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Effect of Alcohol on Bioactivity of Glasses

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One of the CaO,SiO_2-based bioactive glasses($50CaO \cdot 50SiO_2$ in mol%) were soaked for various periods in a simulated body fluid(SBF) with or without containing alcohols such as methanol, ethanol, and 1-propanol. Effect of the alcohols was investigated on the apatite formation on the glass surface with thin-film X-ray diffraction, FT-IR reflection spectroscopy and scanning electron microscopy. Under the presence of alcohols up to 0.1mol/l in the SBF an apatite layer was formed on the glass, while it consisted of crystallites similar in morphology but larger in size than that found for the SBF without the alcohols. It was concluded that the alcohols little influenced the the apatite forming ability of the CaO,SiO_2-based glasses.

1. INTRODUCTION

Since discovery of Bioglass® by Hench *et al.* in the early 1970s[1], various types of glasses and glass-ceramics have been developed that can bond to living bone. Some of them are already clinically used as valuable bone substitutes such as artificial middle ear bones, alveolar ridge maintenance implants, artificial iliac crests and vertebrae[2)-4)], etc. These bioactive glasses and glass-ceramics, however, can not replace a whole piece of femur and tibia, because fracture toughness is lower and Young's modulus is higher than that of the human cortical bone. One may consider obtaining bioactive materials with high fracture toughness by incorporating bioactive glass powders into an organic polymer with sol-gel methods. Bioactive composite materials prepared with the sol-gel methods would contain residual alcohols that are used during preparation. However, the effect of alcohols on bioactivity of glasses has not been revealed yet.

It has been already confirmed[5)] that the essential condition for various kinds of glasses and glass-ceramics to bond to living bone is the formation of a biologically active bone-like apatite layer on their surfaces when they are embedded in the body, and that the apatite layer is reproduced on the surfaces of bioactive glasses and glass-ceramics even in an acellular simulated body fluid(SBF). Moreover, the binary system $CaO-SiO_2$ gives the simplest composition showing bioactivity[6),7)]. In the present study, in order to reveal the effect of alcohols on bioactivity of glasses, the apatite formation on the surface of $50CaO \cdot 50SiO_2$(mol%) glass is thus investigated in the SBF containing alcohols.

2. EXPERIMENTAL PROCEDURE

2.1. Preparation of $50CaO \cdot 50SiO_2$(mol%)

A glass of the nominal composition $50CaO \cdot 50SiO_2$(mol%) was prepared by melting a mixture of appropriate amounts of reagent-grade chemicals $CaCO_3$ and SiO_2 with a 30ml platinum crucible at 1600 ℃ for 1 h in an $MoSi_2$ electric furnace. The melt was poured on to a stainless steel plate to be formed into a plate about 1mm thick, and allowed to cool in an SiC furnace from 700 ℃. Rectangular specimens of 15x10x1 mm^3 were cut from the obtained

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glass. Both surfaces of the specimens were polished with a diamond paste(1μm in diameter) and washed with acetone in an ultrasonic cleaner.

2.2. Soaking in SBF containing various alcohols

The obtained specimen was soaked in the 35 ml of SBF with and without containing various alcohols. SBF has nearly equal ion concentrations(Na^+ 142.0, K^+ 5.0, Ca^+ 2.5, Mg^{2+} 1.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM(=10^{-3}mol·dm^{-3})) and pH to the human blood plasma. It 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 into distilled water. It was buffered at pH7.25 with 50 mM tris(hydroxymethyl)aminomethane($(CH_2OH)_3CNH_2$) and 45 mM hydrochloric acid(HCl), and its temperature was kept at 36.5℃. SBF is already confirmed to be able to well reproduce the apatite formation on the surfaces of glasses and glass-ceramics in the body environment. Methanol(CH_3OH), ethanol(C_2H_5OH) and 1-propanol(C_3H_7OH) were selected as the additives to SBF. A preliminary test showed that precipitation was not observed till the concentration of methanol exceeded 1 mol·dm^{-3}. Thus we fixed the concentration of alcohols in SBF as 0.1 mol·dm^{-3}. The examined fluids are given in Table 1.

Table 1 Examined Solution

Solution	Additive	Concentration(mol·dm^{-3})
SBF	no	0
SBF+MeOH	methanol(CH_3OH)	0.1
SBF+EtOH	ethanol(C_2H_5OH)	0.1
SBF+PrOH	1-propanol(C_3H_7OH)	0.1

2.3. Analysis of surface structure

After soaking in the fluids for various periods, the glass was removed from the fluids and gently washed with acetone. The surface structure was examined with thin-film X-ray diffraction, Fourier-transform infrared(FT-IR) reflection spectroscopy and scanning electron microscopic(SEM) observation. An X-ray diffractometer(RINT-1400, Rigaku, Japan) attached with a thin-film attachment CN2651A was used, and the glancing angle was fixed at 1°, while an infrared spectrometer(FT-IR300, JASCO Co., Japan) was used, and the reflection angle to the normal was set at 75°. Both techniques enabled to detect a layer about 1 μm thick at the surface of the specimen. In the SEM observation, gold film was sputter-coated on the surface of the specimen and electron microscope(JSM-6300, JEOL, Japan) was used.

