

***In Vitro* Estimation with Simulated Body Fluid of OH-Designed Transparent Apatite Ceramics Prepared by Spark Plasma Sintering**

Daisuke Kawagoe, Koji Ioku, Hirotaka Fujimori, Seishi Goto

Division of Applied Medical Engineering Science Graduate School of Medicine, Yamaguchi University

2-16-1 Tokiwadai, Ube 755-8611, Japan

FAX: 81-836-85-9601, e-mail: ioku@po.cc.yamaguchi-u.ac.jp

Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$: HA, is one of the most biocompatibility materials with human bones and teeth. In addition, HA is an interesting substance as column materials for chromatography, sensors and so on. It has been already suggested that the amount of OH^- ions in the structure of HA is closely related to the biocompatibility, the ability for bone formation and the ionic conductivity. Thus, OH-control in the HA structure is necessary for the material designing. In this study, OH^- -controlled HA ceramics were prepared by spark plasma sintering (SPS) at the temperatures from 800 °C to 1100 °C. The HA ceramics prepared at 900 °C and 1000 °C showed transparency. These dense HA ceramics with several OH^- amounts were soaked into the simulated body fluid (SBF), then the behavior of HA in SBF was analyzed up to 14 weeks. After the soaking test in SBF, carbonated HA (CHA) precipitated on the surface of HA ceramics, and then the amount of CHA increased with increasing period of the soaking test. The amount of precipitated CHA depended on the amount of hydroxyl group in HA ceramics.

Key words: hydroxyapatite, simulated body fluid, spark plasma sintering, biocompatibility, transparent

1. INTRODUCTION

Calcium hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$:HA, is the inorganic principle component of natural bones and teeth. This material has the attractive feature of biocompatibility for the human hard tissue. Therefore, many clinical applications of HA were carried out as artificial bones and teeth roots [1-4]. It has been already suggested that the amount of OH^- ion in the crystal structure of HA is closely related to the biocompatibility, the ability for bone formation and the ionic conductivity. The amount of OH^- ion in current HA, however, has not been controlled [5]. In order to prepare more functional HA ceramics, the amount of OH^- ion must be controlled. In the past paper, the amount of OH^- in HA was indicated to be controllable by the control of heating conditions [6, 7].

Previous studies have proven that a simulated body fluid (SBF), which had almost the same ion concentrations as those of human blood plasma [8], can well reproduce the *in vivo* surface changes in certain biomaterials.

In this study, we compared the reaction with SBF of three kinds of HA ceramics with different OH^- amount, prepared by spark plasma sintering.

2. EXPERIMENTAL PROCEDURE

2.1 Sintering Process

A fine powder of HA (High-purity grade Ube Materials, Japan) was used as the starting material. This powder of about 1g was poured into a graphite mold (inner diameter 15 mm), and then sintered by the spark plasma sintering method (SPS: Dr Sinter-511S, Sumitomo Coal Mining, Kanagawa, Japan). The temperatures of the samples during sintering were measured by thermocouples of Rh/Pt-Pt, which was inserted into the wall of the graphite mold to measure the sample temperature. The samples were pressed uniaxially under 10 MPa, and then they were heated at 800 °C, 900 °C and 1000 °C for 10 min with a heating rate of 25 °C · min⁻¹.

SBF was prepared by dissolving reagent chemicals of NaCl, NaHCO₃, KCl, K₂ HPO₄, 3H₂O, MgCl₂ · 6H₂O, CaCl₂ and Na₂ SO₄ in deionized water. The fluid was then buffered at physiological pH 7.25 and at 37°C with tris-(hydroxy-ethyl) aminomethane [$\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3$] and hydrochloric acid [HCl]. The ion concentration of SBF is shown in Table 1.

