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Apatite formation on silica gel in simulated body fluid: effects of structural modification with solvent-exchange

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The prerequisite for glasses and glass-ceramics to bond to living bone is the formation of biologically active bone-like apatite on their surfaces. It has been shown that even a pure silica gel forms the bone-like apatite on its surface in a simulated body fluid. In the present study, pore structure of silica gels prepared by hydrolysis and polycondensation of tetraethoxysilane in an aqueous solution containing polyethylene glycol was modified by 1M HNO_3, and 0.1M and 1M NH_4OH solution treatments. The three kinds of resultant gels all contained large amounts of silanol groups and trisiloxane rings, but differ greatly in pore structure of nanometre pore size. Irrespective of these differences, all the gels formed the bone-like apatite on their surface in the simulated body fluid. It was speculated that a certain type of structural unit of silanol groups, which is easily formed in the presence of the polyethylene glycol, is effective for the apatite formation.

1. Introduction

It is believed that the prerequisite for glasses and glass-ceramics to bond to living bone is the formation of a layer of biologically active bone-like apatite, i.e. carbonate-containing apatite, on their surfaces in the body [1, 2]. It has been proposed that hydrated silica developed on their surfaces in the body plays an important role in nucleation of the apatite [3–6]. In previous studies, it was confirmed experimentally that pure silica gel which was prepared by hydrolysis and polycondensation of tetraethoxysilane in aqueous solution containing polyethylene glycol and subsequently heat treated at 600 °C, induces the formation of the apatite on its surface in a simulated body fluid with ion concentrations nearly equal to those of the human blood plasma [7–11] as well as *in vivo* [12]. The same gel, however, did not form the apatite on its surface in simulated body fluid [11] nor as *in vivo* [12], when heat treated above 900 °C. This was attributed to a decrease in the amount of the silanol groups on the surface of the silica gel by the heat treatment. West and Hench [13] proposed, on the basis of molecular orbital calculation, that trisiloxane rings are effective for the apatite formation. Later, Pereira *et al.* [14, 15] proposed that only pores larger than 2 nm radius of the silica gel are effective for the apatite nucleation. The present authors, however, recently showed that neither the silica gels prepared in a pure water, nor that prepared in an aqueous solution containing polyacrylic acid, form apatite on their surfaces in simulated body fluid, although both of them contain large amounts of trisiloxane rings and pores larger than 2 nm radius [16]. In the present study, in order to investigate the effects of the pore structure of silica gels on apatite-forming ability, the pore structure of silica gel prepared in an aqueous solution containing polyethylene glycol was modified with solvent exchange, and their apatite-forming abilities were examined in the simulated body fluid.

2. Experimental procedure

2.1. Gel preparation and characterization

Silica gel was prepared by the hydrolysis and polycondensation of tetraethoxysilane (TEOS) as follows. First, 0.7 g polyethylene glycol having an average molecular weight of 10 000 was dissolved into 8.0 g ion-exchanged distilled water, and 0.81 g concentrated nitric acid (62 wt %) was added. Then 7.0 ml TEOS was added to the above solution under vigorous stirring. After stirring for 5 min, the solution was transferred in a plastic petri dish, with its top tightly sealed, and was kept at 40 °C in an air-circulating oven for gelation. After ageing for 18 h, the obtained wet gel was immersed in 1M HNO_3, 0.1 M NH_4OH or 1 M NH_4OH for 6 h. The solutions were renewed every 2 h. After this solvent-exchange treatment, the gel was dried at 40 °C for 6 d. The dried gel was heated up to 400 °C at a rate of 100 °C h^{-1}, held at the temperature for 2 h, and then allowed to cool to room temperature.

For silica gels thus obtained, the volume of pores smaller than 200 nm diameter was measured by an

automatic nitrogen adsorption pore-size analyser (ASAP-2000, Micromeritics, Tokyo, Japan). The morphologies of the silica gels were observed by scanning electron microscopy (SEM: S-2500CX, Hitachi Ltd, Tokyo, Japan). Laser Raman spectroscopy (T64000, Jobin-Yvon, France) was employed to examine the structures of the gels. For Raman spectroscopic measurements, the 514.5 nm line of an Ar^+ laser in the 180° reflection configuration was used.

