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Electronic microscopy analysis of HAP single crystals prepared by hydrothermal method①

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Abstract: Hydroxyapatite(HAP, $Ca_{10}(PO_4)_6(OH)_2$) is one of the quite important bone implant materials. The hydroxyapatite crystals were synthesized under hydrothermal condition. The specimen was verified to be HAP crystal by the X-ray powder diffractometry(XRD). Then the specimen was distinguished single crystal from polycrystal by the use of the transmission electron microscope(TEM). The diffraction pattern of the specimen is neatly arranged diffraction spots, that verified the crystals were single crystals. The interplanar distance d calculated from diffraction spot is coincided with that of HAP's JCPDS card. Moreover, crystal face angles calculated from crystal face index are coincided with the values by measuring on the pattern. The HAP crystals are needle-like in shape with about 3 μm in diameter and 180 μm in length. Most of the crystals are separate whiskers. Their length/diameter ratio ranges from 40 to 100. The average ratio is about 60.

Key words: hydroxyapatite; single crystal; hydrothermal method; diffraction pattern
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1 INTRODUCTION

Hydroxyapatite(HAP, $Ca_{10}(PO_4)_6(OH)_2$), with its high biocompatibility and bioactivity, is one of the quite important bone implant materials. From the 1970s, HAP ceramics and HAP composite materials have been widely used for repairing bone defects and filling materials of plastic etc[1].

The strength of single crystal approaches theoretical strength. It is about 1 000 times higher than that of polycrystal of the same material[2]. Owing to their high strength, well crystallized HAP single crystals could be extensively used as the reinforcement for biomaterials.

The hydrothermal method is a method in which reactions take place in a hermetic container that can provide conditions of high temperature and high pressure when water is used as a reaction media[3]. Under such conditions, insoluble or unsolvable materials can be dissolved and re-crystallized. Single crystals, powder, fibers or whiskers can be prepared by the hydrothermal method. They may be ultra-fine and well-crystallized materials without coacervation so that they could be used as the reinforcement materials. Studies of hydroxyapatite single crystals preparation under hydrothermal condition began in the early 1990 s. In 1990, Itatani et al[4] prepared carbonate-containing hydroxyapatite whiskers by hydrothermal method. One year later, Yoshimura et al[5] reported that needle-like HAP was prepared through $Ca(OH)_2$ and H_3PO_4 under hydrothermal condition of 200 ℃, 2 MPa for 5 h. The largest ratio of length to diameter is 11. In 1993, Fujishiro et al[6] found needle-like hydroxyapatite formed when HAP powder prepared by homogenous precipitation method was treated under hydrothermal condition. Hydoxyapatite whiskers with 20－30 μm in length and 0.1－1.0 μm in diameter can be synthesized by β-tricalcium phosphate when citric acid was used as additive under 200 ℃ and 2 MPa. When HAP suspension was used as the initial material and citric acid was used as the additive, HAP whiskers with 10－30 μm in length and 0.5 μm in width can be obtained at 180－220 ℃[7]. In 1996, Atsuo et al[8] prepared carbonate-containing HAP single crystals by hydrothermal method.

This paper describes the process of HAP single crystals preparation under hydrothermal condition. The specimens were identified by X-ray powder diffractometry(D/MAX ⅢA, Rigaku Ltd, Japan), scanning electron microscope(SX-40, Akashi Seisakusho Ltd, Japan), transmission electron microscope(H-600STEM/EDX DV9100, Hitachi Co. Japan) and infrared spectroscopy(60SXB, Nicolet Co. Ltd, America).

2 EXPERIMENTAL

Analytically pure calcium nitrate 4-hydrate and diammonium hydrogen orthophosphate were used as the initial materials. They were dissolved in the third-distilled water(whose resistance is about 17 MΩ/cm, magnesium

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ions are removed because it may inhabit HAP crystals growth[9]). Their concentrations were 0.028 mol·dm^{-3} and 0.025 mol·dm^{-3}. The pH value of the solution was adjusted to a given one. The solution must be clear. A certain amount of carbomide was used as additive. 300 mL of modified mixture solution was poured into a quartz glass tube whose volume is 400 mL. Then the tube with a glass cover was put in a hermetic vessel, whose height/diameter ratio is 12(Fig. 1). The vessel was heated to 150 ℃ fastly. It was maintained about 30 min. Then the top, middle and bottom part of the vessel were respectively heated to 165, 220 and 270 ℃ at heating rate of 0.2 ℃/min. It was kept for 120 h. Finally the heating power was turned off. The vessel was opened when it was cooled down to the room temperature.

