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Bioactivity of $CaO \cdot SiO_2$-based glasses: *in vitro* evaluation

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In order to fundamentally study compositional dependence of bioactivity of glasses, both the surface structural changes of P_2O_5-free $CaO \cdot SiO_2$ glass due to exposure to a simulated body fluid and the effects of adding a third component, such as Na_2O, MgO, B_2O_3, Fe_2O_3, P_2O_5 and CaF_2, were investigated. An acellular aqueous solution which had almost equal ion concentrations to those of the human blood plasma was used as the simulated body fluid. The surface structure changes were examined by electronprobe X-ray microanalysis, thin-film X-ray diffraction (XRD) and Fourier transform infrared (FTIR) reflection spectroscopy. It was found that even P_2O_5-free $CaO \cdot SiO_2$ glass forms an apatite layer on its surface in the simulated body fluid, and that the rate of formation of the surface apatite layer is increased with the addition of Na_2O and P_2O_5 while it decreased with the addition of MgO, B_2O_3, CaF_2 and Fe_2O_3. This indicates that even P_2O_5-free $CaO \cdot SiO_2$ glass can bond to living bone, forming the surface apatite layer in the body and that its bioactivity is increased with the addition of Na_2O and P_2O_5 while it is decreased with MgO, B_2O_3, CaF_2 and Fe_2O_3. It is speculated that a glass of the composition $CaO \cdot SiO_2$ 100, Fe_2O_3 3 in weight ratio does not bond to living bone.

1. Introduction

It was previously shown that glass–ceramic A–W [1, 2] containing crystalline apatite ($Ca_{10}(PO_4)_6(O, F_2)$) and β-wollastonite ($CaO \cdot SiO_2$) in a $MgO–CaO–SiO_2$ glassy matrix bonds to living bone [3] through an apatite layer which is formed on the surface of the glass–ceramic in the body [4–8], and that the apatite layer is formed by a chemical reaction of the calcium and silicate ions dissolved from the glass–ceramic with the phosphate ion in the surrounding body fluid [9–11]. On the basis of these findings, it can be expected that P_2O_5-free $CaO–SiO_2$ glasses will also bond to living bone forming the apatite layer on their surfaces in the body.

In the present study, formation of the apatite layer on the surface of $CaO \cdot SiO_2$ glass and effects of addition of the third components were investigated in a simulated body fluid. An acellular aqueous solution which had almost equal ion concentrations to those of the human blood plasma was used as the simulated body fluid. It was previously shown that this kind of simulated body fluid can reproduce *in vivo*-apatite formation on the surface of glass–ceramic A–W [12]. Surface structural changes of the glasses due to exposure to the simulated body fluid were examined by an electron-probe X-ray microanalysis, thin-film X-ray diffraction and Fourier transform infrared reflection spectroscopy. On the basis of these results, compositional dependence of bioactivity of glasses was discussed fundamentally.

Fundamental studies on compositional dependence of bioactivity of glasses have been reported by Hench *et al.* [13–15] for $Na_2O–CaO–SiO_2–P_2O_5$ glasses and those with various added components. In the present study, a more simple composition, that is, Na_2O- and P_2O_5-free $CaO \cdot SiO_2$ was chosen as the basic composition, and in addition, an aqueous solution nearer to the human body fluid in its ion concentrations was used as the simulated body fluid instead of Tris-buffered pure water used by Hench *et al.* [14, 15].

2. Experimental

Powder mixtures (30 g) of the nominal composition $CaO \cdot SiO_2$ and that with various added third components, which are given in Table I, were prepared by reagent grade chemicals of $CaCO_3$, SiO_2, Na_2CO_3, MgO, H_3BO_3, Fe_2O_3, $CaHPO_4 \cdot 2H_2O$ and CaF_2. They were put into a platinum crucible and melted at 1550–1600° C for 2 h in a $MoSi_2$ electric furnace. The melts were poured onto a stainless steel plate at room temperature and pressed into a plate 1–2 mm thick. The obtained glasses were cut into a rectangular specimen 10 mm × 15 mm × 1 mm and polished with 1 μmΦ diamond paste on their surfaces. They were

