JOURNAL OF MATERIALS SCIENCE. MATERIALS IN MEDICINE 4 (1993) 127–131

Process of formation of bone-like apatite layer on silica gel

P. LI, C. OHTSUKI, T. KOKUBO
Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan
K. NAKANISHI, N. SOGA, T. NAKAMURA*, T. YAMAMURO‡
*Faculty of Engineering, *Research Center for Biomedical Engineering and ‡Faculty of Medicine, Kyoto University, Kyoto City 606, Japan*

It has been proposed that a hydrated silica plays an important role in forming a biologically active apatite layer on the surfaces of bioactive glasses and glass–ceramics in the body. Recent experiments have shown that a silica hydrogel actually induces apatite formation on its surface in a simulated body fluid (SBF). In the present study the process of apatite formation on silica gel was investigated by means of thin-film X-ray diffraction, Fourier-transformed infrared reflection spectroscopy and scanning electron microscopic observation of the surface of the silica gel, as well as the measurement of changes in the ion concentration of the fluid. It was found that the induction period for the apatite nucleation on the surface of the silica gel was about 6 days. Once the apatite nuclei were formed they grew, taking a spherulitic form by consuming the calcium and phosphate ions from the surrounding fluid. Each spherulite consisted of a lot of flake that clustered into a petal-like morphology. The flake was carbonate-containing hydroxyapatite of small-crystallites and/or defective structure. The Ca/P ratio of the apatite was estimated as 1.5–1.6. Thus, the apatite formed was able to induce secondary nucleation of the apatite.

1. Introduction

Research in the past two decades has shown that a group of glasses and glass–ceramics with specific compositions can bond to living bone [1–6]. These bioactive glasses and glass–ceramics are already used clinically for the replacement and reconstruction of bone damaged by disease or accident [7–14]. It has been found that all bioactive glasses and glass–ceramics hitherto known bond to bone through an apatite layer that is formed on their surfaces in the body [5, 6, 15–22]. It is therefore believed that the prerequisite for glasses and glass–ceramics to bond to bone is the formation of an apatite layer on their surface in the body [23, 24].

It has been proposed that hydrated silica that is formed on the surfaces of bioactive glasses and glass–ceramics in the body plays an important role in forming the apatite layer [25–27]. Recently it was shown experimentally by the present authors that pure silica hydrogel induces formation of a bone-like apatite on its surface when the gel is soaked in an SBF with ion concentrations nearly equal to those in human blood plasma [28].

In this study the process of formation of the bone-like apatite layer on the surface of the silica gel in the SBF was investigated.

2. Experimental procedure

A pure silica gel was prepared from commercial tetraethoxysilane (TEOS) and polyethylene glycol (PEG) with average molecular weight of 10 000. The PEG was dissolved in distilled water and a small amount of nitric acid was added. Hydrolysis of TEOS occurred when it was added to this solution with stirring. The resultant solution, the molar ratio of which was TEOS:PEG:H_2O:HNO_3 1:0.002:14.2:0.41, was kept at 40 °C in an oven for hydrolysis and polycondensation which proceeded for 8 h, and was followed by an additional 10 h ageing for gelation. The aged wet silica gel was soaked in an ethanol–water solution to leach out the organic phase remaining in the gel. The gel was heated to 400 °C and held at that temperature for 2 h after the wet gel was dried at 40 °C. The silica gel was cooled in the furnace to room temperature. It had micrometre-range interconnected pores as well as nanometre-range pores [28].

An SBF with ion concentrations nearly equal to those in human blood plasma [29], as shown in Table I, was prepared by dissolving reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$ and Na_2SO_4 in distilled water. The fluid was buffered at pH 7.4 at 37 °C with tris-(hydroxymethyl)aminomethane [Tris; $(CH_2OH)_3CNH_2$] and hydrochloric acid (HCl). This fluid is able satisfactorily to reproduce apatite formation on the surfaces of various kinds of bioactive glasses and glass–ceramics *in vivo* [19, 30–34].

