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EFFECT OF VARIOUS PROPERTIES OF HYDROXYAPATITE CERAMICS ON OSTEOCONDUCTION AND STABILITY

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INDEXING WORDS

hydroxyapatite ceramics; osteoconduction; shape

SYNOPSIS

Hydroxyapatite ceramics (HA) are widely used for clinical applications as a bone substitute or dental implant because they have been shown to be biocompatible and exhibit excellent osteoconductivity when grafted into the bone tissue. However, the influence of the physical properties of HA on the osteoconduction and stability remains unclear. We examined here the effect of various properties of HA granules on osteoconduction and stability using 6 types of HA granules. The HA granules were grafted into the rabbit tibia and the initial bone formation and long-term stability of the new bone were studied histologically. The following results were obtained;

1. Osteoconduction of the HA granule was influenced by the shape. Multi-nuclear granules with continuous pores ranging from 30 to 200 μm in diameter showed both excellent osteoconduction and stability.
2. Solubility behavior of HA granules, which was effected by the relative surface area sizes, also seemed to be an important factor for osteoconduction and stability.
3. Stable bi-phase ceramics with HA and βTCP were produced at the sintering temperature of 1200°C when Mg was added. The βTCP and Mg content did not negatively influence the initial osteoconduction or long-term stability.

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INTRODUCTION

Various artificial materials have been used to repair or reconstruct maxillary and mandibular bone defects instead of autogeneous bone grafts over the past two decades. Among these materials, hydroxyapatite (HA) is widely used for clinical application because it exhibits excellent osteoconduction and attaches directly to the bone when grafted into the bone tissue.[1-5] However, the effects of various properties of HA, such as sintering temperature, chemical composition, shape and solubility behavior, on the osteoconduction and stability are controversial.[6-10] In this study, 6 samples of HA granules were produced with various shapes, relative surface area sizes, solubility behaviors and chemical composition, and the effects of these properties on the initial osteoconduction of HA and the long-term stability of the new bone were investigated in detail.

MATERIALS AND METHODS

1) Sample specimen

Six samples of HA granules were produced and named AP1 to AP6. The procedures for making HA granules are shown in Fig. 1. Hydroxyapatite powders were synthesized by a precipitation method from aqueous solutions, and then granulated and sintered at 1200℃. Sintered granules were sieved from 500 to 1000 μm in diameter. The Ca, P and Mg content, and H2O(+) (combined water) of each sample are shown in Table I. The Ca/P molar ratio of these samples ranged from 1.62 to 1.69. X-ray diffraction analysis showed that β-tricalcium phosphate (βTCP) was contained in AP2 and AP3, while AP6 contained a small amount of free CaO.

Scanning electron microscopic (SEM) findings of each sample are shown in Fig. 2. AP1 was a round and compact spherical granule without pores on the surface. AP2 was an irregular shaped granule with continuous pores smaller than 30 μm in diameter and the surface was coarse at the SEM scale. AP3 was a compact, and multi-nuclear granule with continuous pores ranging from 30 to 200 μm in diameter. AP4 was a multi-nuclear granule with a coarse surface and small continuous pores. AP5 was a compact and multi-nuclear granule similar to AP3. AP6 showed many micropores smaller than 10 μm in diameter.

2) Solubility Test

It has been reported that HA itself stimulates and promotes bone formation around the surface by dissolving Ca. Therefore, a solubility test of each sample was carried out to investigate the effects of solubility behavior on osteoconduction and stability. The solubility test procedures are shown in Fig. 3. A weighed amount of each sample was placed into a flask and 50 ml of an isotonic NaCl solution was added. The flask was capped with a silicon rubber cap, then kept at 37℃ in a water bath equipped with a mechanical shaker. At stipulated times, each flask was removed and the sample solution was filtrated with a membrane filter. The Ca and P contents of the filtrates were analyzed with an Inductively Coupled Plasma Emission Spectrometer.