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TABLE I Nominal compositions of examined glasses (in weight ratio)

Name	$CaO \cdot SiO_2$	Na_2O	MgO	B_2O_3	Fe_2O_3	P_2O_5	CaF_2
CS	100	0	0	0	0	0	0
CS + Na_2O	100	3	0	0	0	0	0
CS + MgO	100	0	3	0	0	0	0
CS + B_2O_3	100	0	0	3	0	0	0
CS + Fe_2O_3	100	0	0	0	3	0	0
CS + P_2O_5	100	0	0	0	0	3	0
CS + CaF_2	100	0	0	0	0	0	6

cleaned with acetone in an ultrasonic cleaner and immersed into 35 ml simulated body fluid which had almost equal ion concentrations to those of the human blood plasma [16] as shown in Table II. The fluid was prepared by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 into a distilled water. It was buffered at pH 7.25 with 50 mM tris(hydroxymethyl)–aminomethane ($NH_2C(CH_2OH)_3$) and 45 mM hydrochloric acid (HCl). The temperature of the fluid was maintained at 36.5° C. Neither living cell nor organic substances were added.

At various daily intervals after the immersion, specimens were gently washed with acetone and embedded in a polyester resin. Their cross-sections were polished with a 1 $\mu m\Phi$ diamond paste, coated with a carbon film and then analysed with an electronprobe X-ray microanalyzer (Shimazu EPM-810). The electron beam was 0.3 μm in diameter and the accelerating voltage was 12.5 kV.

The surfaces of the specimens as-washed with acetone were analysed with a thin-film X-ray diffraction and Fourier transform infrared reflection spectroscopy. An X-ray diffractometer with at thin-film attachment (Rigaku, Model 2651A1) was used for the X-ray diffraction, in which the specimen surface was fixed at the angle of 1° to the incident beam. Fourier transform infrared spectrometer was used for the infrared spectroscopy (Japan Spectroscopic FT-IR5M). Reflection angle was 75°. Both the techniques enable analysis of the thin layer (about 1 μm thick) at the surface.

3. Results and discussion

Figs 1 to 7 show scanning electron microscope (SEM) photographs of a cross-section of the glasses soaked in the simulated body fluid for 20 days, as well as compositional profile determined by the electronprobe X-ray microanalysis on the cross section. Compositional profiles of boron and fluorine are not given in Figs 4 and 7, respectively, since they could not be detected by the present apparatus. It can be seen from Figs 1 to 7 that a layer rich in calcium and phosphorus is formed on the surfaces of all the examined glasses except for Fe_2O_3-containing glass CS + Fe_2O_3 in the simulated

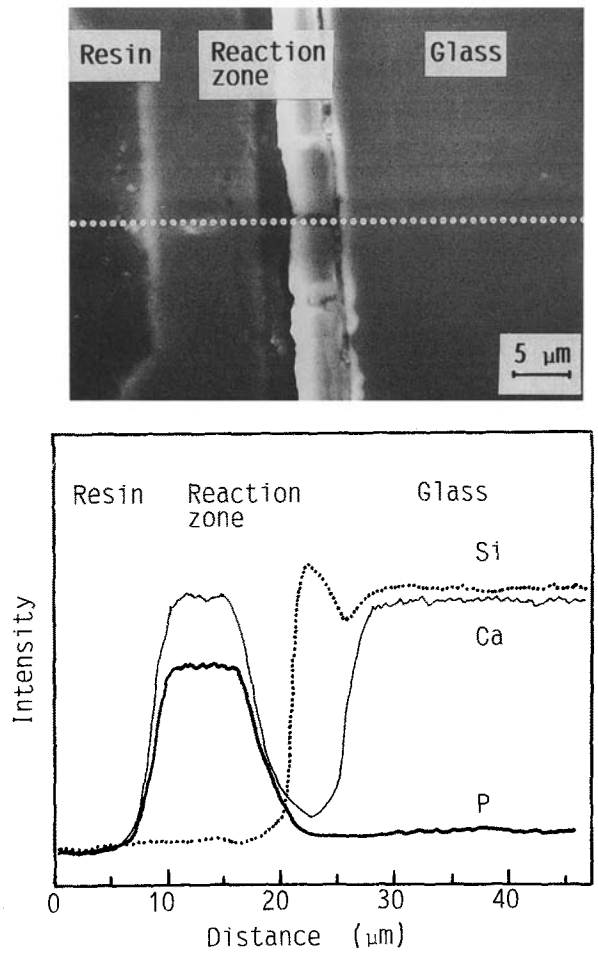


Figure 1 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS soaked in the simulated body fluid for 20 days.

