Synthesis and in vitro evaluation of hydroxyapatite with controlled morphology

M Kamitakahara*, T Saito and K Ioku
Graduate School of Environmental Studies, Tohoku University
6-6-20 Aoba, Aramaki, Aoba-ku, Sendai, 980-8579, Japan

*E-mail: kamitaka@mail.kankyo.tohoku.ac.jp

Received 11 August 2011, Revised 16 November 2011

Abstract. Hydroxyapatite (HA) ceramics have been used as bone-repairing materials. Particles in HA ceramics show different properties depending on their compositions and morphologies. It is expected that the control of the composition and morphology contributes to improvement of biological properties of HA ceramics. In order to reveal the effect of the composition and morphology, the HA ceramics composed of rod-shaped or plate-shaped particles with calcium-deficient composition were prepared by the hydrothermal process. The in vitro evaluation, simulated body fluid (SBF) soaking test, was then conducted for these samples. The calcium-deficient HA ceramics composed of rod-shaped or plate-shaped particles were reacted with SBF and incorporated calcium and phosphate ions from SBF. This implies that these HA ceramics show high affinity to bone in the body.

1. Introduction

The technology which repairs the damaged bone is important because the damage in bones decreases the quality of life of the patient. Some ceramics are used for repairing the damaged bone. Hydroxyapatite (HA; Ca_{10}(PO_4)_6(OH)_2) is a main inorganic component of bone, and HA ceramics are focused as bone-repairing materials. HA ceramics can bond to bone directly and sintered HA ceramics have been clinically used as bone-repairing materials [1]. However, the little resorption [2] and low ability to stimulate bone formation of the sintered HA ceramics are problems. As the properties of particles in HA ceramics depend on their composition and morphology, it is expected that controlling the morphology and composition of HA particles would contribute to an improvement in the biological properties of HA ceramics. We previously prepared calcium-deficient HA ceramics composed of rod-shaped particles, which exposed the a-face preferentially, through a hydrothermal process. These ceramics stimulated bone regeneration and were resorbed completely in rabbit femora [3,4]. This regeneration is likely due to the calcium-deficiency of the composition and the exposure of a specific face of the HA crystal. Bioreosorbable calcium-deficient HA could be expected to contribute to the development of new biodegradable bone substitutes.

However, the knowledge about the effect of the composition and morphology of the HA has been insufficient and it is needed to reveal why calcium-deficient HA ceramics composed of rod-shaped particles show superior properties. In the present study, calcium-deficient HA ceramics composed of rod-shaped or plate-shaped particles were prepared by the hydrothermal process, and in vitro evaluation, simulated body fluid (SBF) soaking test [5], was conducted.
2. Materials and methods

2-1 Preparation of samples

The commercial available alpha-tricalcium phosphate (α-TCP, Ca$_3$(PO$_4$)$_2$, Taihei Chemical Industrial Co., Ltd. Japan) powder 0.10 g was pressed into a pellet 12 mm in diameter by uniaxial pressing. Three pellets were immersed in 30 cm$^3$ of an ammonia solution, whose pH was controlled at 11, in a Teflon®-lined sealed vessel. The vessel was kept at 160 °C for 24 h for the hydrothermal treatment. The sample was named Rod-HA.

In order to obtain the pellets composed of plate-shaped HA particles, octacalcium phosphate (OCP, Ca$_8$H$_2$(PO$_4$)$_6$·5H$_2$O) was used as a starting material. The OCP particles were obtained by the reaction of phosphoric acid and calcium carbonate in distilled water at 60 °C according to the method previously reported [6]. The obtained OCP powder 0.10 g was pressed into a pellet 12 mm in diameter by uniaxial pressing. Three pellets were immersed in 30 cm$^3$ of a nitric acid solution, whose pH was controlled at 4, in a Teflon®-lined sealed vessel. The vessel was kept at 160 °C for 24 h for the hydrothermal treatment. The sample was named Plate-HA.

The crystal phase and surface morphology of the obtained samples were examined by X-ray diffraction (XRD, RINT-2200VL, Rigaku Co. Japan) and scanning electron microscopy (SEM, S-4100, Hitachi Ltd. Japan), respectively. The Ca/P molar ratio of the products was determined based on a previously developed method [7,8]. The resultant HA particles were heated at 900 °C for 3 h to decompose the samples into a mixture of stoichiometric HA and β-TCP. We calculated the Ca/P molar ratio by determining the ratio of HA and β-TCP formed in the decomposition by XRD and then applying the calibration curve of HA and β-TCP [7].

