Bioactivity of M_2O–TiO_2–SiO_2 (M=Na, K) Glasses: An *In vitro* Evaluation

Hyun-Min Kim, Fumiaki Miyaji, Tadashi Kokubo,* Masahiko Kobayashi,† and Takashi Nakamura†

Department of Material Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-01

†Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kyoto 606-01

(Received March 6, 1996)

The formation of biologically active bonelike apatite on the surfaces of glasses and glass-ceramics is an essential condition for their bonding living bone. In the present study, apatite formation on the surfaces of M_2O–TiO_2–SiO_2 (M = Na, K) glasses was examined in a simulated body fluid with ion concentrations nearly equal to those of human blood plasma in order to evaluate their bioactivity. While none of the Na_2O–TiO_2–SiO_2 ternary glasses formed the surface apatite, K_2O–TiO_2–SiO_2 glasses, not only as SiO_2-rich glasses but also as TiO_2-rich glasses, formed the apatite. The alkali ions in the glasses exchanged with H_3O^+ ions in the fluid. The TiO_2-rich or SiO_2-rich hydrogel which was formed on the surfaces of the glasses induced apatite nucleation. The increased degree of the supersaturation of the fluid with respect to the apatite due to the increase in pH provided favorable conditions for apatite nucleation. This indicates that alkali titanates as well as alkali silicates can be basic components of bioactive glasses.

It has been shown for various kinds of glasses, glass-ceramics, and sintered ceramics that an essential requirement for them to bond to living bone is the formation of a biologically active bonelike apatite on their surfaces in the living body.[1—10] In addition, it has also been shown for various kinds of bone bonding, i. e. bioactive glasses, glass-ceramics, and sintered ceramics, that bonelike apatite can be reproduced on their surfaces even in an acellular simulated body fluid with ion concentrations nearly equal to those of human blood plasma.[11—16] Therefore, it is believed that the bioactivity of a material can be evaluated *in vitro* by examining apatite formation on its surface in a simulated body fluid.

It has been generally assumed hitherto that glasses and glass-ceramics showing bioactivity should contain both calcium and phosphorus oxides in their compositions to form an apatite on their surface in the living body. The present authors, however, recently showed that even P_2O_5-free Na_2O–SiO_2 and CaO–SiO_2 glasses form bonelike apatite on their surfaces *in vitro* and *in vivo*.[7,14,15,17] The authors also showed that TiO_2 gel as well as SiO_2 gel prepared by a sol–gel process induced apatite formation on its surface *in vitro*.[18—21] In view of these facts, alkali titanate glasses are also expected to form bonelike apatite on their surfaces in the body environment.

The purpose of the present study is to evaluate the bioactivity of alkali titanate glasses *in vitro*. The alkali titanate binary systems, however, form glasses only in a narrow compositional region. Therefore, in the present study, the bioactivity of glasses were investigated in the M_2O–TiO_2 (M = Na or K) systems with added SiO_2 by examining bonelike apatite formation on their surfaces in a simulated body fluid with ion concentrations nearly equal to those of human blood plasma. The compositional dependence of the apatite formation of the glasses is discussed in terms of the surface reaction of the glasses in the fluid.

Experimental

Preparation of Glasses. Some ternary glasses on the tie lines $2M_2O\cdot3TiO_2$–SiO_2 and $2M_2O\cdot3SiO_2$–TiO_2 (M = Na or K) in the Na_2O–TiO_2–SiO_2[22] and K_2O–TiO_2–SiO_2[23] systems, some glasses in the M_2O–TiO_2 and M_2O–SiO_2 systems, all of which are shown in Fig. 1, and a quaternary glass $16Na_2O\cdot16K_2O\cdot48TiO_2\cdot20SiO_2$ in mol% (NKT20) were prepared by the following method. Powder mixtures of the corresponding compositions were prepared by using reagent-grade Na_2CO_3, K_2CO_3, SiO_2, and TiO_2, and melted in a platinum crucible at 1450—1550 °C for 1 h. The melts were poured on a brass plate to be formed into glass plates 0.5 mm thick, and annealed at 500 °C. The obtained glasses were cut into $8\times8\times0.5$ mm^3 in size, polished with 3—4 μmϕ diamond paste, and washed with acetone in an ultrasonic cleaner.

Bioactivity Evaluation *In vitro*. Each specimen was immersed in 15 mL of an acellular simulated body fluid (SBF) having pH and ion concentrations almost equal to those of human blood plasma (Table 1).[12,24] The fluid was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4\cdot3H_2O$, $MgCl_2\cdot6H_2O$, $CaCl_2$, and Na_2SO_4 into distilled water, and was buffered at pH 7.25 with aminotris (hydroxymethyl) methane ($(CH_2OH)_3CNH_2$) and hydrochloric acid (HCl) at 36.5 °C. It was previously shown that apatite formation on the surfaces of bioactive glasses and glass-ceramics *in vivo* can be reproduced fairly precisely in this fluid.[4—8,11,17]

Analyses of Specimen Surface and SBF. After soaking in SBF for various periods, the specimens were removed from the fluid and washed with acetone. Surface structural changes of the specimens were examined by thin-film X-ray diffraction (TF-XRD: Model 2651 A1, Rigaku, Japan), Fourier-transform infrared reflection spectroscopy (FT-IRRS: Model 5M, JASCO, Japan), and scan-

