean $\pm$ SD) were 20.1 $\pm$ 6.3 MPa (AW70%), 6.7 $\pm$ 2.8 MPa (AW80%), and 2.4 $\pm$ 0.6 MPa (AW90%), respectively.

Cylinders of porous β-TCP (4.4 mm diameter $\times$ 9 mm length, pore size 100$\sim$400 μm) were prepared and provided by OLYMPUS Co., Ltd. (Tokyo, Japan). The chemical composition of β-TCP was $Ca_3(PO_4)_2$. The porosity was 75%, and compressive strength about 2 MPa.

Operative Technique. Thirty eight, Japanese White Rabbits (3 to 4-month-old, male) weighing from 2.5 to 3.0 kg, were used. The rearing and the investigations were carried out according to the animal experimentation established by our institute.

The rabbits were anaesthetized by the intramuscular injection of ketamine HCl (10 mg/kg body weight, Sankyo Co., Tokyo, Japan) and the intravenous injection of pentobarbital sodium (25 mg/kg body weight, Dainippon Co., Osaka, Japan). To prevent infection, an intramuscular injection of cefazolin sodium (20 mg/kgbody weight, Fujisawa Co., Osaka, Japan) was used.

The operations were performed under standard aseptic conditions. Following a lateral incision along the leg, abductor muscles were divided, and the distal aspect of the femur was exposed. A hole 4.5 mm in diameter and 10 mm in depth was drilled in the frontal direction, leaving the medial cortex intact. After irrigation with saline, a cylinder of A-W GC or β-TCP was pushed into the hole, and the periosteum and skin were closed in layers. The incision wound was sprayed with sterile plastic dressing (Nobecutane, Yoshitomi Co., Osaka, Japan).

Experimental Design. Three rabbits were used in each experiment. The animals were euthanized at 0, 2, 4, 8, 12, 24, and 36 weeks after implantation by an intravenous overdose injection of pentobarbital sodium. Three of the original rabbits died, 1 at 2 weeks after the operation and 2 at 20 weeks after, because of local infection and unknown cause and they were replaced. The distal femurs, except those from the animals euthanized at 2 weeks, were radiographed in the antero-posterior direction with soft X-ray (Softex - CMB, SOFTEX Co., Tokyo, Japan). The radiographs were inputted with a scanner (Epson ES-2200; Seiko Epson Co., Suwa, Japan). We chose the central part of the implants (25 $\times$ 10 pixcels) as the ROI area. In addition, serial radiographic changes were examined using NIH image software (U.S. National Institute of Health, Bethesda, MD USA).

The femurs were then cut into 5 mm sections, sagittal to the long axis, using a high speed, water-cooled circular saw with fine diamond coating (BS-3000, EXAKT, Norderstedt, Germany). The samples were fixed in 70% methanol, dehydrated in serial concentrations of methanol, and embedded in polymethylmetacrylate without decalcification. They were ground to a thickness of 30 μm using a grinding machine (MODEL 900, Grinder/Polisher, South Bay Technology INC., San Clemente, CA, USA). The surface was finished with number #2000 sandpaper and rapping films (MARTO Rapping Film 3.0 μm, MARTO, Tokyo, Japan). The specimens were stained by toluidine blue staining and examined under light microscopy. The areas of A-W GC and β-TCP and those of the newly formed bone, were quantitatively evaluated using the NIH Image software.

TRAP staining. The distal femurs harvested at 8 weeks after implantation were fixed in 70% methanol, dehydrated in serial concentrations of methanol, and embedded in glycolmetacrylate without decalcification.