Table I. Chemical composition, relative surface area and mineral phase of HA samples.

sample	Ca (%)	P (%)	Mg (%)	H_2O (%)	Ca/P (molar ratio)	relative surface area (m^2/g)	mineral phase
AP1	39.93	18.65	0.09	<0.01	1.66	0.04	HA
AP2	39.20	19.04	0.42	<0.01	1.62	0.06	HA, βTCP
AP3	39.10	19.05	0.43	<0.01	1.62	0.07	HA, βTCP
AP4	40.13	18.84	0.19	0.14	1.66	0.07	HA
AP5	40.54	18.74	0.16	0.13	1.67	0.07	HA
AP6	40.96	18.74	<0.01	0.38	1.69	0.44	HA, CaO

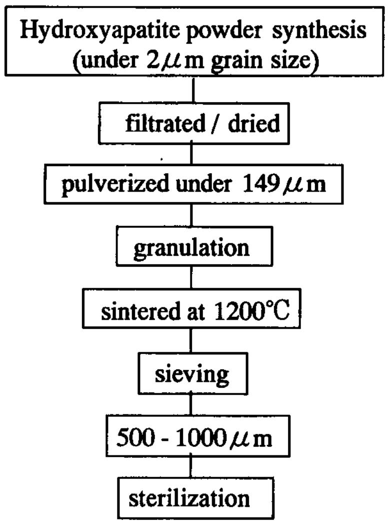

Fig. 1. The procedure to make HA granules.

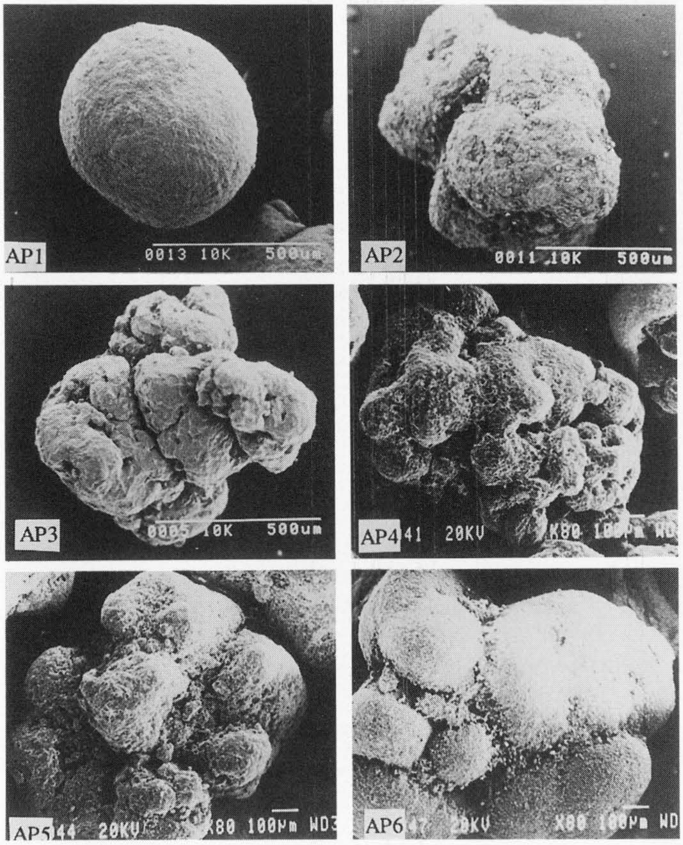

Fig. 2. SEM micrography of HA samples.