2.2. Soaking gel in a simulated body fluid

Rectangular silica gel specimens $6 \times 6 \times 1.5\,mm^3$ in size were immersed in 15 ml simulated body fluid (SBF) with ion concentrations (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM) nearly equal to those of human blood plasma. SBF was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in distilled water and buffered at pH 7.4 at 36.5 °C with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_2$) and 1 M HCl, as reported previously [11, 17]. It has already been confirmed that this fluid can reproduce fairly precisely, *in vivo*, apatite formation on the surfaces of various kinds of bioactive glasses and glass-ceramics [18–22].

2.3. Surface analysis

After the specimens were soaked in SBF for various periods, they were removed from the fluid, immersed in 10 ml ion-exchanged distilled water for 5 min for washing, and dried at room temperature. The surface structural changes of the specimens were analysed by thin-film X-ray diffraction (2651A, Rigaku Co., Tokyo, Japan), laser Raman spectroscopy and SEM observation. In the X-ray diffraction measurements, the surface of a specimen was fixed at an angle of 1° against the direction of the incident beam.

2.4. Analysis of SBF

The concentration of the silicon released from the gels into SBF was measured by inductively coupled plasma (ICP) atomic emission spectroscopy (SPS1500VR, Seiko Instruments Inc., Tokyo, Japan). For ICP measurements, 1 ml of fluid was diluted with 10 ml of ion-exchanged distilled water.

3. Results

Fig. 1 shows scanning electron micrographs of the fractured surfaces of the silica gels which were subjected to different solvent exchange and then heat treatment at 400 °C. All the resultant silica gels showed identical interconnecting structures with pores of about 2 μm diameter. This result indicates that the micrometre-range pores in the silica gel are hardly changed by the solvent exchange.

Fig. 2 shows the nitrogen adsorption–desorption isotherms for the silica gels which were subjected to

