In vivo absorption of porous apatite- and wollastonite-containing glass-ceramic

KUNITAKA OHSAWA, MASASHI NEO*, TSUYOSHI OKAMOTO, JIRO TAMURA, TAKASHI NAKAMURA
Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
E-mail: neo@kuhp.kyoto-u.ac.jp

The behavior of porous apatite- and wollastonite-containing glass-ceramic (AW) in the bone marrow cavity was investigated. Cylinders of porous AW (4 mm in diameter and 20 mm long, mean porosity of 70% and mean pore diameter of 200 μm) were implanted into the bone marrow cavity of rabbit femurs, and analyzed by chronological radiograms and by scanning electron microscopy one, three, six, and 12 months later. The pores of porous AW are interconnected and homogenously distributed, and its compressive strength is nearly equal to that of human cancellous bone. Bone formed in the pores at the center of the material by one month and bonded to the material directly. The volume of newly formed bone in the material pores reached a peak at three months, and decreased gradually after six months. The trabecular structures of AW were gradually remodeled by newly formed bone, while AW–bone bonding was maintained during bone remodeling and material absorption. AW was absorbed continuously, and at six and 12 months the residual material corresponded to about 64 and 30% of the starting material, respectively. Porous AW may therefore be useful as an absorbable bone substitute.

1. Introduction

Apatite- and wollastonite-containing glass ceramic (AW) is highly bioactive [1–4] and has been widely used in clinical applications. It is used mainly in bulk form under weight-bearing conditions, for example, as artificial vertebrae or intervertebral spacers, because of its superior mechanical strength [5–7]. The high bioactivity of AW is attributed to the rapid formation of an apatite layer on its surface [3, 8]. This is formed by partial dissolution of the glassy and wollastonite phases of the surface region and subsequent calcium ion release, resulting in apatite precipitation. Despite its surface solubility, dense AW is stable after bone bonding, and therefore refractory to absorption [9, 10].

In 1994, Neo *et al*. [11] demonstrated the remodeling of AW surfaces by newly formed bone and subsequent absorption of AW during long-term implantation. They concluded, however, that the absorbed width of the surface of AW is less than 50 μm per year and that any influence on clinical applications is minimal when used as a bulk material. Recently, however, a porous type of AW has become available and is used clinically, taking advantage of its high bioactivity [12, 13]. Fujita *et al*. [14] reported that porous AW implanted into the bone-marrow cavity of long bones is absorbed gradually. However, there are only a few basic studies on the absorption of porous AW *in vivo*, although it is very important to clarify these characteristics when it is used clinically. In the present study, we investigated the behavior of porous AW implanted into the bone marrow cavity.

2. Materials and methods

Cylinders made of four kinds of materials, 4 mm in diameter and 20 mm long, were initially used in this experiment. The materials were porous AW, commercially available porous hydroxyapatite (HA), dense AW, and specially made AW (porosity 25%, pore size range 1–100 μm). In the latter three materials, however, no detectable absorption was observed. Although the porous HA had the same mean porosity and mean pore size as the porous AW, its 3-D structure was quite different from AW and it was not appropriate as a control. Therefore, only porous AW is focused on in the present study.

2.1. Materials

Porous AW (porosity 70% and mean pore diameter 200 μm) was provided by Nippon Electric Glass Co. Ltd. (Otsu, Japan). The chemical composition of porous AW is 4.6% MgO, 44.7% CaO, 34.0% SiO_2, 16.2% P_2O_5, and 0.5% CaF, and the glass-ceramic consists of 38% apatite

*Author to whom all correspondence should be addressed.