3) Implantation test into the rabbit tibia

New Zealand White rabbits, 2 kg in weight, were used for the implantation test. All animal experiments were conducted according to the "Guidelines for Animal Experiments at Kobe University School of Medicine". The implant procedures are shown in Fig. 4. Under general anesthesia induced by pentobarbital sodium, a hole about 1.5 $\times$7 mm in size was made in the rabbit tibia, and granules were grafted into the cavity after the cortex and bone marrow were removed. The rabbit tibia was

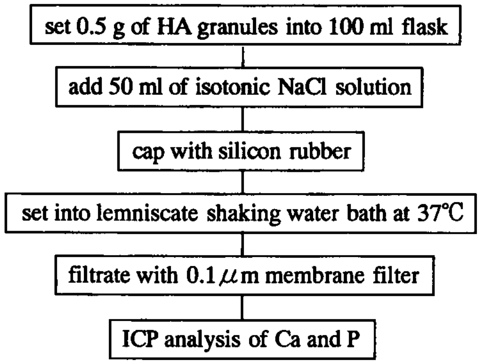

Fig. 3. Schematic flow of solubility test of HA granules.

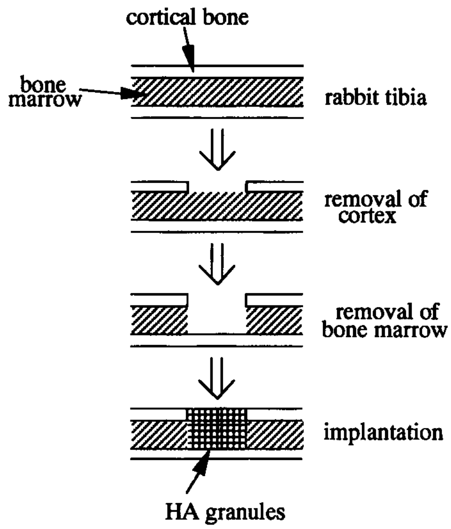

Fig. 4. Scheme showing the implantation procedure of HA granules.

removed and examined 2 weeks and 6 months after implantation to investigate the initial osteoconduction and the stability of the new bone. Four specimens were obtained in each group. The specimens were fixed in 10% formalin solution, decalcified and embedded in paraffin wax. Sections were cut at 4 μm thickness and stained with hematoxylin and eosin. Histologic observation was performed and the percentage of the new bone area was measured by photomicrography of 40 magnifications of the central area of the implant.

RESULTS

1) Solubility behavior

The changes of dissolved Ca and P in an isotonic NaCl solution are shown in Figs. 5 and 6. The amount of dissolved Ca was the lowest in AP1, and increased from AP5, AP3, AP2, AP4 and AP6. There was a close relationship between dissolved Ca and the relative surface area in AP1 to AP5. The rate of dissolution of P closely paralleled the rate for Ca in AP1 to AP5. On the other hand, AP6, which contained free CaO, showed only a small amount of dissolved P in comparison with the large amount of dissolved Ca.

2) Initial osteoconduction

It was reported that bone formation started in the peripheral area initially and extended to the central area of the implantation in this experimental method using rabbit tibia, and that HA granules played an important role in the bone formation.[5)]

In AP1, osteoblasts were attached to the surface of HA granules and the bone formation was active around them 2 weeks after implantation, which showed that AP1 had good osteoconductivity (Fig. 7).

In AP2, bone formation was active in the periphery of the implanted area, but in the central region bone formation was a little delayed and only connective tissue grew into the inner continuous pores (Fig. 8).

AP3 showed the most active bone formation among the 6 samples in the implanted area, and new bone was also formed in the inner continuous pores (Fig. 9).

AP4 and 5 showed almost the same findings as AP2. Active bone formation in the peripheral region, but bone formation in the central part was a little delayed.

AP6 showed the most inferior bone formation, and there was no bone tissue in the inner pores (Fig. 10).

The quantitative analysis of the bone tissue in the central part of the implanted area indicated that AP3, which had multi-nuclear granules with continuous pores ranging from 30 to 200 μm in diameter, demonstrated the best osteoconduction 2 weeks after implantation (Fig. 11).

3) Stability of the HA-bone complex

It was reported that HA granules were connected by new bone 2-4 weeks after implantation in the rabbit tibia and was called the HA-bone complex.[5)]

In AP1, which has a compact spherical shape, a small amount of bone resorption was observed in the central area 6 months after implantation (Fig. 12).