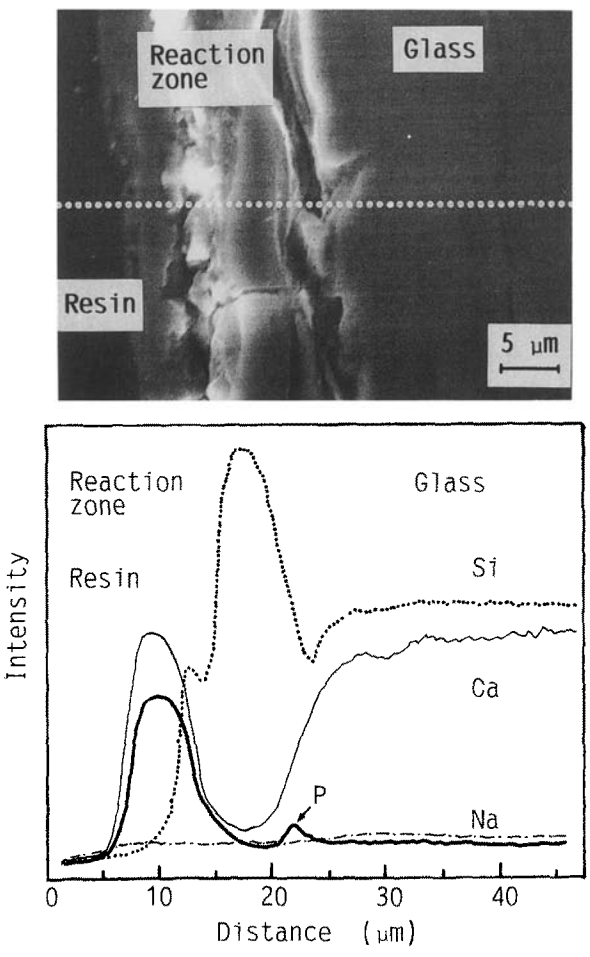


Figure 2 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS + Na_2O soaked in the simulated body fluid for 20 days.

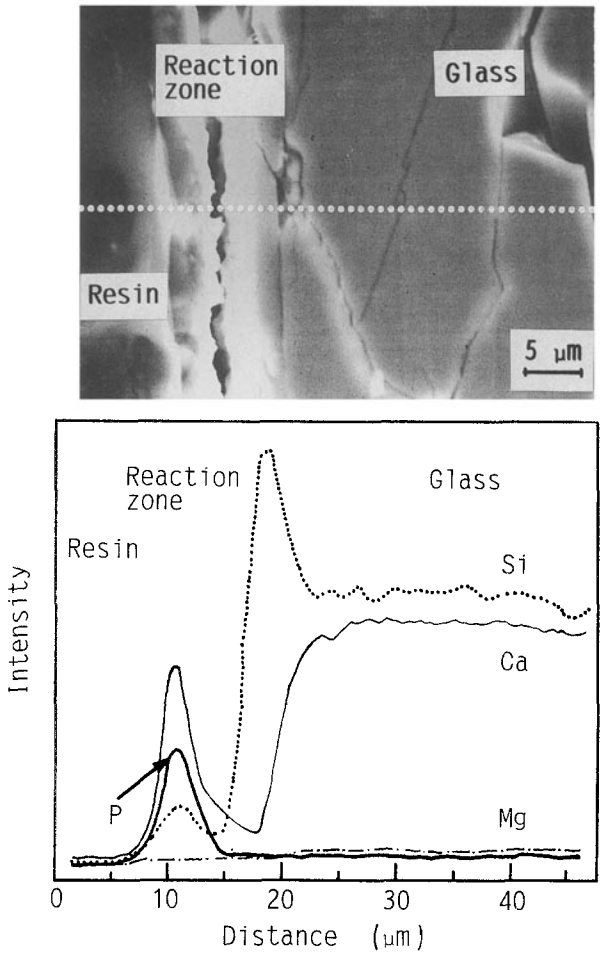


Figure 3 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS + MgO soaked in the simulated body fluid for 20 days.

