Development of non toxic magnetic beads has been strongly desired to cure more effectively for patients having diseases such as cancer and osseosarcoma. In this study, Fe was added during calcium phosphate preparation by conventional wet process, and the condition of Fe ion and the effect of Fe doping on the crystal phase, microstructure and other properties of HAp were investigated. The crystal phase of obtained samples had a HAp structure with low crystallinity, and the crystallinity of samples decreased with increasing the Fe ratio. The obtained powders had granular crystals with 10 nm in diameter. From the XRD and XANES measurement, it is thought that the actual doped amount into the HAp structure was 1, 1.8 and 3 mol % for Ca of HAp in the case of 5, 10 and 20 mol % of addition, respectively, and the most of added Fe existed as an iron oxide phase at the surface of HAp particles.

**Key-words : Fe-doped hydroxyapatite, XAFS, Magnetic property, Drug delivery system**

1. Introduction

Many kinds of magnetic beads such as paramagnetic iron oxide particle and surface-modified ones by polymer have been widely used in a medical field. However, those iron-oxide-based magnetic beads have been pointed out the risk of the elution of iron by long term use. The resolvability of iron strongly depends on its particle size. Since the particle size of magnetic beads is decided by a targeting place, control of degradability is one of the problems that we should overcome to use it in a living body. Because the elution of iron affects proliferation and metabolism of cell, there is a possibility to exhibit some toxicity. Therefore, development of non toxic magnetic bead has been strongly desired.

Calcium phosphate has been well understood as bioactive and biodegradable materials. Especially, hydroxyapatite \([\text{Ca}_10(\text{PO}_4)_{6-(OH)}_2]\), HAp is a chief inorganic component of hard tissue and exhibits excellent bone conductivity and biocompatibility. Therefore, HAp has been widely used as alternates of hard tissue and surface modification film of artificial implants to connect them with remaining bone rapidly. Since HAp also has non toxicity and excellent ion exchange ability, many kinds of studies for some functional elements doped HAp such as Mn, Si, Zn, Fe and Mg have been reported so far. Those doped HAp will be a good candidate for not only alternates of hard tissue but also drug delivery system (DDS) materials and heavy-metal removables. Margin of safety and controllability to transfer to an affected region are required for DDS materials. From that point of view, magnetic element doped HAp could be a good candidate as novel DDS material. There are four magnetic elements, Fe, Co, Ni and Gd, to show ferromagnetism around room temperature. In those elements, Co and Ni have high toxicity. Although Gd has been used as a contrast media for MRI, there is anxiety of cost performance because Gd is rare earth material. From these reasons, in a present study, we paid attention to Fe due to its high saturation magnetization and low cost. It has been reported that the resolvability of HAp decreased by addition of Fe, and HAp is basically understood as a low degradable materials in a body. Considering these reasons, it is expected that non toxicity magnetic bead can be developed by addition of Fe to the HAp structure.

In this study, we doped Fe to the HAp to use as a novel magnetic bead to control its action by magnetic field and investigated condition of Fe and the effects of Fe doping on the crystal phase, microstructure and other properties of HAp.

2. Experimental procedures

0.1 mol/l calcium nitrate \([\text{Ca}(\text{NO}_3)_2]\), ammonium phosphate dibasic \((\text{NH}_4)_2\text{HPO}_4\) and iron chloride (FeCl₃) aqueous solutions were used as starting materials. After adjusting the pH of solutions, phosphate solutions were gently dropped to mixed solution of calcium and iron to become the Ca/P ratio of 1.67. The ratio of Fe was changed in the range from 0 to 20 mol % for the amount of Ca solution. After mixing up each other, obtained solution was aged at room temperature for 1 h, and then, it was filtered and dried at 323 K for 24 h. A part of samples were heat treated at 1073 K for 2 h with heating rate of 4°C/min. After heat treatment, samples were air-cooled in the furnace to room temperature. The crystal phase of samples was indexed by XRD (UltimaIV, Rigaku Co., Japan). Scans were performed at 2θ = 10°–40° with scan rate of 4°/min using Cu-Kα radiation. The morphology was observed by FE-SEM (s-4500, Hitachi, Japan) with applied voltage of 15 kV and TEM (2000FX, JEOL, Japan) with applied voltage of 200 kV. The chemical structure was investigated by FT-IR (ALPHA, Bruker Optics K. K., Japan) using KBr method. The measuring resolution was 4 cm⁻¹ and iterations were performed for 24 times in the range 400–4000 cm⁻¹.

