ULTRASTRUCTURAL ANALYSIS OF SYNTHETIC HYDROXYAPATITE WITH PREFERRED ORIENTATION TO *c*-PLANE USING HIGH-RESOLUTION TRANSMISSION ELECTRON MICROSCOPY

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Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAp) is an inorganic compound which is close to chemical compositions of human hard tissues, and it has good biocompatibility and bioactivity [1]. The HAp can be widely used as bone grafts and adsorbent of bio-related substances [2]. It is well-known as an adsorbent which can not result in degeneration of protein during adsorption operation. The HAp crystal belongs to hexagonal system, and there are two types of crystal planes: *a*(*b*)-planes which charged on positive and *c*-planes which charged on negative [3]. Actually, the HAp crystals in a cortical bone and dental enamel have preferred orientation to the *c*- and *a*(*b*)-axis directions, respectively.

In theory, the oriented HAp has strong specificity of adsorption on negative-charged or positive-charged proteins on the basis of the different electrostatic charge of crystal planes. The HAp crystal planes with preferred orientation may improve the selective specificity to protein adsorptions. Actually, we have successfully synthesized fiber-shaped and plate-shaped HAp single crystals, respectively [4,5]. In our previous study, the ultrastructure of synthetic fiber-shaped HAp was examined by using high-resolution transmission electron microscopy (HR-TEM), the results indicated that the fiber-shaped HAp particle was highly strained single crystal with the *c*-axis orientation parallel to the long axis of the fibre [6]. In this work, the ultrastructure of the synthetic plate-shaped HAp particle was investigated by HR-TEM.

Experimental

Preparation of plate-shaped HAp

The plate-shaped HAp was synthesized from the air-liquid interface of starting solution via enzyme reaction of urea with urease (activity: 143 unit·mg^{-1}). The starting solution 1000 cm^3 was prepared by mixing 5.0 mmol·dm^{-3} $CaCO_3$, 3.0 mmol·dm^{-3} H_3PO_4, 1.0 mol·dm^{-3} $(NH_2)_2CO$, and then added the aqueous HNO_3 solution to adjust at pH 3.0. The starting solution was dispensed in glass petri dishes after just adding the 2.73 cm^3 urease solution (0.1 mass%), and heated in incubator at 50 ºC for 96 h. After reaction, a membranal product was formed from the air-liquid interface of starting solution. The interface product was collected and processed by heating treatment at 600 ºC for 2 h.

Characterization of plate-shaped HAp

The crystalline phase of the resulting powder was identified by powder X-ray diffraction (XRD; MiniFlex, Rigaku) using Cu-K_α radiation generated at 30 kV and 15 mA. The morphology of the obtained powder was observed by scanning electron microscope (SEM; JSM6390LA, JEOL). Furthermore, the ultrastructure of the product was examined by HR-TEM (JEM-2100F, JEOL) instrument at an accelerated voltage of 200 kV. HR-TEM sample was prepared by dispersing the powders in ethanol and collecting them onto carbon coated copper grid.

Results and Discussion

The XRD pattern of resulting HAp was shown in Fig. 1(a) together with commercial HAp (HAp-100, Taihei Chemical Indusreial Co. Ltd.) with isotropic orientation (Fig. 1(b)). The product was identified with HAp single phase, and the (002) and (004) reflections which correspond the *c*-plane were more intense than those of a standard HAp.

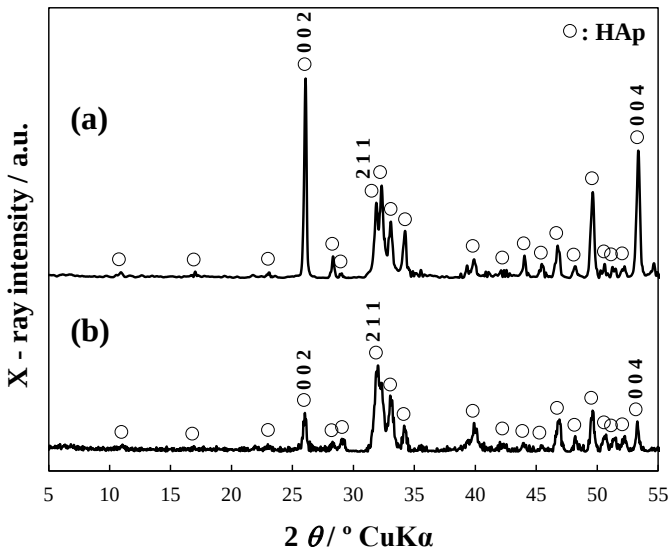

Fig. 1 XRD patterns of (a) resulting HAp and (b) commercial HAp without preferred orientation.

Figure 2 shows the SEM micrograph of the synthetic HAp particles; the hexagonal plate-shaped particle morphology could be observed. The average sizes of

plate-shaped particles were about 10 μm, and they had smooth surfaces.