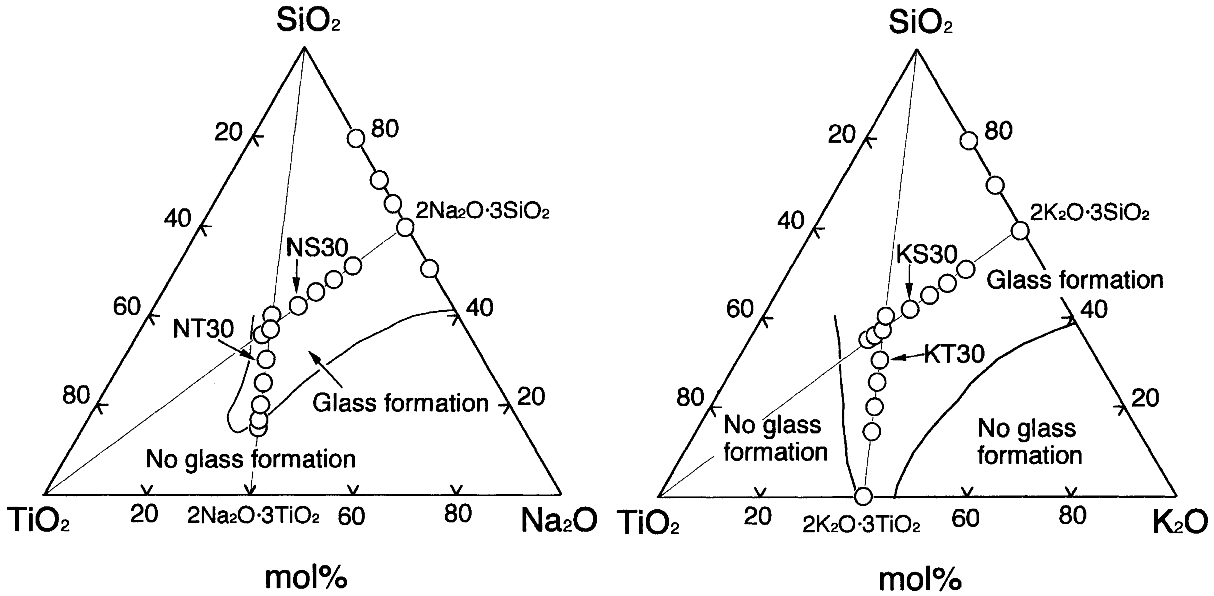

Fig. 1. Compositions of glasses examined in the present study. NS30: $28Na_2O \cdot 30TiO_2 \cdot 42SiO_2$ in mol%; NT30: $28Na_2O \cdot 42TiO_2 \cdot 30SiO_2$ in mol%; KS30: $28K_2O \cdot 30TiO_2 \cdot 42SiO_2$ in mol%; KT30: $28K_2O \cdot 42TiO_2 \cdot 30SiO_2$ in mol%.

Table 1. Ion Concentrations and pH of Simulated Body Fluid (SBF) and Those of Blood Plasma

Ion	Concentration (mM)	
	SBF	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	2.5	2.5
Ca^{2+}	1.5	1.5
Cl^-	148.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.25	7.20—7.40

ning electron microscopy (SEN: Model S2500CX, Hitachi, Japan) equipped with energy-dispersive X-ray spectroscopy (EDX: Model EMAX3700, Horiba, Japan). Changes in element concentrations and pH of SBF due to immersion of specimens were measured by inductively-coupled plasma (ICP) atomic emission spectroscopy (Model SPS1500, Seiko Inst., Japan) and a pH meter (Model P-14, Horiba, Japan).

Results and Discussion

Figures 2, 3, 4, and 5 show the TF-XRD and FT-IRRS patterns of the surfaces of some Na_2O–TiO_2–SiO_2 ternary and K_2O–TiO_2–SiO_2 ternary glasses, which are denoted in Fig. 1, soaked in SBF for various periods. Figure 6 shows those of a Na_2O–K_2O–SiO_2–TiO_2 quaternary glass. The main peaks in the TF-XRD patterns of the K_2O–TiO_2–SiO_2 and Na_2O–K_2O–TiO_2–SiO_2 glasses were ascribed to hydroxyapatite according to Powder Diffraction Data File No. 9-0432. Peaks in the FT-IRRS patterns at about 450 and 1100 cm^{-1} were assigned to Si–O–Si bending and Si–O stretching, respectively, and those at about 950 cm^{-1} to Ti–O–Si bonds.[25] Peaks in the FT-IRRS patterns at about 600 and 1050 cm^{-1} for KS30, KT30, and NKT20 glasses after soaking for 3 d were ascribed to an amorphous calcium phosphate, and combination of two peaks at about 570 and 610 cm^{-1} and at about 1050 and 1120 cm^{-1} for these glasses after further soaking to a crystalline apatite.[6,14—17] The presence of a peak at about 1450 cm^{-1} due to C–O bonds in the IRRS patterns of these glasses indicates that the apatite formed on the surfaces of these glasses is a bone-like carbonate-containing apatite.[6,14—17,26,27] It can be seen from Figs. 2, 3, 4, 5, and 6 that bonelike carbonate-containing apatite is formed on the surfaces of the K_2O–TiO_2–SiO_2 and Na_2O–K_2O–TiO_2–SiO_2 glasses through an amorphous calcium phosphate within 1 week and grows with further soaking in SBF, whereas it was not formed on the Na_2O–TiO_2–SiO_2 glasses even after soaking for 4 weeks.

Apatite formation was also examined for other glasses in Fig. 1 similarly. The results after soaking in SBF for 4 weeks are summarized in the Na_2O–TiO_2–SiO_2 and K_2O–TiO_2–SiO_2 compositional triangles in Fig. 7. Most of M_2O–SiO_2 and M_2O–TiO_2 (M = Na, K) binary and ternary glasses having near-binary compositions showed too high surface reactivity in SBF, and thus dissolved into the fluid. It can be seen from Fig. 7 that none of Na_2O–TiO_2–SiO_2 ternary glasses from the apatite, whereas ternary K_2O–TiO_2–SiO_2 glasses formed it on their surfaces. It should be noted that not only SiO_2-rich glass but also TiO_2-rich glass formed the apatite in the K_2O–TiO_2–SiO_2 system.