A rectangular specimen of the silica gel with dimensions 6 mm × 6 mm × 1.5 mm was immersed in 15 ml

TABLE I Ion concentrations of SBF and human blood plasma

	Ion concentration (mM)							
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	2.5	1.5	147.8	4.2	1.0	0.5
Human plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0	0.5

SBF in a polyethylene bottle maintained at 37 °C. After soaking for various periods, the specimens were removed from the fluid and immersed in 10 ml ion-exchanged water for 5 min. The specimens were then dried at room temperature. Their surfaces were analysed using thin-film X-ray diffraction (TF-XRD), Fourier-transformed infrared reflection spectroscopy and scanning electron microscopy (SEM). The concentrations of Ca, P and Si in the fluid were monitored with inductively coupled plasma (ICP) emission spectroscopy during the soaking. Periodically, 0.5 ml fluid was drawn out from one of the bottles and diluted into 5 ml distilled water for ICP measurement.

3. Results and discussion

Figs 1 and 2 show the TF-XRD patterns and Fourier-transformed infrared reflection spectra of the surfaces of the silica gel as a function of the soaking time in SBF. The assignments of the main peaks, which were made by referring to previously published data [30, 32], are also shown in these figures. It can be seen from Figs 1 and 2 that apatite started to form on the surface of the silica gel about 7 days after the silica gel was immersed in the fluid, and grew with increasing soaking time. The TF-XRD patterns in Fig. 1 show rather broad diffraction peaks for the apatite. This means that the apatite formed had a small-crystallite and/or defective structure, similar to bone apatite. The Fourier-transformed infrared reflection spectra in Fig. 2 show a reflection peak at around 1400 cm^{-1}, which is ascribed to carbonate ion [35, 36]. This means that the apatite formed on the surface of the silica gel was incorporated with carbonate ion, similar to the bone apatite.

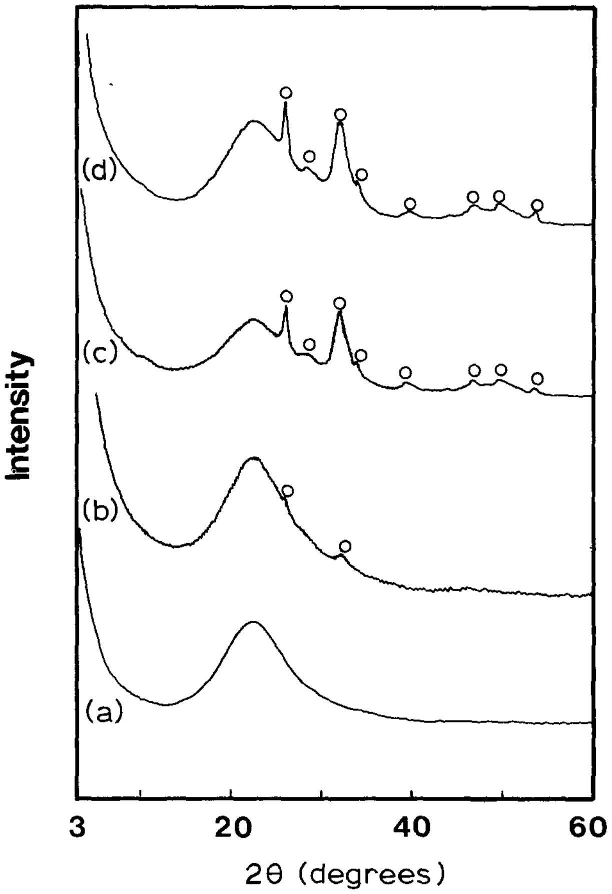


Figure 1 TF-XRD patterns of the surface of the silica gel soaked in SBF for various periods: (a) before immersion and (b) 7 days, (c) 10 days and (d) 14 days. (o) Apatite.