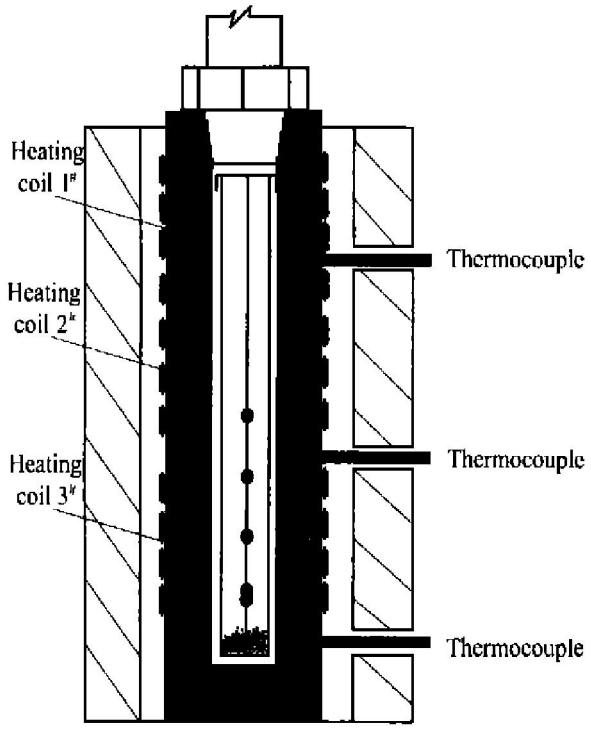

Fig. 1 Skematic of experimental apparatus

The product was filtered quickly using a suction filter, washed by distilled water and anhydrous ethanol and dried at 60 ℃ in vacuum. The product was identified by XRD and IR. SEM is taken to survey morphology of HAP. HAP crystal was also studied by electron diffraction of TEM when the specimen was scattered in distilled water.

3 RESULTS AND DISCUSSION

3.1 XRD analysis of specimen

Fig. 2 shows the XRD spectrum of the specimen. The interplanar spacing d shown in Fig. 1 is accurately coincided with that of HAP's JCPDS card(9-432). To compare Fig. 2 with the card, it can also be found that the relative condensity in figure is almost consistent with that in the card 9-432. On these grounds we can preliminary identified the specimen with hydroxyapatite crystal.

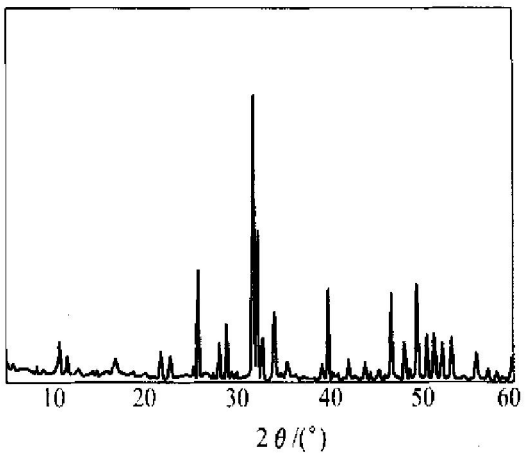

Fig. 2 XRD spectrum of product

3.2 IR spectrum analysis of specimen

Fig. 3 shows IR spectrum of the specimen. Hydroxyapatite has two kinds of anion group, the PO_4^{3-} group and the OH^- group. The spectrum shows bands of phosphate at 474, 570, 602, 962, 1 046 and 1 090 cm^{-1} as well as those of hydroxyl at 631 and 3 572 cm^{-1}. The IR spectrum presents further evidence to identify the product with hydroxyapatite.

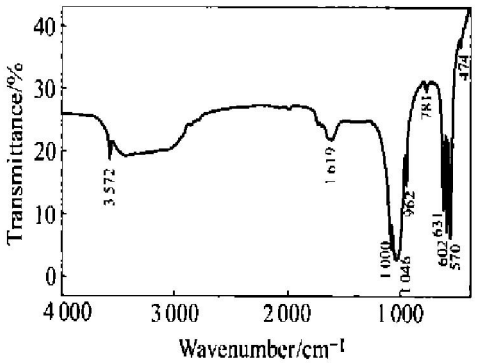

Fig. 3 IR spectrum of product

3.3 Chemical analysis of specimen

It is easy to understand that the specimen only contains CaO, P_2O_5 and H_2O. The following table gives oxide composition of the product. The result indirectly confirms that specimen is HAP, yet it also bears out that of obtained hydroxyapatite is slightly lower than that of standard stoichiometric HAP.