Figures 8 and 9 show changes in pH and element concentrations of SBF with soaking of the Na_2O-containing (NS30 and NT30) and the K_2O-containing (KS30 and KT30) glasses, respectively. Only slight increases in Na concentration and pH are observed in the initial stage of the soaking and no changes in Ca and P concentrations were seen for the Na_2O-containing glasses. On the other hand, the K_2O-

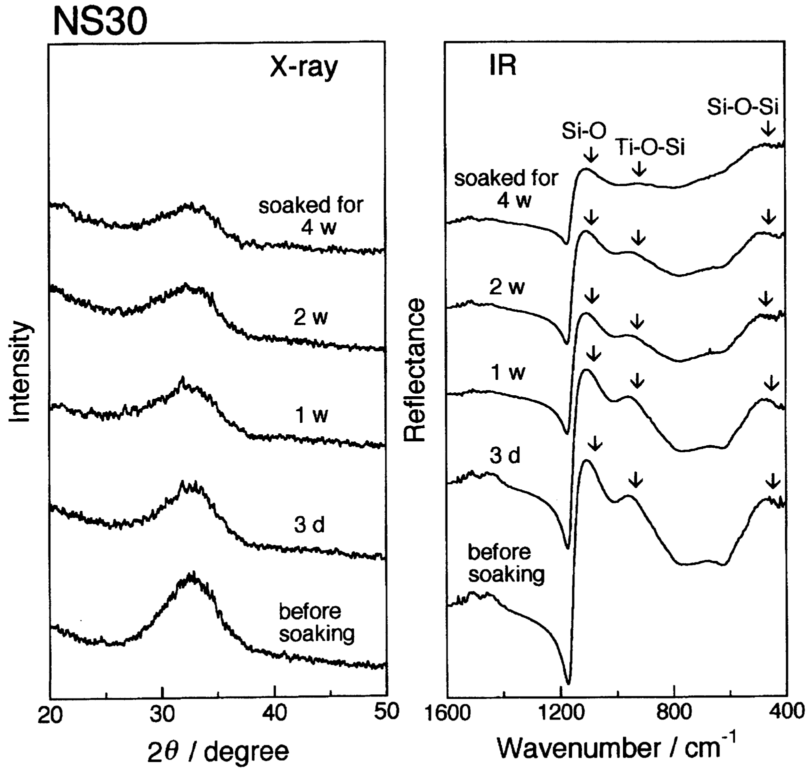

Fig. 2. TF-XRD and FT-IRRS patterns of the surface of NS30 glass before and after soaking in SBF for various periods.

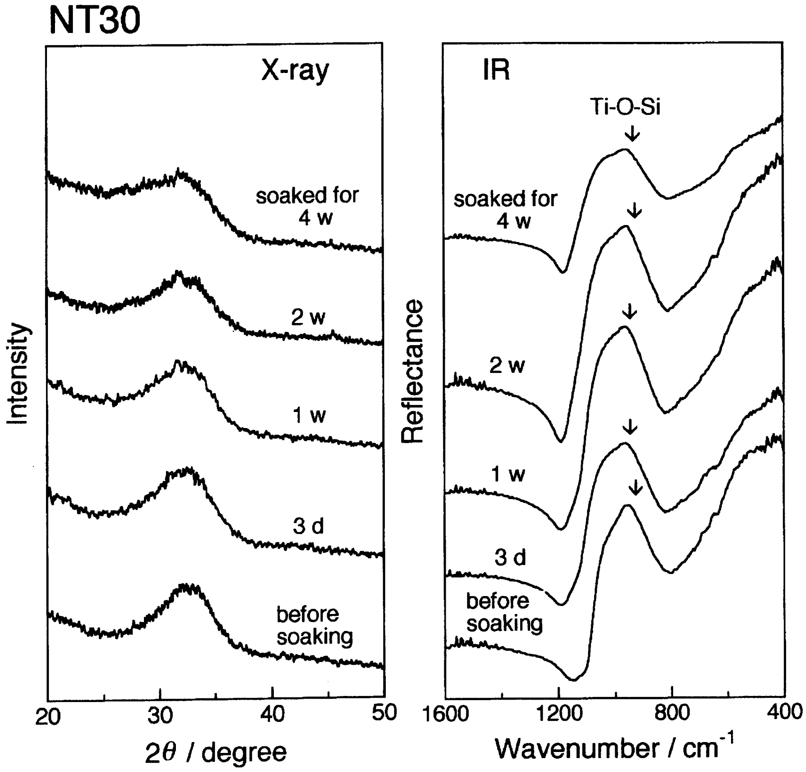

Fig. 3. TF-XRD and FT-IRRS patterns of the surface of NT30 glass before and after soaking in SBF for various periods.

containing glasses showed appreciable increases in K concentration and pH in the initial stage of the soaking and appreciable decreases in Ca and P concentrations for a long period. The former increases are attributed to ion exchange of K^+ ions in the glasses with H_3O^+ ions in the fluid.[28] The latter decreases are attributed to apatite formation on the surfaces of glasses consuming calcium and phosphate ions from the fluid.

Figure 10 shows the SEM-EDX profiles of the cross-sections of KS30 and KT30 glasses soaked in SBF for 2 weeks.