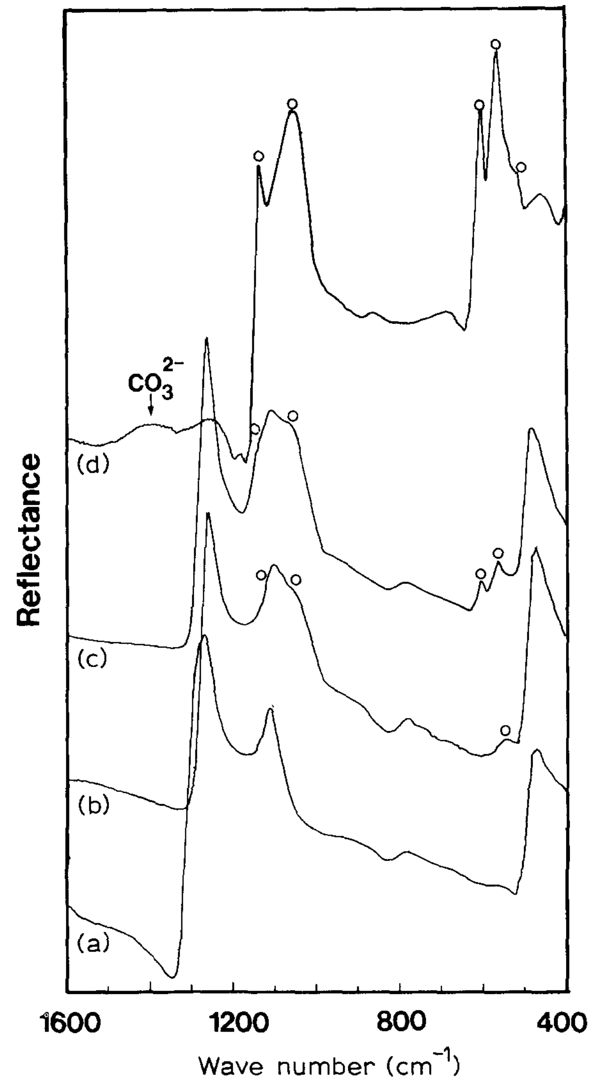


Figure 2 Fourier-transformed infrared reflection spectra of the surface of the silica gel soaked in SBF for various periods: (a) before immersion and (b) 7 days, (c) 10 days and (d) 14 days. (o) Apatite

