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Chemical reaction of bioactive glass and glass–ceramics with a simulated body fluid

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Glass–ceramic A-W containing crystalline apatite and wollastonite in an $MgO–CaO–SiO_2$ glassy matrix bonds to living bone through an apatite layer which is formed on its surface in the body. The parent glass G of glass–ceramic A-W and glass–ceramic A, which has the same composition as glass–ceramic A-W but contains only the apatite, also bond to living bone through the surface apatite layer, whereas glass–ceramic A-W(Al), which contains the apatite and wollastonite in an $MgO–CaO–SiO_2–Al_2O_3$ glassy matrix, neither forms the surface apatite layer nor bonds to living bone. In the present study, in order to reveal the mechanism of formation of the surface apatite layer, changes in ion concentrations of a simulated body fluid with immersion of these four kinds of glass and glass–ceramics were investigated. Bioactive glass G and glass–ceramics A and A-W all showed appreciable increases in Ca and Si concentrations, accompanied by an appreciable decrease in P concentration, whereas non-bioactive glass–ceramic A-W(Al) hardly showed any element concentration change. It was speculated from these results that dissolution of the Ca(II) and Si(IV) ions from bioactive glass and glass–ceramics plays an important role in forming the apatite layer on their surfaces in the body.

1. Introduction

High-strength bioactive glass–ceramic A-W containing crystalline apatite and wollastonite in an $MgO–CaO–SiO_2$ glassy matrix [1–5] bonds to living bone through an apatite layer which is formed on its surface in the body [6–10]. Both the parent glass G of glass–ceramic A-W and glass–ceramic A which has the same composition as glass–ceramic A-W but contains only the apatite also bond to living bone through the surface apatite layer [8, 9, 11], whereas glass–ceramic A-W(Al) which contains the apatite and wollastonite in an $MgO–CaO–SiO_2–Al_2O_3$ glassy matrix neither forms the surface apatite layer nor bonds to living bone [12]. It has been also reported that Bioglass-type glasses [13, 14] and Ceravital-type glass–ceramics [15] bond to living bone through the surface apatite layer. This indicates that the essential condition for glasses and glass–ceramics to bond to living bone is formation of the apatite layer on their surfaces in the body.

The same kind of apatite layer can be formed on the surfaces of bioactive glasses and glass–ceramics even in an acellular simulated body fluid which has almost equal ion concentrations to those of the human blood plasma [16–19]. This indicates that the surface apatite layer is formed by a chemical reaction of the glasses and glass–ceramics with the surrounding body fluid. In the present study, in order to reveal the mechanism of formation of the surface apatite layer, changes in ion concentrations of the simulated body fluid with immersion of bioactive glass G and glass–ceramics A and A-W were investigated in comparison with those for immersion of non-bioactive glass–ceramic A-W(Al).

2. Experimental procedure

2.1. Sample preparation

Glass G was prepared by pouring a melt of the composition MgO 4.6, CaO 44.7, SiO_2 34.0, P_2O_5 16.2 and CaF_2 0.5 wt % on to a stainless steel plate and pressing it into a plate about 1 mm thick [3]. Glass–ceramic A was prepared by heating as-formed glass G up to 870 °C at a rate of 1 °C min^{-1} and keeping it at 870 °C for 4 h [3]. Glass–ceramic A-W was prepared by pulverizing glass G into grains about 3 μm in size, pressing them into a bar, and then heating the powder compact up to 1050 °C at a rate of 1 °C min^{-1} and keeping it at 1050 °C for 4 h [3]. Glass–ceramic A-W(Al) was prepared by heating a glass powder compact of the composition MgO 3.6, CaO 40.4, SiO_2 33.2, P_2O_5 16.5 and Al_2O_3 6.3 wt % up to 1030 °C at a rate of 1 °C min^{-1} and keeping it at 1030 °C for 4 h [20].

2.2. Determination of crystal contents

The contents of crystalline phases in glass–ceramics A, A-W and A-W(Al) were roughly determined in the previous work [3]. In the present study, their contents

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were more precisely determined by the following method for investigating the surface reactions of the glass–ceramics in the simulated body fluid.