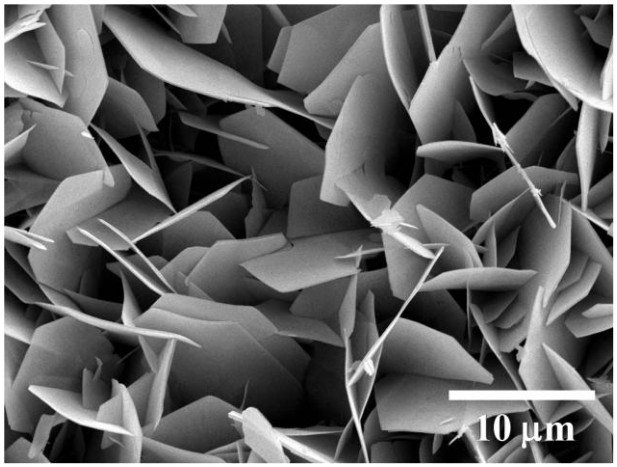

Fig. 2 SEM micrograph of resulting HAp particles.

The HR-TEM micrographs and selected area electron diffraction (SAED) pattern of the resulting plate-shaped HAp particle were shown in the Fig. 3. The SAED observations were performed in three areas (A-C) of low-magnification micrograph Fig. 3(a). The diffraction pattern from area A (Fig. 3(b)) showed clear spots corresponding to HAp hexagonal structure with high crystalline, and the other two diffraction patterns reveal a similar geometry too. From the SAED pattern, the lattice constants were determined to be a=b=0.94 nm, and the axial angle was 120º, it suggests that the observed direction corresponded the [001] zone axis (c-axis direction) of HAp crystal. Fig. 3(c) shows a middle-magnification lattice image of the area A, lattice plane (100) with 0.82 nm spacings can be observed in this lattice image, but no other planes with different orientations can be seen. Figure 3(d) shows a high-magnification HR-TEM lattice image, a highly periodic sequence of atom can be observed. The white closed circles of insert in Fig. 3(d) corresponded to OH^-, which are surrounded by Ca^{2+} and PO_4^{3-}. Around one OH^-, six equivalents OH^- exist and form a hexagon grid. These results indicate that the plate-shaped HAp particles were of single crystals with preferred orientation to the a(b)-axis direction.

Conclusion

The plate-shaped HAp was synthesized from the air-liquid interface of starting solution via enzyme reaction of urea with urease. The crystalline phase, particle morphology and ultrastructure of the resulting HAp were investigated by XED, SEM and HR-TEM. Those results suggest that the resulting hexagonal plate-shaped HAp particles were of single crystals with preferred orientation to the c-plane.

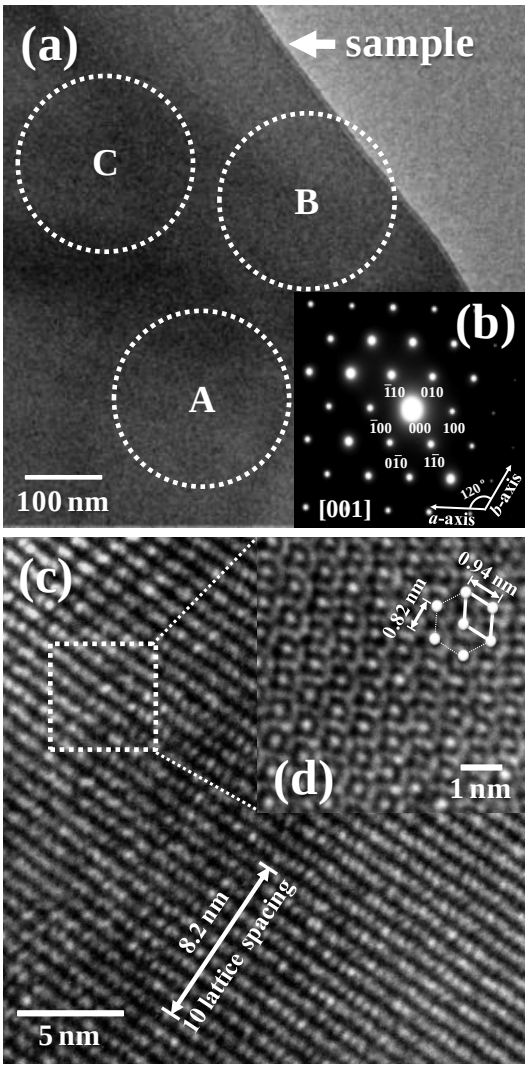

Fig. 3 HR-TEM micrographs and SAED pattern of resulting plate-shaped HAp, (a) low-magnification image, (b) SAED pattern, (c) middle-magnification image and (d) high-magnification image.

References

1. L. L. HENCH, Bioceramics: From concept to clinic, *J. Am. Ceram. Soc*., 1991, **74**, 1487-1510.
2. T. Kawasaki, S. Takahashi and K. Ikeda, Hydroxyapatite high-performance liquid chromatography: column performance for proteins, *Eur. J. Biochem.*, 1985, **152**, 361-367.
3. M. I. Kay, R.A. Young, A.S. Posner, Crystal structure of hydroxyapatite, *Nature*, 1964, **204**, 1050-1052.
4. M. Aizawa, H. Ueno, K. Itatani and I. Okada, Syntheses of calcium-deficient apatite fibres by a homogeneous precipitation method and their characterizations, *J. Euro. Ceram. Soc.*, 2006, **26**, 501-507.
5. Z. Zhuang and M. Aizawa, Adsorption behaviors of proteins on hydroxyapatites with various particle morphologies, *Archives of BioceramicsResearch*, 2010, **10**, 153-156.
6. M. Aizawa, A. E. Porter, S. M. Best and W. Bonfield, Ultrastructural observation of single-crystal apatite fibres, *Biomaterials*, 2005, **26**, 3427-343.