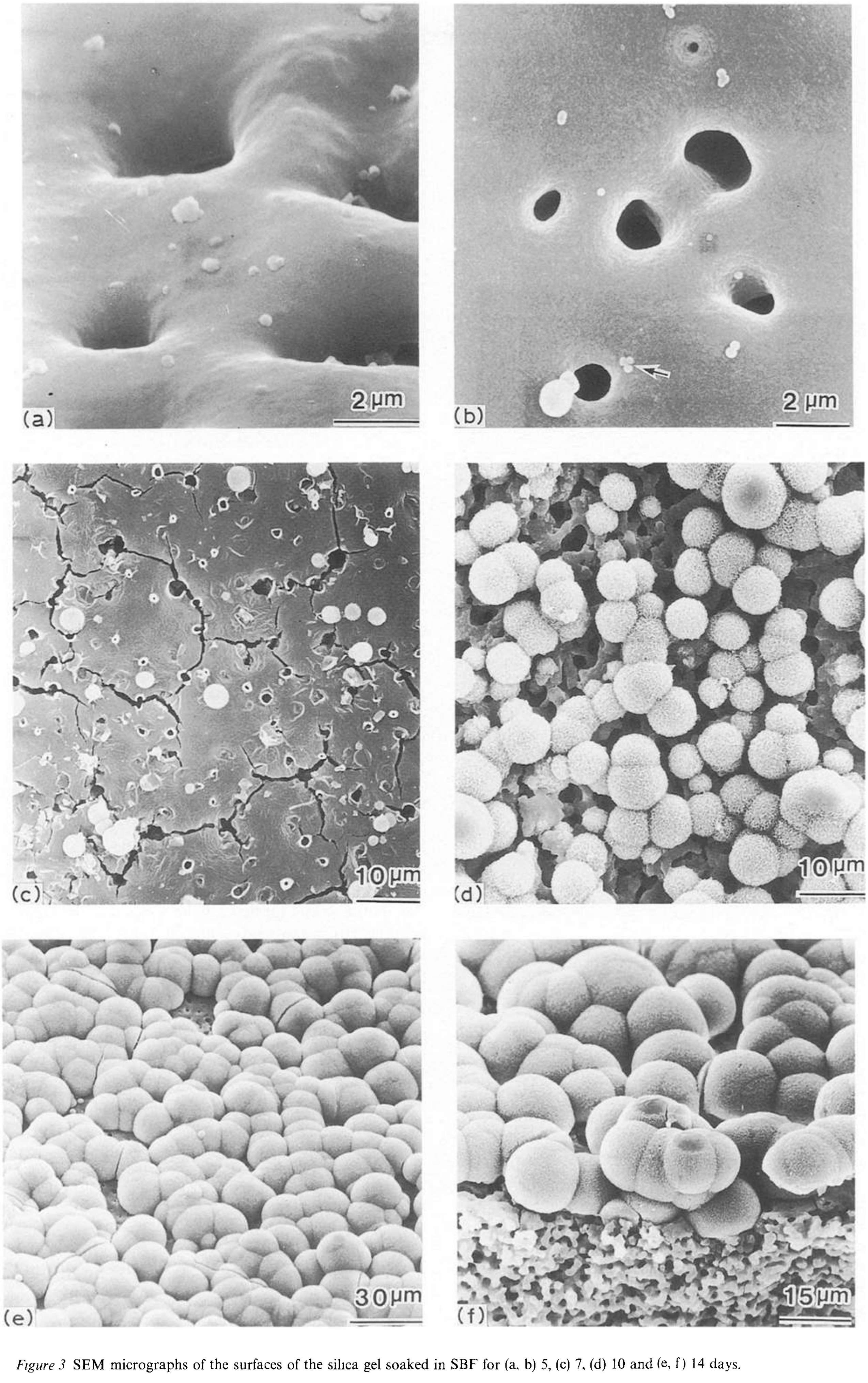
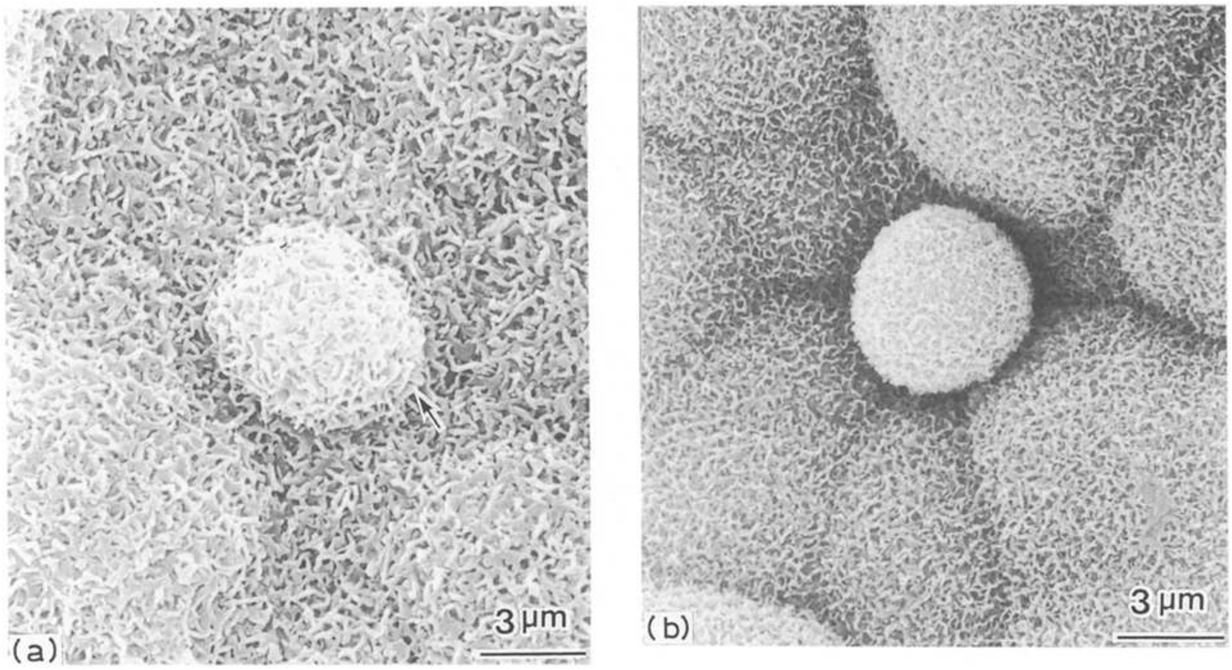