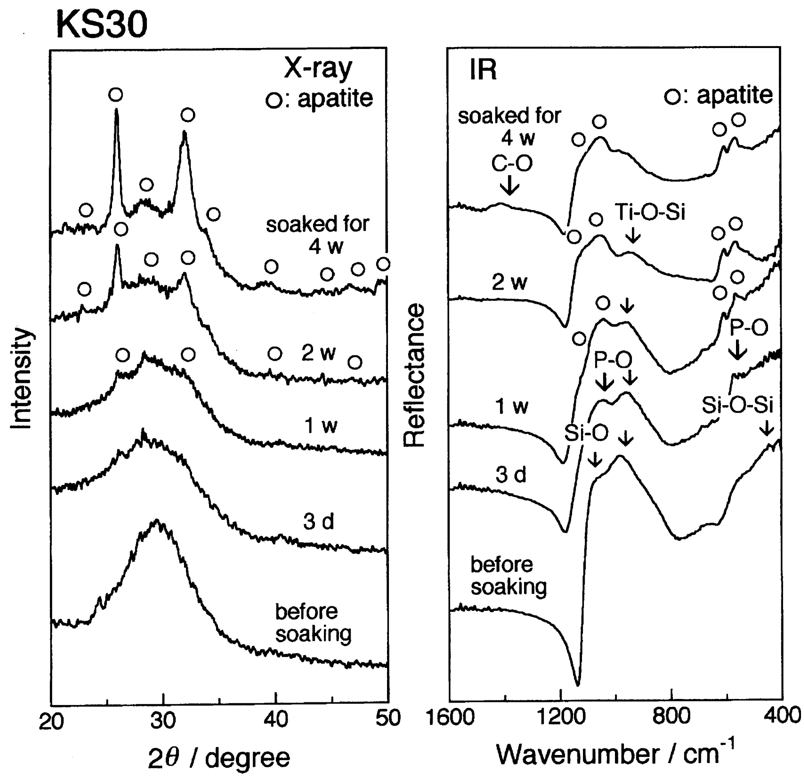

Fig. 4. TF-XRD and FT-IRRS patterns of the surface of KS30 glass before and after soaking in SBF for various periods.

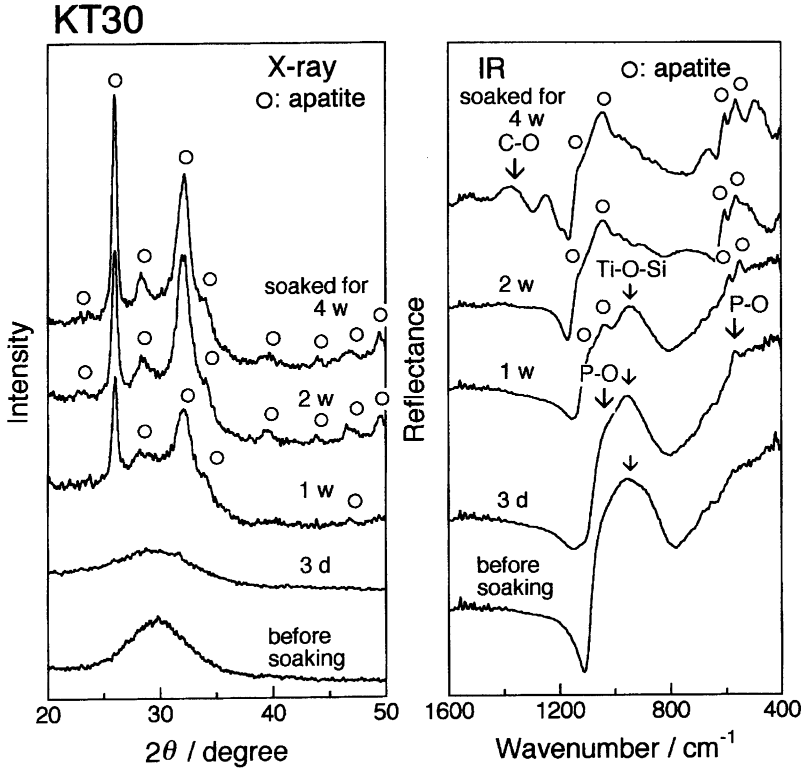

Fig. 5. TF-XRD and FT-IRRS patterns of the surface of KT30 glass before and after soaking in SBF for various periods.

Changes in the compositional profiles from the glass bulks to the surfaces indicate that two kinds of layers with different compositions are formed near the surfaces of the glasses due to soaking in SBF. One is the outermost surface layer rich in Ca and P. The other is a layer rich in Si or Ti and poor in K, which is located beneath the Ca- and P-rich layer. The Ca- and P-rich layer corresponds to the apatite layer detected by TF-XRD in Figs. 4 and 5. The Si- or Ti-rich layer is considered to be formed by ion exchange of K^+ ions in the glasses with H_3O^+ ions in SBF.

