In order to develop non toxic magnetic beads, influence of Fe addition to the hydroxyapatite was investigated, and Fe doped hydroxyapatite was prepared by hydrothermal process at 423 K. The crystal phase of obtained samples had a hydroxyapatite related structure, and $\alpha$-Fe$_2$O$_3$ phase generated as a second phase at the ratio of Fe more than 5 mol % additions. The ratio of $\alpha$-Fe$_2$O$_3$ phase increased depending on the ratio of Fe, and 40 and 73% of added Fe existed as $\alpha$-Fe$_2$O$_3$ phase at 5 and 10 mol % addition, and the estimated doped amount of Fe is about 2.1 mol % for 5 mol % addition and 2.7 mol % for 10 mol % addition, respectively. All samples showed paramagnetic property, and magnetization of sample increased depending on the ratio of Fe. The maximum magnetization of 0.63 Am$^2$/kg was obtained at the Fe ratio of 10 mol %.

1. Introduction

Calcium phosphates, especially hydroxyapatite [Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAp], is a chief inorganic material occupying about 70% of our hard tissue, and HAp is innocuity for a biological body and an environment. In addition, HAp exhibits excellent biocompatibility and bioactivity and also has unique property of ion exchange ability. By using these properties, functional elements doped HAp such as Si, Al, Mg, Mn and Zn has been prepared by many kinds of method and applied to not only artificial bone but also coating films on the contact surface of artificial implants with remaining bone. Besides, due to the stability of HAp in a biological body, application as a DDS (drug delivery system) material is recently considered. Margin of safety and controllability to transfer it 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 circumstances, we paid attention to Fe due to its high saturation magnetization and low cost, and investigated the effect of Fe addition to the HAp by co-precipitation method at room temperature in a previous study. As a result of that, decrement of crystallinity, microparticulation and increment of magnetization were observed by increasing amount of Fe addition. However actual doped amount of Fe into the HAp structure was around 1 mol % for Ca of HAp and most of doped Fe existed as $\alpha$-FeOOH and $\alpha$-Fe$_2$O$_3$ at the surface of particles. Therefore, synthesis in lower Fe concentration and/or synthesis technique with higher reaction efficiency such as hydrothermal processing was required.

2. Experimental procedures

0.1 mol/l calcium nitrate [Ca(NO$_3$)$_2$], ammonium phosphate dibasic [(NH$_4$)$_2$HPO$_4$] and iron chloride (FeCl$_3$) aqueous solutions were used as starting materials. Each solution was mixed up becoming the Ca/P ratio of 1.67. After mixing, they were hydrothermally treated at 423 K for 24 and 72 h. Obtained solution was filtered and dried at 323 K for overnight. The crystal phase of samples was indexed by XRD (UltimaIV, Rigaku Co., Japan). Scans were performed at 2$\theta$ = 10–40$^\circ$ with scan rate of 4°/min using Cu-K$\alpha$ 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 magnetic property was measured by VSM with applied magnetic field of 800 A/m. The local structure of samples was investigated by XANES spectra for Ca-K, P-K and Fe-K edge. Both of Ca-K and Fe-K edge XAFS data were corrected by transmission and fluorescence mode using Si(111) double crystal monochrometer at BL01B1 in the SPring-8, respectively. For the XAFS 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 monochromater 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). Dissolution behavior of samples was measured by immersion test using acetate buffer solution of pH 4 at 311 K for 168 h. For the immersion test, obtained powders were uniaxial pressed and then calcined at 873 K for 2 h. The detailed preparation condition was listed in Table 1.