Figure 3 SEM micrographs of the surfaces of the silica gel soaked in SBF for (a, b) 5, (c) 7, (d) 10 and (e, f) 14 days.



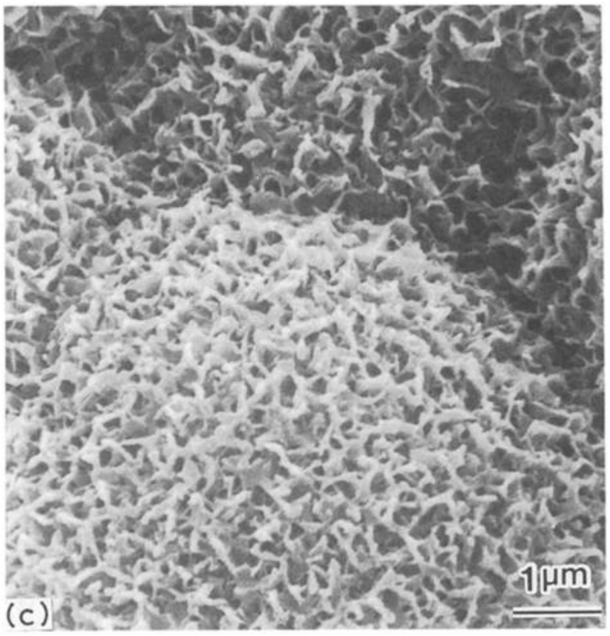


Figure 4 SEM micrographs at higher magnifications of the apatite formed on the silica gel soaked in SBF for 14 days.

Fig. 3 shows SEM micrographs of the surfaces of the silica gel as a function of the soaking time in SBF. It can be seen from Fig. 3 that apatite started to precipitate on the silica gel, taking the form of tiny spherulites about 5 days after the gel was soaked in SBF. The spherulites increased in both number and size with increasing soaking time, and completely covered the surface of the silica gel within 14 days. Fig. 4 shows higher-magnification SEM micrographs of the apatite formed on the silica gel soaked in SBF for 14 days. It can be seen from Fig. 4 that each spherulite consists of a large number of flakes. Some spherulites were formed not only directly on the surface of the silica gel, but also on the surfaces of other growing spherulites (Fig. 4a) or at their interfaces (Fig. 4b). This indicates that the front of a growing apatite can also become a nucleating site of another apatite. Therefore, the spherulites formed were closely connected with each other at their interfaces, as shown in Fig. 4c.

Fig. 5 shows changes in the Ca, P and Si concentrations of SBF with the soaking time of the silica gel. It can be seen from Fig. 5 that the Si concentration increased for the first 8 days and reached a constant value of about 55 p.p.m. Both the Ca and P concentrations started to decrease abruptly 6 days after the silica gel was immersed, then continued to decrease slowly. The constant concentration of 55 p.p.m. for the Si corresponds to the solubility of the amorphous silica in water [37]. The decreases in the Ca and P concentrations in SBF are attributed to the growth of the apatite nuclei formed on the surface of the silica gel. The SBF was already supersaturated with respect to the apatite, similarly to body fluid [38, 39]. Once the apatite nuclei were formed, therefore, they grew spontaneously by consuming the calcium and phosphate ions from the surrounding fluid. The period from the time when the silica gel was immersed until