The glass–ceramics were pulverized into grains below 350 mesh (44 μm opening) in size, mixed with a reagent-grade chemical of calcium fluoride (CaF_2) as the internal standard in a weight ratio of 6 : 1, and then subjected to X-ray diffraction. The contents of apatite and β-wollastonite were determined by intensity ratios of 3 0 0 reflection of the apatite to 1 1 1 reflection of the calcium fluoride, and 3 1 0 and 5 0 1 reflections of the wollastonite to 1 1 1 reflection of the calcium fluoride, respectively, referring to their calibration curves. The calibration curves for the apatite and the β-wollastonite were prepared by using standard powder mixtures of a sintered hydroxyapatite with glass G and of a high-purity natural β-wollastonite (from Kibi-gun, Gifu prefecture, Japan) with glass G, respectively. In determination of the content of the β-wollastonite, in order to avoid the effect of preferred orientation of the wollastonite, all the samples were homogeneously mixed with Lakeside cement (Nition Chikagaku Sha Co., Kyoto, Japan) in a weight ratio of 0.7:1 on a heated slide glass, cooled to room temperature and then pulverized, before X-ray diffraction. At least seven measurements were made for obtaining one set of data.

2.3. Soaking in simulated body fluid

Glass G and glass–ceramics A, A-W and A-W(Al) were pulverized into grains below 32 mesh (500 μm opening) and above 48 mesh (297 μm opening), and washed with pure acetone and ion-exchanged water. The grains in the amount of 4.00 g were immersed in 50 ml of a simulated body fluid which had almost equal ion concentrations to those of human blood plasma as shown in Table I [21]. The fluid was prepared by dissolving reagent-grade chemicals NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$ and $CaCl_2$ in ion-exchanged water contained in a polyethylene bottle and buffered at pH 7.25 with 50 mM trishydroxymethyl aminomethane ($(CH_2OH)_3CNH_2$) and 45 mM HCl. It was maintained at 36.5 °C and shaken at a rate of 120 strokes per min during soaking of the specimens.

2.4. Measurement of element concentrations

At various periods after the specimens were immersed in the simulated body fluid, 1 ml aliquots of the fluid were taken out and the concentrations of magnesium, calcium, silicon, aluminium and phosphorus were analysed by inductively coupled plasma (ICP) emission spectroscopy. The fluorine concentration was measured by lanthanum-ALC (alizarin complexone) absorption spectroscopy. Proton concentration was measured by a pH meter.

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

Ion	Concentration (mM)	
	Simulated fluid	Human plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	148.8	103.0
HCO_3^-	4.2	13.5
HPO_4^{2-}	1.0	1.0

3. Results

3.1. Constituent phases of samples

Contents of the constituent crystalline phases of glass–ceramics A, A-W and A-W(Al) are shown in Table II. Contents and compositions of the residual glassy phases of the glass–ceramics are also shown as well as those of glass G. They were calculated from the contents of the crystalline phases and the composition of the parent glass, assuming that the apatite and the β-wollastonite have the compositions given by the chemical formulae $Ca_{10}(PO_4)_6(O, F_2)$ and $CaO \cdot SiO_2$, respectively. In the case of glass–ceramic A-W(Al), although a trace amount of anorthite ($CaO \cdot Al_2O_3 \cdot 2SiO_2$) was detected by powder X-ray diffraction, it was neglected.

It can be seen from Table II that the content of the apatite is equal among all the examined glass–ceramics. Almost all the P_2O_5 in the glass–ceramics entered into the apatite phase and was absent in the residual glassy phase.

3.2. Element concentration changes of fluid

Changes in concentration of magnesium, calcium, silicon and phosphorus as well as in pH of the simulated body fluid with the immersion of glass G and glass–ceramics A, A-W and A-W(Al) are shown in Figs 1–4 as a function of soaking time. Fluorine and aluminium were not detected for all the fluids examined.

It can be seen from Figs 1–4 that bioactive glass G and glass–ceramics A and A-W show appreciable increases in the calcium and silicon concentrations and a little increase in magnesium concentration, accompanied by an appreciable decrease in the phosphorus concentration, whereas non-bioactive glass–ceramic A-W(Al) hardly shows any change in element concentrations.