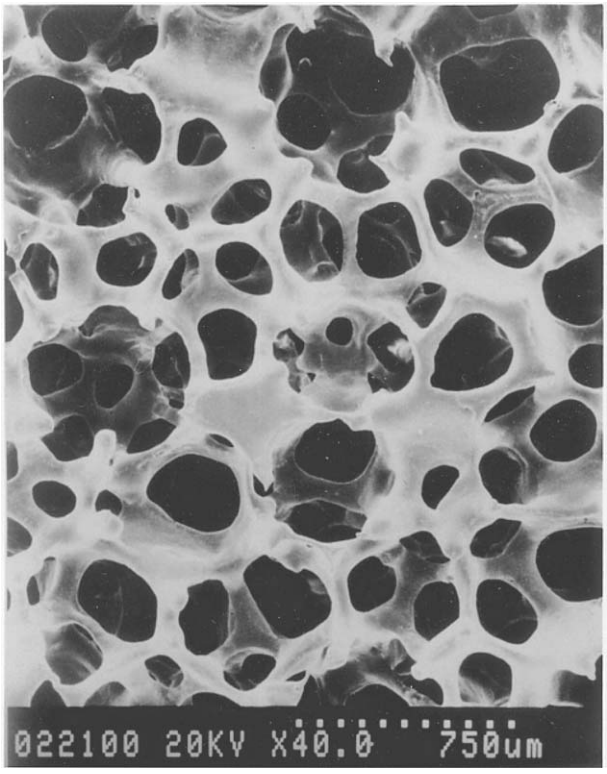

Figure 1 Scanning electron microscopy of the porous AW of 70% porosity.

$[Ca_{10}(PO_4)_6(O, F_2)]$, 34% β-wollastonite ($SiO_2$–CaO), and 28% residual glass, as described previously, and is the same as that of dense AW [15]. The pores of porous AW are interconnected and homogeneously distributed (Fig. 1). Its compressive strength is 16–20 MPa [16, 17].

2.2. Animal experiments

Thirty-two mature male Japanese white rabbits (Japan SLC Inc., Shizuoka, Japan), with an average weight of about 3.0 kg, were used in the original experiment. The rabbits were anesthetized by intravenous injection of pentobarbital at 50 mg/kg body weight, with local administration of 0.5% lidocaine. Surgery was performed under standard aseptic conditions. A 3.0 cm midline skin incision was made on the anterior aspect of the knee, and the joint was exposed with a medial parapatellar approach. A hole, 4 mm in diameter, was drilled retrogradely in the bilateral femoral intercondylar notch. Chosen at random, one of the four kinds of material aforementioned was inserted into the hole, 20 mm from the joint surface, as illustrated in Fig. 2(a). After all surgical procedures, the rabbits were maintained in cages on a regular laboratory diet. After all, porous AW was implanted into the unilateral femur of 16 rabbits.

2.3. Radiological evaluation

Four rabbits containing porous AW received chronological radiological evaluation. Lateral radiograms of the femurs were taken under general anesthesia immediately after implantation and at one, three, six, and 12 months after operation. For the radiograms a Tanka X-ray RT-1005S model (GE Yokokawa Medical System, Co., Ltd., Kanagawa, Japan) was used at 50 kV, 50 mA, for 0.1 s. Fuji medical X-ray film RX-U regular type (Fuji Photo Film Co., Ltd., Kanagawa, Japan) was used.

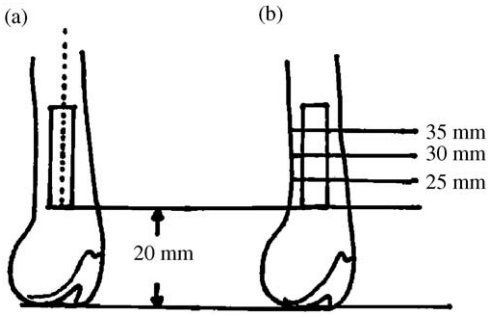

Figure 2 (a) Implantation of the materials. Materials were inserted 20 mm from the joint surface. (b) Each specimen was cut at levels 25, 30, and 35 mm from the joint surface.

2.4. Histological examinations

At each time-point of one, three, six or 12 months, three rabbits with porous AW implants were euthanized with an overdose of pentobarbital and the specimens were then collected.

The specimens were fixed in 10% phosphate-buffered formalin for seven days and dehydrated in serial dilutions of ethanol (70, 80, 90, and 100% v/v) for three days each, after which they were embedded in polyester resin. Horizontal sections were cut using a band-saw (BS-3000CP, EXAKT cutting system, Norderstadt, Germany) perpendicular to the axis of the femur 25, 30, and 35 mm from the knee-joint surface (Fig. 2(b)). These three sections were each polished with diamond paper and sputter-coated with a thin layer of carbon. They were studied using a scanning electron microscope (SEM) (model S-800, Hitachi Ltd., Tokyo, Japan) in backscatter mode. Images were analyzed using an energy-dispersive X-ray microanalyzer (EDX) (EMAX-3000, Horiba Ltd., Kyoto, Japan). The residual material is easily distinguished from newly formed bone in the pores because of its higher density. The areas of residual material and newly formed bone were analyzed on a Macintosh computer, using the public domain NIH Image program (developed at the U.S. National Institute of Health and available from the internet by anonymous FTP from zippy.nih.gov, or on floppy disk from the National Technical Information Service, Springfield, Virginia, part number PB95-500195GEI).

