

Preparation of Hydroxyapatite Material Using Apatite Hydrogel

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Artificial hydroxyapatite (HAp) ceramics are widely used as medical implant, and generally synthesized at high temperature. A high temperature synthesis brings low specific surface area and poor reactivity. Biological apatite is poorly crystalline apatite with high surface reactivity. In this present study, poorly crystalline apatite materials were prepared by caking of the apatite hydrogel. With maturation period, the particle size of apatite hydrogel decreased in air, but increased in N₂. IR spectroscopy indicated that the precipitate was carbonate apatite, and the FT-IR absorbance ratio (CO₃-band/PO₄-band) of the apatite hydrogel prepared in air increased, but those in N₂ decreased. The shrinkage behavior of specimen may involve 2 steps. It does depend on humidity, and seems to be independent of the particle size of apatite hydrogel. The gel-like materials, precipitated from the solution of Na₂HPO₄·12H₂O and CaCl₂·2H₂O solution, containing various contents of polystyrene were dried in air at r.t., and treated with acetone to produce porous materials. The shrinkage of specimen depends on the volume ratio of additives. The apatite materials having macro / micro-pore were obtained and stable in aqueous solution.

Key words: apatite, hydrogel, caking, nanometric crystals, porous materials

1. INTRODUCTION

Artificial hydroxyapatite (HAp) ceramics have been developed, and widely employed as a biomedical implant, primarily because of their bio-compatibility, bio-activity, and osteo-conduction characteristics with respect to host tissue [1,2]. Many implant devices have been developed to have their pore structure specially designed to improve the anchorage of an implant in bone [3-6]. Bone in-growth is thought to require large pores of over 200 μm in diameter and is enhanced by interconnecting micro-pores which enable extracellular fluid to diffuse through the whole implant mass.

Various methods to impart porosity to a ceramic body are known, mainly based on admixing a foreign combustible organic material that burned away during firing. The gel-casting method and the coating of polymer foams with ceramic slurry is also the most widespread processing approach to produce open pore structure. These studies on HAp dense or porous material preparation were also reported, and many steps and the heat-treatment are required to produce HAp materials. A high temperature brings grain growth of ceramic, low specific surface area, and poor reactivity. Another technique of solvent casting and particulate leaching is commonly used to fabricate porous materials. The technique involves dissolving a polymer in a solvent and casting the solution in a mold with a porogen - small crystalline

molecules. The solvent is evaporated and the porogen is leached out [7,8].

Biological apatite is poorly crystalline apatite with high surface reactivity due to their nanometric crystal size and the hydrated layer rich in active environments of mineral ions on the crystal surface. The poorly crystalline apatite can be obtained by precipitation from saturated solution of calcium and phosphate [9]. The purpose of this study is to develop low temperature synthesis of dense or porous apatite materials, through the technique using the apatite precipitates, its caking, and simultaneous forming micro-size polymer.

2. EXPERIMENTAL

Di-sodium hydrogen phosphate dodeca-hydrate (Na₂HPO₄·12H₂O) was dissolved in pure water, and calcium chloride di-hydrate (CaCl₂·2H₂O) in pure water. The latter solution was put into the former solution, and pH was adjusted to 7.40 using sodium hydroxide solution. The mixed solution was stirred at 500 rpm at room temperature in air or under N₂ atmosphere. The stirring period (maturation period) was 1-60 days. The precipitate was filtered off and washed with distilled water. The obtained gel-like material was dried in air.

The additive used to produce porous material was polystyrene. A certain volume of polystyrene beads was dissolved in 150 ml of dichloromethane, and dodecyl sulfate sodium was

dissolved in distilled water. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in distilled water was put into the mixed solution, and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ dissolved in distilled water was added 1 day after, and the mixed solution was stirred at 500 rpm for several days at ambient temperature. The mixture was filtered off, washed, and dried in the same manner described above. The drying specimen was put into acetone for 1 day to remove additives.