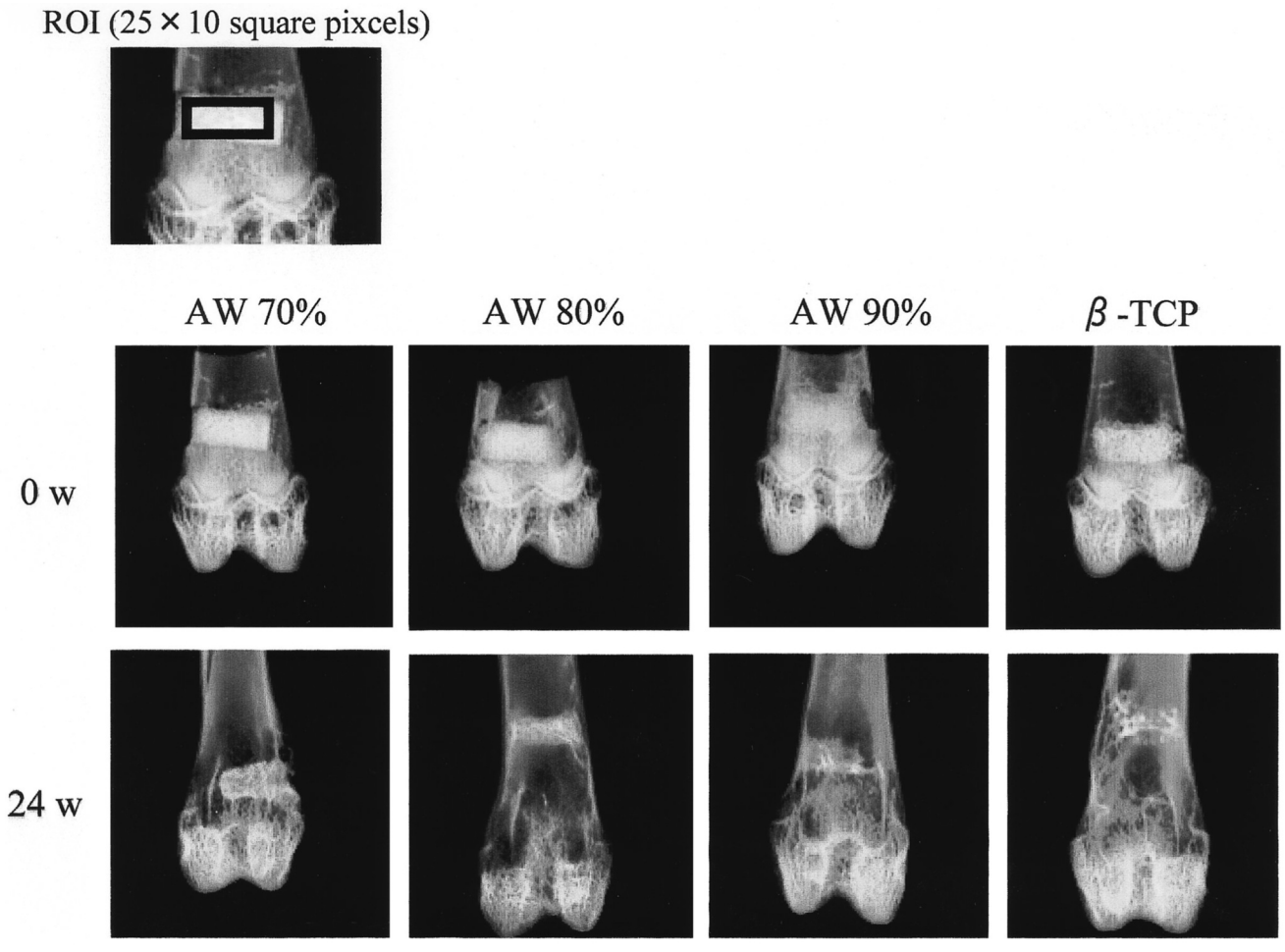

Fig. 1 Radiographic findings of the implants taken immediately and 24 weeks after implantation (40 kv, 10 mA, 10 sec). The radiopacity and the areas of the A-W GC and β-TCP cylinders decreased remarkably at 24 weeks.

They were cut with a microtome (Leica/JUNG, model K, Wetzlar, Germany) in 5 μm thin sections and stained for tartrate-resistant acid phosphatase (TRAP).