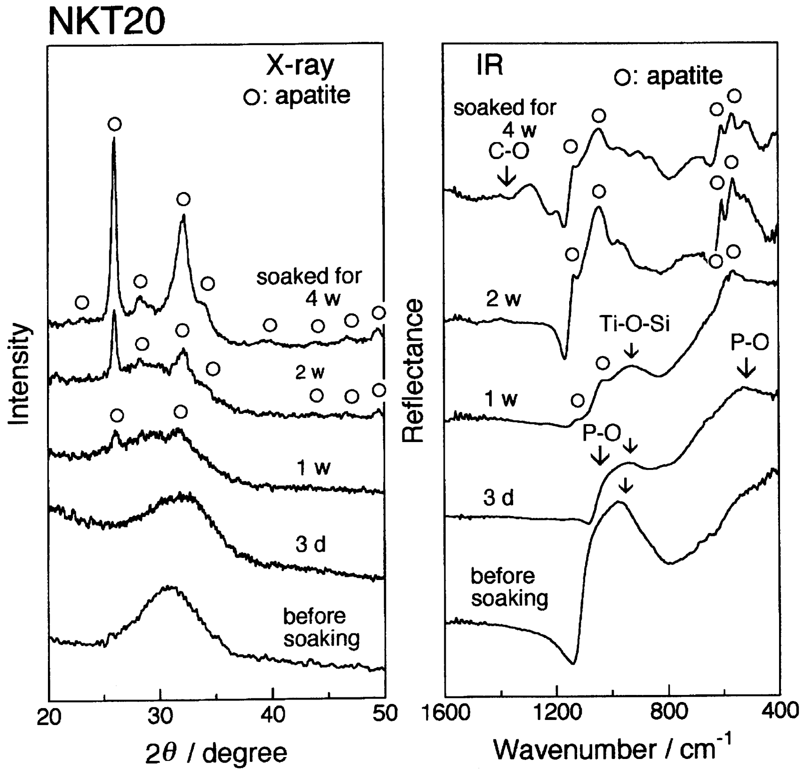

Fig. 6. TF-XRD and FT-IRRS patterns of the surfaces of NKT20 glass before and after soaking in SBF for various periods.

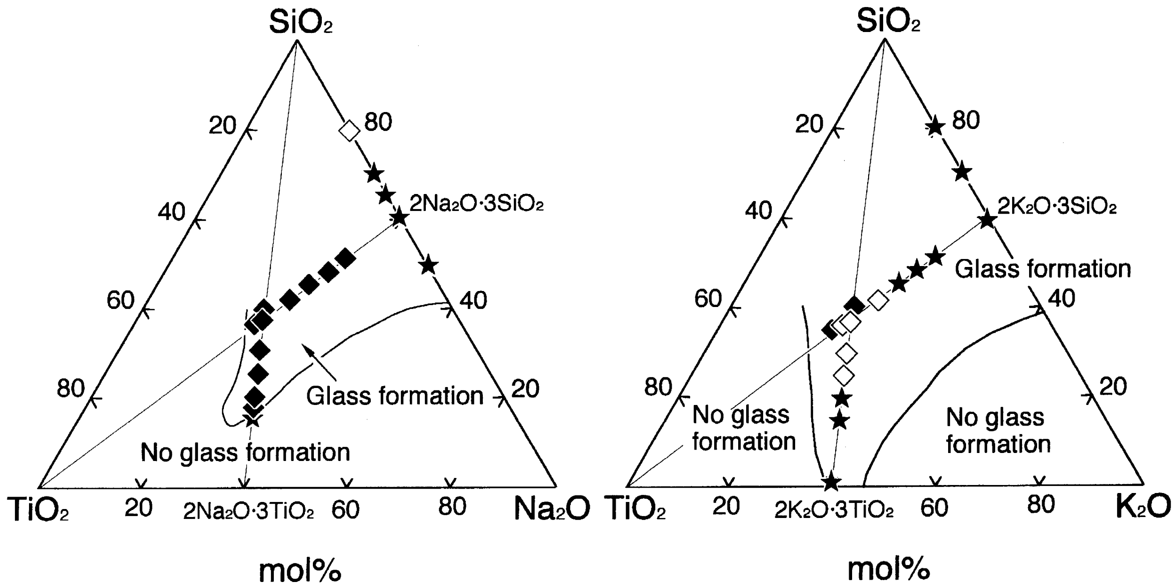

Fig. 7. Compositional dependence of apatite formation on the surfaces of Na_2O–TiO_2–SiO_2 and K_2O–TiO_2–SiO_2 glasses after soaking in SBF for 4 weeks (◇: apatite formation, ◆: no apatite formation, ⋆: dissolution).

From these results, the reason why K_2O–TiO_2–SiO_2 glasses formed the bonelike apatite layer on their surfaces, but Na_2O–TiO_2–SiO_2 glasses did not, is explained as follows. When exposed to SBF, K_2O–TiO_2–SiO_2 glasses first release K^+ ions into the fluid via ion exchange with H_3O^+ ions. This gives an SBF pH increases and formation of a TiO_2-rich or SiO_2-rich hydrogel on the surfaces of the glasses. Although the SBF is already metastably supersaturated with respect to the apatite even under normal conditions,[15,24,29] the pH increase gives rise to a further increase in the degree of supersaturation of SBF with respect to the apatite. Under these favorable conditions, the TiO_2-rich or SiO_2-rich hydrogel induces apatite nucleation.[20] As a result, a large number of apatite nuclei are formed on the surfaces of the glasses. Once the apatite nuclei are formed, they spontaneously grow into a bonelike apatite by consuming the calcium and phosphate ions from the surrounding fluid. On the other hand, although a Na_2O–SiO_2 binary glass forms the apatite via