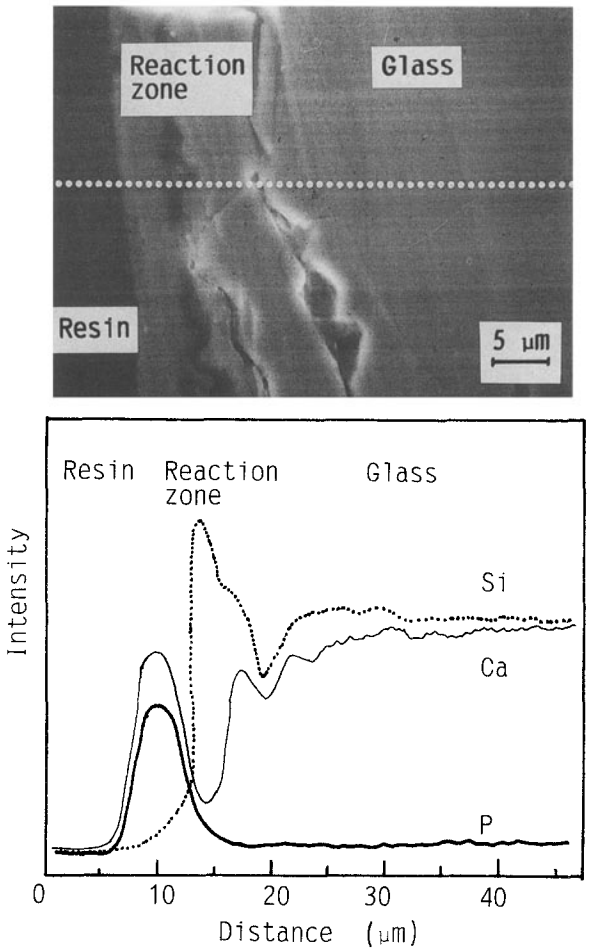


Figure 4 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS + B_2O_3 soaked in the simulated body fluid for 20 days.

body fluid. In the case of glasses forming the Ca, P-rich layer, a Si-rich layer is also formed under the Ca, P-rich layer similar to Bioglass-type glasses [17, 18]. Andersson *et al.* pointed out that silicon penetrates as far as the outer surface of the Ca, P-rich layer for Na_2O–CaO–SiO_2–P_2O_5 glass and proposed a possible mechanism of formation of the Ca, P-rich layer on the bases of the observation [19]. Figs 1 to 7, however, show that there are various types of penetration of Si into the Ca, P-rich layer. In the cases of CS and CS + P_2O_5, Si penetrates slightly.

Figs 8 to 14 show thin-film X-ray diffraction patterns and Fourier transform infrared reflection spectra of the surfaces of the glasses soaked in the simulated body fluid for various time intervals. Assignment of X-ray diffraction peaks and infrared reflection peaks were made according to the procedure described in a previous paper [7]. It can be seen from these figures that a crystalline apatite layer is formed even on the surface of P_2O_5-free CaO · SiO_2 glass, as well as on the surface of other examined glasses except for Fe_2O_3-containing glass, CS + Fe_2O_3 in the simulated fluid. Their X-ray diffraction patterns are characterized with broad lines. This means that the Ca, P-rich layer described above consists of crystalline apatite of defective structure and/or small crystallites, similar to the apatite in the natural bone. It can be also seen from Figs 8 to 14 that the rate of apatite formation on the surface of CaO · SiO_2 glass is increased with the addition of Na_2O and P_2O_5 while decreased with the addition of MgO, B_2O_3, CaF_2 and Fe_2O_3.