On the other hand, AP3, which had multi-nuclear granules with continuous pores ranging from 30 to 200 μm and showed the best initial osteoconduction, demonstrated little bone resorption in the 6-month specimens and maintained a stable HA-bone complex in the whole implanted area (Fig. 13).

AP2, AP4 and AP5 which exhibited less bone formation in the central area 2 weeks after implantation, showed little bone resorption as observed in AP3 after 6 months.

Bone resorption occurred most aggressively in AP6 among the 6 samples in the central area 6 months after implantation (Fig. 14).

Quantitative analysis of the bone tissue also supported these histologic findings (Fig. 11).

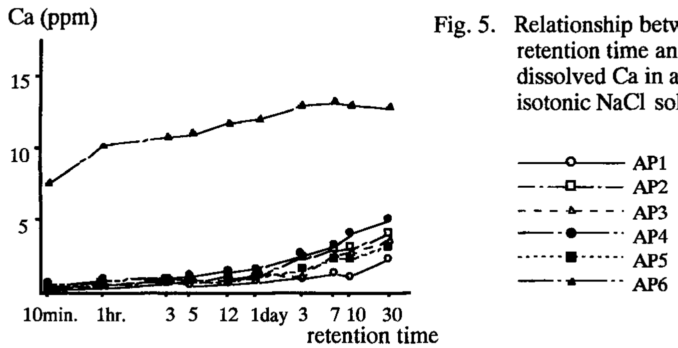

Fig. 5. Relationship between retention time and dissolved Ca in an isotonic NaCl solution.

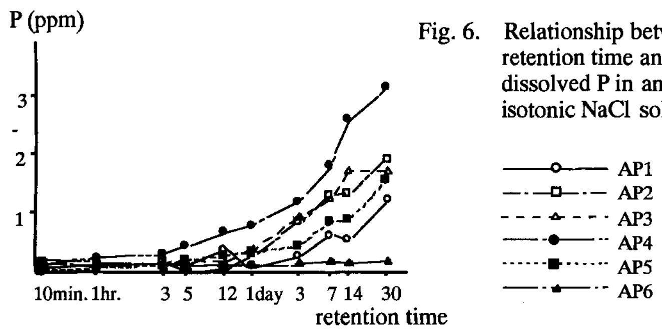

Fig. 6. Relationship between retention time and dissolved P in an isotonic NaCl solution.

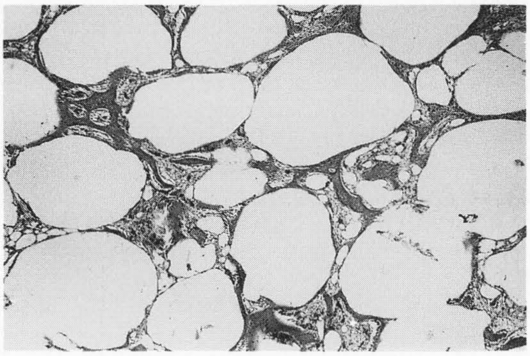

Fig. 7. Histologic features of AP1 2 weeks after implantation. Active bone formation was observed in the central region. Small granules are the fragments of HAP samples which were broken through the implant procedure. HE stain, $\times 40$.

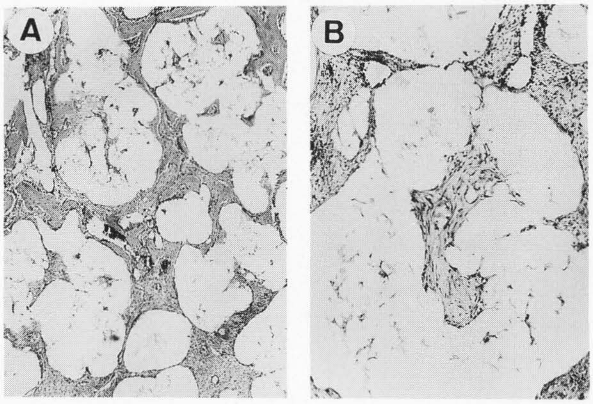

Fig. 8. Histologic features of AP2 2 weeks after implantation. Bone formation in the central area is inferior to that in the peripheral area. There was less bone formation in the inner pores. HE stain, A: $\times 40$., B: $\times 100$.

