

Morphologically Controlled Fibrous Spherulites of an Apatite Precursor Biocrystal

Katsuya Teshima,^{*,†} Mitsuo Sakurai,[†] SunHyung Lee,[‡] Kunio Yubuta,[§] Shun Ito,[§] Takaomi Suzuki,^{†,||} Toetsu Shishido,[§] Morinobu Endo,^{||,⊥} and Shuji Oishi^{†,||}

Department of Environmental Science and Technology, Faculty of Engineering, Shinshu University, Nagano 380-8553, Japan, Faculty of Engineering, Shinshu University, Nagano 380-8553, Japan, Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan, Institute of Carbon Science and Technology, Shinshu University, Nagano 380-8553, Japan, and Department of Electrical and Electronic Engineering, Faculty of Engineering, Shinshu University, Nagano 380-8553, Japan

Received July 21, 2008; Revised Manuscript Received November 10, 2008

ABSTRACT: Unique and characteristically formed spherulitic crystals of octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) were grown for the first time by the gel method, using the system $\text{Ca}(\text{NO}_3)_2-(\text{NH}_4)_2\text{HPO}_4$ -agar. The shape of test tubes crucially affected the morphological development of the OCP crystals. The feature of OCP spherulites grown in U-type test tubes was an aggregation of a relatively ordered, large number of ultralong and flexible whiskers radiating from the center region of the spheres, resulting in high porosity. On the other hand, OCP crystals grown in straight test tubes were a spherulite consisting of a large number of short plate-like, well-faceted crystallites radiating from a common origin. In both cases, OCP crystals grew with spherulitic morphologies even under varied conditions of different growth parameters, including gel concentration, reactant concentration, and growth period.

The hard tissues of the body comprise bone, teeth, and so forth, and are composed of inorganic materials and organic molecules which are in intimate association with each other.^{1,2} The elucidation of the role of macromolecules, such as collagen and proteins, in the nucleation, crystallization, aggregation, and phase transformation of calcium phosphate biominerals in normal and pathological mineralization has been of special interest. Among these biominerals, the crystal structure of octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, $\text{Ca}/\text{P} = 1.33$) is composed of the intercalation of two layers, that is, apatitic and hydrated layers.^{3,4} OCP, therefore, has been suggested to participate in mineralized tissue formation as an initial precursor phase.^{5,6} Here, we report the growth of morphologically controlled OCP crystals in naturally derived gel matrices. Unique and characteristically formed spherulitic crystals of OCP were grown for the first time by the gel method, using the system $\text{Ca}(\text{NO}_3)_2-(\text{NH}_4)_2\text{HPO}_4$ -agar. The feature of OCP spherulites grown in U-type test tubes was an aggregation of a relatively ordered, large number of ultralong and flexible whiskers radiating from the center region of the spheres, resulting in high porosity. OCP spherulites will be favorable for various technological applications, such as biomaterials for a precursor of mineralized tissue and industrial materials for various adsorbents, sensors, or separators.^{7,8}

Calcium phosphate biominerals are usually produced by precipitation in solution,^{5,6} and a few studies^{9,10} have been reported on the gel growth of these biominerals. In our previous study, OCP crystals were grown by a gel method via silica matrices.⁹ They were milky white and spherical in shape with short plate-like crystals radiating from a central nucleus (Figure 1). In this case, the formation of OCP crystals was accomplished by the reaction between phosphate and calcium ions in silica gel matrices. The silica gel was prepared by neutralization of an aqueous solution of sodium metasilicate. Furthermore, the gel itself contained the phosphate ions derived from $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (inner reactant). The gels were prepared in standard straight test tubes. Calcium salt solution (outer reactant) was put on the top of the gels, and calcium ions were allowed to diffuse. Finally, over a period of typically a

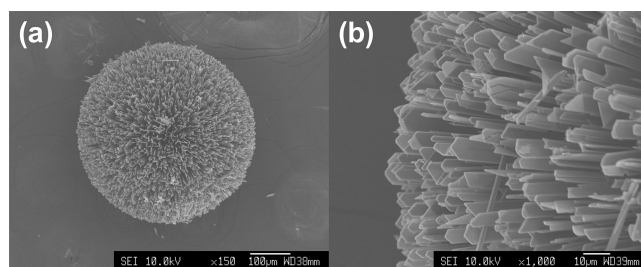


Figure 1. SEM images showing spherulitic morphology of the OCP crystal grown in a straight type test tube. (a) Low and (b) high magnification. This higher magnification shows short plate-like crystals composing the spherulite of highly crystalline OCP crystals.

few days, crystals of visible size grew in these silica matrices as mentioned above (Figure 1). The purpose of this study is the growth of morphologically controlled OCP crystals in naturally derived gel matrices. Original U-type test tubes are used as a reaction field to control Ca^{2+} and PO_4^{3-} ion diffusion, because many crystal faces can be affected simultaneously by changes in solution conditions.