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and Fe ions (Ca$^{2+}$) parameter is caused by the difference of ion radius between Ca and Fe. It is thought that the change of lattice parameter is caused by the difference of ion radius between Ca and Fe. Increasing the ratio of Fe increased the ratio of Ca to P: 0.5–1.67. The ratio of Fe was 0–20 mol%. Heat treatment temperature: 873–1273 K.

Table 1. Preparation condition of Fe doped HAp

| Precursor             | Amount (mol/l) |
|-----------------------|----------------|
| Ca(NO$_3$)$_2$ 4H$_2$O | 0.1            |
| (NH$_4$)HPO$_4$       | 0.1            |
| FeCl$_3$ 6H$_2$O       | 0.1            |
| Ratio of Ca to P       | 0.6884         |

The magnetic property was measured by VSM with an applied magnetic field of 800 A/m. The local structure of samples was investigated from X-ray-absorption near-edge structure (XANES) spectra for Ca-K, P-K and Fe-K edge. The Ca-K XANES data was corrected by transmission mode using Si(111) double crystal monochrometer at BL01B1 in the SPring-8. For the XANES measurement, the samples were prepared as pellets with the thickness varied to obtain a 0.5–1 jump at the both of Ca-K and Fe-K absorption edge. P-K edge XANES spectra were recorded in total-electron yield mode using InSb crystal monochrometer at BL2A in the UVSOR. The data were collected with the ionization chambers. XANES were analyzed by subtracting a linear background computed by least-squares fitting. The analysis of XANES data was conducted using the commercial software “REX2000” (Rigaku Co. Ltd., Japan). The detailed preparation condition was listed in Table 1.

3. Results and discussion

Figure 1 shows XRD patterns of Fe doped HAp prepared at the Ca/P ratio of 1.67 and the ratio of Fe from 0 to 20 mol%. All samples had HAp related structure with low crystallinity, independent of the Ca/P ratio and Fe, and the crystallinity of samples decreased depending on the ratio of Fe. Okazaki et al. have reported that the crystallinity of Fe doped HAp decreased with increasing Fe content in the HAp structure. Similar trend was observed in a present study.

Figure 2 shows XRD patterns of Fe-HAp prepared at the Ca/P ratio of 1.67 and the ratio of Fe from 0 to 20 mol% after heat treatment at 1073 K for 2 h. Although the crystallinity of samples improved by heat treatment at more than 1073 K, they thermally decomposed to $\beta$-TCP phase. Although small peak derived from $\alpha$-TCP phase was detected at 1 mol% of Fe addition, it disappeared at more than 5 mol% of Fe addition. This is thought that the thermal decomposition to $\alpha$-TCP was inhibited by Fe addition. Additionally, the integral intensity of $\beta$-TCP and HAp phases changed depending on the ratio of Fe. The calculated lattice parameter from XRD patterns changed depending on the ratio of Fe, and the lattice parameter of the sample prepared at 0 mol% of Fe addition was $a = 0.9412$ nm and $c = 0.6874$ nm, respectively. This lattice parameter was similar to the data listed in JCPDS (PDF No. 09-0432, $a = 0.9418$ nm and $c = 0.6884$ nm), respectively. However, lattice parameter of $c$-axis decreased with increasing the ratio of Fe. It is thought that the change of lattice parameter is caused by the difference of ion radius between Ca and Fe ions (Ca$^{2+}$ = 99 pm, Fe$^{3+}$ = 66 pm). Besides, it has been also reported that HAp easily decomposes to $\beta$-TCP phase because HAp does not maintain its structure by addition of Fe due to their ion radius mismatch.

In order to estimate the doped ratio of Fe into the HAp structure, quantitative analysis from XRD spectra was performed for heat treated samples by internal reference method using commercial HAp, $\beta$-TCP and $\alpha$-Fe$_2$O$_3$ powder. In the case of 5 mol% addition, about 79% of added Fe existed as $\alpha$-Fe$_2$O$_3$ phase, and this value means that actual amount of doped Fe into HAp structure is 1 mol% for the Ca of HAp. Similarly, estimated actual amount of Fe in HAp structure was 1.8 and 3.1 mol% for the 10 and 20 mol% of addition, respectively.