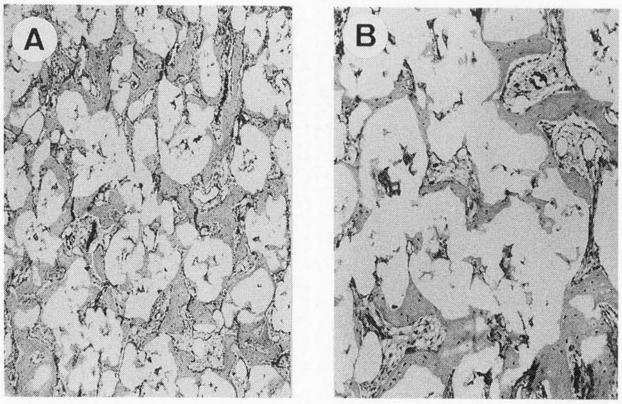

Fig. 9. Histologic features of AP3 2 weeks after implantation. Active bone formation is observed in the inner pores as well as on the surfaces of the granules. HE stain, A: ×40, B: ×100.

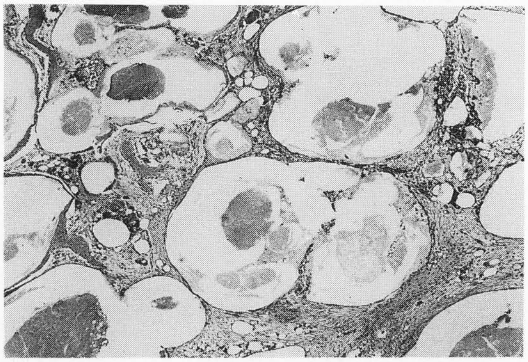

Fig. 10. Histologic features of AP6 2 weeks after implantation. Bone formation was inferior and there was found no bone invasion into the inner pores. HE stain, ×40.

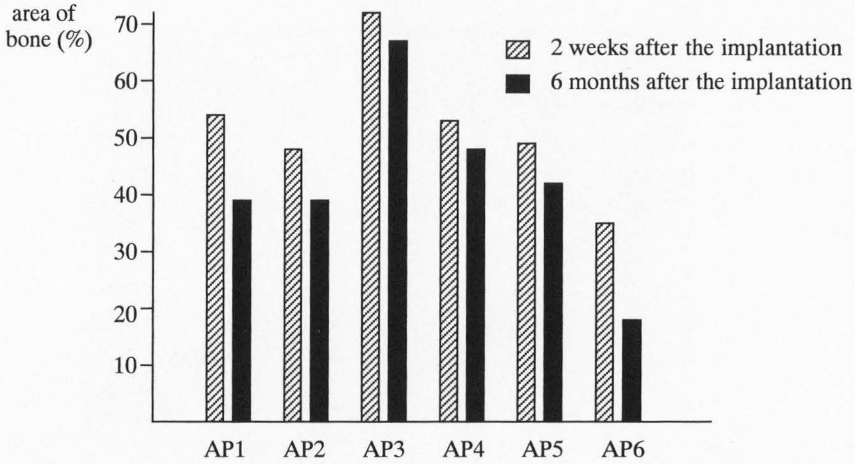

Fig. 11. Quantitative analysis of bone tissue in the central region of the grafted area of AP1 to AP6.

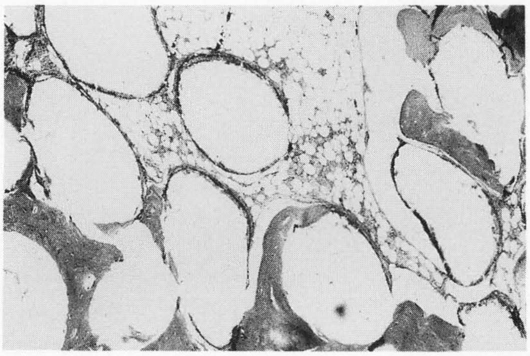

Fig. 12. Histologic features of AP1 6 months after implantation. There is found a little bone resorption in the central region. HE stain, $\times 40$.