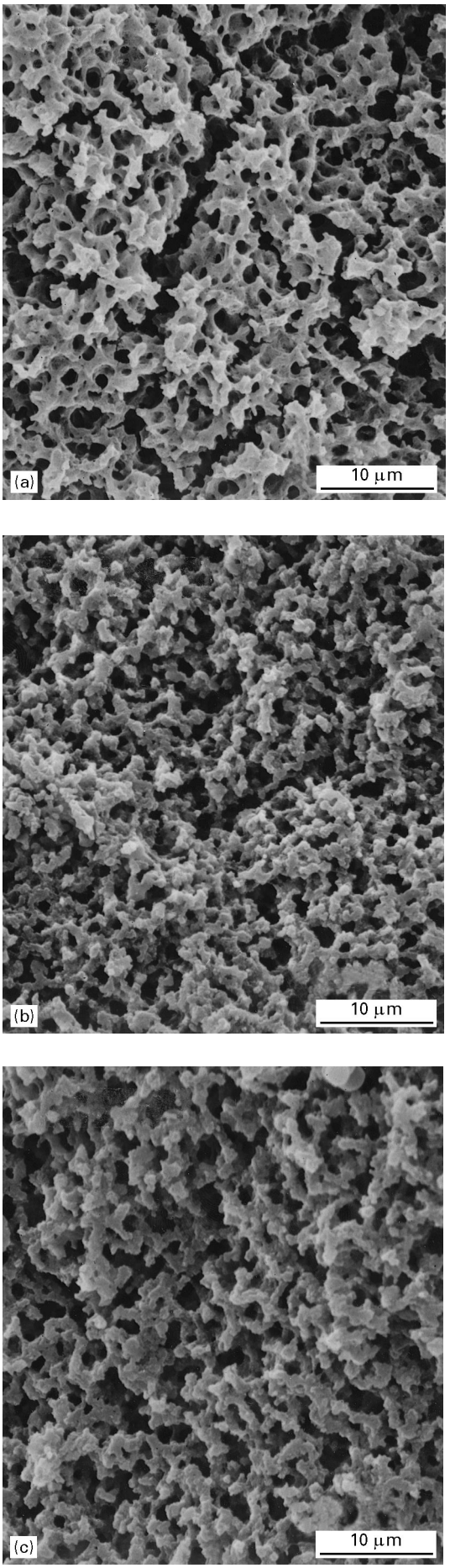


Figure 1 Scanning electron micrographs of fractured surfaces of the silica gels which were subjected to different solvent exchanges and heat treatments at 400 °C. (a) 1 M HNO_3, (b) 0.1 M NH_4OH, (c) 1 M NH_4OH.

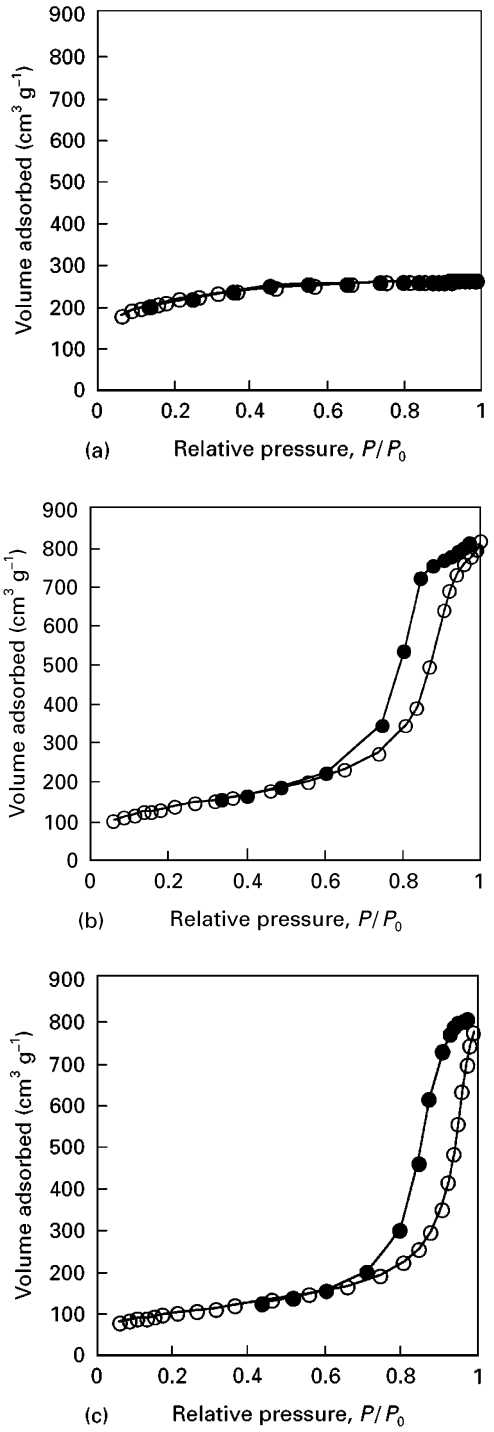


Figure 2 (a–c) Nitrogen adsorption–desorption isotherms at 77 K for the silica gels which were subjected to the different solvent exchanges and heat treatments at 400 °C, as in Fig. 1.

different solvent exchange and heat treatment at 400 °C. According to the Brunauer–Deming–Deming–Teller (BDDT) classification [23], the gel treated with 1 M HNO_3 exhibited a typical type I isotherm which was attributed to the existence of smooth and cylindrical type pores. The silica gels treated with 0.1 M and 1 M NH_4OH exhibited type IV isotherms, which were attributed to the existence of ink-bottle type pores. Fig. 3 shows the volume of nanometre size pores in the silica gels which were subjected to different solvent exchange and heat treatment at 400 °C. It can be seen from Fig. 3 that all the silica gels have mesopores ranging from 1.7–200 nm. The gel treated with 1 M HNO_3 also has micropores less than 1.7 nm, whereas