2.2 Characterization

The chemical composition of HA was analyzed by an inductively coupled plasma spectrometer (ICP-MS:

Seiko Inst., SPQ 9000S, Japan). The starting powder and the obtained ceramics were identified by a powder X-ray diffractometer with graphite-monochromatized CuK α radiation, operated at 40 kV and 20 mA (XRD; Geiger flex 2027, Rigaku, Japan). The ceramics was polished with using a 4000-grid SiC and then polished finely with using a paste containing Al₂O₃ fine particles smaller than 0.5 μ m in size. The hardness of samples was evaluated by an indentation method using a Vickers hardness tester (Model AVK-A II, Akashi, Japan) at the load of 300 gf for 10 s. After etching in 0.2 mol lactic acid solution for 50 s [9], the surface of ceramics was observed by using a field emission scanning microscopy (FE-SEM; JSM 820, JEOL, Japan). The amount of OH⁻ ion in the ceramics was analyzed quantitatively by infrared spectroscopy (FT-IR; Spectrum 2000, Perkin Elmer). Thermogravimetry and differential thermal analysis (TG-DTA; TG-DTA32, Seiko Instruments Inc. Japan) was executed under the following conditions: 10 mg of samples, α -Al₂O₃ reference, and a heating rate of 10 $^{\circ}$ C \cdot min⁻¹ from room temperature to 1200 $^{\circ}$ C.

Table. 1
Ion concentration of human blood plasma and SBF.

Ion	Concentration / mM	
	Blood plasma	SBF
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	103.0	147.8
HCO ₃ ⁻	27.0	4.2
HPO ₄ ²⁻	1.0	1.0
pH	7.20-7.40	7.25

3. RESULTS AND DISCUSSION

The quantitative chemical analysis gave a Ca/P molar ratio very closely to the correct HA stoichiometry (Ca/P=1.67). There were little impurities as follows; Sr<8.9, Si<6.6, Mg<6.0, Na<5.0, K<5.0, Fe<0.9, Cu<0.7, Mn<0.2, Ba<0.1, Ni<0.1 and Cd<0.1 ppm. The particle size along long axis of HA powders was less than 0.1 μ m by TEM observation. The size and shape of particles were relatively homogeneous.

No phases other than HA were revealed by XRD for the starting samples and samples after sintering by SPS at 800 $^{\circ}$ C, 900 $^{\circ}$ C and 1000 $^{\circ}$ C for 10 min (Fig. 1).

The spectrum of FT-IR indicates the presence of OH⁻ in the starting powder and also in the prepared ceramics (Fig. 2). The band due to the stretching vibrations of OH⁻ ion appears at 3571cm⁻¹ [10]. The quantity of OH⁻ in HA ceramics sintered by SPS decreased with increasing temperature of sintering. According to FT-IR and TG-DTA, the HA ceramics prepared at 800 $^{\circ}$ C was OH⁻ fully containing hydroxyapatite and the ceramics prepared at 900 $^{\circ}$ C was oxyhydroxyapatite, Ca₁₀(PO₄)₆(OH)_{1.2}O_{0.4}□_{0.4}, where □ is a neutral vacancy in the OH⁻ site.

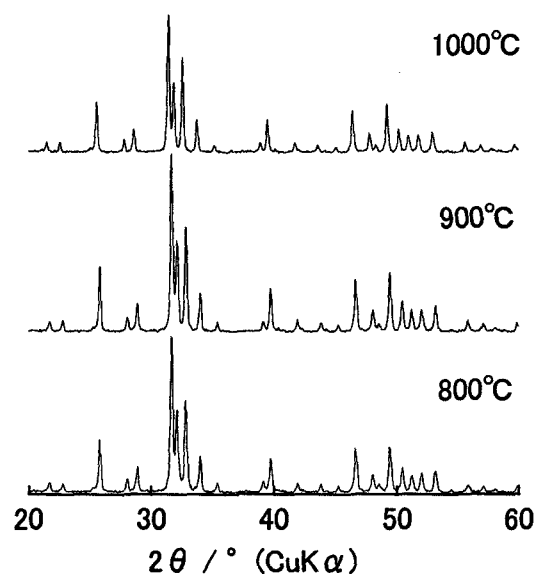


Fig.1 Patterns of XRD of ceramics prepared by SPS at 800 $^{\circ}$ C, 900 $^{\circ}$ C and 1000 $^{\circ}$ C.