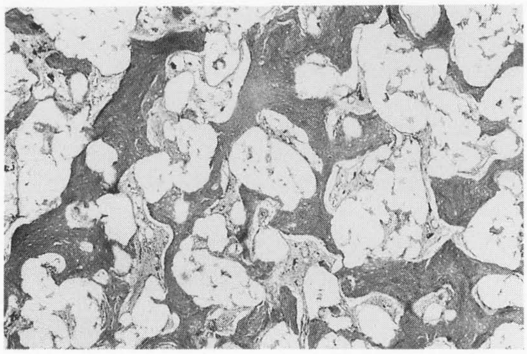

Fig. 13. Histologic features of AP3 6 months after implantation. Little bone resorption and a stable HA-bone complex are observed. HE stain, ×40.

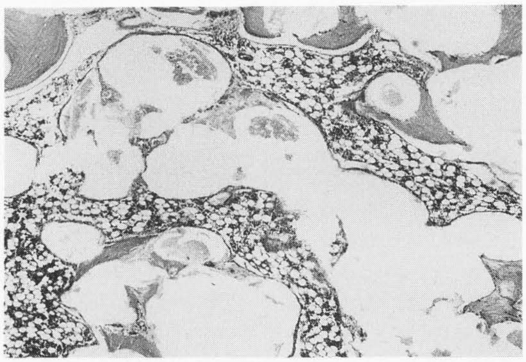

Fig. 14. Histologic features of AP6 6 months after implantation. Bone resorption is seen in the central region. HE stain, ×40.

DISCUSSION

Many investigators have reported that HA is combined directly with the bone tissue.[1-10] However, there were some questions as to the experimental methods in many of these previous studies. HA was implanted into the cavities made in the cortical or cancerous bone of animals in these experiments. It is thought not to be relevant to evaluate the effect of HA on bone formation by these experimental methods because new bone forms later in these cavities regardless of whether HA is implanted or not. Umeda reported that osteoblasts were arranged on the surface of HA granules in the early stage when HA were implanted into the cavity after cortex and bone marrow were removed, like the present implantation procedure, and followed by active bone growth along the surface of HA. These findings indicate that HA granules act as the base or nucleus of the arrangement of osteoblasts and new bone formation, and these properties of HA regarded as "osteoconductivity".

HA is clinically used for the purpose of increasing of bone tissue, such as the alveolar augmentation of atrophied jaws or the reconstruction of mandibular resection, and it is expected that HA conducts new bone to the grafted area. In many cases of alveolar augmentation, loss of height was reported to take place a few years later. Consequently it is also expected that the new bone conducted by HA is stable without any resorption for a long time. This experimental method using the rabbit tibia seems highly suitable for the investigation of these problems. We confirmed in the same experimental method that if bioinert titanium granule, biodegradable βTCP granule or nothing was grafted into the cavity made in the bone marrow of rabbit tibia, active bone formation was observed in the early stage but the new bone was resorbed and the cavity returned to bone marrow as it was before by 3 months after the procedure.[5.11.12] On the other hand, only when bioactive materials such as HA were grafted, new bone remained in the cavity even on 6-months specimens after the implantation. These results show that HA plays an important role in the long-term stability of new bone as well as in the initial osteoconduction. However, there remain several questions as to which types of HA is the most effective for the initial osteoconduction and the long-term stability.

