

Research Article

A Novel Method of Preparing a Nanometer-Scale Rod Array of Hydroxyapatite Crystals

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Abstract This study proposed a new strategy for preparing self-assembling one-dimensional HAp nanorods into organized superstructures. We employed glass of a few specific compositions in the system Na₂O-CaO-SiO₂ 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. A nanometer-scale rod array of HAp having preferred orientation to the *c*-axis was successfully prepared simply by soaking the soda-lime silica glass substrates in Na₂HPO₄ aqueous solution at 80 °C. Those HAp rods grew up perpendicularly to the glass surface, and the crystallites covered glass surface uniformly, resulting in “dental enamel-like” rod array structure. The present procedure is significant as it allows to design biomimetic materials and therapeutic agents with applicable both the biomedical and material science fields because the nano-textured HAp crystals exhibit a hierarchical architecture and may provide specific cell attachment and proliferation with controlled planes of growth and nano- and micrometer-scale topography.

Keywords soda-lime silica glass; hydroxyapatite; crystal; preferred orientation; nanorod

1 Introduction

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 hydroxyapatite (HAp) crystals in the nanoregime. Day et al. proposed a new and simple apatite formation scheme: calcium-containing borate glass spheres were soaked in alkaline phosphate solutions to yield HAp particles, where the glass to HAp conversion was dependent on the glass composition [4]. Unfortunately, the obtained HAp particles consisted of randomly oriented 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. Recently, we employed soda-lime silica glass of specific compositions in the system Na₂O-CaO-SiO₂ for the phase conversion from glass to HAp [1] 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 [2, 3, 5, 7–9]. In this study, our concern is dominated on fabrication of nanometer-scale HAp rod arrays with a preferred orientation that simulate bone or “enamel-like” structure, depending on the presence or absence of carbonate ions.

2 Materials and methods

Soda-lime silica glasses in the system Na₂O-CaO-SiO₂ were prepared via a conventional melt-quench method and were coded based on CaO content such as G25. The representative chemical composition was shown in Table 1. The mixtures of required quantities of reagent grade Na₂CO₃, CaCO₃ and SiO₂ were melted in a platinum crucible in air for 1 hour at 1600 °C. The quenched glass was shaped into square pieces of 0.5 × 0.5 × 0.1 cm³ 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) Na₂HPO₄ 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 mm². For comparison, phosphate aqueous solutions containing carbonate ions (0.001 M NaHCO₃) were also prepared, and then the glass substrates were soaked in these phosphate solutions under the same conditions. The

Sample code	Glass system			HAp	
	Chemical composition (mol%)			002 diffraction (14d)	
	Na ₂ O	CaO	SiO ₂	Lotgering factor	
G25	20	25	55	0.86 ^a	0.61 ^b
Dental enamel	—	—	—	0.86	
HAp	—	—	—	0.06	

^a 0.01 M Na₂HPO₄ solution without NaHCO₃.

^b 0.01 M Na₂HPO₄ solution containing 0.001 M NaHCO₃.

Table 1: Representative chemical composition of Na₂O-CaO-SiO₂ glass and its XRD analysis result. For comparison, XRD analysis results of human dental enamel and commercial HAp were also shown.

change in pH of the phosphate solution was monitored with a pH meter (Horiba, Kyoto, Japan) as a function of the soaking period. After 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; CuK α , 40 kV, 20 mA) in $\theta/2\theta$ -scan mode. 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 nondestructively served to conventional X-ray diffraction analysis because chemical polishing and mechanical polishing severely destroy the surface structure of outermost dental enamel. 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 [00l] direction, the orientation factor f is defined as [6]:

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

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.