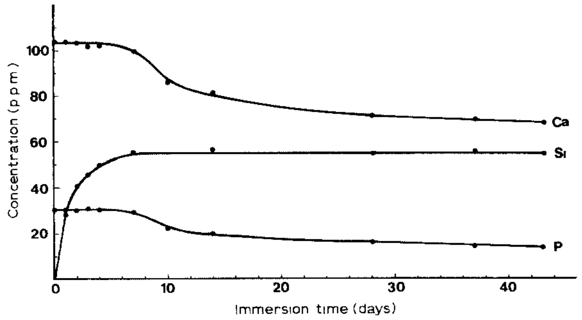


Figure 5 The change in ion concentrations of SBF with time after the silica gel was immersed in SBF.

the time when the Ca and P concentrations started to decrease (i.e. 6 days) corresponds to the induction period for apatite nucleation on the surface of silica gel. This is consistent with the results from the TF-XRD, Fourier-transformed infrared and SEM experiments described above. In the case of a silica glass and a quartz crystal, apatite formation was not observed on their surfaces in the same SBF even 21 days after they were soaked [28]. This indicates that the silanol group, abundant on the surface of the silica gel, accelerates apatite nucleation, although its detailed mechanism is not yet known. The atomic ratio of the Ca and P that were consumed from the fluid corresponds to the Ca/P ratio of the apatite formed on the surface of the silica gel. The Ca/P ratio of the apatite, which was calculated from the decreases in the Ca and P concentrations in the SBF in the period from 14 to 40 days after the silica gel was immersed, was 1.6–1.5. This is lower than the 1.67 for the stoichiometric hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References

