JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 3 (1992) 119–125

Compositional dependence of bioactivity of glasses in the system $CaO–SiO_2–Al_2O_3$: its *in vitro* evaluation

CHIKARA OHTSUKI, TADASHI KOKUBO
Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan
TAKAO YAMAMURO
Faculty of Medicine, Kyoto University, Sakyo-Ku, Kyoto City 606, Japan

In order to investigate fundamentally the effect of Al_2O_3 on the bioactivity of glasses and glass–ceramics, the compositional dependence of bioactivity of glasses in the system $CaO–SiO_2–Al_2O_3$ was studied *in vitro*. It is already known that the essential condition for glasses and glass–ceramics to bond to living bone is the formation of an apatite layer on their surfaces in the body, and that the surface apatite layer can be reproduced even in an acellular simulated body fluid which has almost equal ion concentrations to those of the human blood plasma. In the present study, bioactivity of the glasses was evaluated by examining apatite formation on their surfaces in the simulated body fluid with thin-film X-ray diffraction, Fourier transform infrared reflection spectroscopy and scanning electron microscopic observation. Only $CaO–SiO_2–Al_2O_3$ glasses containing Al_2O_3 less than 1.5 mol% formed the surface apatite as well as Al_2O_3-free $CaO–SiO_2$ glasses, but $CaO–SiO_2–Al_2O_3$ containing Al_2O_3 more than 1.7 mol% did not form it as well as an SiO_2-free $CaO–Al_2O_3$ glass. This indicates that only a small amount of addition of Al_2O_3 to glass compositions suppresses the bioactivity of glasses and glass–ceramics by suppressing apatite formation on their surfaces in the body.

1. Introduction

Glass–ceramic A-W containing apatite and wollastonite in an $MgO–CaO–SiO_2$ glassy matrix bonds to living bone [1], whereas glass–ceramic A-W(Al) containing the same kinds of crystalline phases in an $MgO–CaO–SiO_2–Al_2O_3$ glassy matrix does not bond to the living bone [2]. A similar adverse effect of Al_2O_3 on bioactivity has been also reported for Bioglass-type glasses in the system $Na_2O–CaO–SiO_2–P_2O_5$ [3] and Ceravital-type apatite-containing glass–ceramics in the system $Na_2O–K_2O–MgO–CaO–SiO_2–P_2O_5$ [4]. It has not, however, been revealed fundamentally how Al_2O_3 effectively suppresses the bioactivity of glasses and glass–ceramics.

In the present study, in order to investigate this problem, the compositional dependence of the bioactivity of glasses in the system $CaO–SiO_2–Al_2O_3$ was studied *in vitro*. Binary $CaO–SiO_2$ glasses are the simplest glasses known to show bioactivity [5, 6], and hence are suitable as basic glasses in this kind of fundamental study. It has been shown for various kinds of glass and glass–ceramic including Bioglass-type glasses, Ceravital-type glass–ceramics and A-W-type glass–ceramics that the essential condition for glasses and glass–ceramics to bond to living bone is formation of an apatite layer on their surfaces in the body [7–14], and that the apatite layer can be reproduced on their surfaces even in an acellular simulated body fluid which has almost equal ion concentrations to those of the human blood plasma [15–18]. In the present study, bioactivity of the glasses was evaluated by examining the formation of an apatite layer on their surfaces in the simulated body fluid with thin-film X-ray diffraction, Fourier transform infrared reflection spectroscopy and scanning electron microscopic observation.

2. Experimental procedure

2.1. Preparation of glass

A compositional region in the system $CaO–SiO_2–Al_2O_3$ where glass can be formed by an ordinary melting technique was previously reported by Imaoka and Yamazaki [19]. Powder mixtures in the amount of about 20 g of various compositions in the glass-forming region were prepared using the reagent-grade chemicals $CaCO_3$, SiO_2 and Al_2O_3. They were put into a 50 ml platinum crucible and melted at 1600 °C for 1 h in an $MoSi_2$ furnace. The melts were poured on to a stainless steel plate to be formed into a plate about 1 mm thick, and allowed to cool in an SiC furnace from an appropriate temperature. The cooled substances were examined by visual observation and X-ray diffraction to determine whether they contained crystalline phases or not.

0957–4530

2.2. Soaking in simulated body fluid

The glasses obtained by the method described above were cut into rectangular specimens of 15 mm × 10 mm × 1 mm, polished with 3–4 μm diamond paste, and washed with pure acetone and ion-exchanged water in an ultrasonic cleaner. They were immersed in 35 ml of an acellular simulated body fluid, which had almost equal ion concentrations to those of human blood plasma as shown in Table I [20]. The fluid was prepared by dissolving reagent-grade chemicals NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$and Na_2SO_4 in distilled water. It was buffered at pH 7.25 with 50 mM trishydroxymethyl-aminomethane ($(CH_2OH)_3CNH_2$) and 45 mM HCl, and its temperature was kept at 36.5 °C.