Average particle diameter of the synthesized powder was calculated by a centrifugal sedimentation method with a 0.2% solution of sodium pyrophosphate as a dispersion medium and using SA-CP3 of Shimadzu Co. The specific surface area was measured by the BET method. The rheological property of the slurry was characterized using a digital viscometer (DV-II+ of Brook-field). Thermal analysis of the powder was performed by means of a TG-DTA apparatus (TG8120 of Rigaku Co.). The crystal phase of the synthesized powder and that of the sintered body were each examined by an X-ray powder diffraction method using a X-ray diffractometer at 40KV and 20mA Cu target (MAC Science MXP³). The densities of HAp porous bodies were obtained by the water immersion method based on Archimedeian principle. Pore size distribution was measured using a mercury intrusion porosimetry (AutoPore IV9500 of Shimadzu Co.). Scanning electron microscopy (SEM) and EDX analysis of the powders and sinters were performed using a Hitachi S-3000 SEM, and Horiba EMAX-2200 X-ray analyzer. The bending strength of both the porous and dense HAp sinters was measured by a three-point bending method in which the supporting part were set 40 mm apart from one another and a crosshead speed was 0.5 mm/min. All micro-FTIR spectra were recorded using samples encased in a transparent KBr matrix on a Jasco Micro-FTIR Jansen Fourier transform infrared.

3. RESULT AND DISCUSSION

3.1 Apatite hydrogel

The pH value of the mixed solution was kept at around 7, but slowly changing, as the excess of phosphate and carbonate ions may act as a pH buffer. The particle size of apatite hydrogel is shown in Table 1. With an increase of maturation period, the particle size of the gel decreased in air from 2-3 μm to 0.4 μm for 15 days of maturation period, but increased in N_2 from 1 μm to 4-5 μm for 15 days. The FTIR spectroscopy indicated that the precipitate was carbonate apatite. The excess carbonate ions are coming from air into solution, and carbonate ions in solution may be replaced in phosphate site of apatite. The absorbance ratio of carbonate / phosphate of apatite hydrogel prepared under N_2 was low, as carbonate ions in solution was limited. The ICP derived Ca/P ratios of apatite hydrogel formed in air increased as a function of maturation period. The CO_2 content of apatite hydrogel prepared in air was higher than

that in N_2 atmosphere as shown in Figure 1. Those were analyzed by using CO_2 analyzer. ICP derived Ca/P ratios of apatite hydrogel formed in air increased as a function of maturation period. But the absorbance ratio of carbonate / phosphate of apatite hydrogel prepared under air was slightly up with an increase of maturation period, as carbonate ions in solution was limited.

Table 1 :Particle size (μm) of apatite hydrogel prepared in air or under N_2 atmosphere as a function of maturation period

maturation period (days)	6	9	14
atmosphere air	1.25	0.75	0.37
N_2	1.09	2.74	4.24

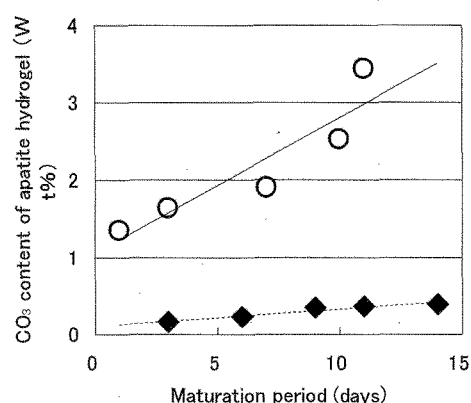


Fig.1 : CO_2 content of apatite hydrogel formed in air (○) and or under N_2 atmosphere (◆) as a function of maturation period.

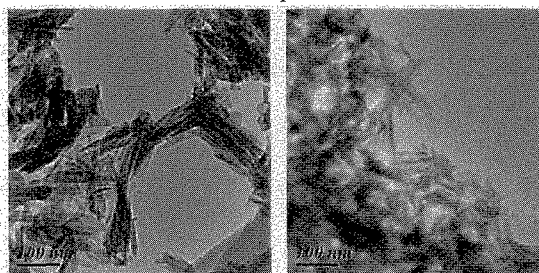


Fig.2 : TEM images of apatite hydrogel, obtained by maturation in air for 6 day (left hand), and in N_2 for 6 days (right hand).

