Apatite Formation on Various Silica Gels in a Simulated Body Fluid Containing Excessive Calcium Ion

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カルシウムを過剰に含む擬似体液中における各種シリカゲル表面でのアパタイト形成

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It was previously shown that a silica gel prepared in a solution containing polyethylene glycol (S-PEG) induces the apatite nucleation in SBF, but those prepared in pure water (S-W) and a solution containing polyacrylic acid (S-PAA) do not induce it. This indicates that a limited type of silanol groups is effective for apatite nucleation. In the present study, apatite-forming ability of these silica gels was examined in SBF containing excessive calcium ion. It was found that all these silica gels formed the apatite on their surfaces in this fluid, although the rate of apatite formation decreased in the order S-PEG > S-PAA > S-W. This indicates that any type of silanol groups has potential for inducing apatite nucleation under this condition. Such environment rich in calcium ion is produced around the surfaces of CaO, SiO₂-based glasses and glass-ceramics when they are implanted into living body. The present results indicate that the CaO-SiO₂ system can be a good basic composition for bioactive glasses and glass-ceramics.

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1. Introduction

It has been shown that bonding of bioactive glasses and glass-ceramics to living bone is achieved through a bonelike apatite layer which is formed on their surfaces in body environment.¹⁾⁻¹⁴⁾ It is, therefore, believed that the prerequisite for glasses and glass-ceramics to bond to bone is the formation of a layer of biologically active bonelike apatite, i.e., carbonate-containing apatite, on their surfaces in the body.^{15),16)}

For the mechanism of apatite formation on the surfaces of bioactive glasses and glass-ceramics, hydrated silica developed on their surfaces in the body has been assumed to induce nucleation of the apatite.¹⁷⁾⁻²⁰⁾ Actually, it was experimentally confirmed that pure silica gel prepared by hydrolysis and polycondensation of tetraethoxysilane in aqueous solution containing polyethylene glycol induces the formation of the apatite on its surface when the gel is soaked in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma.²¹⁾⁻²⁵⁾ The same silica gel heat-treated above 900°C, however, as well as a pure silica glass synthesized by vapor axial deposition and quartz single crystal synthesized by hydrothermal method did not induce it.^{21),25)} These results suggest that the silanol group formed on the surface of the silica gel heat-treated below 800°C in SBF could be responsible for the apatite nucleation.

On the other hand, West and Hench recently

proposed on the basis of molecular orbital calculation that not all kind of the silanol groups but only the silanol groups forming trigonal siloxane can induce the apatite nucleation.²⁶⁾ Present authors showed that a pure silica gel prepared in a solution containing polyethylene glycol induces the apatite nucleation in SBF as mentioned above, but those prepared in pure water and a solution containing polyacrylic acid do not induce it.²⁷⁾ These results suggest that only a certain type of structural unit of the silanol groups is effective for the apatite nucleation.

In the present study, apatite formation on the surfaces of various silica gels prepared in different media was investigated in SBF with excessive calcium ion. The result was discussed in terms of the mechanism of apatite nucleation.

2. Experimental procedure

2.1 Gel preparation

Three kinds of silica gels were prepared by the hydrolysis and polycondensation of tetraethoxysilane (TEOS) in different media. First, 8 g of three kinds of solutions, i.e., as ion-exchanged distilled water, and those added with 0.6 g of polyacrylic acid (30000 in an average molecular weight) or 0.7 g of polyethylene glycol (10000 in an average molecular weight) were prepared. Concentrated nitric acid (HNO₃, 62 mass%) in 0.81 g was added to these solutions. Then 7 ml of TEOS was added to the solutions under vigorous stirring. After stirring for 5

min, the solutions were transferred into plastic petri dishes with its top tightly sealed, and were kept at 40°C in air-circulating oven for gelation for 18h. The obtained wet gels were immersed in 1 M nitric acid for 6h to be washed. In this operation, the polymers contained in the gels were almost leached out. The nitric acid was renewed every 2h. After being dried at 40°C for 6 d, they were heated up to 600°C at a rate of 100°C/h and kept at 600°C for 2h, then allowed to cool to room temperature. The silica gels prepared in pure water and aqueous solutions containing polyacrylic acid or polyethylene glycol are hereafter named S-W, S-PAA and S-PEG, respectively.

2.2 Characterization of gel

The size of macropore contained in the silica gels were examined by a scanning electron microscope (SEM) (S-2500CX, Hitachi, Ltd., Tokyo, Japan) observation, after gold-palladium film was coated on the surface of the specimens. The volume of pore smaller than 200-nm in diameter and specific surface area were measured with an automatic nitrogen adsorption pore size analyzer (ASAP-2000, Micromeritics, Tokyo, Japan). For the pore volume measurement, the silica gels were degassed at 200°C for 30 min under dry nitrogen flow prior to each measurement. The experimental errors in the pore volume measurement were less than 10% for all the samples. Raman spectroscopy (T64000, Jobin-Yvon, France) was employed to examine the tetrasiloxane D1 defect, trisiloxane D2 defect and silanol groups (Si-OH) in the gels. All the Raman spectra were obtained using the 514.5-nm line of an Ar⁺ laser in the 180° reflection configuration.