2.3. Analysis of surface structure

After the specimens were soaked in the simulated body fluid for 7, 20 and 30 days, they were taken out from the fluid and gently washed with acetone. Their surfaces were subjected to thin-film X-ray diffraction, Fourier transform infrared reflection spectroscopy and scanning electron microscopic observation. In the X-ray diffraction experiment, a Rigaku CN2651A2 thin-film attachment was used and the surface of the specimen was fixed at 1° to the incident beam. In the infrared spectroscopy, a Japan Spectroscopic FT-IR5M spectrometer was used and the reflection angle was 75°. These two techniques allowed detection of a layer only about 1 μm thick at the surface. In the scanning electron microscopic observation, a gold–palladium film was coated on the surface of the specimen and a Hitachi S2500CX scanning electron microscope was used.

3. Results and discussion

Fig. 1 shows the compositions of the examined glasses and their appearances. Thin-film X-ray diffraction patterns and Fourier transform infrared reflection spectra of the surfaces of some of the glasses before being soaked in the simulated body fluid are shown in Fig. 2 as references. Their compositions are given in Table II.

Figs 3 and 4 show thin-film X-ray diffraction patterns and infrared reflection spectra, respectively, of the surfaces of the same glasses after being soaked in the simulated body fluid for 7 days. Assignments of the

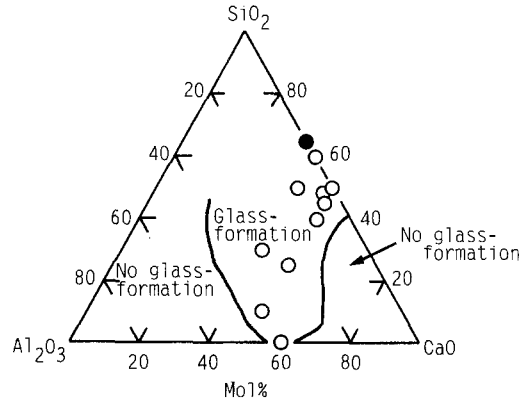

Figure 1 Compositions of the glasses examined and their appearances. The glass-forming region was reported by Imaoka *et al.* [19]; (○) clear transparent glass, (●) immiscible opaque glass.

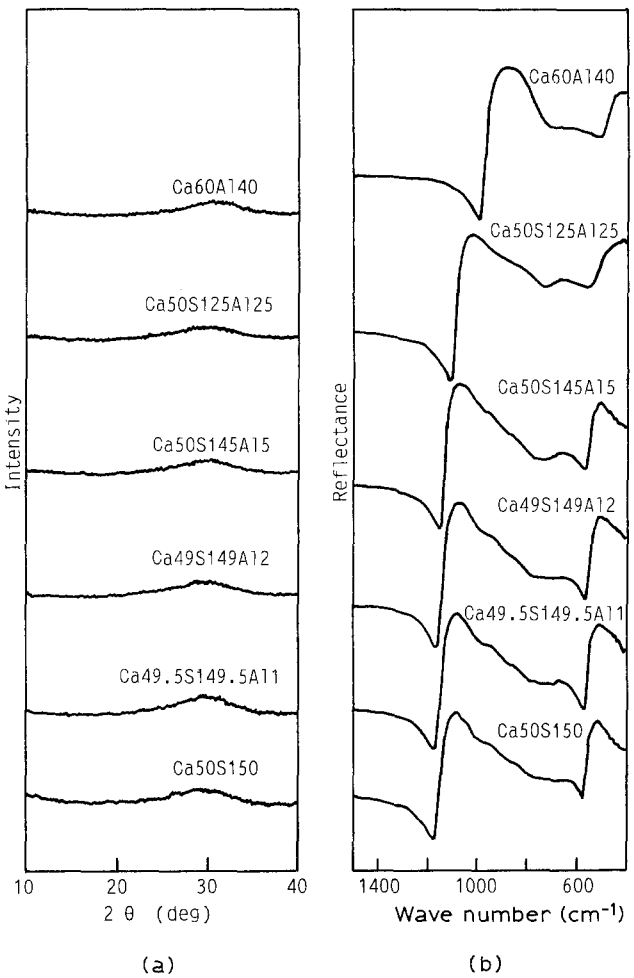

Figure 2 (a) Thin-film X-ray diffraction patterns and (b) Fourier transform infrared reflection spectra of the surfaces of some $CaO–SiO_2–Al_2O_3$ glasses before being soaked.

