J Mater Sci (2006) 41:6134–6137
DOI 10.1007/s10853-006-0444-6

LETTER

Fabrication of high-dispersibility nanocrystals of calcined hydroxyapatite

Masahiro Okada · Tsutomu Furuzono

Received: 7 October 2005 / Accepted: 10 May 2006 / Published online: 12 August 2006

Hydroxyapatite (HAp) is a major inorganic component of bone and teeth. Artificially synthesized HAp has been extensively used in a variety of applications, such as biomaterials, ion exchangers, adsorbents, and catalysts, by exploiting its biocompatibility and adsorbability of many compounds. When low-crystallinity HAp nanoparticles are calcined to increase thermal and chemical stability, the particles typically sinter into a large agglomerate consisting of polycrystal [1–5]. Thus, calcined HAp crystals dispersed in liquid medium on a nanoscale have been difficult to obtain. This paper describes the fabrication of HAp nanocrystals by calcination with an anti-sintering agent interspersed between the particles and the subsequent removal of the agent. The HAp nanocrystals obtained here should be suitable for the above applications owing to their high dispersibility in liquid media, high specific surface area, and high thermal and chemical stability.

We have recently developed a novel inorganic/organic composite for a soft-tissue-compatible material: a flexible silicone elastomer [6] or a silk fibroin [7], whose surface was modified with calcined HAp crystals. After the HAp crystals dispersed in a liquid medium were adsorbed on the substrate, chemical reaction at their interface between the HAp crystals and the substrate was conducted to connect them through covalent bonding. The novel composite retained flexibility of the polymer substrate and showed improved tissue adhesion with the HAp crystals on the surface [8]. Throughout these studies, the HAp crystals were used after calcination at 800 °C to reduce in vivo absorbability. As mentioned above, HAp nanoparticles mostly sinter into large agglomerates of polycrystals during calcination. This made it difficult to control the surface morphology of the composite because the agglomerates had poor dispersibility in liquid media and large size distribution.

Hydrothermal treatment of HAp particles in water medium under high pressure is known to enable the preparation of agglomerate-free HAp crystals [9–11]. However, this treatment generally leads to an increase in crystal size due to Ostwald ripening [12, 13], and is restricted to laboratory-scale products as it is a high-pressure process.

The present study reports the fabrication of nanosized and calcined HAp crystals protected against calcination-induced sintering using an anti-sintering agent interspersed between the particles. Thus, there was no contact between the crystals during calcination. Calcium hydroxide [$Ca(OH)_2$] was selected as an anti-sintering agent because it would not melt at the calcination temperature (800 °C), presumably not dissolve HAp, and could be removed by washing with water after calcination.

Starting HAp particles with low crystallinity were prepared with a modified emulsion system at 25 °C [14]. The resulting product was centrifugally washed and redispersed in water (solid content: 5 wt%). In order to intersperse $Ca(OH)_2$ between the particles, the HAp aqueous dispersion was added into a saturated aqueous $Ca(OH)_2$ solution (0.17 wt%), and the mixture was dried under reduced pressure at 40 °C. The resultant

---

M. Okada · T. Furuzono (✉)
Department of Bioengineering, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan
e-mail: furuzono@ri.ncvc.go.jp

HAp/$Ca(OH)_2$ (1/1, w/w) mixture was calcined at 800 °C for 1 h in air (heating rate: 10 °C/min). After calcination, the mixture was centrifugally washed with water to remove the $Ca(OH)_2$. As a control procedure, the same starting HAp particles were calcined without adding $Ca(OH)_2$.