3. Results and discussion

Figure 1 shows XRD patterns of Fe doped HAp prepared at 423 K for 72 h and the ratio of Fe from 0 to 10 mol %. All samples had HAp related structure, and the crystallinity of samples decreased 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 $a = 0.942$ nm and $c = 0.689$ 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). However, lattice parameter of $c$-axis slightly decreased with increasing the ratio of Fe. It is thought that the change of lattice parameter is caused by the difference of ion radius mismatch between Ca and Fe ions ($Ca^{2+} = 99$ pm, $Fe^{3+} = 66$ pm). In addition, peaks derived from $\gamma-Fe_2O_3$ phase were observed around $2\theta = 27, 33$ and 37° for the ratio of Fe more than 5 mol %, and relative intensity of those peaks getting stronger with increasing the ratio of Fe. In order to estimate the doped ratio of $\alpha-Fe_2O_3$ phase, quantitative analysis from XRD spectra was performed by internal reference method using commercial HAp and $\alpha-Fe_2O_3$ powder. In the case of 5 mol % addition, about 57% of added Fe existed as $\alpha-Fe_2O_3$ phase. Similary, about 73% of added Fe existed as $\alpha-Fe_2O_3$ phase in the case of 10 mol % addition.

Figure 2 shows (a) Ca-K, (b) P-K, and (b) Fe-K edge XANES spectra of Fe-HAp powders prepared at 423 K for 72 h and the ratio of Fe from 0 to 10 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 XANES spectra of all samples were very close to the peak position of commercial HAp as shown in Figs. 2(a) and 2(b). This result indicated that electron state around Ca and P atoms did not change, and the local structure of near Ca and P atoms in samples is very close to commercial HAp. For the Fe-K edge XANES measurement, $\alpha-Fe_2O_3$, $\gamma-Fe_2O_3$, Fe$_3$O$_4$ and $\alpha$-FeOOH were used as references. Since distinct shift in absorption peak did not observed for all samples, quantitative analysis from XRD spectra was performed by internal reference method using commercial HAp and $\alpha-Fe_2O_3$ powder. In the case of 5 mol % addition, about 57% of added Fe existed as $\alpha-Fe_2O_3$ phase. Similary, about 73% of added Fe existed as $\alpha-Fe_2O_3$ phase in the case of 10 mol % addition.

Table 1. Preparation condition of Fe doped HAp by hydrothermal process

| Precursor       | Condition          |
|-----------------|--------------------|
| Ca(NO$_3$)$_2$·4H$_2$O (a): | 0.1 mol/l |
| (NH$_4$)HPO$_4$ (b): | 0.1 mol/l |
| FeCl$_3$·6H$_2$O (c): | 0.1 mol/l |
| (a) + (b) + (c): | 25 ml |
| Ratio of Ca to P: | 1.67 |
| Ratio of Fe: | 0–20 mol % |
| Synthesis Temperature: | 423 K |
| Synthesis Time: | 24, 72 h |

![Fig. 1. XRD patterns of Fe doped HAp powders prepared at 423 K for 72 h and the Fe ratio from 0 to 10 mol %. (a) 0 mol %, (b) 1 mol %, (c) 5 mol %, (d) 10 mol %.

![Fig. 2. XANES spectra of Fe doped HAp powders prepared at 423 K for 72 h and the Fe ratio from 0 to 10 mol %. (A) Ca-K, (B) P-K and (C) Fe-K edge.](image-url)
samples, it is thought that the valence of added Fe did not change and Fe ions exist as trivalent state in the HAp and oxide phase. The obtained Fe-K edge XANES spectra of samples were close to $\alpha$-Fe$_2$O$_3$. Therefore, doped amount of Fe into the HAp structure could be estimated by combining with the amount of $\alpha$-Fe$_2$O$_3$ phase calculated from XRD data. The estimated doped amount of Fe is about 2.1 mol% for 5 mol% addition and 2.7 mol% for 10 mol% addition, respectively.

Figure 3 shows TEM images of Fe-HAp prepared at the ratio of Fe from 0 to 10 mol%. Fe-HAp prepared at the ratio of Fe of 0 mol% had a rod shape with 20 × 50 nm, and the morphology of sample changed with increasing the ratio of Fe. Fe-HAp powders prepared at the ratio of Fe of 10 mol% had a granular structure with 20 nm in diameter. This morphology change from rod to granular is thought that the electrical charge of HAp of a-plane is positive and that of c-plane is negative due to the alignment of Ca$^{2+}$ and PO$_4^{3-}$ ions. Therefore, Fe$^{3+}$ ions are adsorbed to c-plane preferentially, and this phenomenon inhibits the crystal growth taken along the c-axis of HAp structure. 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 and exhibited a good agreement with TEM observation.