The relationship between pore size and bone formation has been discussed by many researchers. Hulbert et al. [13] reported that the size of pores in which calcified bone tissue formed was larger than 100μm in diameter, while osteoid grew into pores ranging from 40 to 100μm. According to Yamazaki,[4] the minimum size of open pores in which bone grew was 100μm in diameter, but the bone also grew into the pores smaller than 10μm when those pores were connected to the pores larger than 100μm in diameter. In the present experiment, AP2, AP3, AP4 and AP5 showed new bone formation in even pores smaller than 50μm, although ingrowth of bone into the micropores smaller than 10μm in AP6 was not observed. The present experiment showed that bone formation was very active and little bone resorption was seen in AP3. These findings indicate that multi-nuclear granules with continuous pores ranging from 30 to 200 μm in diameter show both good initial osteoconduction and long-term stability.

It was reported that HA itself stimulated and promoted bone formation around the implanted HA by dissolved Ca.[2,14,15] The results of solubility test indicated that Ca and P dissolved in constant proportion in AP1 to AP5, and the amount showed a relationship to the relative surface area of each granule. Iwata [16] examined solubility behavior in isotonic NaCl solution and osteoconduction in the rabbit tibia using 3 categories of HA columns with different relative densities, and described that HA columns with low relative density, that is, large relative surface area, showed high solubility and active bone formation.

The question of which chemical composition is the most effective for bone formation has not been well discussed. The chemical composition of HA influences the solubility behavior of Ca and P. HA with a Ca/P molar ratio higher than 1.67 contains CaO. This type of HA very easily releases Ca in an isotonic NaCl solution, and the pH around granules turns into a highly alkaline condition. Kawai et al. [17] reported that dissolving only the Ca seemed to inhibit the biocompatibility and that a CaO content was of no advantage to bone formation. The present experiment also showed that AP6 containing CaO had fairly good osteoconduction but the new bone surrounding HA granules was easily resorbed, and that therefore it was not useful as a bone substitute.

When synthesized by a precipitation method, HA with a Ca/P molar ratio lower than 1.67 contains TCP, and the mineral phase varies according to the sintering temperature and the content of Mg. [18,19] αTCP is easily dissolved in an isotonic NaCl solution, and even more rapidly in the presence of serum albumin,[12] so we think that αTCP is not useful for bone substitute.

βTCP has been studied in connection with biodegradable ceramics by many investigators. It was reported that βTCP showed good osteoconduction, and that after bone tissue was formed, the βTCP itself disappeared. On the other hand, Shimada et al. [11] described that βTCP showed good osteoconduction but the resorption of the new bone surrounding the βTCP granules occurred even sooner than the resorption of this material. Kawai [12,20] reported that stable bi-phase ceramics with HA and βTCP could be produced at a high sintering temperature (1200℃) when Mg was added. AP2 and AP3 were produced in these methods and were stable bi-phase ceramics of HA and βTCP. Solution test showed that the amounts of Ca and P of these bi-phase ceramics were almost equal to those of single phase HA, although βTCP has reported to be more easily dissolved than HA. The reason why AP2 and AP3 were not dissolved more rapidly was thought to be the fact that the presence of βTCP increased the compactness and stability of the granules. Although the exact effects of the βTCP content on the usefulness of HA granule is not clear in the current study, AP3 containing βTCP showed the most excellent initial osteoconduction and long-term stability. Further analysis is needed to clarify these problems.