4. Discussion

It is apparent from Figs 1–3 that bioactive glass G and glass–ceramics A and A-W, which form an apatite layer on their surfaces in the body, dissolve appreciable amounts of the calcium and silicate ions into the surrounding fluid as well as a small amount of the magnesium ions. Glass G also dissolves a small amount of phosphate ions at the initial stage of exposure to the fluid. The appreciable decrease in the phosphorus concentration of the fluid with the immersion of these bioactive glass and glass–ceramics is attributed to the formation of apatite on their surfaces by consuming the phosphate ions from the surrounding fluid. On the other hand, non-bioactive

TABLE II Constituent phases of examined samples

Sample	Constituent phase (wt %)			Composition of glassy phase (wt %)
	Apatite	β-wollastonite	Glassy phase	
Glass G	0	0	100	MgO 4.6, CaO 44.7, SiO_2 34.0, P_2O_5 16.2, CaF_2 0.5
Glass–ceramic A	38	0	62	MgO 7.5, CaO 37.3, SiO_2 55.2
A-W	38	34	28	MgO 16.6, CaO 24.2, SiO_2 59.2
A-W(Al)	38	27	35	MgO 10.3, CaO 16.6, SiO_2 55.0, Al_2O_3 18.1

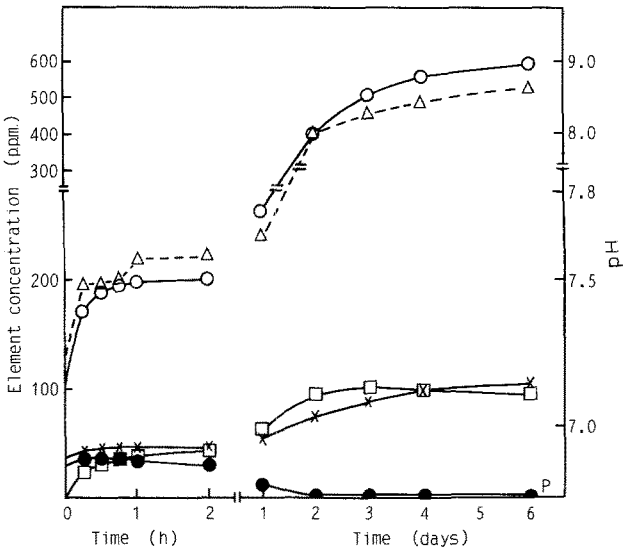

Figure 1 Changes in element concentrations and pH of simulated body fluid due to immersion of glass G: (○) Ca, (×) Mg, (●) P, (□) Si, (△) pH.

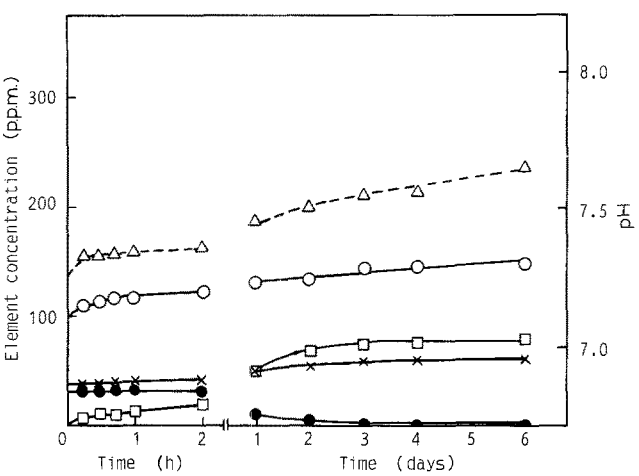

Figure 2 Changes in element concentrations and pH of simulated body fluid due to immersion of glass–ceramic A: (○) Ca, (×) Mg, (●) P, (□) Si, (△) pH.

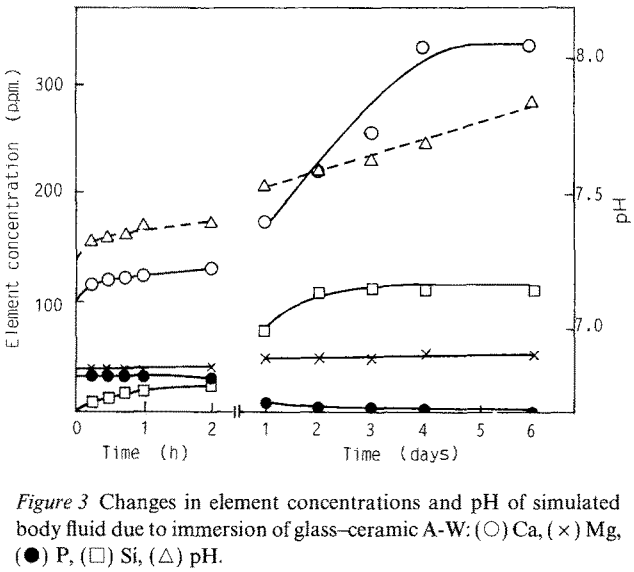

Figure 3 Changes in element concentrations and pH of simulated body fluid due to immersion of glass–ceramic A-W: (○) Ca, (×) Mg, (●) P, (□) Si, (△) pH.