All experiments on the growth of OCP crystals have been carried out in agar gel matrices by employing a U-type test tube. Thermoreversible, naturally derived agarose gels [$(\text{C}_{12}\text{H}_{14}\text{O}_5(\text{OH})_4)_n$, Wako Pure Chemical Industries, Ltd.] were prepared by heating the aqueous suspension of distilled water and the gelling substance to 90 °C in a hot-water bath until clear solutions were obtained. Upon rapid cooling to 0.5 °C in a cold water bath, the solutions formed a gel. A total of 0.18–0.6 g of agarose was dissolved in 60 mL of distilled water so as to have a gel concentration of 0.3–1.0 mass%. As sources of calcium ions and phosphate ions, aqueous solutions of reagent-grade $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$, respectively, were used. After the gel had set, Ca^{2+} ion solution (20 mL, 0.0625–1.00 M) was placed on top of the gel without damaging its surface. Likewise, PO_4^{3-} ion solution (20 mL, 0.0375–0.60 M) was poured on the other side. To compare various effects of ion diffusion, straight-type test tubes were also used for OCP crystal growth. Crystal growth in all cases occurred at 37 °C in an incubator. Over a period of typically a few days, crystals of visible size grew in these agar gel matrices. The crystal products, 5–40 days later, were separated from the gel in warm water. The obtained crystals were investigated by means of X-ray diffraction

* Corresponding author. E-mail: teshima@gipwc.shinshu-u.ac.jp.

[†] Department of Environmental Science and Technology, Shinshu University.

[‡] Faculty of Engineering, Shinshu University.

[§] Tohoku University.

^{||} Institute of Carbon Science and Technology, Shinshu University.

[⊥] Department of Electrical and Electronic Engineering, Shinshu University.

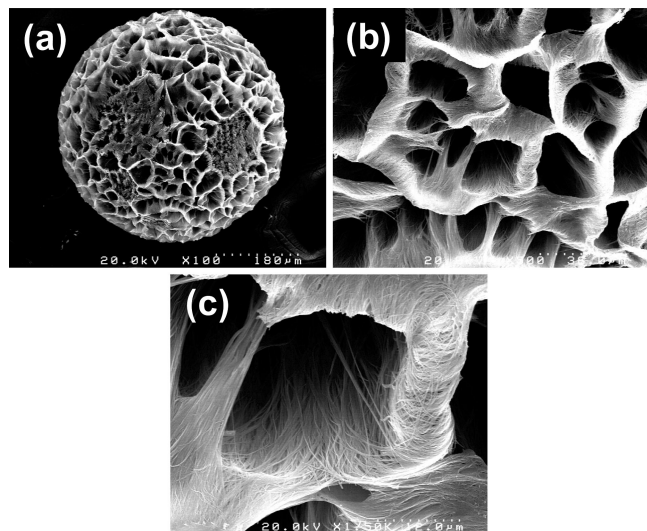


Figure 2. SEM images showing characteristic spherulitic morphology of the OCP crystal grown in a U-type test tube. (a) Overview and (b, c) higher magnification images. The higher magnification shows ultralong and flexible whiskers composing the spherulite of highly crystalline OCP crystals.

(XRD, SHIMADZU, XRD-6000) and Fourier transform infrared spectroscopy (FTIR, JASCO, FT/IR-6100), and observed by use of an optical microscopy and field emission scanning microscopy (FESEM, HITACHI, S-5000 & JEOL, JSM-7000F). The high-resolution transmission electron microscopy (HRTEM, JEOL, JEM-2010) and electron diffraction observations were carried out with JEM-2010 and JEM-2000EXII (JEOL) instruments operated at 200 kV to analyze the crystallinity and elongated direction of the grown crystals.