1. L L HENCH, R J SPLINTER, W C ALLEN and T K GREELEE, *J. Biomed. Mater. Res. Symp.* **2** (1971) 117.
2. B -A VON BLENCKE, H BRÖMER and K. DEUTSCHER, *Med. Orthop. Technol.* **95** (1975) 144.
3. T KOKUBO, M SHIGEMATSU, Y NAGASHIMA, M TASHIRO, T NAKAMURA, T. YAMAMURO and S HIGASHI, *Bull. Inst. Chem. Res., Kyoto Univ.* **60** (1982) 260
4. W HÖLAND, K. NAURNANN, W. VOGEL and J GOMMEL, *Wiss. Zeitschr. Friedrich-Schiller-Univ. Jena, Math.-Nuturwiss. R.* **32** (1983) 571
5. K OHURA, T. YAMAMURO, T NAKAMURA, T KOKUBO, Y. EBISAWA, Y KOTOURA and M. OKA, *J Biomed. Mater Res.* **25** (1991) 357.
6. K OHURA, M IKENAGA, T. NAKAMURA, T. YAMAMURO, Y. EBISAWA, T KOKUBO, Y KOTOURA and M OKA, *J Appl. Biomater.* **2** (1991) 153.
7. R RECK, S STORKEL and A MEYER, *Ann. NY Acad. Sci.* **523** (1988) 100.
8. J. VON GUMMEL, H ZIPPEL and H HÄHNEL, *Z. Klin. Med.* **43** (1988) 179.
9. G E MERWIN, in "Handbook of Bioactive Ceramics", Vol. 1: "Bioactive Glasses and Glass–Ceramics", edited by T. Yamamuro, L. L. Hench and J. Wilson (CRC Press, Boca Raton, Florida, 1990) p. 323.
10. T. YAMAMURO, *ibid.* p. 335.
11. *Idem, ibid.* p. 343.
12. T. YAMAMURO, H OKUMRA, T KITSUGI, Y KAKUTANI, T MATSUI and T. KOKUBO, *J Bone Joint Surg.* **72B** (1990) 889.
13. L L HENCH, H. R STANLEY, A E. CLARK, M HALL and J WILSON, in "Bioceramics", Vol. 4, edited by W. Bonfield, G. W. Hastings and K. E. Tanner (Butterworth/Heinemann, Oxford, 1991) p 232.
14. T. KOKUBO, *J. Ceram. Soc. Jpn.* **99** (1991) 965.
15. L L HENCH and A E CLARK, in "Biocompatibility of Orthopedic Implant", Vol. 2, edited by D. F. Williams (CRC Press, Boca Raton, Florida, 1982) p. 129.
16. T KITSUGI, T YAMAMURO, T NAKAMURA, S HIGASHI, Y KAKUTANI, K HYAKUNA, S. ITO, T KOKUBO, M. TAKAGI and T SHIBUYA, *J. Biomed. Mater. Res.* **20** (1986) 1295.
17. T KITSUGI, T NAKAMURA, T YAMAMURO, T KOKUBO, T SHIBUYA and M TAKAGI, *ibid.* **21** (1987) 1255.
18. T KITSUGI, T. YAMAMURO, T. NAKAMURA and T KOKUBO, *ibid* **23** (1989) 631.
19. T KOKUBO, in "Handbook of Bioactive Ceramics", Vol 1: "Bioactive Glasses and Glass–Ceramics", edited by T. Yamamuro, L. L. Hench and J. Wilson (CRC Press, Boca Raton, Florida. 1990) p. 41
20. T KOKUBO, C OHTSUKI, S KOTANI, T KITSUGI and T. YAMAMURO, in "Bioceramics", Vol. 2, edited by G. Heimke (German Ceramic Society, Cologne, 1990) p. 113.
21. K KANGASNIEMI and A YLI-URPO, in "Handbook of Bioactive Ceramics", Vol. 1: "Bioactive Glasses and Glass–Ceramics", edited by T. Yamamuro, L. L. Hench and J Wilson (CRC Press, Boca Raton, Florida, 1990) p. 97.
22. C OHTSUKI, H KUSHITANI, T KOKUBO, S. KOTANI and T. YAMAMURO, *J. Biomed. Mater. Res.* **25** (1991) 1363.
23. T. KOKUBO. *Biomaterials* **12** (1991) 155.
24. L. L HENCH, *J. Amer. Ceram. Soc.* **74** (1991) 1487.
25. *Idem, Ann. NY Acad. Sci.* **523** (1988) 54.
26. K. H KARLSSON, K FRÖBERG and T RINGBOM, *J Non-Cryst. Solids* **112** (1989) 69.
27. T KOKUBO, *ibid.* **120** (1990) 138.
28. P LI, C OHTSUKI, T KOKUBO, K NAKANISHI, N SOGA, T. NAKAMURA and T. YAMAMURO, *J. Amer. Ceram. Soc.* submitted.
29. J GAMBLE, in "Chemical Anatomy, Physiology and Pathology of Extracellular Fluid", 6th Edn (Harvard University Press, Cambridge, Massachusetts, 1967) p. 1.
30. T KOKUBO, S. ITO, Z. T HUANG, T. HAYASHI, S SAKKA, T. KITSUGI and T YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 331.
31. T KOKUBO, T HAYASHI, S SAKKA, T KITSUGI, T. YAMAMURO, M TAKAGI and T SHIBUYA, in "Ceramics in Clinical Applications", edited by P. Vincenzini (Elsevier, Amsterdam, 1987) p. 175.
32. T KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 721.
33. Y EBISAWA, T KOKUBO, K. OHURA and T YAMAMURO, *J. Mater. Sci Mater. Med.* **1** (1990) 239.
34. C. OHTSUKI, T KOKUBO, K TAKATSUKA and T YAMAMURO, *Nippon Seramikkusu Kyokai Gakujutsu Ronbunshi* **99** (1991) 1.
35. D. G A NELSON and J. D B FEATHERSTONE, *Calcif. Tissue Int* **34** (1982) S69.
36. C REY, B COLLINS, T GOEHL, I R DICKSON and M J GLIMCHER, *ibid.* **45** (1989) 157.
37. R K ILER, in "The Chemistry of Silica" (Wiley, New York, 1979) p. 40.
38. W NEUMAN and M NEUMAN, in "The Chemical Dynamics of Bone Mineral" (University of Chicago, Chicago, 1958) p. 34.
39. C OHTSUKI, T KOKUBO and T YAMAMURO, *J. Non-Cryst. Solids*, in press.

Received 28 January
and accepted 19 February 1992