The percentage area of residual material was calculated as follows:

$$\%\ \text{area} = (\text{area of residual material at a specific time} / \text{area of pre-implantation material}) \times 100.$$

The percentage area of new bone was calculated as follows:

$$\%\ \text{area} = (\text{area of bone in the 4-mm diameter hole at a specific time} / \text{area of the 4-mm diameter hole}) \times 100.$$

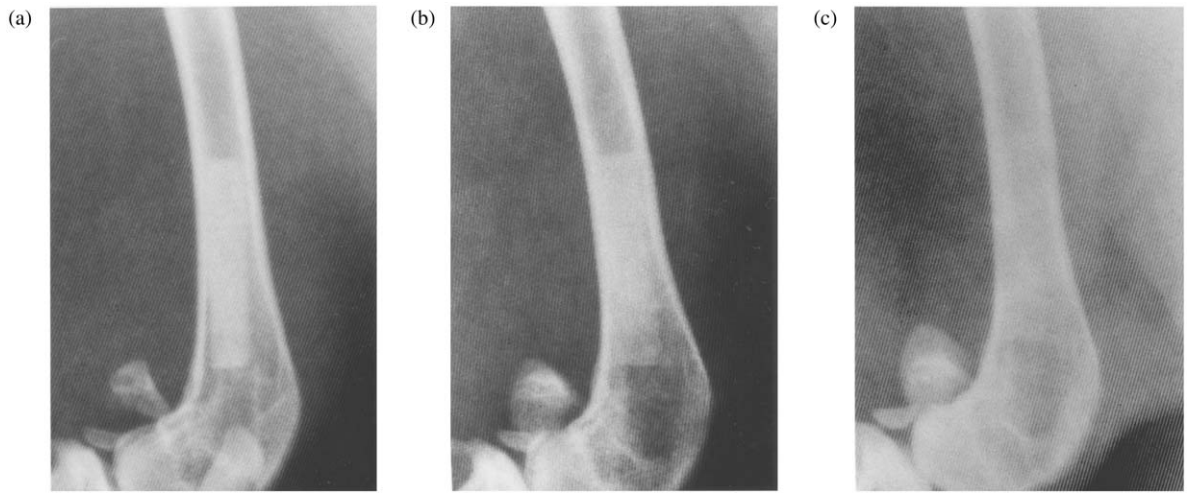

Figure 3 Radiographic changes in AW (a–c). (a) Day of operation; (b) at six months, and (c) 12 months after operation. AW became unclear and disappeared gradually.

3. Results

3.1. Radiographic evaluations

Porous AW appeared to show higher radiopacity at one month after implantation than on the day of the operation, which may mean that new bone had formed in and around the materials. AW became unclear by three months and then gradually disappeared (Fig. 3(a)–(c)).

3.2. SEM evaluations

Bone formation was visible in the pores of the center of the material by one month (Fig. 4(b)). Newly formed bone could be distinguished clearly from the material (Figs. 4 and 5). We could also distinguish AW by its silicon content using EDX. Gradual absorption of the porous AW was observed (Fig. 4). The walls of the AW