These findings might be explained as follows. According to previous investigations by the present authors [9–11], apatite layer on the surface of glass–ceramic A–W is formed, by a chemical reaction of the calcium and silicate ions which were dissolved from the glass–ceramic with the phosphate ion in the surrounding simulated body fluid, where the calcium ion increases the degree of supersaturation of the surrounding body fluid which is already supersaturated with respect to the apatite, while the silicate ion provides favourable sites for nucleation of the apatite on the surface of the glass–ceramic. Formation the apatite layer on the surface of the present P_2O_5-free

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

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

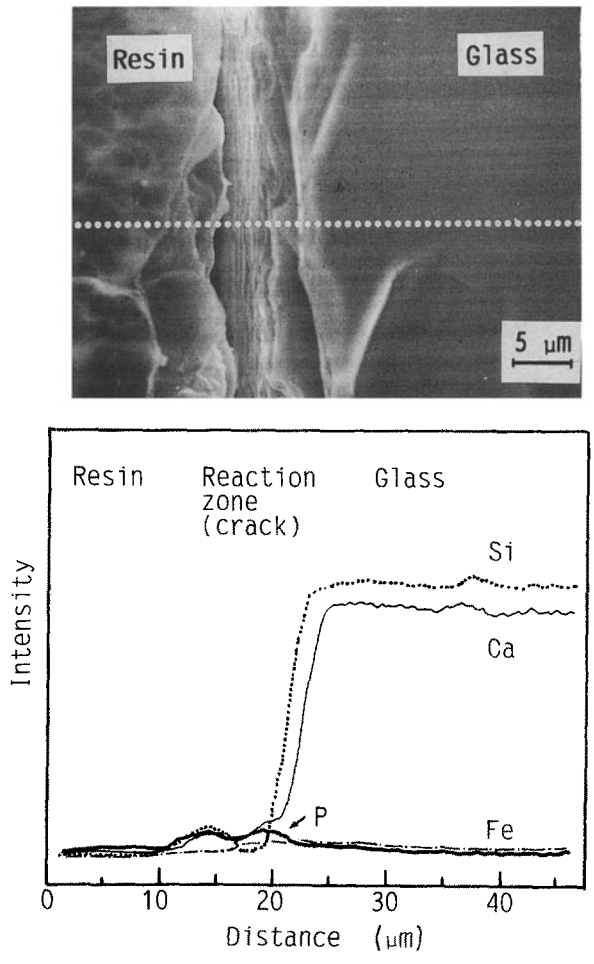


Figure 5 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS + Fe_2O_3 soaked in the simulated body fluid for 20 days.

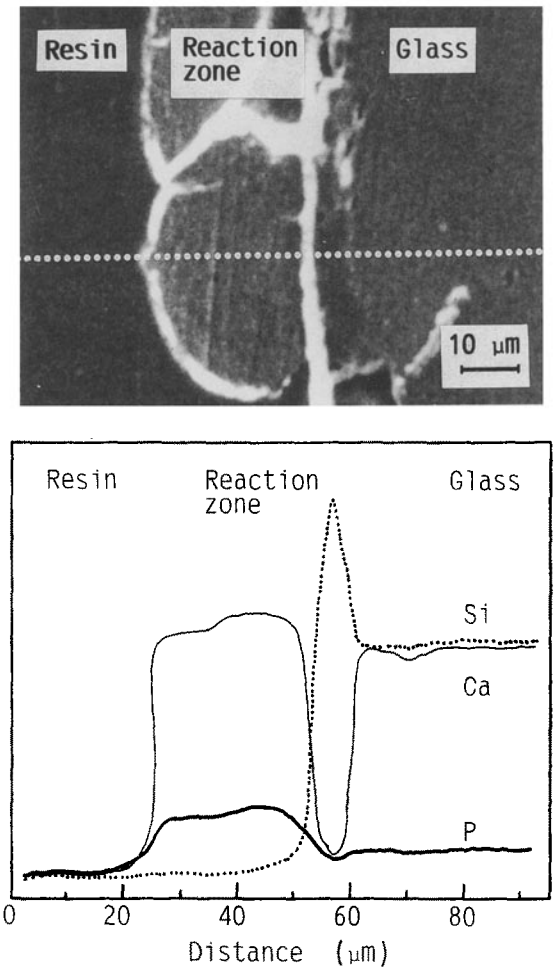
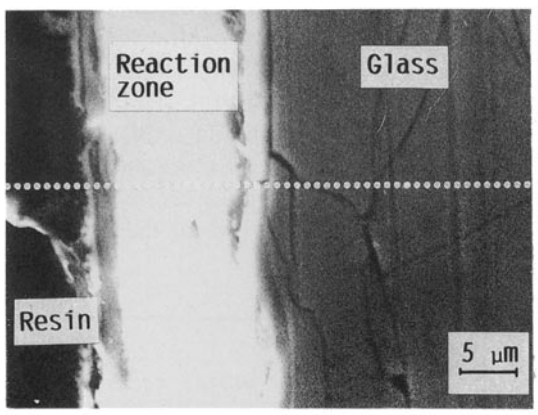