TABLE I Ion concentrations of simulated body fluid and human blood plasma

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

TABLE II Compositions of some of the glasses examined

Name	Composition (mol %)		
	CaO	SiO_2	Al_2O_3
Ca60Al40	60.0	0	40.0
Ca50Si25Al25	50.0	25.0	25.0
Ca50Si45Al5	50.0	45.0	5.0
Ca49Si49Al2	49.0	49.0	2.0
Ca49.5Si49.5Al1	49.5	49.5	1.0
Ca50Si50	50.0	50.0	0

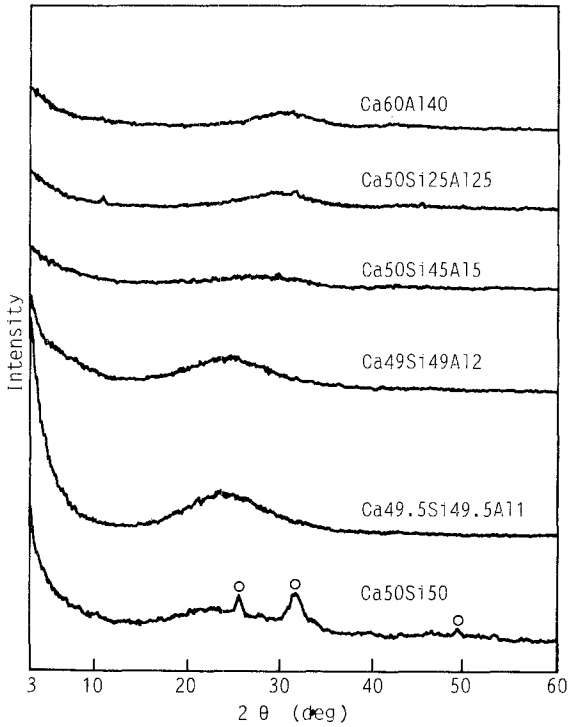

Figure 3 Thin-film X-ray diffraction patterns of the surfaces of CaO–SiO_2–Al_2O_3 glasses soaked in the simulated body fluid for 7 days: (○) apatite peak.

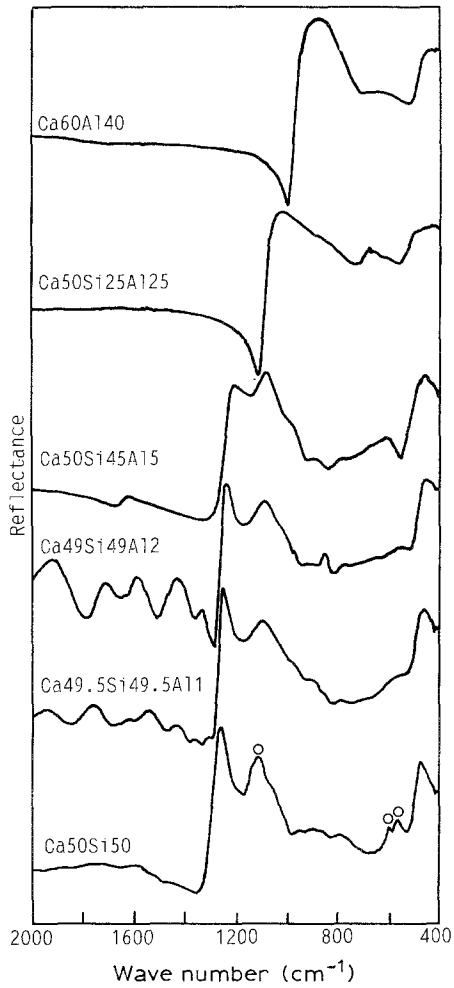

Figure 4 Fourier transform infrared reflection spectra of the surfaces of CaO–SiO_2–Al_2O_3 glasses soaked in the simulated body fluid for 7 days: (○) apatite peak.

main peaks based on the data previously reported [16] are also shown in Figs 3 and 4. It can be seen from Figs 3 and 4 that Ca50Si50 glass forms an apatite phase on its surface in the simulated body fluid in 7 days, whereas Ca60Al40, Ca50Si25Al25, Ca50Si45Al5, Ca49Si49Al2 and Ca49.5Si49.5Al1 do not form it.

Figs 5 and 6 show thin-film X-ray diffraction patterns and infrared reflection spectra, respectively, of the surfaces of the same glasses after being soaked for 20 days. It can be seen from Figs 5 and 6 that Ca49.5Si49.5Al1 glass in addition to Ca50Si50 glass form the apatite phase on their surfaces in the simulated body fluid by 20 days, whereas Ca60Al40, Ca50Si25Al25, Ca50Si45Al5 and Ca49Si49Al2 glasses do not form it even after 20 days.