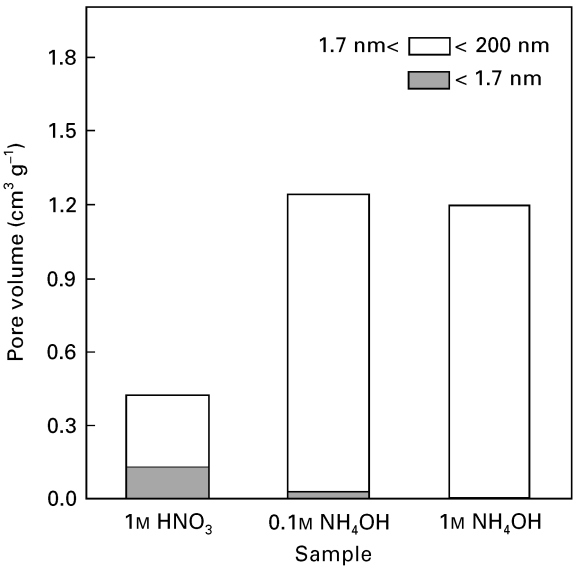


Figure 3 The volume of nanometre-range pores in the silica gels which were subjected to different solvent exchanges and heat treatments at 400 °C.

those treated with 0.1 M and 1 M NH_4OH have few of them.

Fig. 4 shows the Raman spectra of the silica gels which were subjected to different solvent exchange and heat treatment at 400 °C before and after soaking in SBF for 1 d. The Raman band at 800 cm^{-1} is assigned to a symmetric Si–O–Si stretching mode. The broad 430 cm^{-1} band is assigned to a symmetric ring-breathing mode involving mainly oxygen motion. The presence of these bands reflects the formation of a silica gel skeleton. The peak at 495 cm^{-1} is assigned to tetrasiloxane D1 defect, that at 605 cm^{-1} to the trisiloxane D2 defect, and that at 980 cm^{-1} to bulk Si–OH stretching vibration [24]. It can be seen from Fig. 4 that all the silica gels have large amounts of silanol group as well as tetrasiloxane D1 and trisiloxane D2 defects. After soaking in SBF for 1 d, however, the D2 defect present at the surfaces of the silica gels disappeared on hydrolysis.

Figs 5 and 6 show the thin-film X-ray diffraction patterns and scanning electron micrographs of the surfaces of the silica gels which were subjected to different solvent exchange and heat treatment at 400 °C after soaking in SBF for 2 wk, respectively. It can be seen from Figs 5 and 6 that apatite was formed on all the silica gels within 2 wk in SBF.

Fig. 7 shows the changes in silicon concentration of SBF due to the soaking of the silica gels which were subjected to different solvent exchange and heat treatment at 400 °C as a function of soaking time. The increase in silicon concentration is attributed to dissolution of the silicate ions from the gels. It can be seen from Fig. 7 that the rates of dissolution of silicate ions is almost the same irrespective of the kind of exchanged solvent.

4. Discussion

The present authors first showed that even a silica gel which was prepared by hydrolysis and polycondensation of tetraethoxysilane in an aqueous solution