Table 1 Chemical compositions of specimen

Sample No.	x(CaO)/%	x(P_2O_5)/%	x(Ca):x(P)
1#	54.03	42.58	1.61
2#	54.99	42.72	1.62

3.4 Qualitative analysis of single crystal

The qualitative analysis above verified the product was hydroxyapatite crystal, but what we expect is to gain HAP single crystals, that is, the specimen should not only be HAP crystal but also be single crystal whose strength is much higher.

When polycrystal specimen is analyzed by electron diffraction, lots of little crystal grain orientations are different. Once an interplanar distance d of a certain crystal plane group $\{hkl\}$ matches the diffraction requirements, a cone face of diffraction beam formed. The incident ray is axis of the cone and 2 θ is semiangle. The intersection line of the cone face with photo film is a circle ring with $R = L\lambda/d$ in radius. Crystal plane groups with different d produce rings in different radius, so the diffraction pattern of polycrystal is a series of diffraction rings whose centers are the same. The authors confirmed the diffraction pattern of HAP polycrystal was concentric circles[10].

As far as single crystal is concerned, a crystal plane group $\{hkl\}$ orientation is the same in general, so it forms a diffraction spot in case of matching the diffraction requirements. Lots of crystal groups in different interplanar distances form a series of diffraction spots that are arranged in a certain geometric figure.

Fig. 4 shows electron diffraction pattern of the specimen. Diffraction spots are regularly arranged rectangle with center. Fig. 5 shows diagram for calculation. When diffraction happens, the diffraction condition is satisfied, that is:

$$d = L\lambda/R \qquad (1)$$

where $L\lambda = k$, it is the camera constant. R is the distance between the calibration spot and the crystal plane (000) (the brightest spot).

The camera constant k was 31.4 when the specimens were tested. R was directly measured from the figure. A basic parallelogram was selected: the shorter couple of laterals is R_1, the other one is R_2. The angle between R_1 and R_2 must be no more than 90°. The following parameters are gained when distances between the brightest spot and other three spots of the parallelogram are measured: $R_1 = 6.6$ mm, $R_2 = 9.1$ mm, $R_3 = 11.2$ mm. Put k and R into Eqn. (1), d is gotten. $d_1 = 4.76$, $d_2 = 3.45$, $d_3 = 2.80$. The calculation results of d are accordingly consistent with those of the HAP's JCPDS card. ($d_{10} = 4.72$, $d_{20} = 3.44$, $d_{30} = 2.778$)

$$\Delta d_1/d_{10} = (4.76 - 4.72)/4.72 < 0.03$$

$$\Delta d_2/d_{20} = (3.45 - 3.44)/3.44 < 0.03$$

$$\Delta d_3/d_{30} = (2.80 - 2.778)/2.778 < 0.03$$

Crystal face indexes of three diffraction spots are (110), (002), (112) according to the d value. Obviously they meet the relationship: $\boldsymbol{h}_3 = \boldsymbol{h}_1 + \boldsymbol{h}_2$, $\boldsymbol{k}_3 = \boldsymbol{k}_1 + \boldsymbol{k}_2$, $\boldsymbol{l}_3 = \boldsymbol{l}_1 + \boldsymbol{l}_2$, so there is no need to change the signs of indexes h, k and l.