Figs 7 and 8 show thin-film X-ray diffraction patterns and infrared reflection spectra, respectively, of the surfaces of the same glasses after being soaked for 30 days. These patterns and spectra are essentially same as those for the glasses soaked for 20 days.

Fig. 9 shows scanning electron micrographs of the surfaces of the same glasses after being soaked in the simulated body fluid for 30 days. It can be seen from Fig. 9 that leaf-like particles are deposited on the surfaces of Ca50Si50 and Ca49.5Si49.5Al1 glasses, but not on the surfaces of Ca49Si49Al2, Ca50Si45Al5, Ca50Si25Al25 and Ca60Al40 glasses. The morphology of the leaf-like particles is very similar to that of the apatite formed on the surface of glass–ceramic A-W [16] and Ceravital-type glass–ceramic [21]. Ca49Si49Al2 glass shows only a slight track of chemical corrosion. The latter three kinds of glass show no track of any kind of corrosion.

These results are summarized in Fig. 10 together with those of the glasses of other compositions, as a

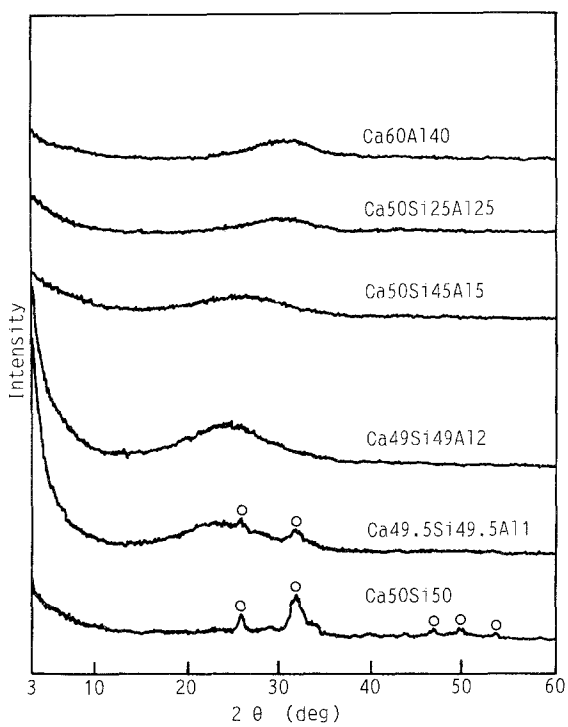

Figure 5 Thin-film X-ray diffraction patterns of the surfaces of CaO–SiO_2–Al_2O_3 glasses soaked in the simulated body fluid for 20 days: (○) apatite peak.

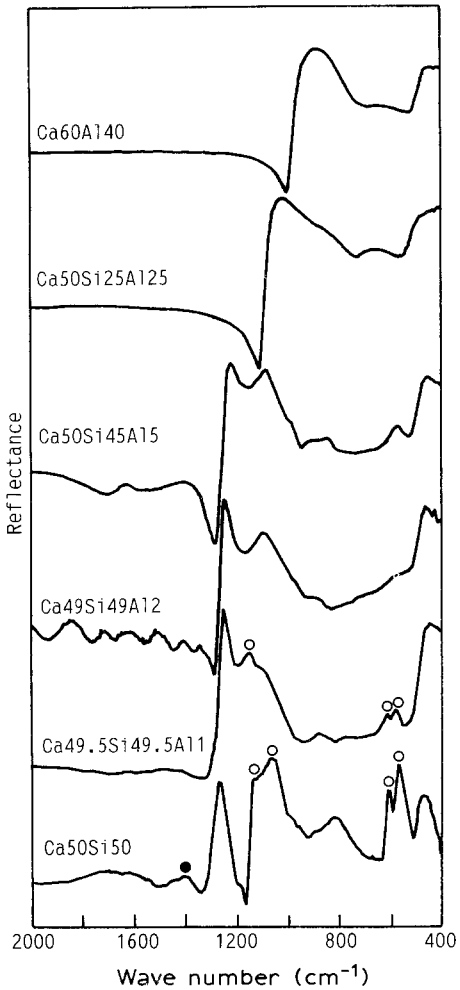

Figure 6 Fourier transform infrared reflection spectra of the surfaces of $CaO–SiO_2–Al_2O_3$ glasses soaked in the simulated body fluid for 20 days: (○) apatite, (●) CO_3^{2-} peak.