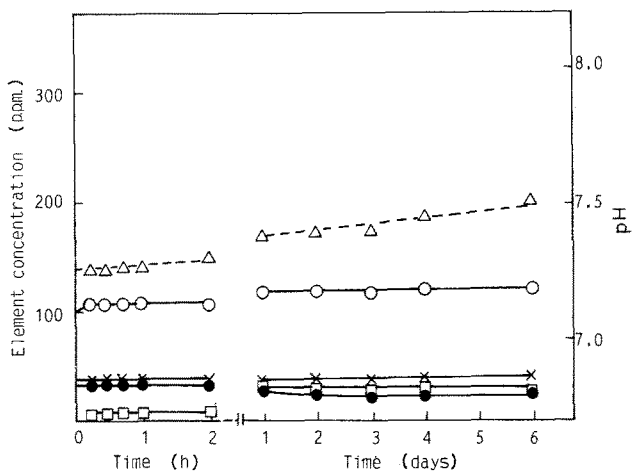

Figure 4 Changes in element concentrations and pH of simulated body fluid due to immersion of glass–ceramic A-W(Al): (○) Ca, (×) Mg, (●) P, (□) Si, (△) pH.

glass–ceramic A-W(Al) hardly dissolves any ions into the fluid and takes few phosphate ions from the fluid (Fig. 4). This indicates that the calcium and/or silicate ions dissolved from the bioactive glass and glass–ceramics play an important role in forming the apatite layer on their surfaces.

It can be also seen from Figs 1–3 that the rate of dissolution of calcium ions increases in the order of glass–ceramic A < glass–ceramic A-W < glass G. This indicates that the calcium ions are dissolved from the glassy phase and wollastonite. The rate of dissolution of silicate ions increased in the order of glass–ceramic A < glass G < glass–ceramic A-W. This indicates that silicate ions are also dissolved from both the glassy phase and wollastonite. The magnesium ion mainly remained in the glassy phase and hence dissolved from the glassy phase. Phosphate ions are dissolved only from glass G, since most of the P_2O_5 in the glass–ceramics entered into the apatite phase and the surrounding fluid is already supersaturated with respect to the apatite, even before the immersion of the glass–ceramics [22]. The phosphate ions required for forming the apatite layer on the

surfaces of glass–ceramics are supplied only from the surrounding fluid. Thus, the mechanism of apatite formation on the surface of glass–ceramic A-W is schematically represented as shown in Fig. 5.

In the case of non-bioactive glass–ceramic A-W(Al), the dissolution of the calcium and silicate ions from both the glassy and wollastonite phases is suppressed by the Al_2O_3 concentrated in the glassy phase (see Table II). It is already well known that Al_2O_3 effectively improves the chemical durability of glasses [23].

Among the calcium and silicate ions dissolved from the bioactive glass and glass–ceramics, the calcium ions might increase the degree of supersaturation of the surrounding fluid with respect to apatite, which is already supersaturated even before the immersion of the glass and glass–ceramics.

Formation of the hydroxyapatite from the constituent ions in an aqueous solution is given by the formula

$$Ca_{10}(PO_4)_6(OH)_2 \rightleftharpoons 10Ca^{2+} + 6PO_4^{3-} + 2OH^- \quad (1)$$

The ionic activity product IP in the solution is given by

$$\begin{aligned} IP &= (a_{Ca^{2+}})^{10}(a_{PO_4^{3-}})^6(a_{OH^-})^2 \\ &= (\gamma_{Ca^{2+}})^{10}(\gamma_{PO_4^{3-}})^6(\gamma_{OH^-})^2 \\ &\quad \times [Ca^{2+}]^{10}[PO_4^{3-}]^6[OH^-]^2 \end{aligned} \quad (2)$$

where a is activity, γ is activity coefficient, and [ ] is the concentration of each ion. According to Neuman and Neuman [22], $\gamma_{Ca^{2+}}$, $\gamma_{PO_4^{3-}}$ and γ_{OH^-} at physiological ionic strength ($\mu = 0.16$) are 0.36, 0.06 and 0.72, respectively. Substituting these values for $\gamma_{Ca^{2+}}$, $\gamma_{PO_4^{3-}}$ and γ_{OH^-} in Equation 2, and referring to the ion concentrations and pH given in Figs 1 to 4, we can calculate the change in ionic activity product of the apatite in the simulated body fluid with the immersion of glass G and glass–ceramics A, A-W and A-W(Al). In this calculation, it is assumed that the following equilibria are maintained among H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} and H^+:

$$H_3PO_4 \overset{K_1}{\rightleftharpoons} H^+ + H_2PO_4^- \quad (3)$$

$$H_2PO_4^- \overset{K_2}{\rightleftharpoons} H^+ + HPO_4^{2-} \quad (4)$$

$$HPO_4^{2-} \overset{K_3}{\rightleftharpoons} H^+ + PO_4^{3-} \quad (5)$$

where the equilibrium constants K_1, K_2 and K_3 are 6.22×10^{-3}, 6.58×10^{-8} and 6.61×10^{-13}, respectively, at 37 °C [24–26], and the activity coefficients γ_{H^+}, $\gamma_{H_2PO_4^-}$ and $\gamma_{HPO_4^{2-}}$ are 0.81, 0.62 and 0.23, respectively [22]. In addition, the following equilibria are assumed to be maintained among Ca^{2+}, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} ions:

$$Ca^{2+} + H_2PO_4^- \overset{K_4}{\rightleftharpoons} CaH_2PO_4^+ \quad (6)$$

$$Ca^{2+} + HPO_4^{2-} \overset{K_5}{\rightleftharpoons} CaHPO_4 \quad (7)$$

$$Ca^{2+} + PO_4^{3-} \overset{K_6}{\rightleftharpoons} CaPO_4^- \quad (8)$$

where the equilibrium constants K_4, K_5 and K_6 are 31.9, 6.81×10^2 and 3.46×10^6, respectively, at 37 °C [27], and activity coefficients $\gamma_{CaH_2PO_4^+}$ and $\gamma_{CaPO_4^-}$, which are approximated by the following Debye–Hückel equation, are both 0.72.

$$\log \gamma_i = \frac{-0.5 Z_i^2 \mu^{1/2}}{1 + \mu^{1/2}} \quad (9)$$

where Z_i is the electric charge of the ion.

The results of the calculation for the first 2 h after the samples were immersed into the fluid are shown in Fig. 6. The solubility product of apatite in aqueous solution is reported to be 5.5×10^{-118} at 37 °C [28]. It can be seen from Fig. 6 that the ionic activity product of the apatite in the simulated body fluid, which is already higher than the solubility product even before immersion of the samples, further increases with the immersion of all the kinds of examined sample. The increase in the ionic activity product for bioactive glass–ceramic A does not appreciably differ from that for non-bioactive glass–ceramic A-W(Al). This suggests that some factor, other than the degree of supersaturation of the surrounding fluid, also plays an important role in forming the apatite layer on the surfaces of bioactive glass and glass–ceramics. The

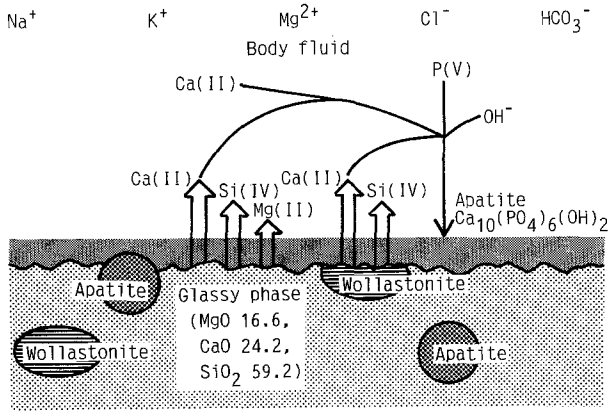

Figure 5 Schematic representation of surface reaction of glass–ceramic A-W in the body.

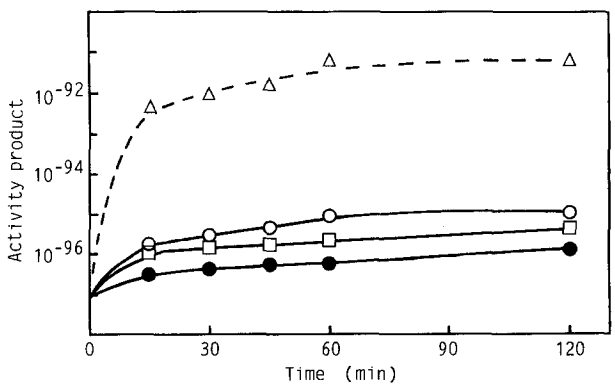

Figure 6 Changes in ionic activity product of the apatite in simulated body fluid with immersion of (△) glass G and glass–ceramics (□) A, (○) A-W and (●) A-W(Al).

amount of silicate ion dissolved from bioactive glass–ceramic A is appreciably larger than that from non-bioactive glass–ceramic A-W(Al) (see Figs 2 and 4). This indicates that the dissolved silicate ions play an important role in forming nuclei of the apatite on the surfaces of bioactive glass and glass–ceramics. It is also reported that silicon plays an essential role in the initial stage of apatite formation in the bone of mammals [29]. The role of the dissolved calcium and silicate ions in forming the surface apatite layer will be discussed in more detail elsewhere.