Figure 6 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS + P_2O_5 soaked in the simulated body fluid for 20 days.

$CaO \cdot SiO_2$ glass will be also similarly interpreted. The Si-rich layer under the apatite layer might be formed by a preferential dissolution of the calcium ion from the glass surface. The phosphate ion required for formation of the apatite was supplied only from the surrounding body fluid.

When the Na_2O is added to the $CaO \cdot SiO_2$ composition, the Na^+ ion will also be dissolved from the glass, increasing the pH of the surrounding simulated body fluid and hence accelerate the apatite formation. When the P_2O_5 is added, the $P^{(V)}$ ion will be also dissolved, increasing the degree of supersaturation of the surrounding fluid with respect to the apatite, and hence accelerate the apatite formation. Decrease in the rate of apatite formation with the addition of the MgO, B_2O_3, CaF_2 and Fe_2O_3 might be interpreted in terms of the suppressing effect of these third components on the dissolution of the calcium ion. Consequently, the Si-rich layer is small or not observed for the glass with added CaF_2 and Fe_2O_3, respectively, as shown in Figs 7



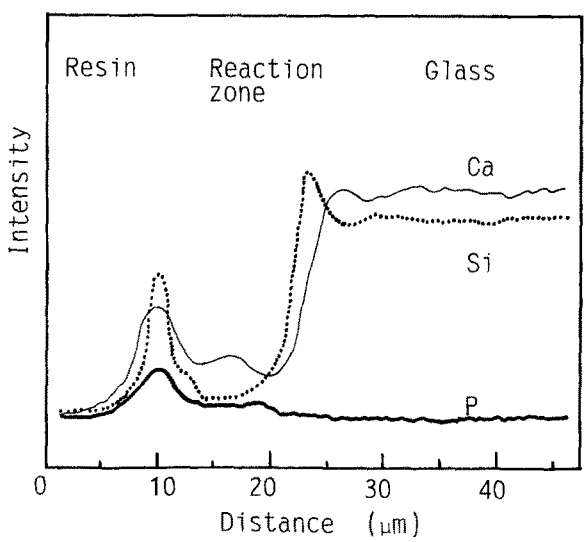


Figure 7 SEM photograph (upper) and compositional profile (lower) of a cross-section of glass CS + CaF_2 soaked in the simulated body fluid for 20 days.

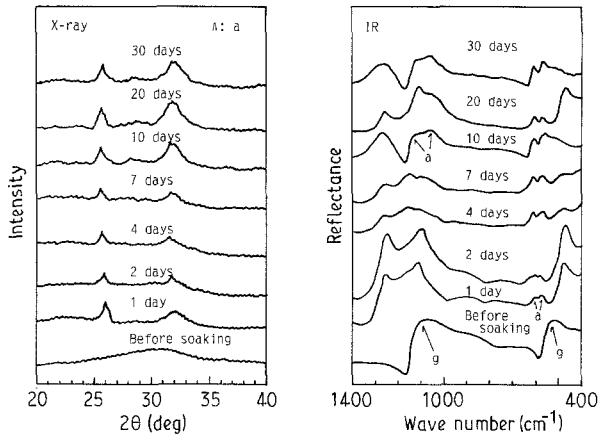


Figure 8 Thin-film XRD patterns and FTIR reflection spectra of glass CS soaked in the simulated body fluid for various periods. a: apatite, g: glassy phase.