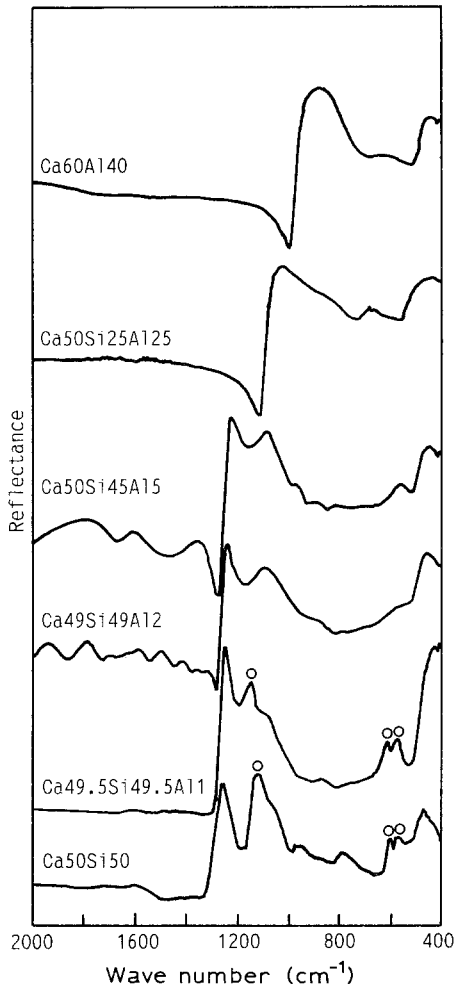

Figure 8 Fourier transform infrared reflection spectra of the surfaces of $CaO–SiO_2–Al_2O_3$ glasses soaked in the simulated body fluid for 30 days: (○) apatite peak.

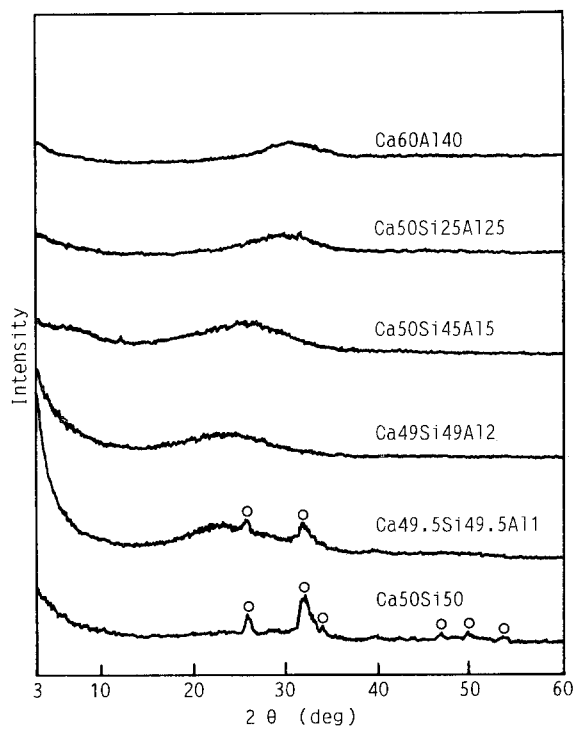

Figure 7 Thin-film X-ray diffraction patterns of the surfaces of $CaO–SiO_2–Al_2O_3$ glasses soaked in the simulated body fluid for 30 days: (○) apatite peak.

function of soaking time in the simulated body fluid. It can be seen from Fig. 10 that $CaO–SiO_2–Al_2O_3$ glasses containing Al_2O_3 less than 1.5 mol % form the apatite layer on their surfaces in the simulated body fluid by 30 days as well as Al_2O_3-free $CaO–SiO_2$ glasses, whereas $CaO–SiO_2–Al_2O_3$ glasses containing Al_2O_3 more than 1.7 mol % do not form it (even after 30 days) as well as an SiO_2-free $CaO–Al_2O_3$ glass. The present authors previously reported that $CaO–SiO_2–Al_2O_3$ glass containing 1.7 mol % (2.9 wt %) of Al_2O_3 barely formed a Ca, P-rich layer on its surface and bonded to living bone at 25 weeks after being implanted into a tibia of rabbit [6]. This is consistent with the present result. These results indicate that only a small amount of addition of the Al_2O_3 to glass compositions suppresses the bioactivity of glasses and glass–ceramics, by suppressing formation of an apatite layer on their surfaces in the body.