Results

Radiological study. The radiographic findings of the implants taken immediately and 24 weeks after implantation are shown in Fig. 1. The radiopacity and the areas of all the porous A-W GC and β-TCP cylinders decreased remarkably at 24 weeks. Quantitative image analyses showed that the radiopacity of all the A-W GC cylinders decreased gradually and approached that of normal cancellous bone with time (Fig. 2). Although the initial values of the radiopacity differed according to the porosity (70% > 80% > 90%), they began to decrease as early as 4 weeks after implantation, and became almost

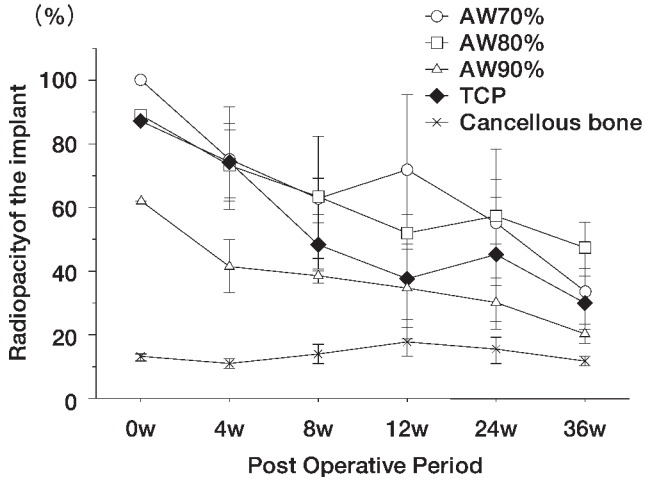

Fig. 2 Chronological change of the radiopacity of the implants. The value of 70% porosity A-W GC immediately after implantation was regarded as 100%.

same values at 36 weeks of implantation. The radiopacity of β-TCP cylinders showed similar changes as those of A-W GC, but the changes were more rapid and marked changes were observed within 12 weeks after implantation (Fig. 2).

Histological study. Histologic examination revealed that the walls of A-W GC pores became thinner with time after implantation. Fig. 3 shows the serial changes of the areas of the implanted A-W GC and β-TCP matrices measured by NIH Image software. The areas of the implants decreased continuously with time, and approached that of 20% of the original ones 36 weeks after implantation. In compared with β-TCP, the resorption rates of porous A-W GC were relatively gradual. The mean resorption rates (% area/week) of the 70%, 80%, 90% porosities A-W GC and 75% porosity β-TCP cylinders were 2.56, 1.49, 0.97, and 3.17, respectively. The resorption rate of β-TCP was about

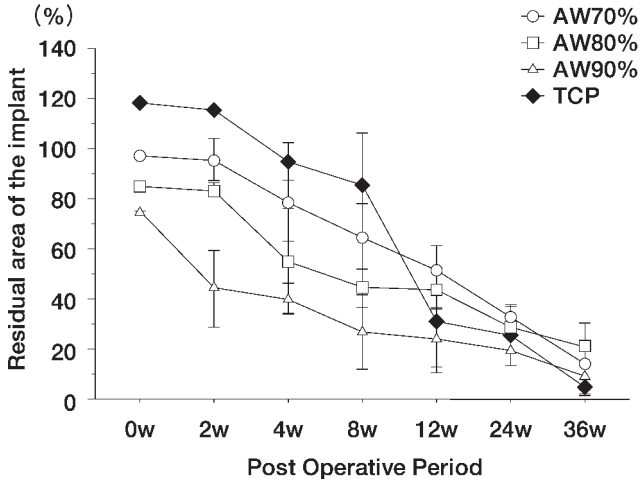

Fig. 3 Chronological change of the residual area of implants. The value of 70% porosity A-W GC immediately after implantation was taken as 100%.