2-2 In vitro test

To estimate the chemical reaction of the obtained HA samples in the body, the obtained pellets were soaked in a simulated body fluid (SBF) which has ion concentrations nearly equal to those of human blood plasma [5]. The ion concentrations are shown in Table 1. This solution does not contain proteins and cells, and is widely used to reveal the chemical reaction between the materials and the body fluid. The pellets were soaked in SBF with pH 7.40 for 3 d, and then removed from the SBF. The changes in the pellet surfaces were examined by SEM. The changes in the element concentrations of SBF were examined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, iCAP 6200, Thermo Scientific Inc, USA).

| Table 1. Ion concentrations of human blood plasma and simulated body fluid (SBF) | Concentration / mol·m$^{-3}$ |
|---|---|
| | Na$^+$ | K$^+$ | Mg$^{2+}$ | Ca$^{2+}$ | Cl$^-$ | HCO$_3^-$ | HPO$_4^{2-}$ | SO$_4^{2-}$ |
| Blood plasma | 142.0 | 5.0 | 1.5 | 2.5 | 103.0 | 27.0 | 1.0 | 0.5 |
| SBF | 142.0 | 5.0 | 1.5 | 2.5 | 147.8 | 4.2 | 1.0 | 0.5 |

3. Results and Discussion

Figures 1 and 2 show the XRD patterns and SEM photographs of the obtained pellets. The XRD patterns indicated that the crystal phase of both the pellets was HA. However, the morphologies of the particles which construct the pellets were different. For the sample Rod-HA which was prepared from α-TCP, rod-shaped HA particles were observed. It has been reported that the hydrothermal treatment of α-TCP provides the rod-shaped HA particles [9]. On the other hand, for the sample Plate-HA which was prepared from OCP, plate-shaped HA particles were observed. The morphology of the starting material, OCP particle, was plate-shaped and this plate-shaped morphology was maintained even after the hydrothermal treatment. This result is consistent with the previous study [10], in which plate-shaped HA was obtained by the hydrothermal treatment of OCP particles. The Ca/P molar ratios of Plate-HA and Rod-HA were 1.61 and 1.62, respectively. This indicates that the obtained HA samples have calcium-deficient compositions.
Figure 1. XRD patterns of the pellets.

Figure 2. SEM photographs of the surfaces of the pellets.

Figure 3 shows the SEM photographs of the pellets after soaking in SBF for 3 d. The changes by soaking in SBF were not observed from SEM observation under this magnification. However, the calcium (Ca) and phosphorus (P) concentrations of SBF were significantly decreased after soaking the Rod-HA and Plate-HA pellets. The Ca and P concentrations of SBF after soaking the pellets for 3 d are shown in Table 2. These imply that Ca-deficient HA ceramics composed of rod-shaped or plate-shaped particles were reacted with SBF and incorporated calcium and phosphate ions from SBF. It is speculated that the HA particles prepared by the hydrothermal treatment were highly reactive with SBF and small precipitates or crystal growth of the original HA particles occurred. It has been reported that the materials which bond to bone can form the HA layer on their surfaces in the body and bond to bone through this HA layer. This HA formation on the materials can be reproduced in SBF [5]. Therefore, the high reactivity of calcium-deficient HA ceramics composed of rod-shaped or plate-shaped particles implies that these ceramics show high affinity to bone in the body. The high reactivity of the calcium-deficient HA ceramics composed of rod-shaped particles in the body might be one of the reasons why the ceramics show superior properties in body.
4. Summary
Pellets composed of HA particles with controlled morphology and composition were prepared, and they were soaked in SBF to evaluate the behaviors under physiological conditions. Calcium-deficient HA ceramics composed of rod-shaped or plate-shaped particles were reacted with SBF and incorporated calcium and phosphate ions from SBF. This implies that these ceramics show high affinity to bone in the body.

5. Acknowledgement
This research was partially supported by JSPS KAKENHI (21300175). We are grateful for the experimental help of Profs. K. Tohji and N. Tsuchiya of Tohoku University.

6. References
[1] Hench L L 1991 J. Am. Ceram. Soc. 74 1487
[2] Hoogendoorn H A, Renooij W, Akkermans L M, Visser W and Wittebol P 1984 Clin. Orthop. Relat. Res. 187 281
[3] Okuda T, Ioku K, Yonezawa I, Minagi H, Gonda Y, Kawachi G, Kamitakahara M, Shibata Y, Murayama H, Kurosawa H and Ikeda T 2008 Biomaterials 29 2719
[4] Gonda Y, Ioku K, Shibata Y, Okuda T, Kawachi G, Kamitakahara M, Murayama H, Hideshima K, Kamihira S, Yonezawa I, Kurosawa H and Ikeda T 2009 Biomaterials 30 4390
[5] Kokubo T and Takadama H 2006 Biomaterials 27 2907
[6] Kamitakahara M, Okano H, Tanihara M and Ohtsuki C 2008 J. Ceram. Soc. Japan 116 481
[7] Ioku K, Murakami T, Ikuma Y and Yoshimura M 1992 J. Ceram. Soc. Japan 100 1015 [in Japanese].
[8] Ishikawa K, Duchene P and Radin S 1993 J. Mater. Sci.: Mater. Med. 4 165
[9] Ioku K, Kawachi G, Sasaki S, Fujimori H and Goto S 2006 J. Mater. Sci. 41 1341
[10] Kamitakahara M, Ito N, Murakami S, Watanabe N and Ioku K 2009 J. Ceram. Soc. Japan 117 385