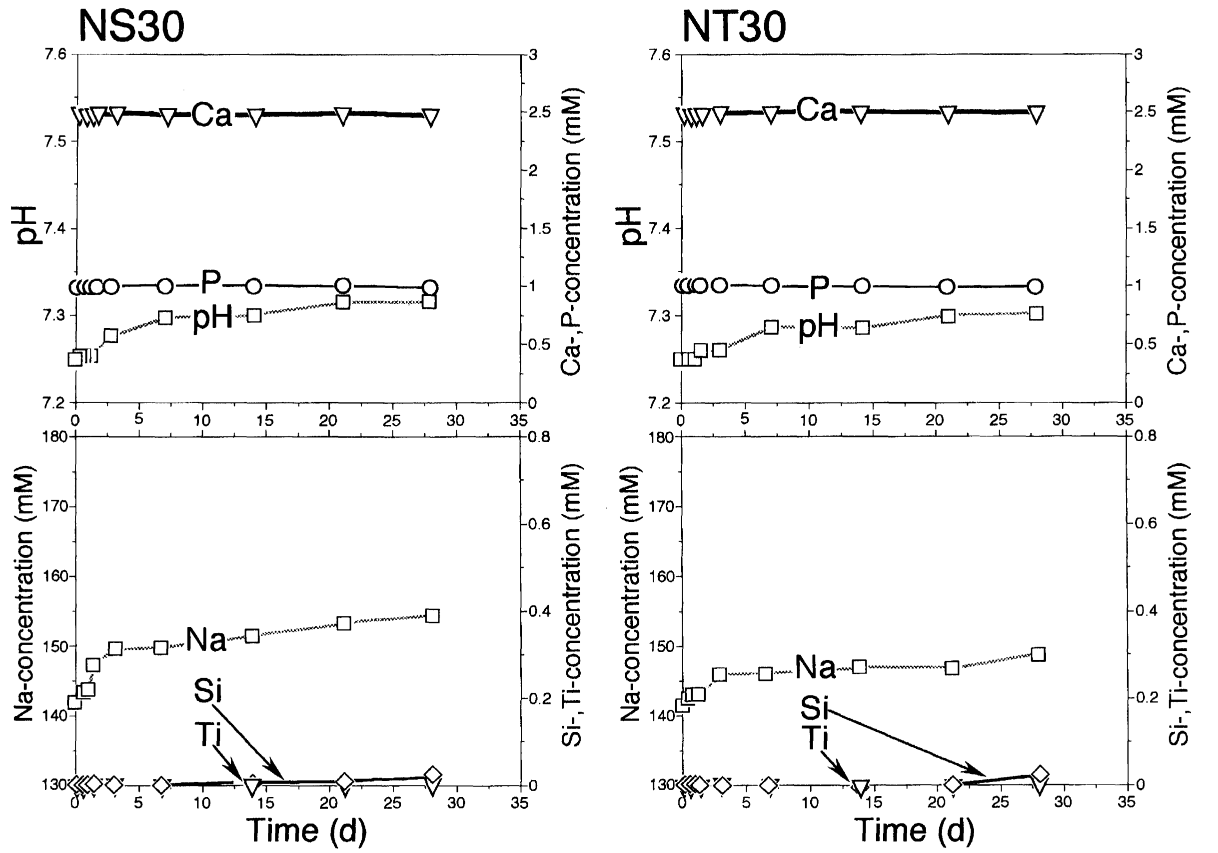

Fig. 8. Changes in pH and element concentrations of SBF with soaking of NS30 and NT30 glasses.

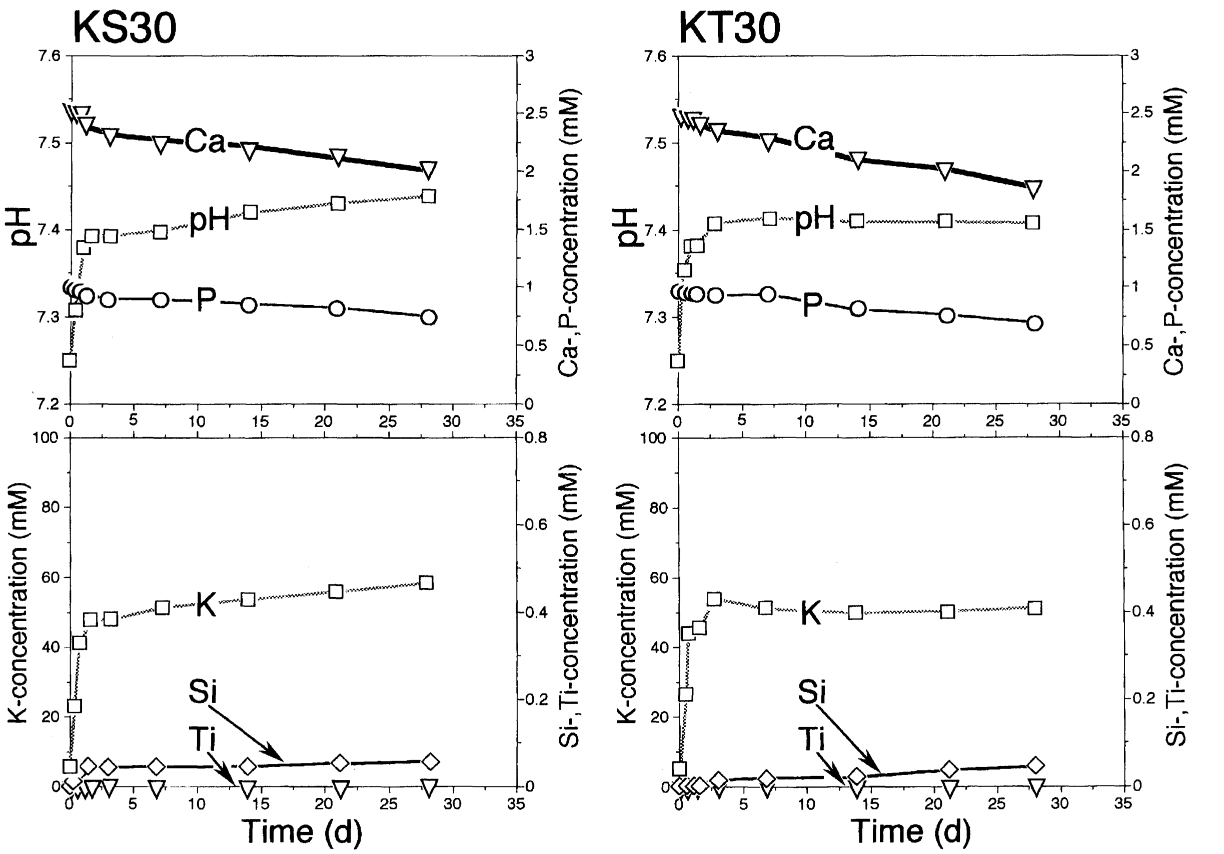

Fig. 9. Changes in pH and element concentrations of SBF with soaking of KS30 and KT30 glasses.