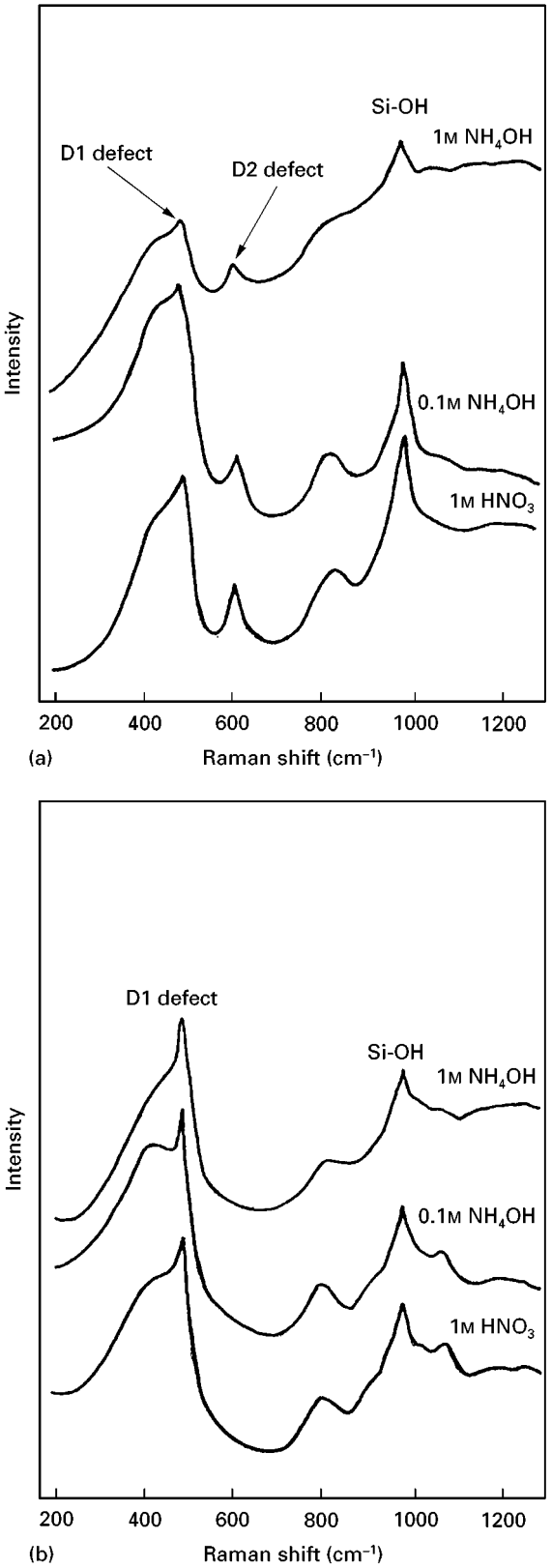


Figure 4 Raman spectra of the silica gels which were subjected to different solvent exchanges and heat treatments at 400 °C (a) before and (b) after soaking in SBF for 1 d.

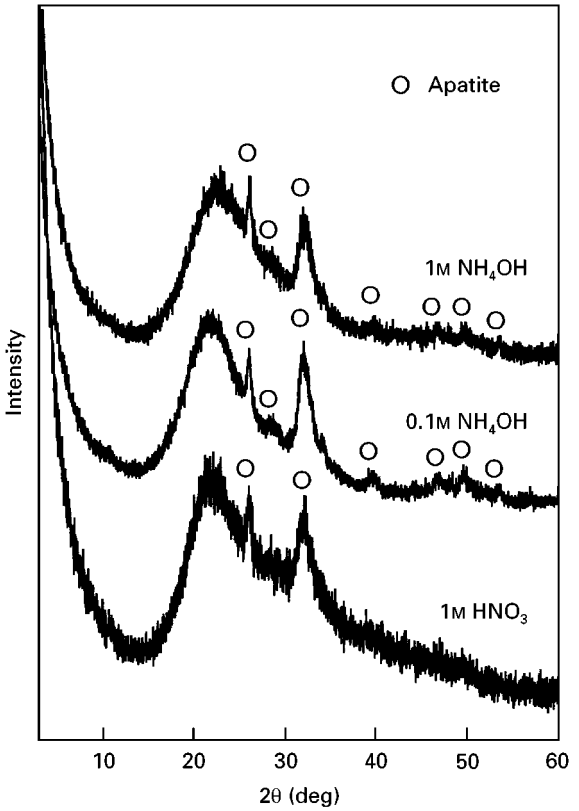
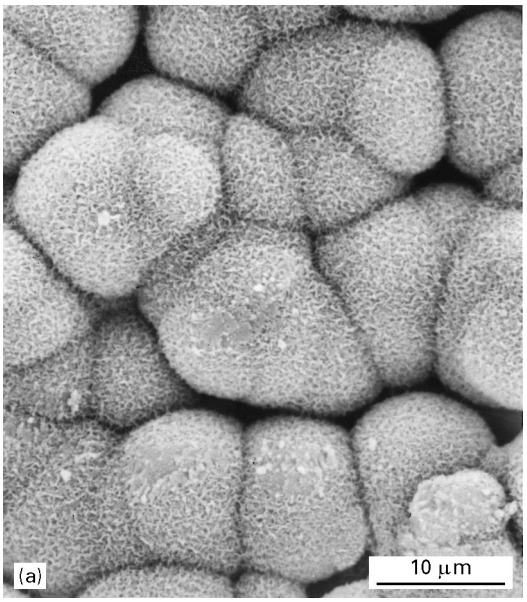