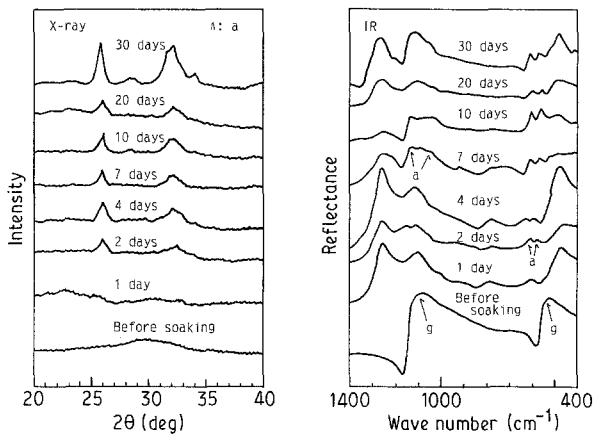


Figure 11 Thin-film XRD patterns and FTIR reflection spectra of glass CS + B_2O_3 soaked in the simulated body fluid for various periods. a: apatite, g: glassy phase.

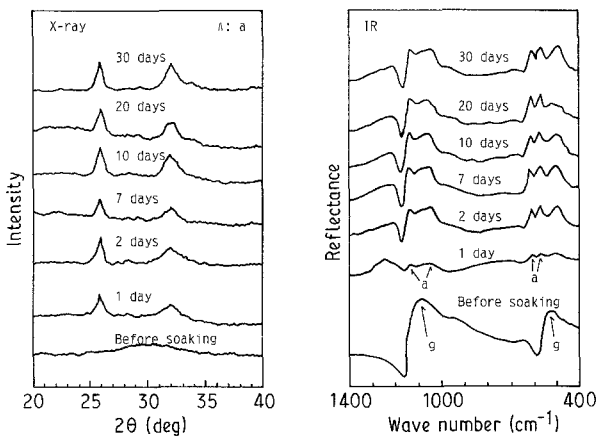


Figure 9 Thin-film XRD patterns and FTIR reflection spectra of glass CS + Na_2O soaked in the simulated body fluid for various periods. a: apatite, g: glassy phase.

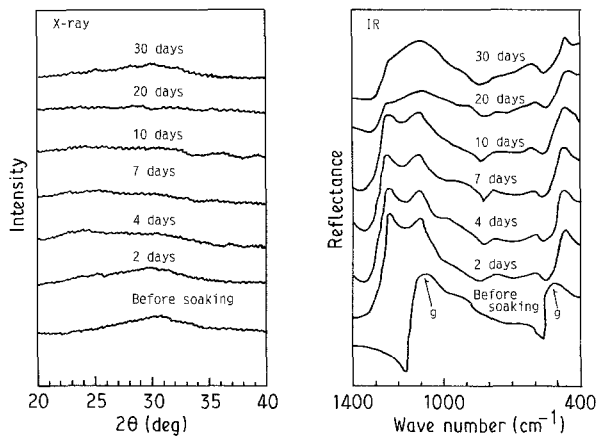


Figure 12 Thin-film XRD patterns and FTIR reflection spectra of glass CS + Fe_2O_3 soaked in the simulated body fluid for various periods. g: glassy phase.

and 5. Ferric oxide is known as an effective component for improving the chemical durability of glasses [20]. Krajewski *et al.* [21] also reported that bioactivity of Bioglass-type glasses is much reduced by incorporation of the Fe^{3+} ion. This is consistent with present results.

Ogino *et al.* previously reported that a P_2O_5-free Na_2O–SiO_2 glass forms an apatite layer on its surface in an aqueous solution containing $Ca^{(II)}$ and $P^{(V)}$ ions [22]. Kasuga *et al.* reported that $CaO \cdot SiO_2$ glass–ceramic forms an apatite layer on its surface in a simulated body fluid [23]. These findings are also consistent with the present results.