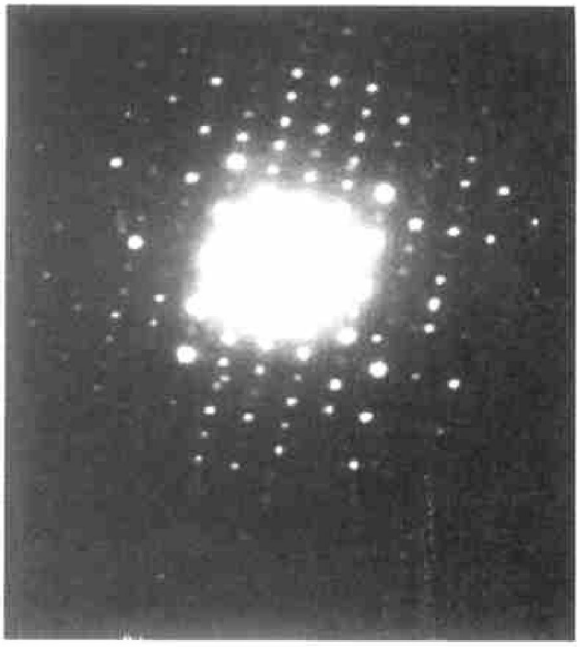

Fig. 4 Diffraction pattern of specimen

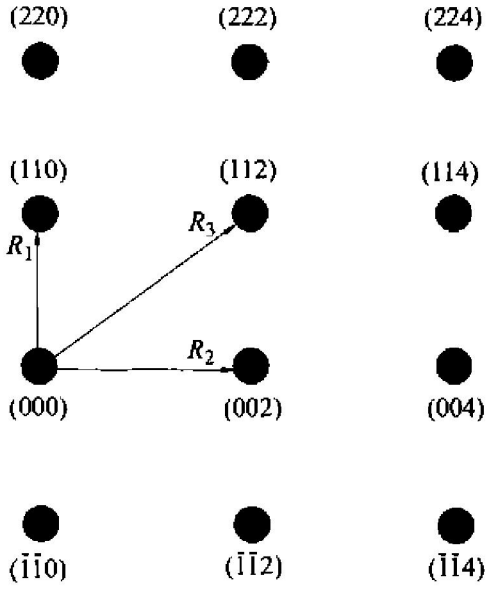

Fig. 5 Calculation diagram (2: 1)

Hydroxyapatite belongs to hexagonal system. $a = 0.943$ nm, $c = 0.688$ nm[11]. According to the formula of crystal face angle(Eqn. (2) as an example)[12], the angle $\alpha_{1,2}$, $\alpha_{1,3}$ and $\alpha_{2,3}$ can be calculated.

$$\cos\alpha_{1,2} = [h_1h_2 + k_1k_2 + (h_1k_2 + h_2k_1)/2 + \frac{3a^2}{4c^2}l_1l_2]/[(\sqrt{h_1^2 + k_1^2 + h_1k_1 + \frac{3a^2}{4c^2}l_1^2}) \times (\sqrt{h_2^2 + k_2^2 + h_2k_2 + \frac{3a^2}{4c^2}l_2^2})] \qquad (2)$$

Based on the calculated $\cos\alpha_{1,2} = 0$, $\cos\alpha_{1,3} = 0.5894$, $\cos\alpha_{2,3} = 0.8078$, the angle $\alpha_{1,2} = 90°$, $\alpha_{1,3} = 54°$, $\alpha_{2,3} = 36°$ an be determined. All of three are of great consistency with diffraction figure. This gives a fur-

ther proof that the obtained specimen is hydroxyapatite single crystal.

3.5 Morphology analysis of specimen

Seen from the SEM image (Fig. 6), most of the specimens are separated single crystals. They are needle-like, whose length ranges from 80 to 300 μm while the average length is about 180 μm. The width ranges from 1 μm to 8 μm. The ratio of length/width lies in the range from 40 to 100. The average ratio is 60.

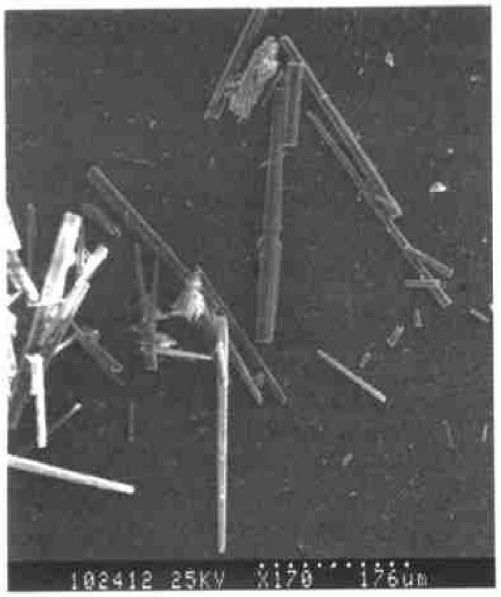

Fig. 6 SEM image of product

From Fig. 7, it can be seen that the crystal has its neat border. Its cross section is regular hexagon in shape. It is well crystallized and distributes separately.

Fig. 7 SEM image of product

4 CONCLUSIONS

Needle-like hydroxyapatite single crystals with high crystallinity can be batch-bulk prepared under hydrothermal condition. The shape of crystals are uniform. They are about 180 μm long and 3 μm wide. Most of the crystals are separate single crystals. The interplanar distance d calculated from diffraction pattern is closely coincided with that of HAP's JCPDS card. Moreover, crystal face angles calculated from crystal face indexes, which are calibrated from diffraction pattern, are consistent with the value by measuring on the pattern. The products are well-crystallized HAP single crystals.

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