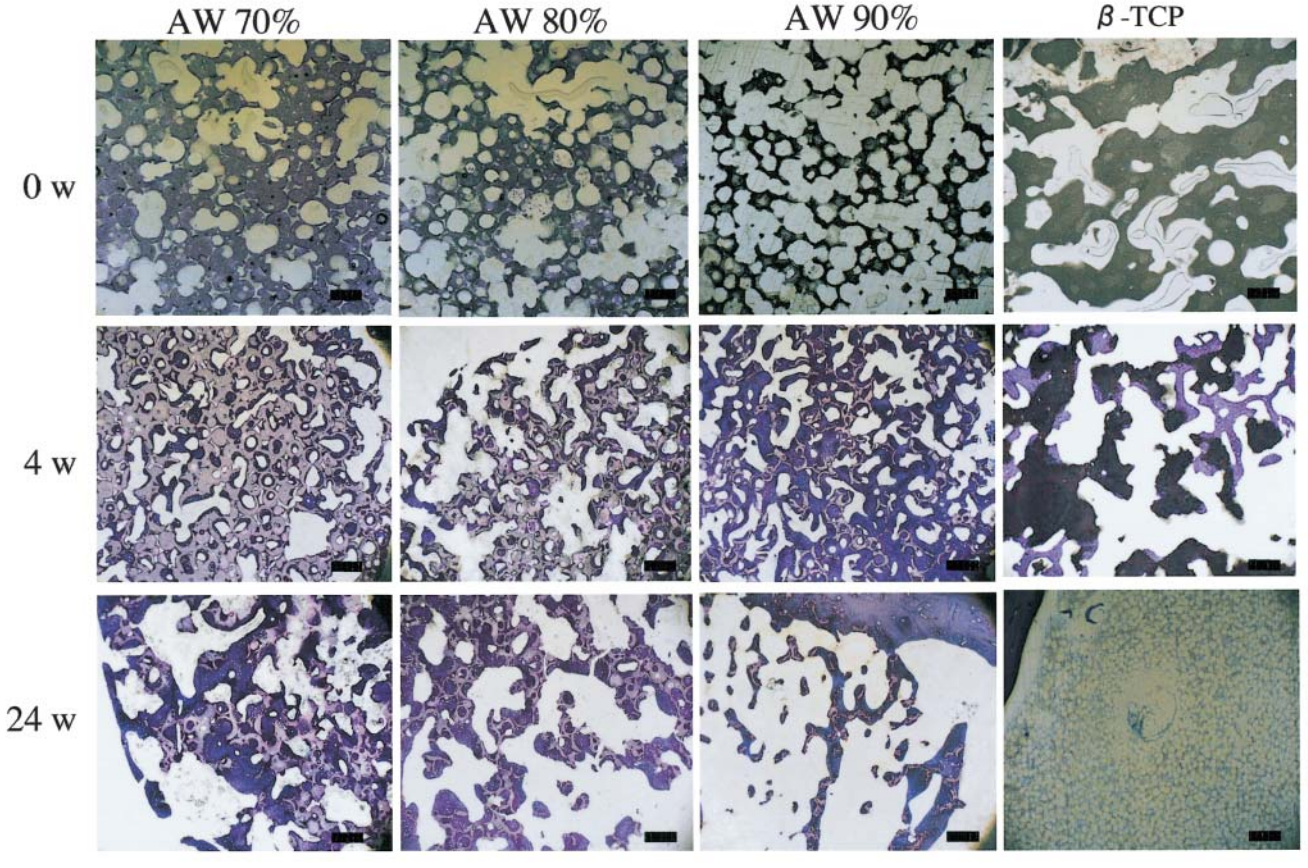

Fig. 4 Histologicalal findings of the implants (Toluidine blue staining, bars indicate 100 μm, × 40) New bone formation was observed in pores of all the implants, especially in the 90% porosity A-W GC, at 4 weeks. The pore size of β-TCP was remarkably enlarged by 4 weeks. At 24 weeks, all implants bonded with newly formed bone, but the implant areas decreased, especially in the 90% porosity A-W GC and in β-TCP.

1.2 times as that of AW70% and 3 times as that of the AW90% cylinders.

Toluidine blue surface staining showed new bone formation, mainly in the peripheral areas of the AW70% and AW80% cylinders, at 2 weeks after implantation. In AW90%, newly formed bone was observed even at the center of the cylinders at 2 weeks. Four weeks after implantation, newly formed bone had appeared in the pores of the center of the AW70% and AW80% cylinders (Fig. 4). Direct contact between the newly formed bone and A-W GC was observed. In AW90%, new bone formation was most evident at 4 weeks after implantation, while, it was most prominent at 24 weeks in the AW70% cylinders.