According to previous studies on the mechanism of apatite formation on the surface of glass–ceramic A-W [22, 23], chemical reaction of the Ca(II) and Si(IV) ions dissolved from the glass–ceramic with the P(V) ion in the surrounding body fluid gives the apatite formation on the surface of the glass–ceramic. In this reaction, the Ca(II) ion increases the degree of supersaturation of the surrounding body fluid with

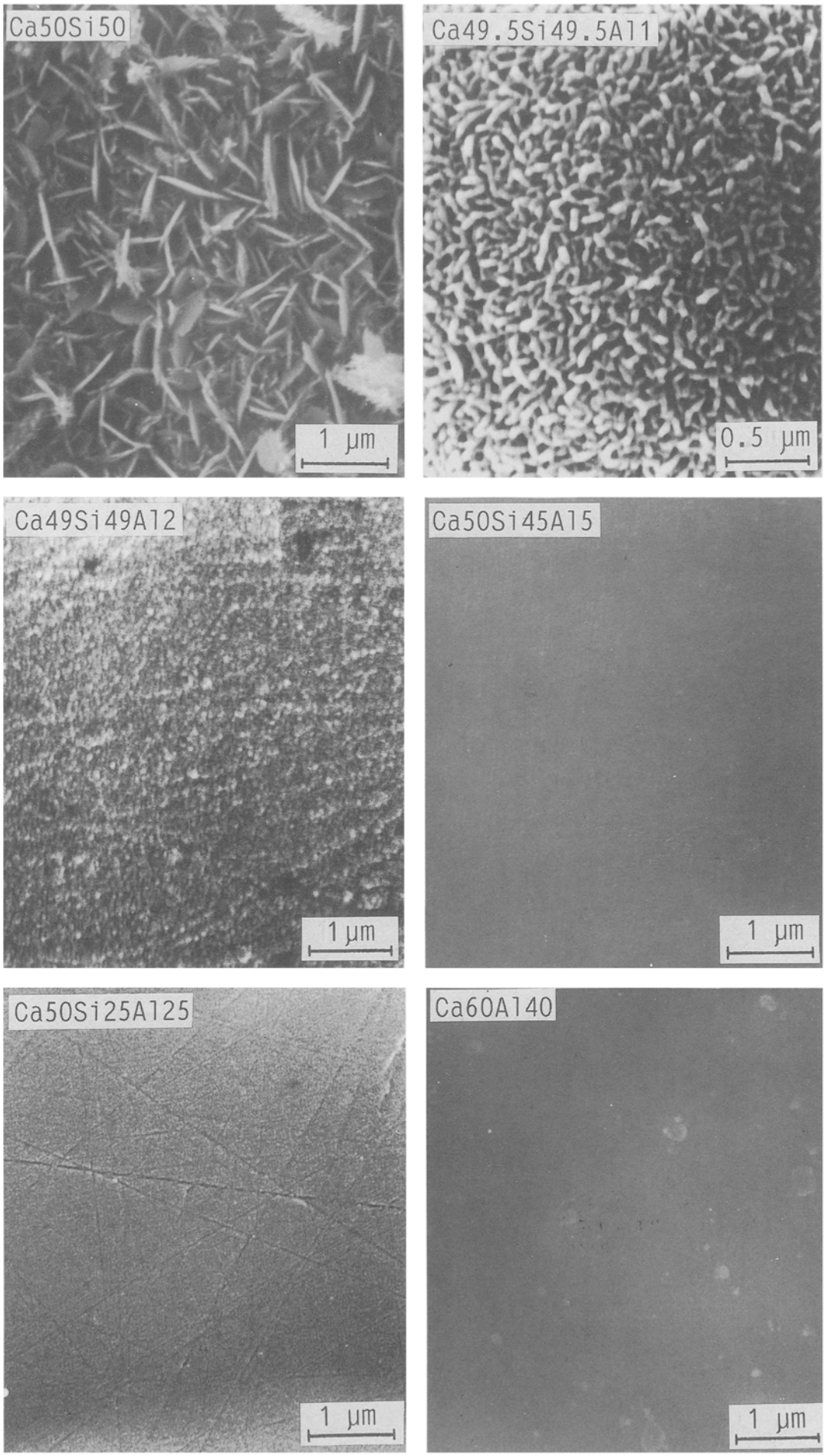

Figure 9 SEM photographs of the surfaces of $CaO–SiO_2–Al_2O_3$ glasses soaked in the simulated body fluid for 30 days.