2.3 Soaking gel in a simulated body fluid

A simulated body fluid (SBF1.5Ca) with excessive calcium ion, i.e., Ca concentration of which is 1.5 times that of the simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma as given in Table 1, was prepared by dissolving reagents NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄ in distilled water. The fluid was buffered at pH 7.4 at 36.5°C with 50 mM of tris (hydroxymethyl) aminomethane ((CH₂OH)₃CNH₂) and 45 mM hydrochloric acid (HCl) as was reported previously.²⁵⁾ Rectangular specimens of 6×6×1.5 mm³ in size were cut from the gels. For comparison, similar specimens were cut from a highly pure silica glass (Sumiquartz SK-1300, Sumitomo Metal Industries Ltd., Tokyo, Japan) synthesized by vapor axial deposition. At

least, three pieces of each kind of specimen were immersed in 15 ml of SBF1.5Ca. After being soaked in SBF1.5Ca for various periods, the specimens were removed from the fluid and gently washed with distilled water. The specimens were dried at room temperature.

2.4 Surface analysis

Surface structures of the specimens before and after soaking in SBF1.5Ca were analyzed using thin-film X-ray diffraction (2651A, Rigaku Co., Tokyo, Japan), Fourier-transformed infrared (FT-IR) reflection spectroscopy (FT-IR5M, JASCO, Tokyo, Japan) and SEM observation. In the X-ray diffraction, the surface of a specimen was fixed at an angle of 1° against the direction of the incident beam. The FT-IR reflection spectroscopy was performed with the reflection angle of 75°.

2.5 Measurement of silicon dissolution

Concentrations of the silicon released from the gels and silica glass into SBF1.5Ca were measured with inductively coupled plasma (ICP) atomic emission spectroscopy (SPS1500VR, Seiko Instruments Inc., Tokyo, Japan). For ICP atomic emission spectroscopy, one milliliter of the solution was drawn from the bottle and added to 10 ml of ion-exchanged distilled water.

3. Results

Figure 1 shows the Raman spectra of the surfaces of various silica gels prepared in different media, and silica glass. On the basis of the previous study, each observed Raman peak is assigned as follows.²⁸⁾ The broad weak peaks at about 800 and 1070 cm⁻¹ are assigned to a mode of Si-O-Si symmetric stretching vibration and a transverse optical (TO) mode of Si-O asymmetric stretching vibration, respectively. The broad large peak at about 430 cm⁻¹ is assigned to a symmetric ring-breathing mode involving mainly oxygen motion. The sharp peaks at 495 and 605 cm⁻¹ are assigned to tetrasiloxane D1 defect and trisiloxane D2 defect, respectively. The 980 cm⁻¹

Fig. 1. Raman spectra of the surfaces of various silica gels prepared in different media and silica glass.

Table 1. Ion Concentrations of SBF and SBF1.5Ca in Comparison with Those of Human Blood Plasma

	Concentration / mM							
	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	148.8	4.2	1.0	0.5
SBF1.5Ca	142.0	5.0	1.5	3.75	148.8	4.2	1.0	0.5

peak is assigned to bulk Si-OH stretching vibration. It can be seen from Fig. 1 that the intensity of the 980 cm^{-1} -peak ascribed to Si-OH stretching vibration of silica gels is almost same irrespective of kind of media used in preparation, whereas the peak is not observed for silica glass. This indicates that all the silica gels contain a large amount of silanol

Table 2. Microstructures of the Silica Gels

Gel	Medium for gel preparation	Size of macropore ²⁷⁾ (μm)	Volume of pore (cm^3/g)		Specific surface area (cm^2/g)
			*micro-	**meso-	
S-W	H_2O	-	0.0	0.5710	836.52
S-PAA	$\text{H}_2\text{O}+\text{PAA}$	1	0.0	0.3841	626.79
S-PEG	$\text{H}_2\text{O}+\text{PEG}$	2	0.106	0.2752	680.83

*pores with size less than 1.7 nm

**pores with size ranging 1.7 - 200 nm

groups on their surfaces irrespective of the preparation history, although silica glass does not.