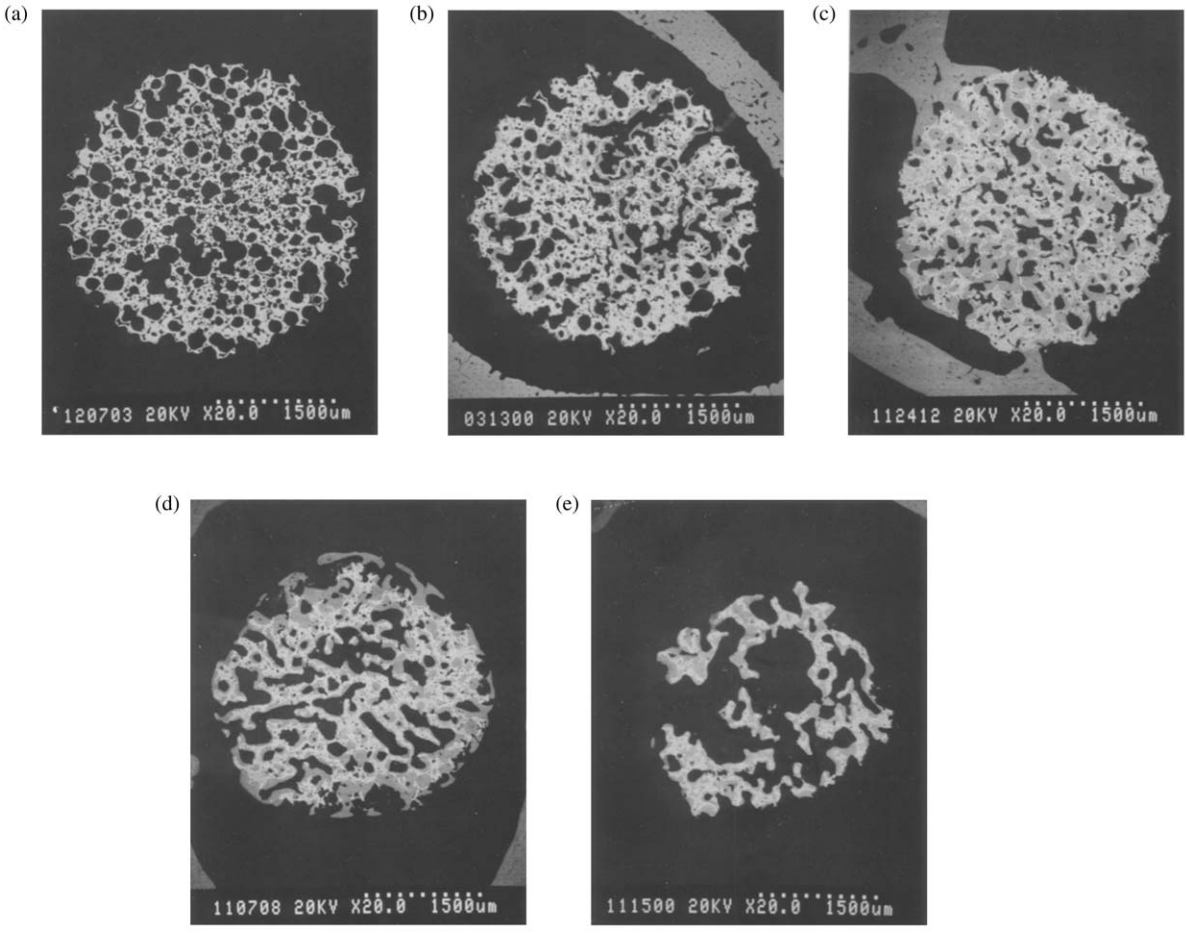

Figure 4 Scanning electron microscopy observations. (a) Before implantation and (b) at one, (c) three, (d) six, and (e) 12 months after operation. Newly formed bone (gray area) is visible in the pores at one month after operation. Gradual absorption of AW (white area) can be seen. The original magnification × 20; the dotted line indicates 1500 μm.