First, in order to examine the effect of $Ca(OH)_2$ on the crystal phase and composition of HAp, X-ray diffraction (XRD; RAD-X, Rigaku International Co., Tokyo, Japan) with CuKα radiation and Fourier-transform infrared (FTIR) spectroscopy (Spectrum One, Perkin-Elmer Inc., MA, USA) were performed as shown in Fig. 1. In Fig. 1a and b, both XRD profiles showed highly crystalline HAp, and no other calcium phosphate phases could be detected. In the FTIR spectrum of HAp calcined with $Ca(OH)_2$ shown in Fig. 1d, a peak at 3,640 cm^{-1} due to stretching of OH in $Ca(OH)_2$ was not observed, indicating complete removal of $Ca(OH)_2$. Bands at 877 and 1,413/1,456 cm^{-1} observed in both the FT-IR spectra are attributed to CO_3^{2-} substituting phosphate positions in HAp lattice [15], and came from atmospheric carbon dioxide under high solution pH during the preparation of the starting HAp. A new peak at 3,544 cm^{-1} in Fig. 1d seems to be due to the formation of calcium-rich apatite (Ca/P atomic ratio > 1.67) [16]. It is worth pointing out that the Ca/P atomic ratio of the HAp calcined with $Ca(OH)_2$ (Ca/P = 1.58) slightly increased as compared with that without additives (Ca/P = 1.56) measured by inductively coupled plasma-atomic emission spectrometry (SPS4000, Seiko Instrument Inc., Chiba, Japan). These results might suggest the formation of calcium-rich apatite from the crystal surface during calcination, by migration of calcium ions from $Ca(OH)_2$ on the calcium-deficient crystal.

The HAp crystals were observed by scanning electron microscopy (JSM-6301F, JEOL Ltd., Tokyo, Japan) and transmission electron microscopy (JEM-2000 EXII, JEOL Ltd.) as shown in Fig. 2. Single-crystal size (or grain size of polycrystal) was measured from the micrographs, and is presented as "mean ± SD" ($N = 100$). The crystal sizes were not statistically different between the HAp crystals calcined without and with $Ca(OH)_2$ (52.5 ± 15.4 nm for calcination without additives; 55.7 ± 15.1 nm for calcination with $Ca(OH)_2$). Although some agglomerates were observed in Fig. 2b, it is difficult to judge whether the agglomerates were polycrystals or not. This is because the micrograph was taken under dry condition and nanosized particles generally tend to gather due to a capillary force of the medium between the particles during the drying of the medium.

Therefore, the dispersibility of the HAp crystals was evaluated form the dispersed-particle size, which was measured in ethanol medium by dynamic light scattering (DLS; ELS-8000, Otsuka Electronics Co., Ltd., Kyoto, Japan) at 10-ppm concentration and a light-scattering angle of 90° (Fig. 3). In the case of calcination without

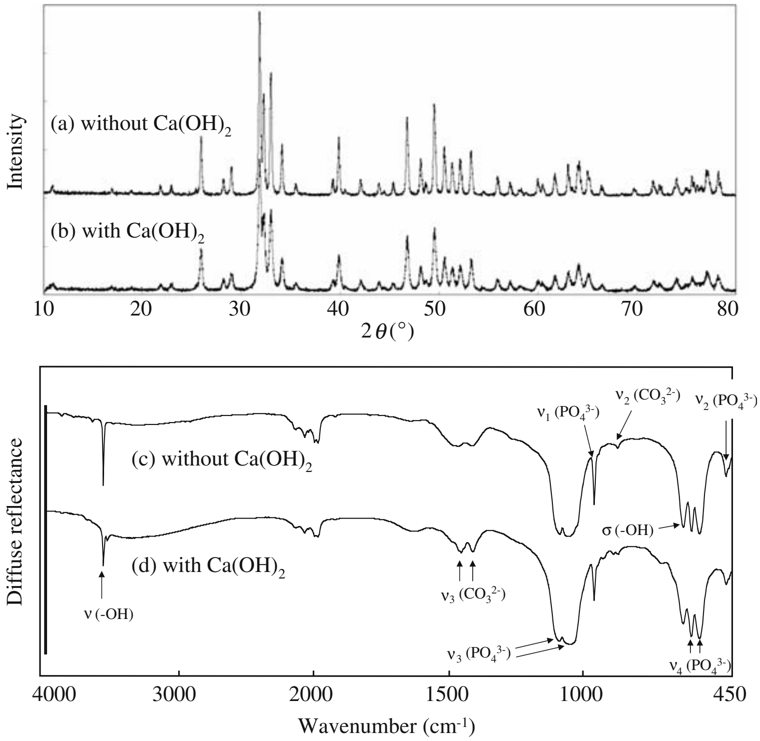

Fig. 1 X-ray diffraction patterns (**a**, **b**) and FTIR spectra (**c**, **d**) of hydroxyapatite (HAp) particles calcined at 800 °C for 1 h without (**a**, **c**) and with (**b**, **d**) $Ca(OH)_2$ (HAp/$Ca(OH)_2$ = 1/1 w/w) interspersed between the particles. $Ca(OH)_2$ was centrifugally washed off with an aqueous solution after calcination