2.4. Measurement of element concentration

After the specimen was removed from the fluids, the concentrations of calcium, silicon and phosphorus in the fluids were measured with an inductively coupled plasma(ICP) emission spectroscopy. pH of the fluids was also measured.

3. RESULTS AND DISCUSSION

Figure 1 shows thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of the glass before and after soaking for various periods in (a)SBF, (b)SBF+MeOH, (c)SBF+EtOH and (d)SBF+PrOH. The spectra for a apecimen without soaking are denoted as "0h". The main peaks in Fig. 1 were assigned to as described on the bases of the data previously reported[3),8)]. The IR reflection peaks at 500, 650, 1100 and 1250 cm^{-1} were ascribed to Si-O bending vibration[3)], P-O bending vibration[3)], transverse optical mode of Si-O stretching vibration and longitudinal optical mode of Si-O stretching vibration[8)], respectively. Fig. 1(a) shows that the intensities of X-ray scattering at small angles increases and IR reflection peak at 1250 cm^{-1} appears in the first day of immersion in SBF.

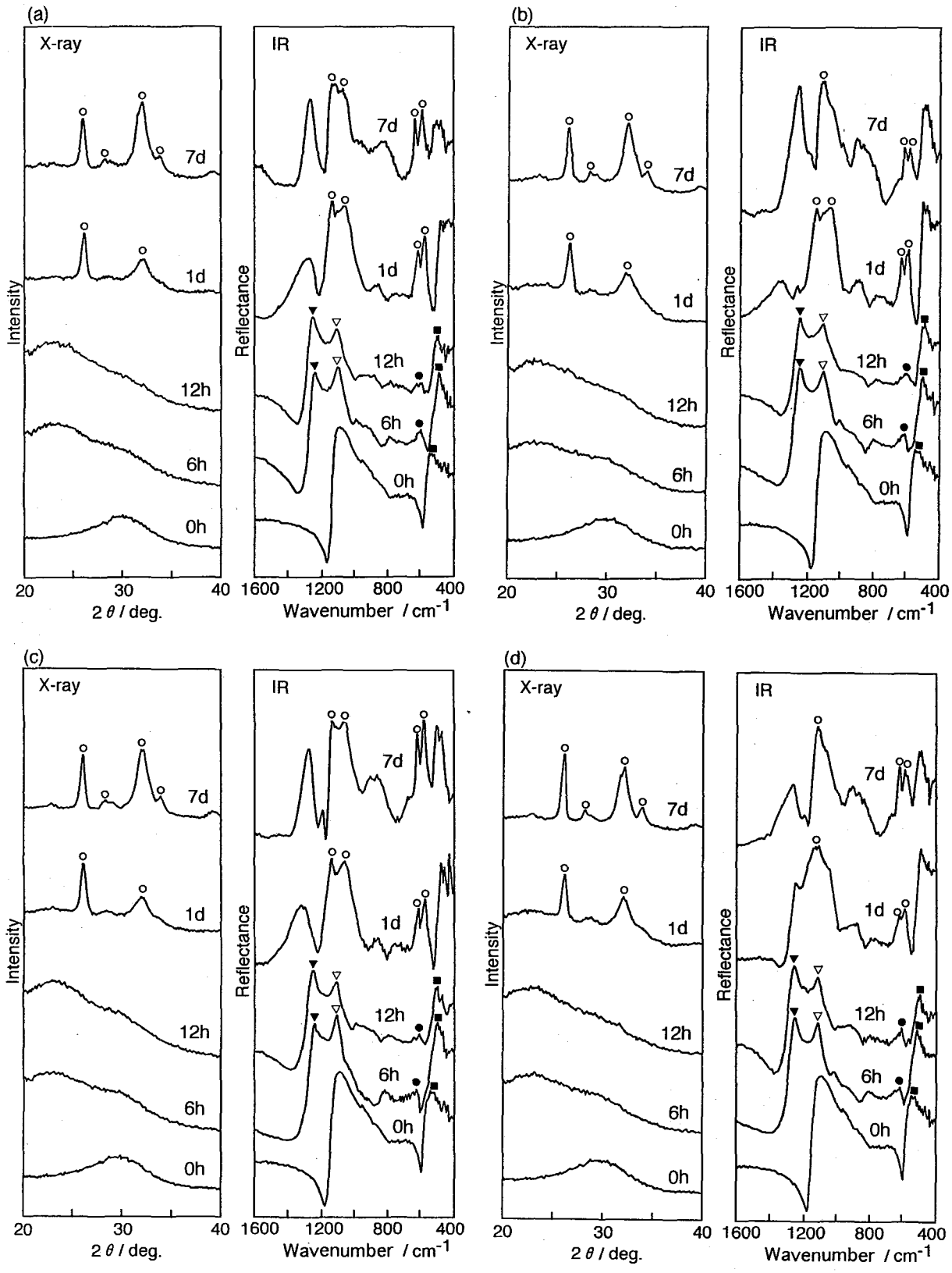

Fig. 1 Thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surface of the glass soaked in (a)SBF, (b)SBF+MeOH, (c)SBF+EtOH and (d)SBF+PrOH for various periods.
○: apatite, ●: P-O bending vibration, ■: Si-O bending vibration, ▽: transverse optical mode of Si-O stretching vibration, ▼: longitudinal optical mode of Si-O stretching vibration.

This indicates that a silica hydrogel layer is formed on the surface of the glass. The IR reflection peak at 650 cm^{-1} simultaneously grew. This suggests that a type of amorphous phosphate such as a calcium phosphate is formed on the surface of the silica gel layer. Both the X-ray diffraction patterns and IR reflection spectra in Fig. 1(a) show that an apatite layer is formed by 1 day and it grows with increasing soaking time. The same results of the surface structural changes of the glass were observed for the glass soaked in SBF containing methanol, ethanol or 1-propanol(Fig. 1(b)-(d)). These results indicates that the glass forms an apatite layer on its surface even if the alcohols are present in SBF. Figure 2 shows SEM photographs of the surface of the glass soaked for 7 days in (a)SBF and (b)SBF+PrOH. Fine flake-like particles are observed, while the size of crystallites formed in SBF+PrOH is larger than that formed in SBF. These particles are the crystals of apatite after Fig. 1. Similar flake-like particles are also observed on the glass soaked in SBF+MeOH and SBF+EtOH. That is, the morphology of the apatite crystal did not change with coexistence of the alcohol.