Because of its rapid resorption, only a faint β-TCP matrix was observed at 24 weeks. The area of the newly formed bone was less abundant in β-TCP in compared with A-W GC during the entire experiment period (Fig. 5).

TRAP staining. Numerous TRAP-positive multinucleate giant cells were observed on surface of the A-W GC and β-TCP matrices at 8 weeks after implantation (Fig. 6).

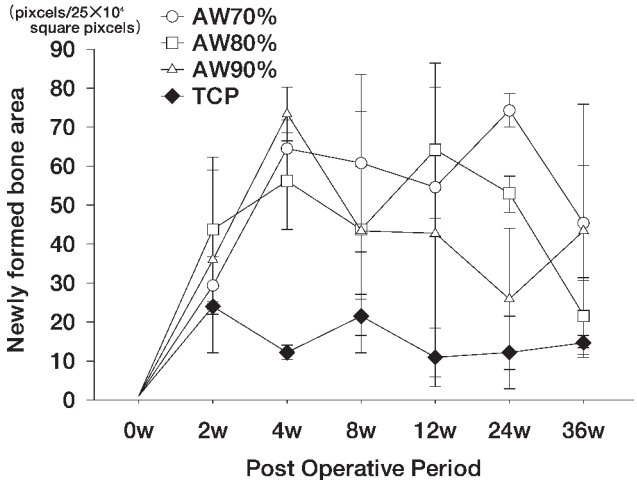

Fig. 5 Chronological change of the areass of newly formed bone. Abundant new bone formation was observed in all the A-W GC cylinders. The quantity of newly formed bone was less abundant in β-TCP than in A-W GC.

Discussion

Bioactive ceramics, defined as ceramics that form a direct bond with living bone tissue, include surface-bioactive ceramics [*e.g.*, Bioglass®, Ceravital®, hydroxyapatite] and resorbable ceramics [*e.g.*, β-TCP, calcite] [3, 6].

Dense A-W GC (0.7% porosity) is classified as a surface-bioactive ceramics and has been thought to be difficult to resolve *in vivo* [1–3]. Conversely, A-W GC granules disappeared when they were implanted in bone defect as a porous material (70% porosity). However, there have been no report examining the process of resorption of porous A-W GC *in vivo* [4, 5]. In the present study, we showed that the A-W GC was resorbed *in vivo* when it was implanted in bone as a porous