Figure 4 shows FT-IR spectra of Fe-HAp prepared at 423 K for 72 h and the ratio of Fe from 0 to 10 mol%. The obtained spectra were similar to commercial HAp, and there are several HAp related bands of OH stretching band at 3570 cm$^{-1}$ and librational band at 638 cm$^{-1}$. The weak CO$_3$ v3 and the carbonate v2 bands were observed at 1400–1550 and 873 cm$^{-1}$ respectively and these CO$_3$ bands were caused by dissolved carbon oxide. The phosphate bands were observed in the range from 950 to 1200 cm$^{-1}$. There are PO$_4$ v1 mode at 963 cm$^{-1}$, PO$_4$ v3 mode at 1033 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 PO$_4$ v4 mode at 566 and 605 cm$^{-1}$, PO$_4$ v4 mode at 470 cm$^{-1}$, and labile PO$_4$ mode at 638 cm$^{-1}$ as a minor band. The prevailing of the CO$_3$ v2 band at 873 cm$^{-1}$ indicates the formation of the B-type carbonate.$^{23,24}$

Figure 5 shows M-H loops of Fe-HAp prepared at 423 K for 72 h and the ratio of Fe from 0 to 10 mol%. The all samples showed paramagnetic behavior. The magnetization of the 0 mol% Fe doped sample at the applied magnetic field of 800 A/m was $1.0 \times 10^{-2}$ Am$^2$/kg, and this value was similar to commercial HAp. The magnetization of samples increased with increasing the ratio of Fe and maximum value of 0.63 Am$^2$/kg was obtained at the ratio of Fe of 10 mol% and the applied magnetic field of 800 A/m. Since quantity of iron oxide phase increases with increasing the ratio of Fe as shown in Fig. 1, it is suggested that the generation of iron oxide phase strongly affected the increment of magnetization.$^{25,26}$
Fig. 6. Degradability of Fe doped HAp in acetate buffer solution.

Figure 6 shows degradation behavior of Fe-HAp in acetate buffer solution of pH 4. The degradability of sample was measured from weight loss before and after immersion. The rate of mass decrease of Fe-HAp samples decreased from 3.8 to 1.8% with increasing the ratio of Fe. Although there is the effect of iron oxide phase, the trend of degradation behavior and magnetic behavior as shown in Fig. 5 provides a possibility to use the Fe-HAp as novel magnetic beads.

From these results, solid solubility of Fe into the HAp structure is inferred less than 2 mol% for the Ca of HAp in a present study. Therefore, in order to clarify the effect of Fe doping into the HAp structure, synthesis in lower Fe concentration region is required in a further study.

4. Conclusions

Fe doped HAp powders were prepared by hydrothermal process using Ca(NO$_3$)$_2$, (NH$_4$)$_2$HPO$_4$ and FeCl$_3$ solutions. All samples had HAp-related structure and relatively high crystallinity by comparing with Fe-doped HAp powders prepared by co-precipitation method. The crystallinity of samples strongly depended on the ratio of Fe, and it decreased with increasing the ratio of Fe. $\alpha$-Fe$_2$O$_3$ phase was generated as a secondary phase at the ratio of Fe more than 5 mol%, and the ratio of $\alpha$-Fe$_2$O$_3$ phase increased with increasing the ratio of Fe. The estimated doped amount of Fe from XRD and XANES analysis is about 2.1 mol% for 5 mol% addition and 2.7 mol% for 10 mol% addition, respectively. The microstructure of powders changed from rod shape with 20 $\times$ 50 nm at the Fe ratio of 0 mol% and granular shape with 20 nm in diameter at the Fe ratio of 10 mol%. From the VSM measurement, the magnetization of 0 mol% Fe doped HAp was $1.0 \times 10^{-2}$ Am$^2$/kg which is similar value to commercial HAp, and the magnetization of samples increased with increasing the ratio of Fe. The degradability of sample decreased with increasing the ratio of Fe.