Figure 3 shows TEM images of Fe-HAp prepared at the Ca/P ratio of 1.67 and the ratio of Fe from 0 to 20 mol%. All samples had granular morphology consisted of smaller grains. The grain size of sample prepared at the ratio of Fe of 0 mol% was in the range from 10 to 20 nm, and the drastic change of grain size by increasing the ratio of Fe was not observed. On the other hand, the specific surface area of Fe-HAp decreased with increasing the ratio of Fe as listed in Table 2. The grain size of sample can be estimated from the specific surface area. As shown in Table 2, the specific surface area of sample increased with increasing the ratio of Fe. This result indicated that the grain size of sample decreased with increasing the ratio of Fe.
Figure 4 shows FT-IR spectra of Fe-HAp prepared at the Ca/P ratio of 1.67 and the ratio of Fe from 0 to 20 mol%. There are several HAp related bands of OH stretching band at 3570 cm⁻¹ and librational band at 638 cm⁻¹. The CO₃ v3 and the carbonate ν2 band were observed at 1400–1550 and 873 cm⁻¹, respectively. The phosphate bands were also observed between 450 and 700 cm⁻¹. There are PO₄ ν4 mode at 566 and 605 cm⁻¹, PO₄ ν3 mode at 1070 cm⁻¹ and band for asymmetric HAp at 1107 cm⁻¹. The phosphate bands were also observed between 450 and 700 cm⁻¹. There are PO₄ ν4 mode at 470 cm⁻¹, and labile PO₄ mode at 638 cm⁻¹ as a minor band. The prevailing of the CO₃ ν2 band at 873 cm⁻¹ indicates the formation of the B-type carbonate.¹⁹,²⁰)

Figure 5 shows (a) Ca-K, (b) P-K, and (b) Fe-K edge XANES spectra of Fe-HAp powders prepared at the ratio of Ca to P of 1.67 and the ratio of Fe from 0 to 20 mol%. The local structure of near Ca and Fe atoms were evaluated by XAFS to circumstantially examine the state of doping Fe to HAp. The Ca-K and P-K edge spectra of all samples were very close to the peak position of commercial HAp. This result indicated that the local structure of near Ca and P atoms in samples were similar to HAp. For the Fe-K edge XANES measurement, α-Fe₂O₃, γ-Fe₂O₃, Fe₃O₄ and α-FeOOH were used as a reference. As shown in Fig. 6(c), the Fe-K edge spectra samples of changed by increasing the ratio of Fe. The Fe-K edge spectra of samples prepared in low Fe ratio region was close to α-FeOOH and that prepared in high Fe ratio region was close to lapped spectra of α-Fe₂O₃ and α-FeOOH. This result also indicated that a small amount of Fe ions could be substituted into the HAp structure and most of Fe exists as α-FeOOH and α-Fe₂O₃ at the surface of powders.

Figure 6 shows M-H loops of Fe-HAp prepared at the Ca/P ratio of 1.67 and the ratio of Fe from 0 to 20 mol%. The all samples showed paramagnetic behavior. The magnetization of the

| Ratio of Fe (mol%) | Specific Surface area (m²/g) |
|-------------------|-----------------------------|
| 0                 | 105.1                       |
| 1                 | 113.1                       |
| 5                 | 137.4                       |
| 10                | 158.0                       |
| 20                | 171.0                       |

Table 2. Specific surface area of Fe doped HAp
crystallinity of samples increased by heat treatment at more than 5 mol% Fe doping sample at the applied magnetic field of 800 A/m was $1.0 \times 10^{-2}$ Am²/kg, and this value was similar to commercial HAp. The magnetization of samples increased with increasing the ratio of Fe and maximum value of 0.8 Am²/kg, and this value was similar to commercial HAp. The magnetization of samples increased with increasing the ratio of Fe.

From these results, solid solubility of Fe into the HAp structure is inferred about 1 mol% for the Ca of HAp. Therefore, in order to clarify the effect of Fe doping into the HAp structure, synthesis technique with higher reaction efficiency such as hydrothermal processing is required in a further study.

4. Conclusions

Fe doped HAp powders were prepared by conventional wet process using Ca(NO₃)₂, (NH₄)₂HPO₄ and FeCl₃ solutions. All samples had HAp-related structure and low crystallinity. The crystallinity of samples strongly depended on the ratio of Fe and it decreased with increasing the ratio of Fe. The Fe doping affected the thermal decomposition behavior of HAp, and thermal decomposition to β-TCP was accelerated. Although the crystallinity of samples increased by heat treatment at more than 1073 K, α-Fe₂O₃ phase generated in the ratio of Fe more than 5 mol%. The particle size of powders was about 20 nm in diameter and independent of the ratio of Fe. From the XANES and XRD analysis, actual amount of doped Fe into the HAp structure was estimated as 1, 1.8 and 3.1 mol% for 5, 10, 20 mol% of addition, respectively, and the most of Fe exists as α-FeOOH and α-Fe₂O₃ at the surface of samples. From the VSM measurement, all samples exhibited paramagnetic behavior, and the magnetization of 0 mol% Fe doped HAp was $1.0 \times 10^{-2}$ Am²/kg, which is similar value to commercial HAp, and the magnetization of samples increased with increasing the ratio of Fe.

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