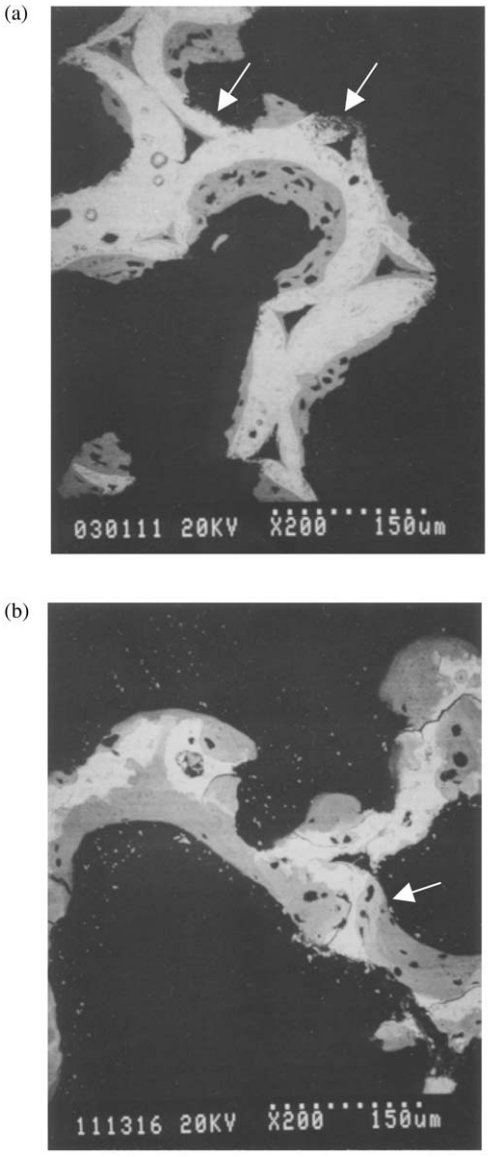

Figure 5 Scanning electron microscopy observations with higher magnification. (a) At one and (b) 12 months after operation. (a) Newly formed bone covered the almost surfaces. Surfaces of the pore walls have become rough in some places (arrows). (b) The trabeculae of AW are fragmented and the surface of AW has become irregular. The arrow indicates the intermediate density zones between AW and bone. The original magnification × 200; the dotted line indicates 150 μm.

pores were 30–150 μm thick before implantation. These became thinner and the reticulated structures were fragmented at three months. Absorption occurred homogeneously but the diameter of the residual AW implant at three months was still about 4 mm. However, after six months the diameter had become smaller as bone remodeling proceeded. The segment also became smaller, but each was always covered with bone. Higher magnification of the AW after one month revealed that the surface of the pore wall became rough especially where bone had not covered the material. (Fig. 5(a)). Furthermore, higher magnification of the AW at six and 12 months showed that the trabecular structures of AW were fragmented, and the surface of AW had become irregular, suggesting remodeling by bone (Fig. 5(b)). At the interface between AW and bone, an intermediate density zone was observed in some places (Fig. 5(b)). This zone had osteocyte lacunae and the same element

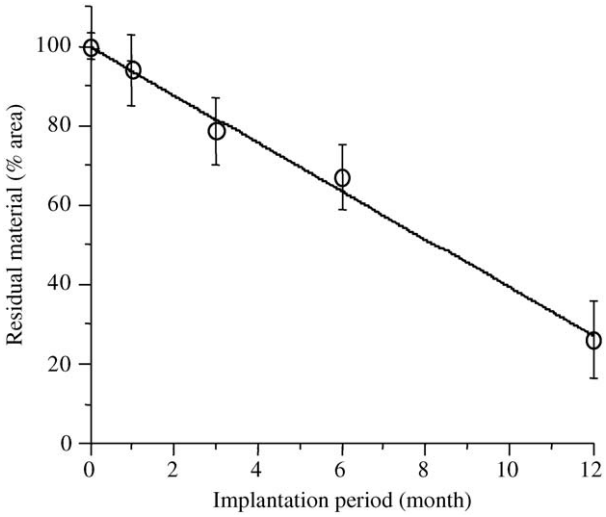

Figure 6 The time course of changes in the residual materials (% area). Results are expressed as means ± standard deviations.

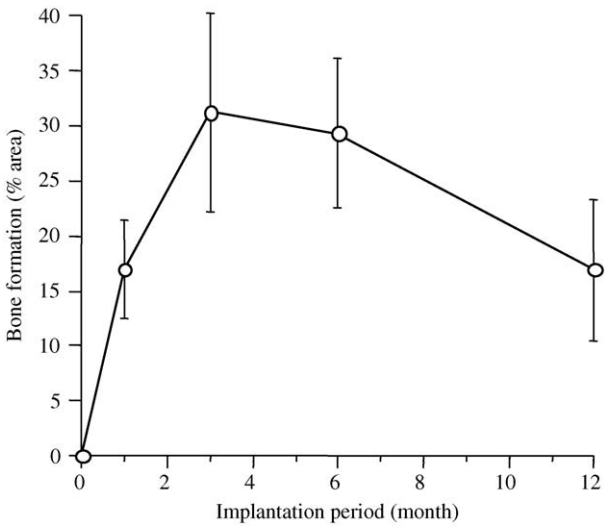

Figure 7 Bone area in the materials. Results are expressed as means ± standard deviations.

levels as bone were demonstrated by EDX. AW–bone bonding was maintained at all points observed.

Fig. 6 shows the serial changes in the residual materials. The residual areas of AW decreased linearly and were approximately 64 and 30% of the initial area after six and 12 months, respectively. Fig. 7 shows the rate of bone formation. Bone formation in the pores had increased by three months, and then decreased gradually after six months.