The microstructures of the silica gels are summarized in Table 2. The S-PAA and S-PEG gels have interconnected macropores in micrometer-size, whereas the S-W gel does not have it. The size of macropores of the S-PAA and S-PEG gels are about 1 and $2\text{ }\mu\text{m}$ in diameter, respectively.²⁷⁾ Among the gels, the S-W and S-PAA gels have only mesopore ranging 1.7–200 nm, whereas the S-PEG gel has micropores less than 1.7 nm in addition to the mesopore. The volume of mesopore decreased in the order $\text{S-W} > \text{S-PAA} > \text{S-PEG}$. The specific surface area is considerably larger in the S-W gel than in the S-PAA and S-PEG gels.

Figures 2, 3, 4 and 5 show the thin-film X-ray diffraction patterns and FT-IR reflection spectra of

Fig. 2. Thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of S-W gel soaked in SBF1.5Ca for various periods.

Fig. 4. Thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of S-PEG gel soaked in SBF1.5Ca for various periods.

Fig. 3. Thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of S-PAA gel in SBF1.5Ca for various periods.

Fig. 5. Thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of silica glass soaked in SBF1.5Ca for various periods.

the surfaces of various silica gels and silica glass soaked in SBF1.5Ca for various periods, respectively. Assignment of main peaks based on the previous papers^{9),20)} are also shown in Figs. 2, 3, 4 and 5. Figure 2 shows that no new phase appears after soaking for 3 d and an apatite begins to form on the S-W gel after soaking for 5 d. On the other hand, Fig. 3 shows that amorphous calcium phosphate forms on the S-PAA gel after the soaking for 3 d, and it transforms into the crystalline apatite and it grows with increasing soaking period. It can be seen from Fig. 4 that the apatite is formed on the S-PEG gel within 3 d and it grows with increasing soaking period. Silica glass, however, did not form the apatite on its surface in SBF1.5Ca even after the soaking for 2 weeks as shown in Fig. 5.

Figures 6 and 7 show the SEM photographs of the surfaces of various silica gels soaked in SBF1.5Ca for 3 and 7 d, respectively. It can be seen from Fig. 6 that a leaflike particles are deposited on the surfaces of the S-PAA and S-PEG gels, whereas no such particles are deposited on the S-W gel. The morphology of the leaflike particles deposited on the S-PEG gel is much clearly observed compared with that of S-PAA gel. That is, the particles deposited on the surface of the S-PEG gel are well crystallized rather

than those on the surface of the S-PAA gel. After the soaking in SBF1.5Ca for 7 d, the leaflike particles are deposited on the surfaces of all the silica gels, as shown in Fig. 7. The morphology of such leaflike particles is quite similar to that of the apatite formed on the surfaces of bioactive glasses and glass-ceramics.^{9),20)} The results of SEM observation

Fig. 8. Changes in silicon concentration of the SBF1.5Ca due to the soaking of various silica gels and silica glass as a function of soaking time.

Fig. 6. SEM photographs of the surfaces of various silica gels soaked in SBF1.5Ca for 3 d.

Fig. 7. SEM photographs of the surfaces of various silica gels soaked in SBF1.5Ca for 7 d.

as well as thin film X-ray diffraction and FT-IR spectroscopy indicate that apatite-forming ability of the silica gels decreases in the order S-PEG > S-PAA > S-W.

Figure 8 shows the changes in silicon concentration of SBF1.5Ca due to the soaking of various silica gels and silica glass as a function of soaking time. It can be seen from Fig. 8 that all the examined silica gels show the steep increase in silicon concentration up to about 4 d, and it reached about 2 mM, which is the solubility of the silica in water,²⁹⁾ whereas the silica glass shows almost no increase in silicon concentration. The behavior of release of silicon from the various silica gels into SBF1.5Ca is similar to the case of the same gels when soaked in the SBF.²⁷⁾

4. Discussion

The previous study showed that among the silica gels examined in the present study, only S-PEG gel forms the bonelike apatite on its surface in SBF.²⁷⁾ In contrast with this, the present study showed that all the examined silica gels form it on their surfaces in SBF1.5Ca, although the rate of the apatite formation decreased in the order S-PEG > S-PAA > S-W. The silica glass did not form the apatite on it both in SBF and SBF1.5Ca. These results are interpreted as follows.

The rate, I , of nucleation of a crystal on a substrate in a supersaturated solution at temperature, T , is generally given by the following equation³⁰⁾

$$I = I_0 \exp\left(\frac{-\Delta G^*}{kT}\right) \exp\left(\frac{-\Delta G_m}{kT}\right) \quad (1)$$

where I_0 is a constant, k the Boltzmann constant, ΔG^* the free energy for formation of an embryo of critical size, and ΔG_m the activation energy for transport across the nucleus-solution interface. The free energy ΔG^* is given by the equation

$$\Delta G^* = \frac{16\sigma^3 f(\theta)}{3(kT/V_\beta \ln(IP/K_0))^2} \quad (2)$$

where σ is an interface energy between the nucleus and the solution, IP the ionic activity product of the crystal in the solution, K_0 the value of IP at equilibrium, i.e., solubility product of the crystal, $f(\theta)$ a function of contact angle between the nucleus and the substrate, and V_β the molecular volume of the crystal phase.