In the U-type test tube experiment, unique microstructured OCP crystals were successfully grown for the first time in agar gels. The grown crystals were milky white and spherical in shape. They were identified as OCP by their powder XRD patterns (Supporting Information), using literature data,¹¹ and by their FTIR spectra. The characteristic diffraction line, which is evidence of OCP growth, was clearly observed at $d = 18.7 \text{ \AA}$ ($2\theta = 4.7$) in the XRD patterns. The typical OCP crystal grown in the U-type agar gel is shown in Figure 2 (growth period = 20 days). OCP crystal balls, up to approximately $500 \mu\text{m}$ in diameter, were grown in the agar gel matrices. The crystal forms and sizes were dependent on various growth conditions, such as the growth period, the solution concentration, and the gel matrices. As clearly shown in Figure 2, the OCP spherulite is an aggregation of numerous dense, ultra long, flexible, relatively ordered whiskers. The OCP crystals grown in the U-type tubes (Figure 2) were more porous than those grown in the straight tubes (Figure 1), since there were many pores on the surface of the sphere, and each wall of the pores consisted of fibrous crystals. In general, each wall of pores that exist in various porous materials is relatively smooth.^{12,13} Furthermore, the grown crystal was found to be spherical in shape with whiskers radiating from the center region of the spherulites (cross-sectional SEM observation). The OCP spherulites appear to result from aggregation of ultralong and flexible whiskers orienting themselves in a spherical envelope. Here, whisker is a needle-shaped single crystal with mostly theoretical strength due to their perfect geometry, not fiber.

Figure 3a shows a bright field TEM image and the corresponding selected area diffraction (SAD) pattern of a typical crystal. The lattice image (Figure 3b) of a typical whisker was taken from the area indicated by the arrow in Figure 3a. Pulverized crystallites were used for the TEM observations. The SAD pattern indicated that its crystal plane of the whisker is confirmed to be in accordance with a OCP structure, an apatite-type columnar structure. The elongated direction clearly corresponded to the $\langle 0001 \rangle$ directions.

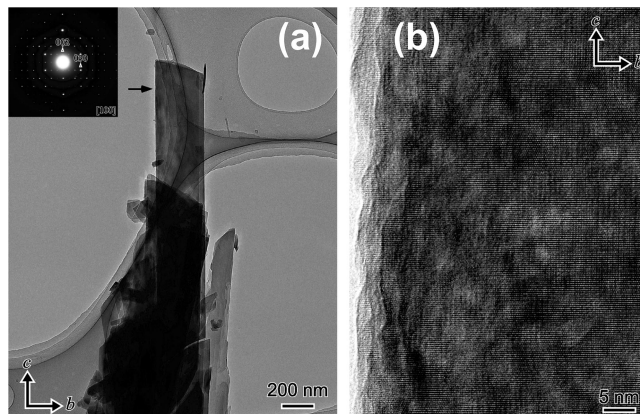


Figure 3. (a) Bright field TEM micrograph and selected area diffraction pattern and (b) lattice image of a typical OCP whisker.

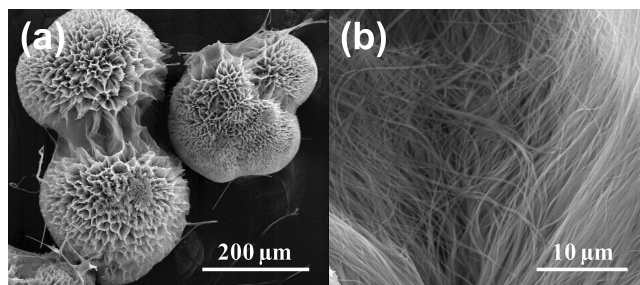


Figure 4. SEM images showing spherical complexes which consist of relatively small OCP crystals. (a) Low and (b) high magnification.

For apatite-type crystals, the $\{10\bar{1}0\}$ face is more stable than the $\{0001\}$ or the $\{10\bar{1}1\}$ end faces and the crystal elongates predominantly in the $\langle 0001 \rangle$ directions.^{14,15} In Figure 3a, the existence of some facets is evidently at the upper region. The whisker was of very good crystallinity because no defects were observed in this lattice image.