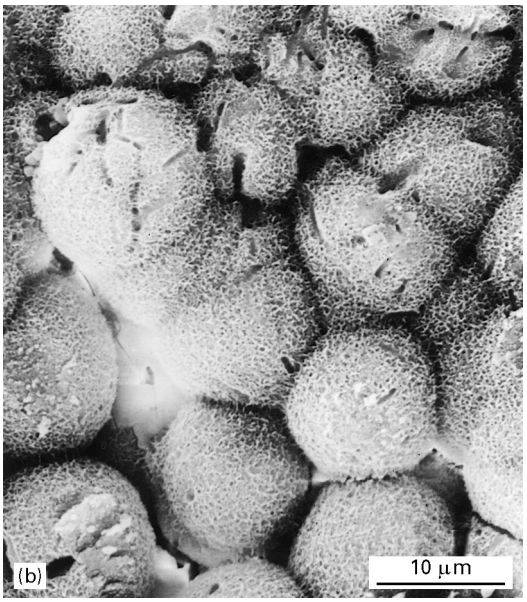
Figure 5 Thin-film X-ray diffraction patterns of the silica gels which were subjected to different solvent exchanges and heat treatments at 400 °C and soaking in SBF for 2 wk.

containing polyethylene glycol, forms bone-like apatite on its surface in SBF within 2 wk, whereas neither a silica glass nor quartz single crystal do. It was then speculated that apatite formation on silica gel is induced by silanol groups on its surface [7]. The same silica gel did not form apatite within the same period in SBF when it was heat treated above 900 °C. This was attributed to the decrease in the concentration of the silanol groups on the surface of the gel soaked in SBF, that is the total amounts of the silanol groups present before soaking in SBF and that newly formed in SBF by the heat treatment [11]. The same gel heat treated at 1000 °C, however, recovered its apatite-forming ability, when it was subjected to plasma treatment in H_2O gas for 30 s [25]. This was attributed to recovery of the silanol groups by the H_2O plasma treatment.

When the silica gel was heat treated, not only the concentration of the silanol groups but also the volume of the pores decreased [11]. Therefore, Pereira *et al.* [14, 15] proposed that pores play an important role in nucleating the apatite, rather than the silanol groups. They showed that the induction time for the apatite formation on a silica gel in SBF increased with decreasing pore volume as well as pore size, for silica gels prepared in different media but heat treated at 600 °C. From these results, they concluded that negatively charged pores larger than 2 nm radius provide favourable conditions for the apatite nucleation and hence act as nucleation sites. On the other hand, West and Hench [13] proposed, on the basis of molecular orbital calculation, that surface defects such as trisiloxane rings are effective for the apatite nucleation.

From these backgrounds, the present authors examined the apatite-forming ability of silica gels prepared in different media, that is, in pure water, aqueous solution containing polyacrylic acid and aqueous solution containing polyethylene glycol in SBF, in the previous paper [16]. All three kinds of gel had many