Acknowledgement This work was partially supported by Grant-in-Aid for Young Scientists (B) (25870061) from the Japan Society of the Promotion of Science (JSPS). The synchrotron radiation experiments were performed at the BL01B1 of SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI), BL-9C of the Photon Factory with the approval of the Photon Factory Program Advisory Committee (Proposal No. 2011G659) and BL-2A of the UVSOR, respectively.

References
1) T. Kokubo, H. M. Kim, M. Kawashita and T. Nakamura, J. Mater. Soc.: Mater. Med., 15, 99–107 (2004).
2) B. B. Ferris, R. A. Dodds, L. Klenerman, L. Bitensky and J. Chayen, J. Bone Joint Surg. Br., 69, 234–237 (1987).
3) C. F. O. Ossa, S. O. Rogero and A. P. Tschiptschin, J. Mater. Soc.: Mater. Med., 17, 1095–1100 (2006).
4) L. L. Hench, J. Am. Ceram. Soc., 81, 1705–1728 (1998).
5) G. Negrouiu, R. M. Pitecesu, G. C. Chitamu, I. N. Mihaiusca, L. Zdrenu and M. Miroiu, J. Mater. Soc.: Mater. Med., 19, 1537–1544 (2008).
6) K. Matsunaga, H. Inamori and H. Murata, Phys. Rev. B, 78, 094101 (2008).
7) M. Sato, R. Tu and T. Goto, Mater. Trans., 48, 3149–3153 (2007).
8) M. Sato, R. Tu, T. Goto, K. Ueda and T. Narushima, J. Ceram. Soc. Japan, 117, 461–465 (2009).
9) A. Nakahira, S. Nakamura and M. Horimoto, IEEE Trans. Magn., 43, 2465–2467 (2007).
10) K. Nakata, T. Kubo, C. Numako, T. Onoki and A. Nakahira, Mater. Trans., 50, 1046–1049 (2009).
11) L. Medvecky, R. Stulajterova, L. Pärlak, J. Trpevska, S. F. Durisin and S. M. Barinov, Physicochem. Eng. Aspects, 281, 211–219 (2006).
12) J. Wang, T. Nonami and K. Yubata, J. Mater. Soc.: Mater. Med., 19, 2663–2667 (2008).
13) E. I. Get’man, N. V. Yablochkov, S. N. Loboda, V. V. Prisedsky, V. P. Antonovich and N.A. Chivireva, J. Solid State Chem., 181, 2386–2392 (2008).
14) M. Valko, H. Morris and T. M. D. Cronin, Curr. Med. Chem., 12, 1161–1208 (2005).
15) P. Caravan, J. J. Ellinson, T. J. McMurry and R. B. Lauffer, Chem. Rev., 99, 2293–2352 (1999).
16) L. M. De Leon-Rodriguez, A. J. M. Lubag, C. Malloy, G. V. Martinez, R. J. Gillies and A. D. Sherry, Acc. Chem. Res., 42, 948–957 (2009).
17) K. W.-Y. Chan and W.-T. Wong, Coord. Chem. Rev., 251, 2428–2451 (2007).
18) E. Terreno, D. D. Castelli, A. Viale and S. Aime, Chem. Rev., 110, 3019–3042 (2010).
19) M. Sato and A. Nakahira, J. Ceram. Soc. Japan, in press. (2013).
20) Y. Wang, S. Zhang, K. Wei, N. Zhao, J. Chen and X. Wang, Mater. Lett., 60, 1484–1487 (2006).
21) E. Lester, S. V. Y. Tang, A. Khlobystov, V. L. Rose, C. Butterly and C. J. Roberts, CrystEngComm, in press. (2013).
22) T. Kubo, M. Hogiri, H. Kagata and A. Nakahira, J. Am. Ceram. Soc., 92, S172–S176 (2009).
23) M. C. Chang and J. Tanaka, Biomaterials, 23, 4811–4818 (2002).
24) T. Moriguchi, K. Yano, S. Nakagawa and F. Kaji, J. Colloid Interface Sci., 260, 19–25 (2003).
25) T. S. Lin, Phys. Rev., 116, 1447–1452 (1959).
26) G. Kletetschka and P. J. Wasilewski, Phys. Earth Planet. Inter., 129, 173–179 (2002).