3 Results and discussion

Figure 1 shows XRD patterns of G25 glass substrates after soaking in 0.01 M Na₂HPO₄ solutions with or without 0.001 M NaHCO₃ at 80 °C for 14 days. All diffraction peaks were assigned to hydroxyapatite (HAp) (#09-0432). Thus, the glass surface was converted to HAp in the 0.01 M Na₂HPO₄ solution with or without 0.001 M

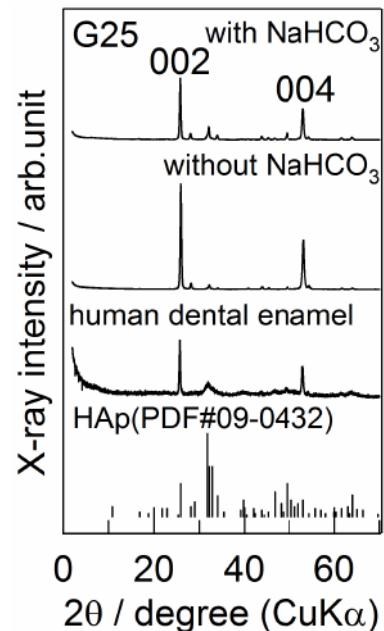


Figure 1: XRD patterns of G25 glass substrates soaked in 0.01 M Na₂HPO₄ solutions with or without 0.001 M NaHCO₃ for 14 days.

NaHCO₃ at 80 °C. No intermediate compounds such as CaHPO₄ · 2H₂O(DCPD) or Ca₈(HPO₄)₂(PO₄)₄ · 5H₂O (OCP) were detected. The XRD patterns of HAp formed on the G25 glass substrates were very similar to that of human dental enamel. That is, the relative intensity of 002 and 004 diffraction peaks is remarkably stronger than that of the other diffractions. Thus, the present glass substrates yielded the *c*-axis oriented HAp crystals. The Lotgering orientation factor f was calculated from applying equation (1) to XRD patterns and was shown in Table 1. Depending on the presence or the absence of carbonate ions, the Lotgering orientation factors f of G25 glass samples were ca. 0.86 and ca. 0.61, respectively, the former is in good agreement with f of human dental enamel (ca. 0.86).

The SEM image in Figure 2 is birds eye view of the HAp rods grown on G25 glass substrate when soaked in the 0.01 M Na₂HPO₄ solution at 80 °C for 24 hours. This image seems to show that needle-like HAp rods are randomly oriented on the glass entire surface. Similarly, SEM image of G25 glass substrates soaked in the 0.01 M Na₂HPO₄ solution with 0.001 M NaHCO₃ at 80 °C for 24 hours showed that smaller HAp rods are randomly oriented on the glass entire surface (not shown here). From the results of XRD analysis and SEM observation, it was confirmed that a nanometer-scale rod array of HAp crystal was successfully prepared.

When soaked in an alkaline phosphate aqueous solution, a soda-lime silica glass substrate is forced to corrosion to release the component ions into the surrounding medium. The reactions can be described as follows:

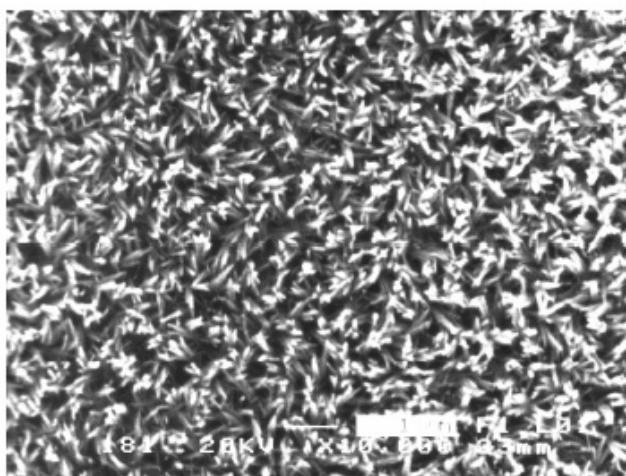
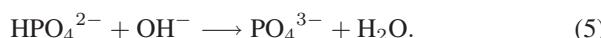
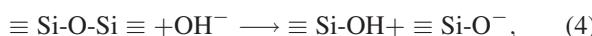
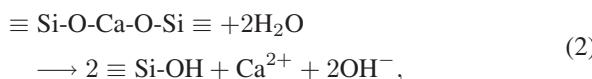


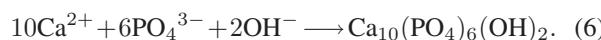
Figure 2: SEM image of the surface of G25 glass substrate after soaking in 0.01 M Na_2HPO_4 solution at 80 °C for 24 hours. Bar: 1 μm .