It was already shown for several kinds of glass and glass–ceramics that apatite formation on their surfaces in the body is an essential requirement for them to bond to living bone [9–11, 13, 15, 24]. Therefore, it can be speculated from the present results that P_2O_5-

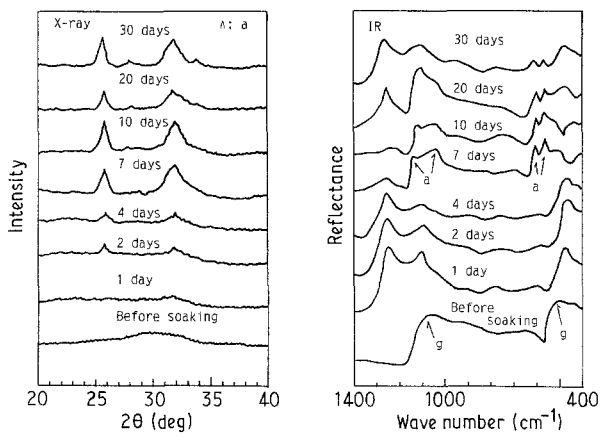


Figure 10 Thin-film XRD patterns and FTIR reflection spectra of glass CS + MgO soaked in the simulated body fluid for various periods. a: apatite, g: glassy phase.

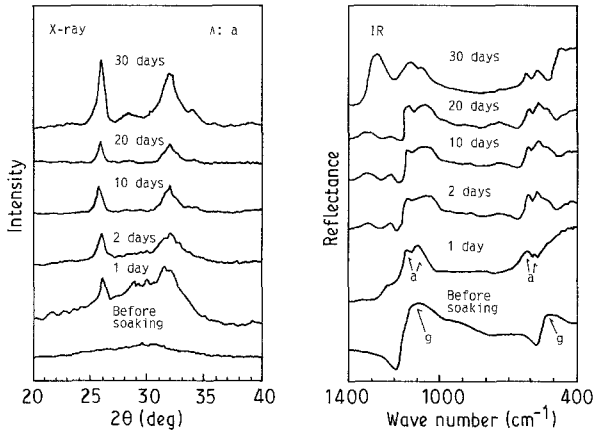


Figure 13 Thin-film XRD patterns and FTIR reflection spectra of glass CS + P_2O_5 soaked in the simulated body fluid for various periods. a: apatite, g: glassy phase.

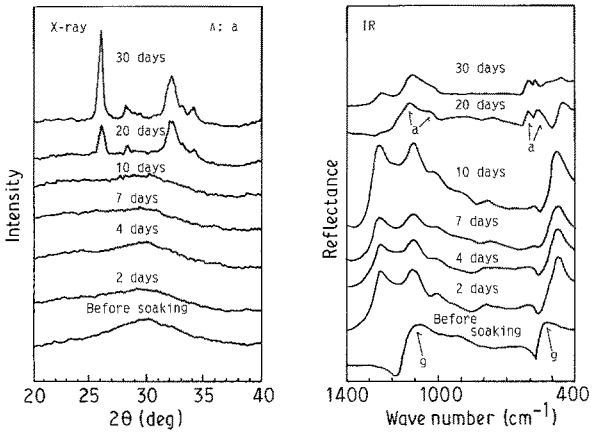


Figure 14 Thin-film XRD patterns and FTIR reflection spectra of glass CS + CaF_2 soaked in the simulated body fluid for various periods. a: apatite, g: glassy phase.

free $CaO \cdot SiO_2$ glass also bonds to living bone forming an apatite layer on its surface in the body, and that the bone-bonding ability of the glass will be increased with the addition of Na_2O and P_2O_5, while it will be decreased with the addition of MgO, B_2O_3, CaF_2 and Fe_2O_3. It is considered that Fe_2O_3-containing $CaO \cdot SiO_2$ glass does not bond to living bone. Correlation of these speculations with *in vivo* studies will be published elsewhere [25].

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