Figure 3 shows changes in concentration of the calcium, silicon and phosphorus as well as in pH of (a)SBF, (b)SBF+MeOH, (c)SBF+EtOH and (d)SBF+PrOH due to immersion of the glass. Fig. 3(a) shows that the calcium and silicon concentration increase for the glass soaked in the SBF until 12 hours. After immersion for 1 day, the calcium concentration decreased, accompanied by an appreciable decrease in the phosphorus concentration. Increase in the calcium and silicon concentration is due to dissolution of Ca(II) and Si(IV) ions from the glass. Then decrease in the calcium and phosphorus concentrations is due to formation of calcium phosphate on the glass. Similar changes in element concentrations were observed for the SBF+MeOH, SBF+EtOH and SBF+PrOH. From the results, it is concluded that the alcohols did not influence on the rate of dissolution of the glass.

The mechanism of apatite formation on CaO,SiO_2-based glasses is reported as follows[9]: The calcium ion dissolved form CaO,SiO_2-based glasses increases the degree of supersaturation of the surrounding body fluid with respect to apatite, and the hydrated silica formed on their surfaces provides specific sites favorable to the apatite nucleation. As a result, the apatite nuclei are easily formed on the surfaces of the CaO,SiO_2-based glasses and grow spontaneously by consuming the calcium and phosphate ions from the surrounding body fluid. We also reported in previous papers that a specific structural unit of the hydrated silica is required for inducing the apatite nucleation[10], and that the apatite nucleation ability of the hydrated silica is suppressed by only a small amounts of impurities such as Al_2O_3 and TiO_2[11]. The present results have indicated that the presence of alcohols in SBF up to 0.1 $mol \cdot dm^{-3}$ do not influence the increasing rate of degree of the supersaturation of the surrounding body fluid with respect to the apatite. Furthermore, the apatite nucleation ability of silica hydrogel formed on the glass is not depressed by the presence of alcohols with a low molecular weight. This suggests that CaO,SiO_2-based bioactive glasses are useful for designing various kinds of bioactive composites utilizing sol-gel methods.