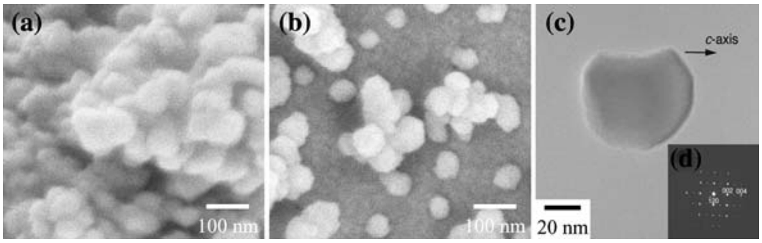

Fig. 2 SEM photographs (**a**, **b**) of HAp crystals calcined without (**a**) and with (**b**) $Ca(OH)_2$. A TEM photograph (**c**) and the associated electron diffraction pattern corresponding to the [210] zone (**d**) of a HAp crystal calcined with $Ca(OH)_2$, showing that the crystal consisted of a single HAp phase

additives, the dispersed-particle size (664.0 ± 382.0 nm) was much larger than the crystal size (52.5 ± 15.4 nm), which indicates the formation of polycrystals of sintered HAp. On the other hand, the dispersed-particle size of the HAp calcined with $Ca(OH)_2$ was 126.3 ± 79.0 nm. This indicates that sintering between the HAp nanocrystals can be mostly prevented by interspersing $Ca(OH)_2$ between the crystals prior to calcination.

However, the dispersed-particles size (126.3 ± 79.0 nm) was larger than the crystal size (55.7 ± 15.1 nm) measured by electron microscope. This suggest that some particles contacted with each other before the deposition of $Ca(OH)_2$ from the aqueous solution. In order to avoid the contact, poly(acrylic acid) (PAA) was used. PAA can act as a dispersion stabilizer by adsorbing on HAp surfaces [17, 18], and an addition of calcium ions into an aqueous PAA solution induces a rapid precipitation of poly(acrylic acid calcium salt) (PAA-Ca). Therefore, it is expected that PAA-Ca precipitates onto the separated HAp particle by addition of calcium ions into the PAA-adsorbed HAp particles. Although the organic component of PAA-Ca will be decomposed during calcination at 800 °C, the thermally decomposed product (CaO) remains on the crystals and presumably acts as an anti-sintering agent.

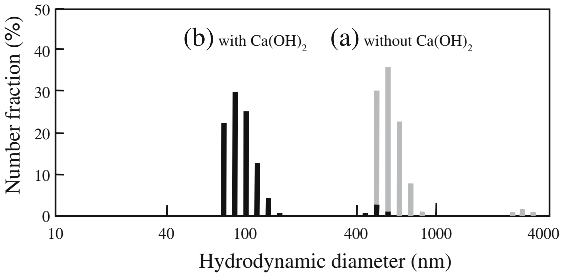

Fig. 3 The dispersed-particle sizes of HAp crystals calcined without (**a**) and with (**b**) $Ca(OH)_2$, measured in ethanol medium

In the present study, PAA (Sigma-Aldrich Co.; weight-average molecular weight = 15,000; HAp/PAA = 1/1 w/w) was added at pH 10, and $Ca(OH)_2$-saturated aqueous solution ($Ca(OH)_2$/COOH in PAA = 1/1 molar ratio) was added to precipitate PAA-Ca. The resultant HAp/PAA-Ca mixture was calcined in the same manner described above, and then washed with water to remove CaO.

The resultant HAp crystals are shown in Fig. 4. The dispersed-particle size of the HAp crystals in ethanol (45.0 ± 14.9 nm) was not statistically different from the crystal size (53.4 ± 16.2 nm) measure by electron microscope. This result indicates that HAp nanocrystals calcined with PAA-Ca can be dispersed as single crystals. IR spectrum and XRD pattern of the HAp single crystals calcined with PAA-Ca were almost the same as those shown in Fig. 1b and d, and Ca/P atomic ratio was 1.58. The achieved high dispersibility of HAp single crystals should be due to the absence of the calcination-induced sintering and might be due to cationic charge of the calcium-rich surface.