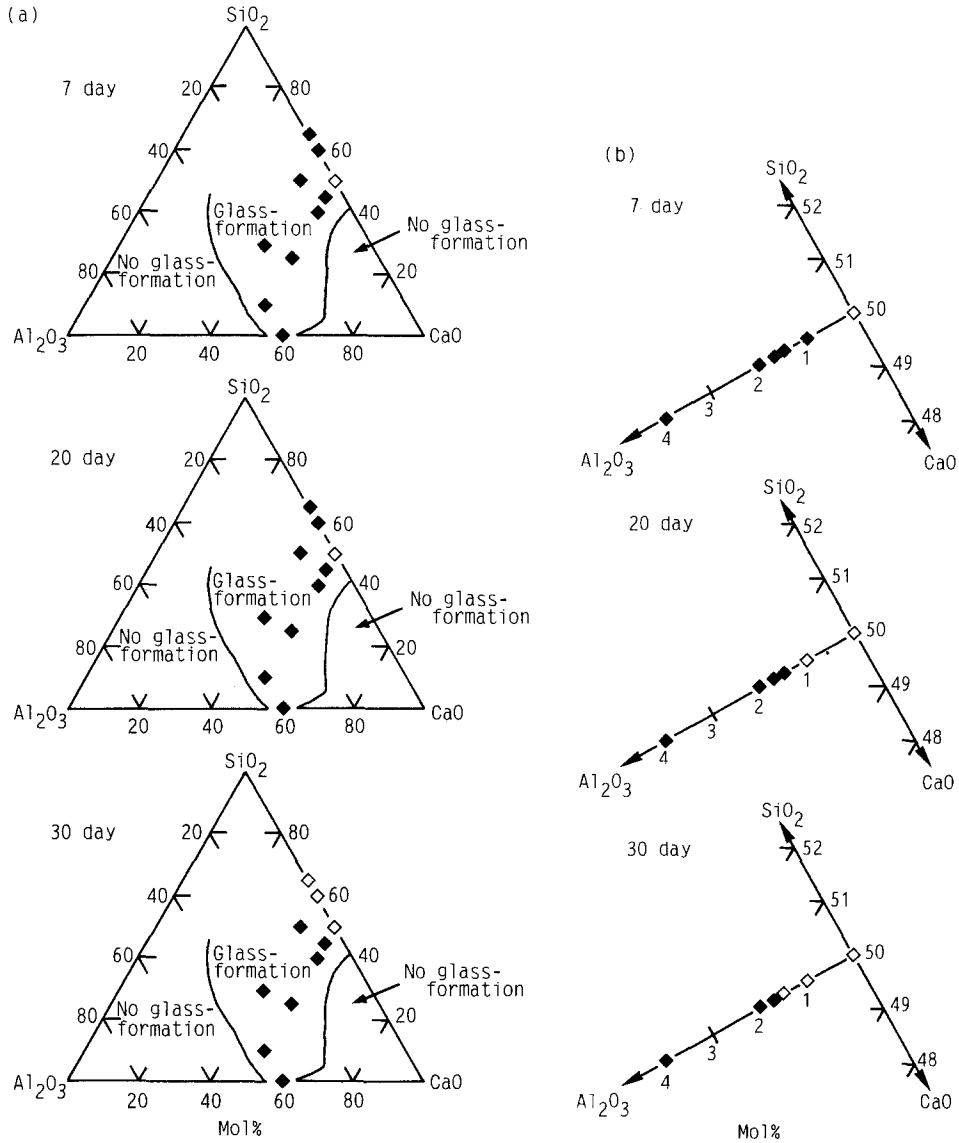

Figure 10 Compositional dependence of apatite formation on the surface of $CaO–SiO_2–Al_2O_3$ glasses as a function of soaking time in the simulated body fluid; (b) is a part of (a). (◇) Apatite formation, (◆) no apatite formation.

respect to apatite, which is already supersaturated even in the normal condition [24], and the Si(IV) ion provides favourable sites for nucleation of the apatite on the surface of the glass–ceramic. On the basis of these findings, apatite formation on the surfaces of $CaO–SiO_2–Al_2O_3$ glasses containing small amounts of Al_2O_3 as well as Al_2O_3-free $CaO–SiO_2$ glasses in the body environment might by similarly interpreted. The phosphate ion required for formation of the apatite is supplied only from the surrounding fluid.

In the case of $CaO–SiO_2–Al_2O_3$ glasses containing appreciable amounts of Al_2O_3 as well as SiO_2-free $CaO–Al_2O_3$ glass, dissolution of the Ca(II) and Si(IV) ions from the glasses might be suppressed by the Al_2O_3 present in the glasses, and hence apatite formation on the surfaces might be suppressed. This speculation is supported by the scanning electron micrographs in Fig. 9. Ca50Si45Al5, Ca50Si25Al25 and Ca60Al40 glasses did not show any track of chemical reaction even after 30 days of soaking in the simulated body fluid. Al_2O_3 is generally known as a component which can improve the chemical durability of glasses [25]. The lower rate of apatite formation on the surface of Ca49.5Si49.5Al1 glass than on the surface of Ca50Si50 glass might be also interpreted in terms of the suppressing effect of Al_2O_3 on the dissolution of Ca(II) and Si(IV) ions from the glass.