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REFERENCES

1. Peelen, J.G.J., Rejda, B.V., Vermcjden, J.P.W., and de Groot, K.: Science of Ceramics.1977. 11. 226/236. Sintered tricalcium-phosphate as bioceramics.
2. Ogiso, M.: The J Jpn Oral Pathol.1978. 45. 170/221. Histological changes in mandibular tissue by implantation of apatite ceramics.
3. Denissen, H.W., de Groot, K., Makkes, P.C., van den Hooff, A., and Klopper, P.J.: J Biomed Mater Res.1980. 14. 716/721. Tissue response to dence apatite implants in rat.
4. Yamazaki, Y.: The J Jpn Oral Pathol.1984. 51. 184/218. Experimental study on porous apatite as artificial bone -Experiment on implantation in the mandible.
5. Umeda, M.: Med J Kobe Univ.1987. 48. 45/62. Histological and biochemical study on the osteoconductivity of hydroxyapatite ceramics.
6. Le Gerous, R.Z., Parsons, J.R., Daculsi, G., Driessens, F., Lee, D., and Liu, S.T., et al.: Ann NY Acad Sci.1988. 523. 268/271. Significance of the porosity and physical chemistry of calcium phosphate ceramics: biodegration-bioresorption.
7. Bauer, G. and Hohenberger, G.: Ceram Forum Int.1989. 66. 23/27. Causes of behavioral variation of bioactive calcium phosphate ceramics in living organisms.
8. Teranobu, O., Naito, I., Takahashi, N., Fujiwara, M., Iwata, K., and Umeda, M., et al.: In: Oral Implantology and Biomaterials (Elsevier, Amsterdam).1989. 233/238. The influence of sintering condition and surface shape of hydroxyapatite ceramics for the osteoconductivity.
9. Kawai, T., Imanishi, N., Teranobu, O., Umeda, M., Iwata, K., and Takahashi, N., et al.: Proceedings of the 33rd Japan Congress on Materials Research.1990. 147/152. Effect of solubility behavior and shape of hydroxyapatite granules on osteoconduction.
10. Umeda, M., Iwata, K., Eida, K., Take, Y., Naito, I., and Takahashi, N., et al.: J Jpn Stomatol Soc.1992. 41. 256/267. Osteoconductivity of HAP and stability of HAP-bone complex -A comparative study among newly developed HAP granule (Kobeceram) and others.
11. Shimada, K., Chin, M., Take, Y., Naito, I., Takahashi, N., and Iwata, K., et al.: J Jpn Soc Oral Implant.1989. 2. 236/243. Histological study on the osteoconductivity and stability of HAP and βTCP: Long-term observation of experimental implantation into the rabbit tibia.
12. Kawai, T.: Dissertation for the degree of doctor of philosophy in the Tohoku University.1994. Study on the development and application as living material of bi-phase hydroxyapatite ceramics.

13. Hulbert, S.F., Morrison, S.T., and Klawitter, J.J.: J Biomed Mater Res.1972. 6. 347/374. Tissue reaction to three ceramics of porous and non-porous structures.
14. Kurashina, K., Kotani, A., Tanaka, H., Toda, Y., and Ono, M.: Shinshu Med J. 1982. 30. 161/173. Studies on the clinical use of ceramic materials. 1: Bone reaction to dense calcium hydroxyapatite ceramics.
15. Nagai, N., Takeshita, N., Shirasuga, H., and Akiba, M.: Jpn J Oral Maxillofac Surg.1985. 31. 1352/1361. Histopathological studies of implantation of hydroxyapatite in the rat jaw bone.
16. Iwata, K.: J Jpn Stomatol Soc.1990. 39. 1039/1065. Effect of chemical composition, surface shape and solubility behavior of hydroxyapatite (HAP) on the osteoconductivity and biocompatibility -Evaluation *in vivo* and *in vitro* study.
17. Kawai, T., Matsushita, T., Teranobu, O., and Shimada K.: J Jpn Soc Dent Mat Dev.1993. 12. 74/83. Surface reaction of bi-phase ceramics sintered from hydroxyapatite powders of different Ca/P in various media.
18. Dikens, B., Schroeder, L.W., and Brown, W.E.: J Solid State Chem.1974. 10. 232/248. Crystallographic studies of the role of Mg as a stabilizing of impurity in β-$Ca_3(PO_4)_2$, I. The crystal structure of pure β-$Ca_3(PO_4)_2$.
19. de Groot, K., Klein, C.P.A.T., Wolk, J.G.C., and de Blieck, H.: In: CRC handbook of bioactive ceramics II (CRC Press).1990. 3/16. Chemistry of calcium phosphate bioceramics.
20. Kawai, T., Takahashi, N., Teranobu, O., and Shimada, K.: JJSMgR.1997. 16. 17/25. Effect of magnesium addition to hydroxyapatite ceramics for new bone tissue formation.