TEM images of the crystals in apatite hydrogel are shown in Fig.2. The crystals of apatite hydrogel formed for 1 day are angular and irregular in shape. Those formed by maturation both in air and in N_2 for several days showed rod-like shape, and seemed to grow somewhat with maturation period, but almost be the same in size, independent of maturation period and atmospheric condition. The X-ray diffraction data of the precipitates showed an apatitic pattern, and the broadness of peaks was observed, which is analogous with bone minerals. The broadening of

002 and 310 lines allowed a determination of crystal dimensions. The crystallite size of the obtained gels was almost constant, independent of maturation period. So the particle size of apatite hydrogel should depend on the dispersibility of apatite crystallites, and the aggregate of apatite crystallites may be strongly affected by the carbonate ions in apatite hydrogel.

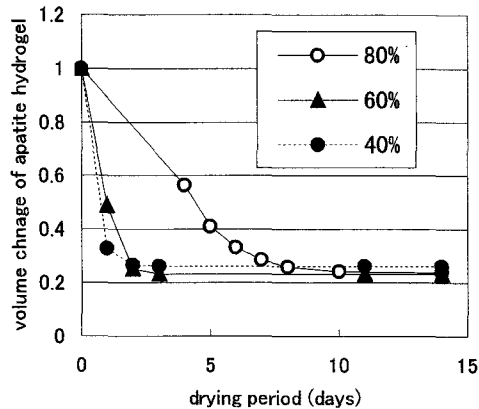


Fig. 3 : The volume change of apatite hydrogel, prepared in air, dried at 40 % (●), 60 % (▲) and 80 % (○) of relative humidity as a function of drying period.

3.2 Caking behavior

The filtered apatite hydrogel was put in the plastic container of 33 mm diameter and 5mm in depth. After 1 day, the specimen was taken outside plastic container, and placed on the paper. The volume changes of the specimens are shown in Fig.3. The volume of specimens decreased exponentially, and the shrinkage of specimens seems to stabilize. At 80% of relative humidity, the volume of the specimen dropped from 2 to 8 days of drying period and the shrinkage seems to stabilize after 10 days of drying period. The specimen set firm, and was difficult to be broken by hand. The shrinkage seems to be relatively slow for the apatite hydrogel obtained for short maturation period, so the apatite hydrogel with large particle size has slow shrinkage rate. The shrinkage behavior of specimen may involve 2 step, as re-aggregation process should be included in drying process. So at high relative humidity, the specimens with different particle size show different shrinkage behavior. At 60% of relative humidity, the volume of the specimens dropped within 2 days, and it seemed to stabilize in 3 days of drying period. The shrinkage is so fast that the behavior seems to be similar, independent of the maturation period. At 40 % of relative humidity, the volume change of the specimens show the same manner as at 60 % of relative humidity. It is clear that the shrinkage behavior strongly depends on the relative humidity, and the particle size of apatite hydrogel affects the shrinkage behavior at high relative

humidity.

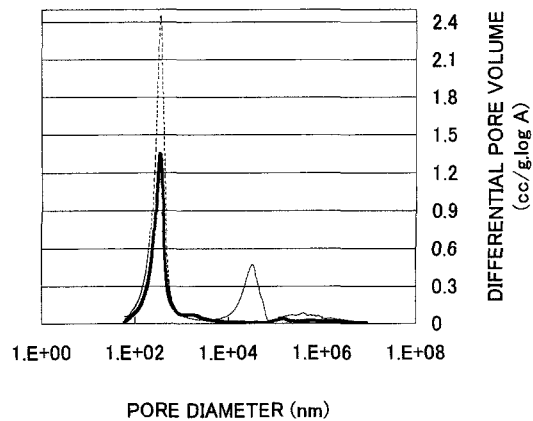


Fig.4 : Pore size distribution of the specimen prepared with 50 vol% of polystyrene (dashed line), and without polystyrene at 70 % of relative humidity.

3.3 Porous Apatite Materials

HAp materials were prepared with 0.1, 0.5, 0.7 as a volume ratio of polystyrene and treated with acetone. The pores of around 30 μm in diameter were observed, which correspond to the size of polystyrene beads in gel. The pore size distribution of specimen prepared with 50 vol% of polystyrene and without polystyrene after treatment with acetone are shown in Fig.4. The large pores of over 500 μm in diameter are there in specimen prepared with over 0.5 of additives. The micropores of around several μm in diameter were also measured using a porosimeter. The co-existence of macro / micro-pore in specimen is believed to be effective in bone in-growth. The specimen dried in air for 2 weeks set firm and are difficult to break, which may be due to hydration effect. The bending strength of specimen decreased with an increase of additive content. With an increase of drying period, the volume of specimen decreased. The volume of the specimens dropped from 2 to 7 days of drying period and the shrinkage seems to have stabilized after 7 days of drying period. The shrinkage of specimen depends on the volume ratio of additives. The specimens after 2 or 3 days' drying procedure tend to break after acetone treatment, while the specimens prepared after 7 day's drying were set firm and not easy to be broken.