- glass corrosion involving hydration and hydrolysis reactions:



The increase in Ca(II) and OH⁻ in the glass surface favors HAp formation as many P(V) are available nearby;

- precipitation of HAp on the glass surface in the phosphate aqueous solution:



The calcium ions are consumed for apatite crystallization as soon as they are released from the glass to the phosphate aqueous solution. The amount of precipitated HAp crystals should depend on the corrosion-precipitation kinetics involving reactions (2), (5), and (6) and on the solubility of soda-lime silica glass substrates in the phosphate aqueous solution. G25 glass was completely converted to silicate-doped HAp. The corrosion involving hydration and hydrolysis reactions for G25 glass was not disturbed by the formation of the hydrated silica layer and the HAp layer. The formation and corrosion of hydrated silica layer may be regulated by both the reaction between Ca(II) and P(V) ions and by pH at the glass-crystal layer interface [1]. Since the crystal growth of HAp was suppressed by the presence of carbonate ions, HAp rods became smaller and more randomly oriented on the glass surface in 0.01 M Na_2HPO_4 solutions with 0.001 M NaHCO_3 .

4 Conclusions

A nanometer-scale rod array of hydroxyapatite crystal was successfully prepared by soaking calcium-containing silicate glass substrates in Na_2HPO_4 aqueous solution at 80 °C for various periods up to 14 days. Nanometer-scale HAp rods grew up perpendicularly to the glass surface, and the crystallites were arranged on the glass entire surface uniformly with preferred orientation to the *c*-axis. The addition of carbonate ions in the alkaline phosphate solution suppressed the crystal growth of HAp and derived smaller Lotgering orientation factor.

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References

- [1] S. Hayakawa, Y. Li, K. Tsuru, A. Osaka, E. Fujii, and K. Kawabata, *Preparation of nanometer-scale rod array of hydroxyapatite crystal*, *Acta Biomater*, 5 (2009), pp. 2152–2160.
- [2] S. Hayakawa, K. Tsuru, O. C., and O. A., *Mechanism of apatite formation on a sodium silicate glass in a simulated body fluid*, *J Am Ceram Soc*, 82 (1999), pp. 2155–2160.
- [3] L. L. Hench, *Bioceramics: from concept to clinic*, *J Am Ceram Soc*, 74 (1991), pp. 1487–1510.
- [4] W. Huang, D. E. Day, K. Kittiratanapiboon, and M. N. Rahaman, *Kinetics and mechanisms of the conversion of silicate (45S5), borate, and borosilicate glasses to hydroxyapatite in dilute phosphate solutions*, *J Mat Sci Mater Med*, 17 (2006), pp. 583–596.
- [5] H.-M. Kim, F. Miyaji, T. Kokubo, C. Ohtsuki, and T. Nakamura, *Bioactivity of $\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2$ glasses*, *J Am Ceram Soc*, 78 (1995), pp. 2405–2411.
- [6] F. K. Lotgering, *Topotactical reactions with ferrimagnetic oxides having hexagonal crystal structures—I*, *J Inorg Nucl Chem*, 9 (1959), pp. 113–123.
- [7] A. Osaka, S. Hayakawa, and C. Ohtsuki, *Effects of cations on surface reactions and apatite nucleation on silicate glasses in a body environment*, in *Bioceramics*, R. Z. LeGeros and J. P. LeGeros, eds., vol. 11, World Scientific Publishing, Singapore, 1998, pp. 489–492.
- [8] H. Takadama, H.-M. Kim, T. Kokubo, and T. Nakamura, *Mechanism of biomineratization of apatite on a sodium silicate glass: TEM-EDX study in vitro*, *Chem Mater*, 13 (2001), pp. 1108–1113.
- [9] K. Tsuru, M. Kubo, S. Hayakawa, C. Ohtsuki, and A. Osaka, *Kinetics of apatite deposition of silica gel dependent on the inorganic ion composition of simulated body fluids*, *J Ceram Soc Japan*, 109 (2001), pp. 412–418.