Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 2152–2160
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Preparation of nanometer-scale rod array of hydroxyapatite crystal

Satoshi Hayakawa [a,b,*], Yaqi Li [a], Kanji Tsuru [a,b], Akiyoshi Osaka [a,b], Eiji Fujii [c], Koji Kawabata [c]

[a] Biomaterials Laboratory, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima, Okayama 700-8530, Japan
[b] Research Center for Biomedical Engineering, Okayama University, 3-1-1 Tsushima, Okayama 700-8530, Japan
[c] Industrial Technology Center of Okayama Prefecture, Haga, Okayama 701-1296, Japan

Received 3 August 2008; received in revised form 5 February 2009; accepted 10 February 2009
Available online 20 February 2009

Abstract

Fabrication of nano- or micro-structured scaffolds to mimic structural and three-dimensional details of natural bone or teeth has been the subject of much interest, and this study proposes a new strategy for self-assembling one-dimensional hydroxyapatite (HAp) nanorods into organized superstructures. A nanometer-scale rod array of HAp having preferred orientation to the *c*-axis was successfully prepared simply by soaking calcium-containing silicate glass substrates in Na_2HPO_4 aqueous solution at 80 °C for various periods. Those HAp rods grew perpendicularly to the glass surface, and the crystallites covered the glass surface uniformly, resulting in a "dental enamel-like" rod array structure consisting of "pine-leaf-like" structure units.

Keywords: Nano-texture; Hydroxyapatite; Self-assembly; Rod array; Dental enamel

1. Introduction

Fully developed mature dental enamel is a highly organized structure of enamel prisms that consist of bundles of nanorod-like hydroxyapatite (HAp) crystals arranged roughly parallel to each other [1–5]. The characteristic structure determines the unique physicochemical properties of the enamel [6–8]. Many attempts have been reported to fabricate nano- or micro-structured scaffolds to mimic structural and three-dimensional details of natural bone or teeth consisting of tiny HAp crystals in the nano-regime, in order to understand the natural biomineralization process and relationship between their unique structure and physicochemical properties [9–19]. However, no one has been successful in arranging such HAp nanocrystals to mimic the fine and complex structure of real bone and teeth.

Carbonated HAp deposits in vivo on some calcium-containing silicate glasses when implanted in bone tissues, and such apatite deposition is well reproduced in vitro in a simulated body fluid (SBF of Kokubo's recipe [20]), which has inorganic ion concentration similar to that of the human plasma. Yet, those apatite crystallites are randomly oriented and diverse in size without exhibiting any organized, ordered microstructure found in the dental enamel. Although many studies are concerned with the apatite formation in SBF, very few have paid attention to the control of the grain size, orientation, and morphology of the HAp crystal planes and arrangement in the apatite layer formed on the calcium-containing silicate glass. Recently, Day et al. [21] proposed a new and simple apatite formation scheme without using SBF: they just soaked calcium-containing borate glass spheres in alkaline phosphate solutions to yield HAp particles, where the glass to HAp conversion was dependent on the glass composition. Unfortunately, the obtained HAp particles consisted of randomly oriented

* Corresponding author. Address: Biomaterials Laboratory, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima, Okayama 700-8530, Japan. Tel.: +81 86 251 8213; fax: +81 86 251 8263.
E-mail address: satoshi@cc.okayama-u.ac.jp (S. Hayakawa).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.02.018

nanometer-scale grains with no preferred orientation. Yet Day's process of the phase conversion from glass to HAp inspired us to try fabricating nano-textured HAp scaffolds to mimic structural and three-dimensional details of natural bone or teeth.

In this study, we employed a soda-lime silica glass in the system Na_2O–CaO–SiO_2 because it may yield Si–OH groups on the surface when soaked in an aqueous system, and their formation is one of the key factors to induce apatite nucleation, and because we can take reference to the past studies of the apatite formation on the silicate systems [22–27]. Our concern is focused on the fabrication of nanometer-scale HAp rod arrays with a preferred orientation that simulates bone or "enamel-like" structure.