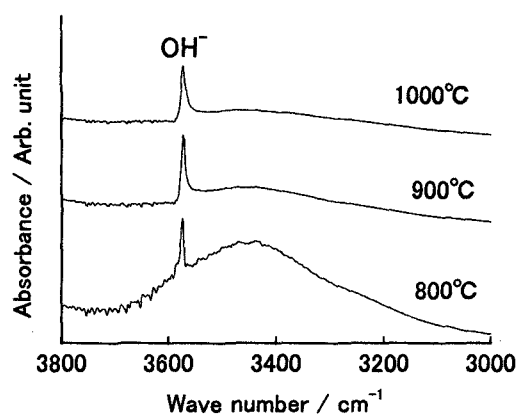


Fig.2 IR spectra of HA ceramics prepared by SPS at 800 $^{\circ}$ C, 900 $^{\circ}$ C and 1000 $^{\circ}$ C.

In the sintering process by SPS, the densification started at about 630 $^{\circ}$ C, and then proceeded with increasing temperature to result in line shrinkage of about 2.2 mm at 900 $^{\circ}$ C, which means the end of

densification.

The HA ceramics sintered by SPS at 800 °C showed about 90 % of a relative density, and the ceramics sintered at 900 °C and 1000 °C showed over 99 % of a relative density. Whereas in the case of normal sintering in air, densification started at about 900 °C [9]. These results proved that SPS is a potential method for fabricating highly densified HA ceramics at much lower temperature like hot-pressing (HP) and hot isostatic pressing (HIP) [11-15].

In particular, sintering period is quite short time. Transparent HA ceramics were obtained by SPS at 900 °C and 1000 °C for 10 min (Fig. 3).

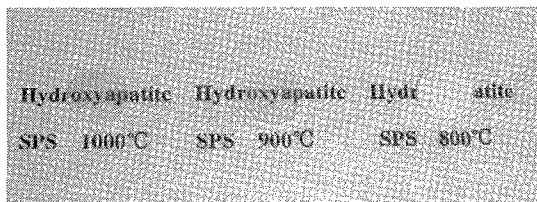


Fig.3 OH-controlled HA ceramics prepared by SPS at 800 °C, 900 °C and 1000 °C.

According to the FE-SEM observation, the ceramics prepared at 800 °C had a few pores of about 0.1 μm in size. The FE-SEM photograph of transparent ceramics prepared at 900 °C after etching is shown in Fig.4. Average grain size of the ceramics sintered at 900 °C were around 0.15 μm. There were almost no pores in the ceramics.

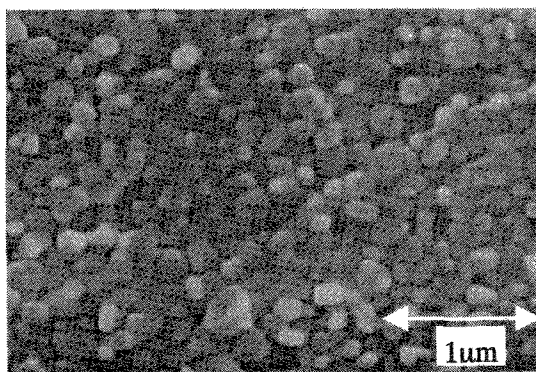


Fig.4 FE-SEM photograph of transparent HA ceramics prepared by SPS at 900 °C.

After the soaking test in SBF, weight of samples increased with increasing period of the soaking test and depended on the amount of hydroxyl

group in HA ceramics (Fig.5). And all the peaks of the XRD patterns were assigned to those of hydroxyapatite.