In discussing the dependence of the apatite nucleation rate on the kind of substrate and solution, V_β is constant, and ΔG_m , σ and K_0 are assumed to be almost equal between in SBF and in SBF1.5Ca. The ionic activity product IP of the apatite varies with the kind of the solution and $f(\theta)$ varies with the kind of the substrate. The former is given by the following equation²⁰⁾

$$IP = (\gamma_{Ca^{2+}})^{10} (\gamma_{PO_4^{3-}})^6 (\gamma_{OH^-})^2 [Ca^{2+}]^{10} [PO_4^{3-}]^6 [OH^-]^2 \quad (3)$$

where γ is the activity coefficient and square bracket

is the concentration of each ion, since the formation of the hydroxyapatite is given by the following equation.

The IP of the apatite calculated for SBF and SBF1.5Ca are 2.12×10^{-96} and 8.97×10^{-95} , respectively. The K_0 of the apatite in an aqueous solution is reported to be 5.5×10^{-118} at 37°C.³¹⁾ This means that both SBF and SBF1.5Ca are highly supersaturated with respect to the apatite and the degree of the supersaturation is higher in SBF1.5Ca than in SBF. Therefore, SBF1.5Ca gives higher apatite nucleation rate for all the substrates than SBF. This is the reason why all the examined gels formed the apatite on their surfaces in SBF1.5Ca whereas only S-PEG gel formed it in SBF.

Even under such favorable environment, the apatite-forming ability largely varied with the substrates. It decreased in the order S-PEG > S-PAA > S-W >> silica glass. This difference attributed to the difference in $f(\theta)$ among the substrates, because other parameters in Eq. (2) are constant. The function $f(\theta)$ for the silica glass is almost 1, since the silica glass did not induce the heterogeneous nucleation. Those for the silica gels are considerably less than 1 and decrease in the order S-W > S-PAA > S-PEG. The surface electrochemical properties such as zeta potential might affect the $f(\theta)$ value, although there is no evidence at present.

It is important in practical applications to know that the silica gel provides fairly low $f(\theta)$ against the apatite, irrespective of its structure, and can induce the heterogeneous nucleation of the apatite, even if it is in an environment rich in calcium. Such an environment is easily achieved around the surfaces of CaO, SiO₂-based glasses and glass-ceramics when they are implanted into a living body. The calcium ion released from the glasses and glass-ceramics increases the degree of the supersaturation of the surrounding fluid with respect to the apatite, and a hydrated silica formed on their surfaces induces the apatite nucleation. Therefore, the present result indicates that the CaO-SiO₂ system can be good basic composition of bioactive glasses and glass-ceramics. This prediction is consistent with the previous finding that in the system CaO-SiO₂-P₂O₅, CaO, SiO₂-based glasses forms the bonelike apatite on them in SBF whereas CaO, P₂O₅-based glasses do not form it.²⁰⁾

The appreciably lower $f(\theta)$ for the silica gels in comparison with the silica glass indicates that these silica gels provide favorable sites for the apatite nucleation, but the silica glass does not it. According to the Raman spectra in Fig. 1, the silica gels have a lot of silanol (Si-OH) groups on their surfaces, whereas the silica glass has not it. On the other hand, Fig. 8 shows that the silica gels dissolve into SBF1.5Ca at high rates, whereas the silica glass little dis-

solves. This indicates that silanol groups are formed on the surfaces of the silica gels furthermore after the soaking in SBF1.5Ca, since the dissolution proceeds via hydrolysis of SiO_2 , whereas it is not formed on that of the silica glass even after the soaking. In view of these facts, it is believed that the silanol groups abundant on the surfaces of the silica gels are responsible for the apatite nucleation. All the silanol groups, however, have unequal ability for the apatite nucleation, since the apatite-forming ability varied with the kind of the gels.

5. Summary

Apatite formation on the surfaces of various silica gels prepared in different media and silica glass was investigated in SBF1.5Ca. After the soaking in SBF1.5Ca for 7 d, the bonelike apatite was formed on the surfaces of all the silica gels having abundant silanol groups. It was not, however, formed on the surface of the silica glass having little silanol groups even after the soaking for 2 weeks. From the results, it can be concluded that all the kind of silanol groups developed on the silica gels can induce the apatite nucleation, although there is some differences in their nucleating abilities. It is interesting to know what type of the silanol groups is most effective for the apatite nucleation. Unfortunately, however, Raman spectra in Fig. 1 give no information about difference in surface structure among the gels. Further study is needed for revealing the most effective structural unit.

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