2. Experimental procedure

Soda-lime silica glasses in the system Na_2O–CaO–SiO_2 were prepared via a conventional melt–quench method and were coded based on CaO content as G45, G36 and G25, respectively and their chemical compositions were shown in Table 1. The mixtures of required quantities of reagent grade Na_2CO_3, $CaCO_3$ and SiO_2 were melted in a platinum crucible in air for 1 h at 1600 °C. The quenched glass was shaped into square pieces of $0.5 \times 0.5 \times 0.1$ cm^3 in size, and mirror-polished by using polishing cloth with 1 μm diamond slurry. The glass substrates were soaked in 0.01 M (mol l^{-1}) Na_2HPO_4 aqueous solution (the initial pH 8.5 ± 0.1) at 80 °C up to 14 days, where the solution volume to specimen surface area ratio was set to be 0.5 ml to 1 mm^2. The change in pH of the soaking solution was monitored with a pH meter (Horiba, Kyoto, Japan) as a function of the soaking period. After being dried at room temperature in air, they were subjected to crystalline phase identification due to X-ray diffraction (XRD; RINT 2500, Rigaku Co., Tokyo, Japan; CuKα ($\lambda = 1.5406$ Å), 40 kV, 200 mA) in both $\theta/2\theta$-scan and 2θ-scan modes. In the former case, the step-scanned range was 2–70° in 2θ, while in the latter mode, the incidence angle (θ) was fixed at 1°, and the 2θ was step-scanned in the range of 2–70° in 2θ. A wisdom tooth (mandibular third molar extracted in dental clinics) was obtained after informed consent from healthy human donor in order to examine the crystalline phase and orientation of HAp crystals of dental enamel. The flat side of a wisdom tooth was non-destructively served to conventional X-ray diffraction analysis because chemical polishing and mechanical polishing severely destroy the surface structure of outermost dental enamel. Crystallite size (CS) can be estimated from the Scherrer equation [28]:

$$D = K\lambda/\beta_D \cos\theta \quad (1)$$

where D is the average crystallite size, K is a constant depending on lattice direction and crystallite morphology, and β_D is the peak width due to finite size and lattice distortion. It is common to assume that the lattice distortion is negligible and $K = 0.9$ while the pure full width of the diffraction peak at the half of the maximum intensity (FWHM) is taken for β_D. Furthermore, for the materials in which crystal growth is seen in a specific direction, degree of the crystalline orientation is represented in terms of the Lotgering orientation factor f from the X-ray diffraction measurements. Suppose the crystallites are preferred-oriented in [001] direction, the orientation factor f is defined as [29]

$$f = (P - P_0)/(1 - P_0) \quad (2)$$

where $P = \sum I(00l)/\sum I(hkl)$ (from sample) and $P_0 = \sum I_0(00l)/\sum I_0(hkl)$ (from the standard data like ICDD-PDF).

The Ca(II), P(V) and Si(IV) concentrations of the phosphate solution were measured due to the inductively coupled plasma emission spectroscopy (ICP-OES; ICPS-7500, Shimadzu, Kyoto, Japan). Field emission scanning electron microscopy (SEM; S-4700, Hitachi, Japan) was used to examine the structural changes of the reaction product on the glass surface. Prepared samples were broken off by hand in order to observe the cross-section image. Energy dispersive X-ray spectroscopy (EDX; DX-4, Edax, Tokyo, Japan) was used to examine the chemical composition of the cross-section of the reaction product. The molecular structure of the reaction product was examined by Fourier transform infrared (FT-IR) absorption spectroscopy (FT/IR300, Jasco, Tokyo, Japan) using KBr disk method. Specific surface area (SSA) of the reaction product

Table 1
Chemical composition of Na_2O–CaO–SiO_2 glass system and their XRD analysis results. For comparison, XRD analysis results of human dental enamel and commercial HAp were also shown.

Sample code	Glass system Chemical composition (mol.%)			HAp 002 diffraction (12 h)		
	Na_2O	CaO	SiO_2	Lotgering factor	CS (nm)[a]	SSA (m^2 g^{-1})[b]
G45	0	45	55	0.46	27	–
G36	10	36	54	0.85	25	–
G25	20	25	55	0.75	26	35 (14d)[c]
Dental enamel	–	–	–	0.86	30	–
HAp	–	–	–	0.06	32	49

[a] CS, crystallite size.
[b] SSA, specific surface area.
[c] 14d, long-term soaking in 0.01 M Na_2HPO_4 solution.