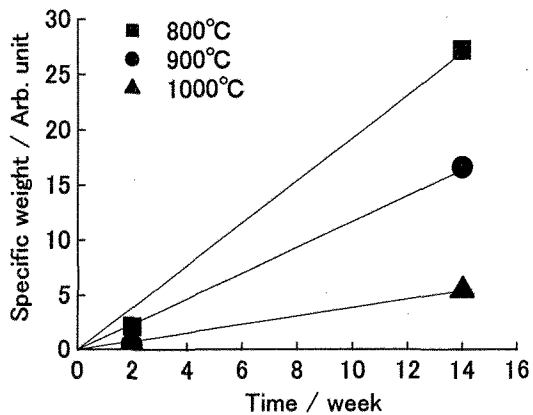


Fig.5 Changes in weight of ceramics prepared by SPS at various temperatures soaked in SBF for 2 and 14 weeks.

The FE-SEM photograph of transparent ceramics prepared at 900 °C after soaking in SBF for 2 weeks is shown in Fig.6. Some precipitates were newly formed after soaking for 2 weeks and then numerous depositions of leaf-like particles were observed.

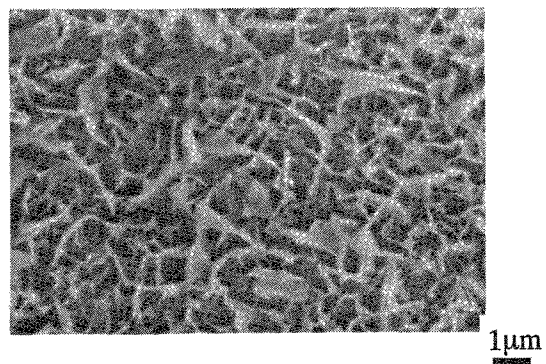


Fig.6 FE-SEM photograph of transparent HA ceramics prepared by SPS at 900 °C after soaking in SBF for 2 weeks.

The spectra of FT-IR indicate the presence of CO_3^{2-} in the ceramics after soaking in SBF (Fig.7). The newly formed phase on the surface after the soaking is considered to be carbonate hydroxyapatite (CHA), judging from changes in weight, morphological change, XRD and the spectra of FT-IR.

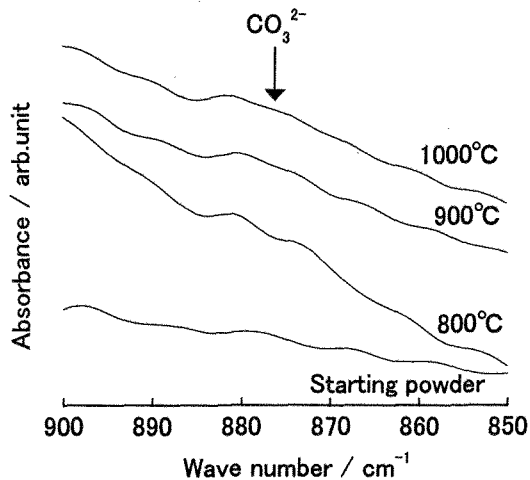


Fig. 7 IR spectra of transparent HA ceramics after soaking in SBF for 2 weeks.

4. SUMMARY

OH controlled dense hydroxyapatite ceramics were prepared by spark plasma sintering at the temperatures from 800 °C to 1000 °C for 10 min. Transparent hydroxyapatite ceramics were prepared by SPS at 900 °C and 1000 °C. The quantity of OH⁻ ion in the structure of hydroxyapatite was decreased with increasing temperature of sintering. After the soaking test in SBF, CHA precipitated on the surface of HA ceramics, and then the amount of CHA increased with increasing period of the soaking test. The amount of precipitated CHA depended on the amount of hydroxyl group in HA ceramics.

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