4. Discussion

These results demonstrated that porous AW with 70% porosity is absorbed fairly quickly. Despite its solubility, AW has been reported to hamper absorption *in vivo* when used as a bulk material. Kitsugi *et al*. [9] demonstrated that the width of the Ca–P-rich layer formed on AW had not changed after 60 days of implantation and speculated that the dissolution of the materials decreases once the bone-like apatite layer and the new bone form on its surface. Yamada *et al*. [10] reported that cultured osteoclasts can absorb the bone-like apatite formed on

the surface of AW, but cannot absorb AW itself under the bone-like apatite. On the other hand, Neo *et al*. [11] demonstrated that the AW surface is remodeled by newly formed bone and that absorption of AW occurs after long-term implantation. They showed that particles of dense AW (100–220 μm in diameter) implanted into rat tibia remained after 96 weeks, indicating that the rate of degradation of their surfaces, which resulted from leaching of calcium and phosphate ions and biological absorption, was less than 50 μm per year. They concluded that any influence on clinical application is minimal when it is used as a bulk material.

However, the behavior of porous AW has not been clarified, even though it has been used clinically [12, 13]. Fujita *et al*. [14] reported that porous AW (porosity: 70%; mean pore diameter: 200 μm) implanted into the intramedullary canal of the canine long bone is absorbed gradually and disappears almost totally after two years. As we demonstrated by SEM, the trabecular structures of AW with 70% porosity are only 30–150 μm thick. Therefore, absorption from the surface of trabecular structures of 50 μm per year may result in division of the trabecular structures and fragmentation of the material in a year. Therefore, the absorption of porous AW demonstrated in the present study may be a natural consequence. Recently, Teramoto *et al*. [17] also demonstrated that porous AW is absorbed continuously *in vivo* using a similar experimental model. They implanted 70, 80, and 90% porous AW into rabbit femurs and examined them radiologically and histologically. Their light microscopic observation of the undecalcified specimens showed that the residual AW of 70% porosity was about 50% of the original after 24 weeks, which is consistent with our result of 64% after six months. They suggested that osteoclasts are associated with the absorption of AW.

Another important point demonstrated in the present study was fast bone formation into pores of AW. AW has higher bioactivity than HA, which means that bone formation on the AW surface is faster [2, 4, 18]. Our results showed that the porous form of AW also has high bioactivity. Further, AW–bone bonding was maintained during bone remodeling and material absorption. The intermediate density zones observed in the present study were also demonstrated by Neo *et al*. [11] during the absorption of AW particles. These zones are considered to be fundamentally bone, because they include osteocyte lacunae and have the same elemental levels as bone as shown by EDX. Neo *et al*. [11] also demonstrated, using transmission electron microscopy, that acicular crystals of bone and macrocrystals of AW intermingle at the AW–bone interface. These findings possibly indicate remodeling of the material surface by bone, suggesting that AW is treated by living tissue as if it were bone. Some crystals of AW were probably incorporated into the bone matrix, resulting in higher density than normal bone.

The compressive strength of 70% porous AW is about 16–20 MPa [16, 17] which is several times higher than that of synthetic porous HA of the same porosity (2–3 MPa) and is nearly equal to that of human cancellous bone. The demonstrated characteristics of porous AW, that is fast bone ingrowth and remodeling of AW-bone as a unit, may mean that porous AW minimizes the reduction of its initial superior mechanical strength until it is completely replaced by bone when it is used under weight bearing conditions. This makes it ideal as an absorbable bone substitute. In the present study, only 70% porous AW was analyzed. Teramoto *et al*. [17] showed the higher the porosity, the higher the absorption rate. Further, the relation between material porosity, absorption rate, and bone formation rate should be clarified. The chronological change of the mechanical strength of the AW–bone complex should also be investigated. Only then can the most appropriate porosity be decided depending on individual clinical situations.

5. Conclusions

We have demonstrated the absorption behavior of AW with 70% porosity. New bone formed into the center of the material of 4 mm in diameter by one month. Once bone formed in the pores, AW–bone bonding was maintained during bone remodeling and material absorption. The material was absorbed linearly and the volume of the residual material was 30% of the original after one year. These characteristics may make porous AW a promising absorbable bone substitute.

Acknowledgments

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