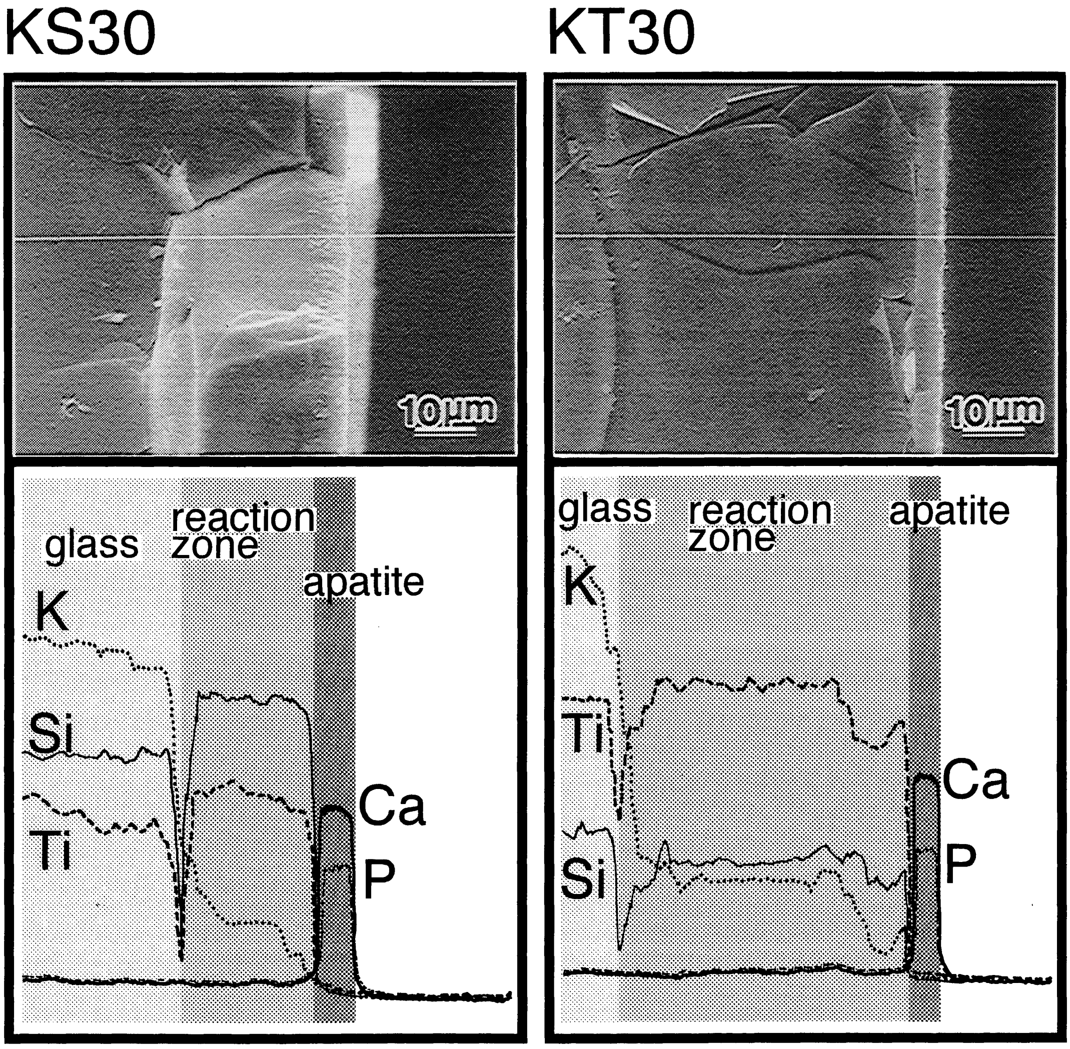

Fig. 10. SEM-EDX profiles of the cross-sections of KS30 and KT30 glasses soaked in SBF for 2 weeks.

Na^+ ion release resulting in SiO_2 hydrogel formation and an SBF pH increase, Na_2O–TiO_2–SiO_2 ternary glasses rarely release Na^+ ions due to the addition of TiO_2. Therefore, the degree of the supersaturation of SBF with respect to the apatite scarcely increases, and a TiO_2-rich or SiO_2-rich hydrogel, which can induce apatite nucleation, is also scarcely formed. Consequently, an apatite layer is not formed on the surfaces of Na_2O–TiO_2–SiO_2 glasses. In the case of Na_2O–K_2O–TiO_2–SiO_2 glasses, Na^+ ions as well as K^+ ions are released, and hence an apatite layer is formed on their surfaces. Although M_2O–TiO_2 (M = Na, K) binary glasses dissolved into SBF after soaking for 4 weeks, it can be seen from the tie line $2K_2O{\cdot}3TiO_2$–SiO_2 in Fig. 7 that the glasses showed an apatite-forming ability with decreasing SiO_2 content. This indicates that the M_2O–TiO_2 binary glasses could form the apatite on their surfaces in an early soaking period in SBF, but this then dissolved into the fluid.