From the results of FT-IR spectroscopy measurement of the specimen prepared with polystyrene upon immersion in acetone for 1 day, it is obvious that additives still remain in the specimen after acetone treatment. For the specimen prepared with polystyrene, these of 0.70 as a volume ratio of polystyrene, the peaks at 1492, 1451, 750 cm^{-1} were observed in addition to the peaks of the precipitates without the additives. These peaks were observed in specimen of 0.5. Considering the results, additives may be removed from the specimen at around 0.4 volume

ratio. The X-ray diffraction data agreed with these results.

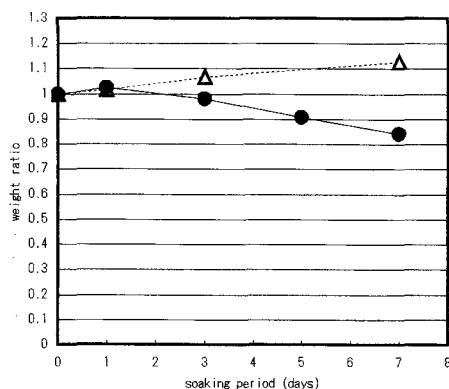


Fig. 5 : Weight change of specimen prepared with 40-50 vol% polystyrene, dried for 2 weeks, treated with acetone, and soaked in pure water (●) and SBF solution (Δ) for 1 week at ambient temperature.

3.4 Stability in aqueous solution

The soaking of specimens in pure water at room temperature was conducted in order to evaluate the stability and released ions of the dried specimen in aqueous solution. Specimens of about 15 mm in diameter and 2 mm in thickness were used. With an increase in soaking time, the weight of specimen soaked in pure water was gradually decreased, which may be due to dissolution into pure water, as shown in Fig. 5. The solubility of these specimens was very high, compared to the HAP sinters [8]. The solubility of HAP depends on the specific surface area and CO₂ content [9]. These HAP materials are not sinters with high surface area, and 2 wt% CO₂ content. The shape of specimens, although seems to be unchanged after soaking in pure water for 2 weeks, and be stable in aqueous solution. The weight of the specimen soaked in SBF solution increased with increasing in soaking period. It may be due to the formation of new apatite layer and on/in the apatite material. The dried specimens slowly dissolve in aqueous solution, but are stable for a certain period. So the adsorptive materials included in this apatite cake may be released into solution for a certain period.

4. CONCLUSION

Apatite hydrogel was prepared from a mixture of di-sodium hydrogen phosphate dodeca-hydrate (Na₂HPO₄ · 12H₂O) and calcium chloride di-hydrate (CaCl₂ · 2H₂O), and its caking behavior, and preparation of porous apatite materials having macro-/micropore was studied. With maturation period, the particle size of apatite hydrogel decreased in air, but increased in N₂. The FT-IR absorbance ratio (CO₃-band/PO₄-band) of the apatite hydrogel prepared in air increased, but those in N₂ decreased. TEM observation revealed

that the crystal size of the apatite hydrogel seems to be almost similar, independent of the maturation period and atmospheric condition. So carbonate ion in apatite hydrogel is believed to strongly affect the aggregation of particles. The caking behavior of apatite hydrogel depended on the relative humidity, and the particle size. At 40-60% of relative humidity, the contraction of apatite hydrogel in volume was completed in 2 days, and the specimen set firm. The gel-like materials, precipitated from the solution of Na₂HPO₄ · 12H₂O and CaCl₂ · 2H₂O solution, containing various contents of polystyrene were dried in air at r.t. The shrinkage of specimen depends on the volume ratio of additives, and the volume of the specimen dropped from 2 to 7 days of drying period and the shrinkage seems to have stabilized after 7 days of drying period. The dried specimen slowly dissolves in aqueous solution, but seems be stable for a certain period. So the apatite adsorptive materials included in the apatite cake may be released into solution for a certain period.

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