Figure 4 shows spherical complexes which consisted of relatively small OCP crystals (growth period = 5 days). Here, unique-textured complexes are, in particular, shown in this paper. Since the growth period of the crystals shown in Figure 4 is much shorter than that shown in Figure 2, their crystal structure was not sufficiently developed; however, the complexes were obviously composed of ultralong and flexible whiskers. In Figure 4, each whisker diameter (width) of 5 days growth was approximately 100 nm and was almost the same as that of 20 days growth. It was clearly found that an isotropic growth of OCP whiskers occurred in the agar gel matrices. In an interface between both of individual OCP spherulites, ultralong and flexible whiskers were completely intertwined with each other. These aggregated spherulites, therefore, could not be separated at all, and had a very unique structure. In the viewpoint of aggregation, OCP whiskers and various matrices (e.g., collagen, cellulose, and porous materials) are thought to be able to form highly complicated composites.

In conclusion, the growth of octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) crystals was accomplished by allowing diffusion of calcium and phosphate ions through naturally derived agar gel matrices. OCP crystals, which were up to approximately $500 \mu\text{m}$ in diameter and milky white, grew as spherulitic morphologies even under various growth conditions, including gel concentration, reactant concentration, and gel age. The shape of test tubes greatly influenced morphological development of OCP crystals. The form of OCP crystals grown in the straight tubes was spherulite consisting of a large number of short plate-like, well-faceted crystallites radiating from a common origin, whereas usage of the U-type tubes modified the feature of OCP spherulites drastically. The charac-

teristic and unique feature of OCP spherulites grown in the U-type tubes was an aggregation of numerous ultralong and flexible whiskers radiating from the center region of the spheres. While the feature of the spherulites grown in the U-type tubes was dependent on the growth period, it was not so in the case of the usage of straight tubes.

Acknowledgment. This research was supported by a Grant-in-Aid for Young Scientists (B) (No. 19760466) from the Ministry of Education, Culture, Sport, Science and Technology. This research was partially supported by CLUSTER (the second stage) of Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: XRD patterns are available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Lowenstam, H. A. *Science* **1981**, *211*, 1126–1131.
- (2) Busch, S.; Dolhaine, H.; DuChesne, A.; Heinz, S.; Hochrein, O.; Laeri, F.; Podebrad, O.; Vietze, U.; Weiland, T.; Kniep, R. *Eur. J. Inorg. Chem.* **1999**, 1643–1653.
- (3) Brown, W. E. *Nature* **1962**, *196*, 1048–1050.
- (4) Mathew, M.; Takagi, S. *J. Res. Natl. Inst. Stand. Technol.* **2001**, *106*, 1035–1044.
- (5) Bigi, A.; Boanini, E.; Walsh, D.; Mann, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 2163–2166.
- (6) Ngankam, P. A.; Lavalle, Ph.; Voegel, J. C.; Szyk, L.; Decher, G.; Schaaf, P.; Cuisinier, F. J. G. *J. Am. Chem. Soc.* **2000**, *122*, 8998–9005.
- (7) Graham, S.; Brown, P. W. *J. Cryst. Growth* **1996**, *165*, 106–115.
- (8) Suzuki, O.; Kamakura, S.; Katagiri, T.; Nakamura, M.; Zhao, B.; Honda, Y.; Kamijo, R. *Biomaterials* **2006**, *27*, 2671–2681.
- (9) Teshima, K.; Takano, A.; Suzuki, T.; Oishi, S. *J. Soc. Inorg. Mater. Jpn.* **2005**, *12*, 335–337.
- (10) Ohta, M.; Tsutsumi, M. *J. Cryst. Growth* **1982**, *56*, 652–658.
- (11) ICDD PDF 26-1056.
- (12) Vlasov, Y. A.; Bo, X. Z.; Sturm, J. C.; Norris, D. J. *Nature* **2001**, *414*, 289–293.
- (13) Lee, W.; Lee, J. K. *Adv. Mater.* **2002**, *14*, 1187–1190.
- (14) Oishi, S.; Michiba, N.; Ishizawa, N.; Rendon-Angeles, J. C.; Yanagisawa, K. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2097–2101.
- (15) Suzuki, T.; Kumeda, I.; Teshima, K.; Oishi, S. *Chem. Phys. Lett.* **2006**, *421*, 343–347.

CG800791S