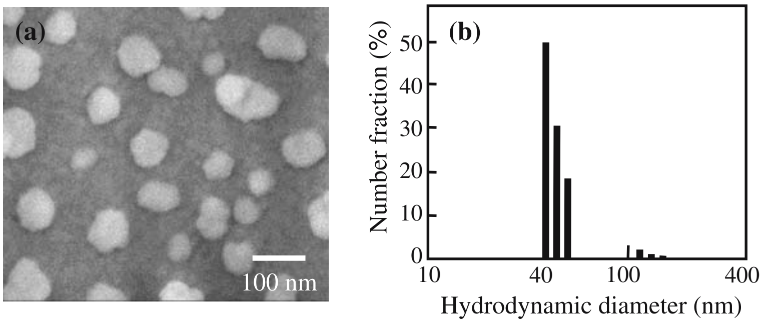

Fig. 4 A SEM photograph (**a**) and the dispersed-particles size (**b**) of HAp crystals calcined at 800 °C for 1 h with poly(acrylic acid calcium salt) (PAA-Ca) surrounding the particles. CaO, the thermally decomposed product of PAA-Ca, was centrifugally washed off with an aqueous solution after calcination

In summary, calcined HAp nanocrystals were successfully fabricated by calcination using an anti-sintering agent interspersed between or surrounding the particles, followed by removal of the agent. The HAp nanocrystals obtained here should be suitable for the various applications mentioned above, and also as dental and orthopedic ultrafine fillers for microporosity owing to their high dispersibility in liquid media and high thermal and chemical stability. Calcination with an anti-sintering agent has a potential application to a wide range of calcined nanoceramic powders, such as alumina, titania, and magnesia, and offer significant benefits over existing technologies because the technique is simple, inexpensive, and amenable to scale-up and processing.

Acknowledgments We thank Dr. K. Sato of the Advanced Manufacturing Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), for helpful discussions. This work was partially supported by a grant from PRESTO, Japan Science and Technology Agency, and a Research Grant for Cardiovascular Diseases from the Ministry of Health, Labour and Welfare, Japan.

References

1. Frenkel J (1945) J Phys USSR 9:385
2. Kuczynski GC (1949) Trans AIME 185:169
3. Barralet JE, Best SM, Bonfield W (2000) J Mat Sci Mater Med 11:719
4. Landi E, Tampieri A, Celotti G, Sprio S (2000) J Eur Ceram Soc 20:2377
5. Bernache-Assollant D, Ababoua A, Championa E, Heughebaertb M (2003) J Eur Ceram Soc 23:229
6. Furuzono T, Sonoda K, Tanaka J (2001) J Biomed Mater Res 56:9
7. Furuzono T, Kishida A, Tanaka J (2004) J Mater Sci Mater Med 15:19
8. Furuzono T, Wang P, Korematsu A, Miyazaki K, Oido-Mori M, Kowashi Y, Ohura K, Tanaka J, Kishida A (2003) J Biomed Mater Res B Appl Biomater 65B:217
9. Somiya S, Ioku K, Yoshimura M (1988) Mater Sci Forum 34–36:371
10. Yoshimura M, Suda H, Okamoto K, Ioku K (1994) J Mater Sci 29:3399
11. Papargyris AD, Botis AI, Papargyri SA (2002) Key Eng Mater 206–213:83
12. Carless JE, Foster AA (1966) J Pharm Pharmacol 18:697
13. Wei M, Ruys AJ, Milthorpe BK, Sorrell CC (1999) J Biomed Mater Res 45:11
14. Furuzono T, Walsh D, Sato K, Sonoda K, Tanaka J (2001) J Mater Sci Lett 20:111
15. Emerson WH, Fisher EE (1962) Arch Biol 7:671
16. Bonel G, Heughebaert J-C, Heughebaert M, Lacout JL, Lebugle A (1988) Ann NY Acad Sci 523:115
17. Misra DN (1993) J Dent Res 10:1418
18. Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, Lambrechts P, Vanherle G, Okazaki M (2001) J Dent Res 80:1565