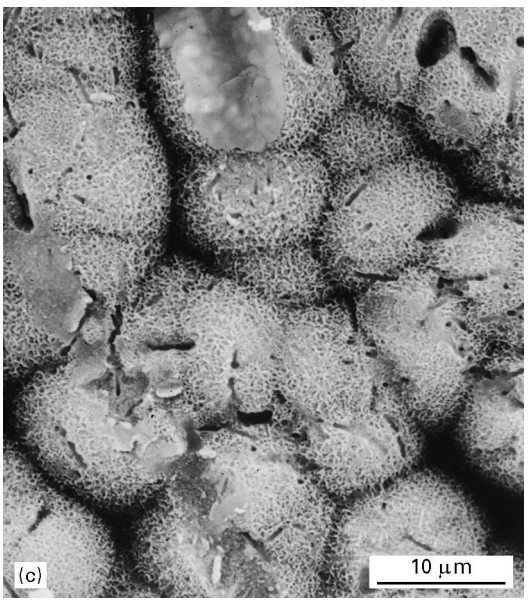


Figure 6 (a–c) Scanning electron micrographs of the surfaces of the silica gels which were subjected to different solvent exchanges and heat treatments at 400 °C as in Fig. 1, after soaking in SBF for 2 wk.

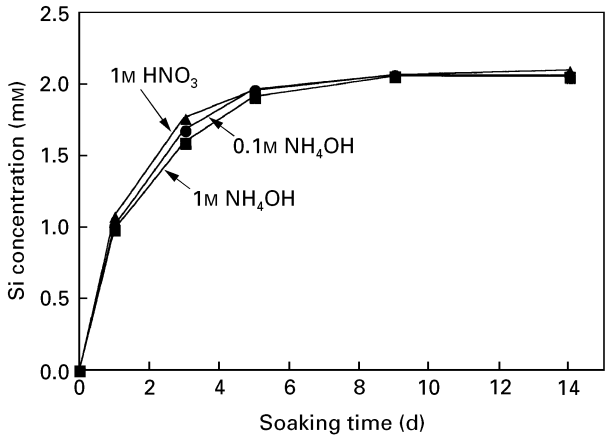


Figure 7 Changes in silicon concentrations of SBF due to the soaking of the silica gels which were subjected to different solvent exchanges and heat treatments at 400 °C as a function of soaking time.

silanol groups as well as trisiloxane rings on their surfaces. Despite this, only the last gel formed the apatite in SBF, whereas the former two kinds of gel did not form apatite within 2 wk. This indicates that neither the presence of the silanol groups nor that of the trisiloxane rings is a sufficient condition for apatite nucleation. The former two kinds of gel contained larger amounts of pores larger than 1.7 nm in size than did the last gel. Despite this, they did not form apatite. This indicates that the presence of pores larger than 1.7 nm is also an insufficient condition for the apatite nucleation. The main difference in pore structure between the gels was the presence of micropores less than 1.7 nm only in the last gel. In order to investigate the effect of these micropores on apatite formation, the pore structure of the silica gel prepared in an aqueous solution containing polyethylene glycol, was modified by solvent exchange in the present study.

Three kinds of gel obtained by different solvent exchanges in the present study all contained many silanol groups as well as trisiloxane rings (D2 defects) on their surfaces (see Figs 4 and 7). The gel treated with HNO_3 solution contained appreciable amounts of micropores less than 1.7 nm in size, and a lesser amount of mesopores larger than 1.7 nm in size (see Fig. 3). The two kinds of gel treated with NH_4OH solutions contained larger amounts of mesopores, but only small amounts of micropores. Irrespective of these differences in pore structure, all the gels formed the apatite in SBF within 2 wk (see Fig. 6). This indicates that pore structure is not related to apatite nucleation. It is speculated that a certain type of structural unit of silanol groups effective for apatite formation is easily formed when hydrolysis and polycondensation of tetraethoxysilane is perormed in the aqueous solution containing polyethylene glycol, although it cannot be identified by IR and Raman spectroscopies.

5. Conclusion

Pore structure of a silica gel prepared by hydrolysis and polycondensation of tetraethoxysilane in an

aqueous solution containing polyethylene glycol was modified by solvent exchange. The three kinds of gel obtained, formed bone-like apatite on their surfaces in simulated body fluid, irrespective of their pore structure. The indicates that a certain type of structural unit of silanol groups, which is easily formed in the presesnce of the polyethylene glycol, is effective for apatite formation.

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