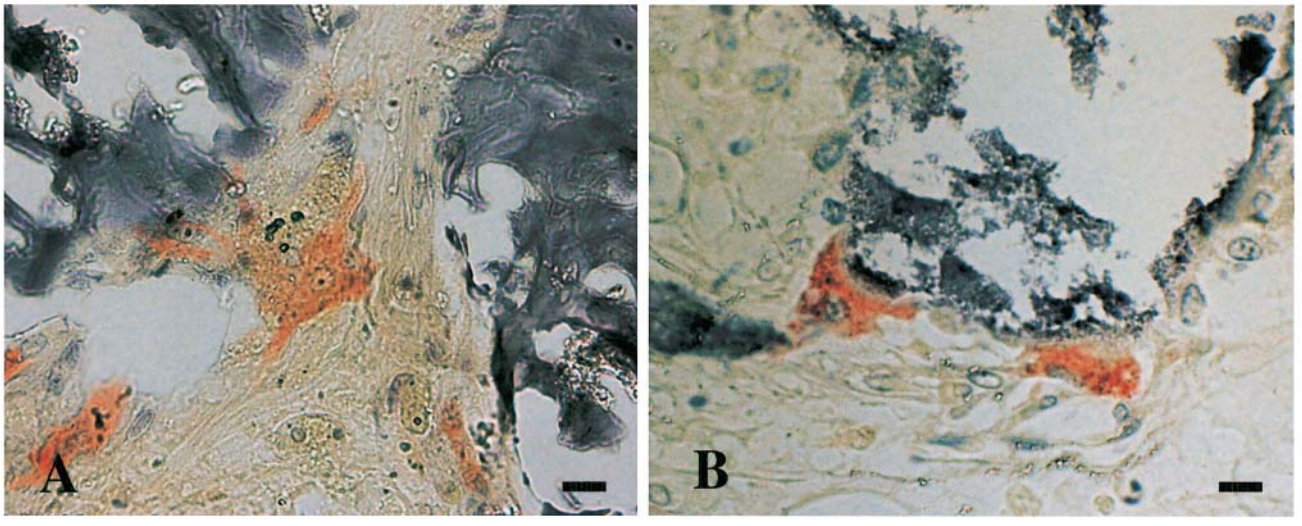

Fig. 6 TRAP staining. TRAP-positive multinucleate cells with lengthening filopodia were observed both in the A-W GC (**A**) (× 400) and β-TCP (**B**) (× 400) ceramics. (Bars indicate 10 μm).

material. This process was examined histologically and radiographically over time.

Under the experiment conditions used, most of the porous A-W GC matrix was resorbed *in vivo* by 36 weeks. In compared with β-TCP, a resorbable bioactive ceramics, however, the resorption rates were gradual. β-TCP (75% porosity) had almost disappeared completely by 24 weeks. Due to its rapid resorption and relatively poor new bone formation, only a faint mineralized matrix remained in the β-TCP cylinders at 24 weeks. In contrast, an abundance of newly formed bone and residual A-W GC matrix were observed at 24 weeks, especially in the A-W GC with 70% porosity.

From the clinical point of view, a desirable artificial bone substitute should have mechanical properties as close as possible to those of the recipient bone, and after implantation, the artificial substitute should, hold its strength for an appropriate period and then be gradually replaced by the recipient skeletal tissue. Of the 4 materials that we examined (A-W GC with 70%, 80%, 90% porosities, and β-TCP with 75% porosity), A-W GC with 70% porosity had the closest mechanical strength as that of normal human cancellous bone [7–9]. Considering its proper mechanical strength, relatively gradual resorption characteristics and good osteochonductive activity, A-W GC with 70% porosity could make a suitable artificial bone substitute.

Two major processes participate in the resorption of bioactive ceramics, a solution process and a cell-mediated process [10]. Partial dissolution of the ceramic in the solution process initiates the accumulation of phagocytosing cells such as macrophages or osteoclasts around the material, and these cells play a central role in the resorption of the material (cell-mediated process).

As for A-W GC, the partial dissolution of the ceramics releases silicon and calcium ions in the surrounding fluid and results in the precipitation of new apatite crystals on the surface of A-W GC [11–14]. In an *in vitro* experiment model, Yamada *et al*. found that actively moving osteoclasts produced many tracklike resorption lacunae on the bonelike apatite layer formed on A-W GC by a simulated body fluid [15]. However, the role of cell-mediated resorption process in the *in vivo* incorporation of A-W GC was still unknown. On the one hand, Neo *et al*. reported that macrophages phagocyting crystals of A-W GC were rarely observed *in vivo* by transmission electron microscopy [12]. On the other hand, Ohsawa *et al*. reported that acid phosphatase positive cells were observed on porous A-W GC *in vivo* [5]. In the present study, many TRAP-positive multinucleate giant cells (osteoclasts) were observed on the surface of A-W GC 8weeks after implantation. These cells were in direct contact with A-W GC, and some contained granules of A-W GC. These findings indicate that the porous A-W GC is resorbed *in vivo* when it is implanted in bone, both by the solution and cell-mediated processes.

In summary, the resorption of porous A-W GC has been demonstrated to take place *in vivo,* and the process has been compared quantitatively with that of β-TCP, a resorbable bioactive ceramics. In comparison with β-TCP, cylinders of porous A-W GC were resorbed more gradually *in vivo*. Histologically, many TRAP-positive multinucleate giant cells (osteoclasts) were observed on the surface of A-W GC. Concurrently, abundant new bone formation was observed on A-W GC. Considering its mechanical strength, relatively gradual resorption characteristics and good osteochonductive activity, A-W GC with 70% porosity could make a suitable artificial bone substitute.

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