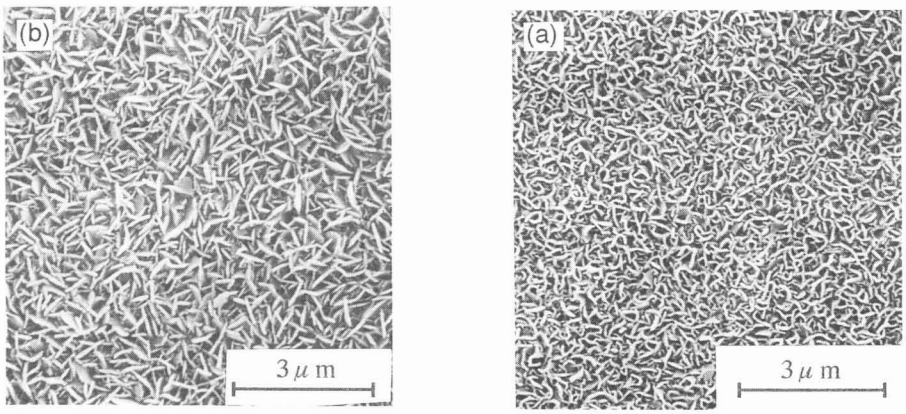

Fig. 2 SEM photographs of the surface of the glass soaked in (a)SBF and (b)SBF+PrOH for 7days.

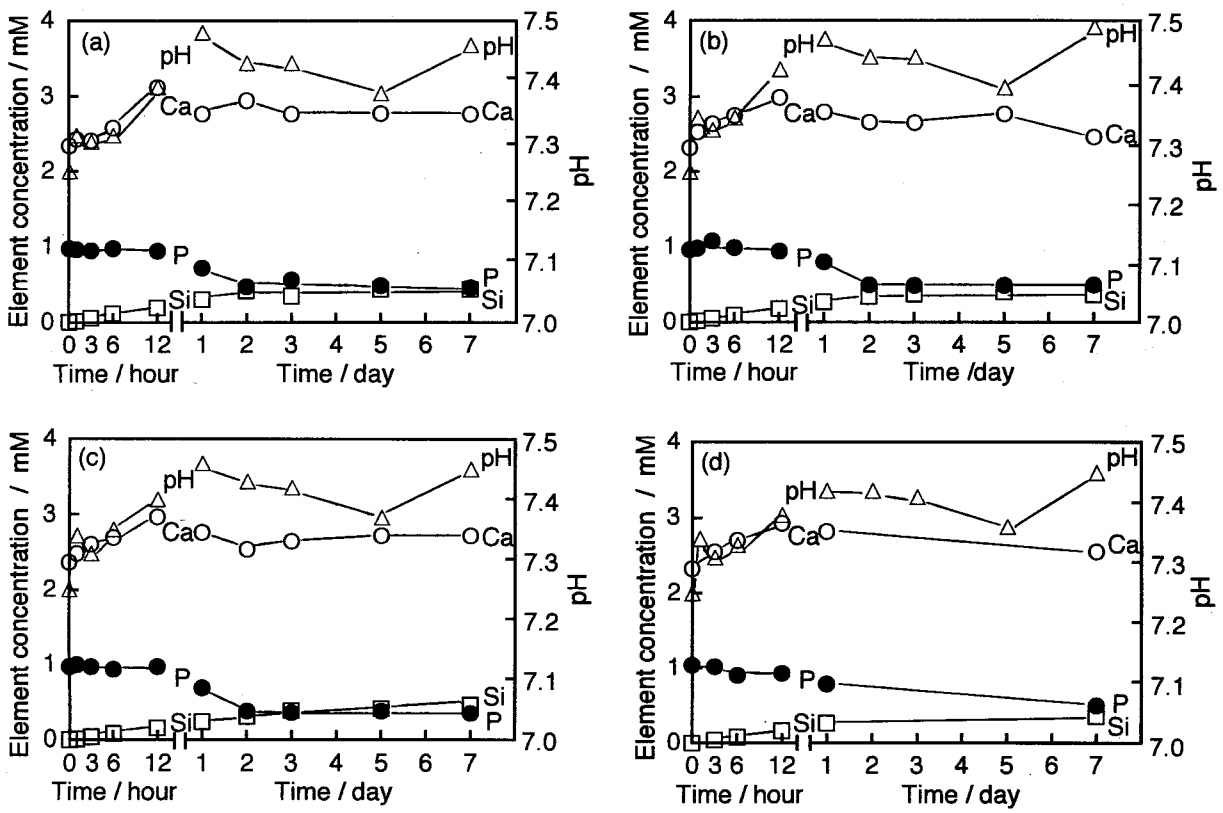

Fig. 3 Changes in element concentrations and pH of (a)SBF, (b)SBF+MeOH, (c)SBF+EtOH and (d)SBF+PrOH due to immersion of the glass.

4. SUMMARY

In order to investigate the effect of alcohols on bioactivity of glasses, we examined the apatite formation on the surface of $50CaO \cdot 50SiO_2$ (mol%) glass in SBF containing methanol, ethanol or 1-propanol. The apatite formation ability of the glass was not influenced by coexistence of the alcohols up to 0.1 $mol \cdot dm^{-3}$ in SBF. CaO,SiO_2-based bioactive glasses show bioactivity even in the presence of alcohols with low molecular weight in the body environment.

Acknowledgment

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