As already mentioned, glasses which form the bonelike apatite layer on their surfaces in SBF can form the same layer even in a living body and bond to living bone.[4,8,11—17] The present results indicate that alkali titanates as well as alkali silicates can be a basic composition for bioactive glasses.

Conclusions

The compositional dependence of apatite formation on the surfaces of M_2O–TiO_2–SiO_2 (M = Na or K) glasses in SBF showed that not only SiO_2-rich glasses but also TiO_2-rich glasses form a bonelike apatite layer on their surfaces, when the rate of alkali ion release is high. Under these conditions, hydrated TiO_2 or SiO_2, which can induce apatite nucleation, is formed and the increased degree of the supersaturation of the fluid with respect to the apatite provides favorable conditions for apatite nucleation. Therefore, it can be concluded that alkali titanates as well as alkali silicates can be basic components of bioactive glasses.

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

References

1) W. Höland, W. Vogel, K. Naumann, and J. Grummel, *J. Biomed. Mater. Res.*, **19**, 303 (1985).

2) T. Kitsugi, T. Nakamura, T. Yamamuro, T. Kokubo, T. Shibuya, and M. Takagi, *J. Biomed. Mater. Res.*, **21**, 1255 (1987).

3) T. Kitsugi, T. Yamamuro, T. Nakamura, and T. Kokubo, *J. Biomed. Mater. Res.*, **23**, 631 (1989).

4) T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi, and T. Yamamuro, *J. Biomed. Mater. Res.*, **24**, 331 (1990).

5) T. Kokubo, "Handbook of Bioactive Ceramics; Bioactive Glasses and Glass-Ceramics," ed by T. Yamamuro, L. L. Hench, and J. Wilson, CRC Press, Boca Raton (1990), Vol. 1, p. 41.

6) L. L. Hench, *J. Am. Ceram. Soc.*, **74**, 1487 (1991).

7) K. Ohura, T. Yamamuro, T. Nakamura, T. Kokubo, Y. Ebisawa, Y. Kotoura, and M. Oka, *J. Biomed. Mater. Res.*, **25**, 357 (1991).

8) C. Ohtsuki, H. Kushitani, T. Kokubo, S. Kotani, and T. Yamamuro, *J. Biomed. Mater. Res.*, **25**, 1363 (1991).

9) T. Kokubo, "Bone-Bonding Biomaterials," ed by P. Ducheyne, T. Kokubo, and C. A. Blitterswijk, Reed Healthcare Communications, Leiden, Netherlands, (1992), p. 31.

10) N. Neo, S. Kotani, T. Nakamura, T. Yamamuro, C. Ohtsuki, T. Kokubo, and Y. Bando, *J. Biomed. Mater. Res.*, **26**, 1419 (1992).

11) T. Kokubo, H. Kushtani, Y. Ebisawa, T. Kitsugi, S. Kotani, K. Ohura, and T. Yamamuro, "Bioceramics," ed by H. Oonishi, H. Aoki, and K. Sawai, Euro America, Tokyo (1989), Vol. 1, p. 157.

12) T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi, and T. Yamamuro, *J. Biomed. Mater. Res.*, **24**, 721 (1990).

13) Y. Ebisawa, T. Kokubo, K. Ohura, and T. Yamamuro, *Mater. Med.*, **1**, 239 (1990).

14) C. Ohtsuki, T. Kokubo, K. Takatsuka, and T. Yamamuro, *J. Ceram. Soc. Jpn.*, **99**, 1 (1991).

15) C. Ohtsuki, T. Kokubo, and T. Yamamuro, *J. Non-Cryst. Solids*, **143**, 84 (1992).

16) M. R. Filgueiras, G. R. Torre, and L. L. Hench, *J. Biomed. Mater. Res.*, **27**, 445 (1993).

17) H. M. Kim, F. Miyaji, T. Kokubo, C. Ohtsuki, and T. Nakamura, *J. Am. Ceram. Soc.*, **78**, 2405 (1995).

18) P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura, and T. Yamamuro, *J. Am. Ceram. Soc.*, **75**, 2094 (1992).

19) P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura, and T. Yamamuro, *Mater. Med.*, **4**, 127 (1993).

20) P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura, T. Yamamuro, and K. de Groot, *J. Biomed. Mater. Res.*, **7**, 28 (1994).

21) P. Li, I. Kangasniemi, K. de Groot, and T. Kokubo, *J. Am. Ceram. Soc.*, **77**, 1307 (1994).

22) M. Imaoka and T. Yamazaki, *Rep. Inst. Ind. Sci., Univ. Tokyo*, **18**, 1 (1968).

23) Bh. V. J. Rao, *Phys. Chem. Glasses*, **4**, 22 (1963).

24) J. Gamble, "Chemical Anatomy. Physiology and Pathology of Extracellular Fluid," 6th ed, Havard University Press, Cambridge, MA (1967), p. 1.

25) A. Matsuda, T. Kogure, Y. Matsuno, S. Katayama, T. Tsuno, N. Touge, and T. Minami, *J. Am. Ceram. Soc.*, **76**, 2899 (1993).

26) I. Rehman, L. L. Hench, and W. Bonfiled, "Bioceramics," ed by P. Ducheyne and D. Christiansen, Butterworth-Heinemann, Philadelphia (1993), Vol. 6, p. 31.

27) I. Rehman, R. Smith, L. L. Hench, and W. Bonfiled, *J.Biomed. Mater. Res.*, **29**, 1287 (1995).

28) N. P. Bansal and N. H. Dormus, "Handbook of Glass Properties," Academic Press, New York (1986), p. 646.

29) W. Neuman and M. Neuman, "The Chemical Dynamics of Bone Mineral," University of Chicago, IL (1958), p. 34.