Gross and Strunz [4] previously interpreted the adverse effect of Al_2O_3 on bioactivity of Ceravital-type glass–ceramic in terms of the inhibiting effect of Al(III) ion dissolved from the glass–ceramic on normal mineralization of the surrounding bony tissue, without mentioning the surface apatite layer. The relation between the surface apatite formation of $CaO–SiO_2–Al_2O_3$ glasses and the dissolution of ions from the glasses will be discussed elsewhere in more detail.

Acknowledgement

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

References

1. T. NAKAMURA, T. YAMAMURO, S. HIGASHI, T. KOKUBO and S. ITOO, *J. Biomed. Mater. Res.* **19** (1985) 685.
2. T. NAKAMURA, T. YAMAMURO, S. HIGASHI, Y. KAKUTANI, T. KITSUGI, T. KOKUBO and S. ITO, in "Treatise on Biomedical Materials", Vol. 1, edited by T. Yamamuro (Kyoto University, Kyoto, 1983) p. 109.
3. R. L. FOLGER, C. S. KUCHERIA, R. E. WELLS and G. E. GARDINER, in "Biomaterials '84", Transactions of the 2nd World Congress on Biomaterials, Vol. 7, edited by J. M. Anderson (Society for Biomaterials, Washington, DC, 1984) p. 352.
4. U. GROSS and V. STRUNZ, *J. Biomed. Mater. Res.* **19** (1985) 251.
5. C. OHTSUKI, T. KOKUBO, K. TAKATSUKA and T. YAMAMURO, *Nippon Seramikkusu Kyokai Gakujutsu Ronbunshi* **99** (1991) 1.
6. K. OHURA, T. NAKAMURA, T. YAMAMURO, T. KOKUBO, Y. EBISAWA, Y. KOTOURA and M. OKA, *J. Biomed. Mater. Res.* **25** (1991) 357.
7. L. L. HENCH, in "Fundamental Aspects of Biocompatibility", Vol. 1, edited by D. F. Williams (CRC Press, Boca Raton, 1981) p. 67.
8. L. L. HENCH and A. E. CLARK, in "Biocompatibility of Orthopaedic Implants", Vol. II, edited by D. F. Williams (CRC Press, Boca Raton, 1982) p. 129.
9. 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.
10. T. KITSUGI, T. NAKAMURA, T. YAMAMURO, T. KOKUBO, T. SHIBUYA and M. TAKAGI, *ibid.* **21** (1987) 1255.
11. 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.
12. T. KITSUGI, T. YAMAMURO, T. NAKAMURA and T. KOKUBO, *J. Biomed. Mater. Res.* **23** (1989) 631.
13. S. KOTANI, T. YAMAMURO, T. NAKAMURA, T. KITSUGI, Y. FUJITA, K. KAWANABE, T. KOKUBO and C. OHTSUKI, in "Bioceramics", Vol. 2, edited by G. Heimke (German Ceramic Society, Cologne, 1990) p. 105.
14. T. KITSUGI, T. YAMAMURO, T. NAKAMURA and T. KOKUBO, *Internatl Orthopaedics (SICOT)* **13** (1989) 199.
15. 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.
16. T. KOKUBO, S. ITO, Z. T. HUANG, T. HAYASHI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 331.
17. T. KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *ibid.* **24** (1990) 721.
18. Y. EBISAWA, T. KOKUBO, K. OHURA and T. YAMAMURO, *J. Mater. Sci. Mater. Medicine* **1** (1990) 239.
19. M. IMAOKA and T. YAMAZAKI, in "Handbook of Glass Data", Part C: Ternary Silicate Glasses, edited by O. V. Mazurin, M. V. Streltsina and T. P. Shvaiko-Shvaikovskaya (Elsevier, Amsterdam, 1987) p. 721.
20. J. GAMBLE, in "Chemical Anatomy, Physiology and Pathology of Extracellular Fluid", 6th Edn (Harvard University Press, Cambridge, 1967) p. 1.
21. C. OHTSUKI, H. KUSHITANI, T. KOKUBO, S. KOTANI and T. YAMAMURO, *J. Biomed. Mater. Res.* **25** (1991) 1363.
22. T. KOKUBO, *J. Non-Cryst. Solids* **120** (1990) 138.
23. *Idem*, in "CRC Handbook of Bioactive Ceramics", Vol. I, edited by T. Yamamuro, L. L. Hench and J. Wilson (CRC Press, Boca Raton, 1990) p. 41.
24. W. NEUMAN and M. NEUMAN, in "The Chemical Dynamics of Bone Mineral" (University of Chicago, Chicago, 1958) p. 34.
25. A. PAUL, *J. Mater. Sci.* **12** (1977) 2246.

Received 6 February
and accepted 5 March 1991