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References

1. T. KOKUBO, M. SHIGEMATSU, Y. NAGASHIMA, M. TASHIRO, T. NAKAMURA, T. YAMAMURO and S. HIGASHI, *Bull. Inst. Chem. Res., Kyoto Univ.* **60** (1982) 260.
2. T. KOKUBO, S. ITO, S. SAKKA and T. YAMAMURO, *J. Mater. Sci.* **21** (1986) 536.
3. T. KOKUBO, S. ITO, M. SHIGEMATSU, S. SAKKA and T. YAMAMURO, *ibid.* **20** (1985) 2001.
4. *Idem, ibid.* **22** (1987) 4067.
5. T. KITSUGI, T. YAMAMURO, T. NAKAMURA, Y. KAKUTANI, T. HAYASHI, S. ITO, T. KOKUBO, M. TAKAGI and T. SHIBUYA, *J. Biomed. Mater. Res.* **21** (1987) 467.
6. T. NAKAMURA, T. YAMAMURO, S. HIGASHI, T. KOKUBO and S. ITO, *ibid.* **19** (1985) 685.
7. S. YOSHII, Y. KAKUTANI, T. YAMAMURO, T. NAKAMURA, T. KITSUGI, M. OKA, T. KOKUBO and M. TAKAGI, *ibid.* **22** (1988) 327.
8. T. KITSUGI, T. YAMAMURO, T. NAKAMURA, S. HIGASHI, Y. KAKUTANI, K. HYAKUNA, S. ITO, T. KOKUBO, M. TAKAGI and T. SHIBUYA, *ibid.* **20** (1986) 1295.
9. T. KITSUGI, T. NAKAMURA, T. YAMAMURO, T. KOKUBO, T. SHIBUYA and M. TAKAGI, *ibid.* **21** (1987) 1255.
10. 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.
11. T. KITSUGI, T. YAMAMURO, T. NAKAMURA and T. KOKUBO, *J. Biomed. Mater. Res.* **23** (1989) 631.
12. *Idem, Internatl Orthopaedics* **13** (1989) 199.
13. L. L. HENCH, in "Fundamental Aspects of Biocompatibility", Vol. 1, edited by D. F. Williams (CRC Press, Boca Raton, 1981) p. 67.
14. 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.
15. 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.
16. 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.
17. T. KOKUBO, S. ITO, Z. T. HUANG, T. HAYASHI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 331.
18. T. KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *ibid.* **24** (1990) 721.
19. C. OHTSUKI, H. KUSHITANI, T. KOKUBO, S. KOTANI and T. YAMAMURO, *ibid.* **25** (1991) 1363.
20. T. KOKUBO, S. ITO, M. SHIGEMATSU, T. NAKAMURA, T. YAMAMURO and S. HIGASHI, *Glastechn. Ber.* **56K 1** (1983) 695.
21. J. GAMBLE, in "Chemical Anatomy, Physiology and Pathology of Extracellular Fluid", 6th Edn (Harvard University Press, Cambridge, 1967) p. 1.
22. W. NEUMAN and M. NEUMAN, in "The Chemical Dynamics of Bone Mineral" (University of Chicago, Chicago, 1958) p. 175.
23. A. PAUL, *J. Mater. Sci.* **12** (1977) 2246.
24. R. G. BATES, *J. Res. Natl. Bur. Stand.* **47** (1951) 127.
25. R. G. BATES and S. F. ACREE, *ibid.* **30** (1943) 129.
26. C. E. VADERZEE and A. S. QUIST, *J. Phys. Chem.* **65** (1961) 118.
27. A. CHUGHTAI, R. MARSHALL and G. H. NANCOLLAS, *ibid.* **72** (1968) 208.
28. H. McDOWELL, T. M. GREGORY and W. E. BROWN, *J. Res. Natl. Bur. Stand.* **81A** (1977) 273.
29. E. M. CARLISLE, in "Silicon Biochemistry", Ciba Foundation Symposium 121 (Wiley, Chichester, 1986) p. 123.

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