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Effect of solid/solution ratio on apatite formation from $CaSiO_3$ ceramics in simulated body fluid

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The effect of the solid/solution (S/S) ratio on apatite formation from $CaSiO_3$ ceramics in simulated body fluid (SBF) was investigated. $CaSiO_3$ ceramics with a Ca/Si ratio of 0.91 were prepared by sintering $CaSiO_3$ powder coprecipitated from ethanol solutions of $Ca(NO_3)_2{\cdot}4H_2O$ and $Si(OC_2H_5)_4$ using NH_4OH as the precipitant. These ceramics were reacted with SBF at S/S ratios of 1.0, 2.5 and 8.3 mg/ml at 36.5 °C for various times. Formation of apatite was observed at all the S/S ratios after soaking for 1 day. The amount and microstructure of the apatite obtained at a S/S ratio of 8.3 mg/ml, however, differed largely from the product formed at the other two S/S ratios. The apatite formed at S/S = 8.3 mg/ml was of smaller particle size, formed in smaller amount and with less preferred orientation of the (00*l*) of apatite crystals compared with those formed at S/S = 1.0 and 2.5 mg/ml. An increase of Ca and decrease of the P components occurred in the soaked SBF at S/S = 8.3 mg/ml, the changes being much more marked than with the other two S/S ratios. These differences in the concentration changes in SBF at different S/S ratios are attributed to the difference in the apatite formation from the $CaSiO_3$ ceramics.

1. Introduction

Various bioactive materials such as glasses [1, 2], glass ceramics [3–5], sintered hydroxyapatite [6], composite materials [7–9], etc., have been developed for medical applications. For these applications it is very necessary to enhance the generation and growth of apatite in a short reaction time in body environment. We [10] have investigated the apatite formation behavior on $CaSiO_3$ ceramics under *in vitro* condition using simulated body fluid (SBF) developed by Kokubo [3–5], which has similar ion concentrations to human blood plasma. The $CaSiO_3$ ceramics were found to show very fast apatite formation (within 1 day) and a high growth rate of the apatite layer (>10 μm in thickness/day) in SBF [11]. This apatite formation behavior, however, varied fairly largely with the chemical composition, porosity and microstructure of the $CaSiO_3$ ceramics [12, 13]. Ceramics showing rapid and copious apatite formation are obtained when the microstructures contain elongated α-$CaSiO_3$ grains (pseudowollastonite; the high temperature phase of $CaSiO_3$) with thick glassy grain boundaries which are formed by liquid phase sintering of a composition with Ca/Si < 1. These samples showed the excellent apatite formation even under flowing SBF conditions [14]. Apatite formation was also found on the $CaSiO_3$ ceramics in SBF containing proteins such as those found in human blood plasma [15, 16].

In all these investigations, the solid/solution (S/S) ratio for the soaking experiments was set at ≈0.25 g in 30 or 35 ml of SBF; these S/S ratios (≈7–8) are similar to the ordinary experimental conditions used by many workers. Samples showing very fast dissolution in SBF very quickly adsorbed the P component from the SBF, becoming full consumption of P after 1 day of soaking at these S/S ratios. This very fast consumption of the P component from the SBF, however, did not result in apatite formation [13]. These results may mean that the S/S ratio in the soaking experiments should have a strong influence on apatite formation in SBF.

In this paper, apatite formation on $CaSiO_3$ ceramics in SBF was investigated by soaking at three different S/S ratios (1.0, 2.5 and 8.3 mg/ml) to elucidate the effect of S/S ratio on apatite formation.

2. Experimental

2.1. Sample preparation and characterization

$CaSiO_3$ powders were prepared by coprecipitation using NH_4OH as the precipitant. The starting materials were $Ca(NO_3)_2{\cdot}4H_2O$ and $Si(OC_2H_5)_4$ (TEOS). The

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former reagent (0.0455 mol) was dissolved in 100 ml of ethanol solution containing a small amount of distilled water (2 ml) while the latter reagent (0.05 mol) was dissolved in 100 ml of absolute ethanol. The small amount of water added to the former solution is to adjust the total H_2O/TEOS $\cong 6$ for hydrolysis of the TEOS. The Ca/Si ratio was set at 0.91, a slightly more SiO_2-rich composition than stoichiometric one, to cause liquid phase sintering [12]. These two solutions were mixed by stirring for 2 h. A precipitate was obtained by rapidly adding 200 ml of NH_4OH (25 mass%) to the solution. The precipitate was dried by evaporating at 60 °C then oven dried at 110 °C overnight. The dried powder was calcined at 600 °C for 2 h to evaporate the residual NH_4-salt, nitrate and ethanol, then fired at 1000 °C for 2 h to crystallize β-$CaSiO_3$ (wollastonite; the low temperature phase). The particle size of the fired powder was measured by particle size analyzer (Microtrack, NIKKISO, Tokyo, Japan).

The fired powder was pressed uniaxially to form green bodies (0.25 g) with dimensions of 10 mm $\phi \times t$ mm. These were sintered at 1400 °C for 2 h at a heating rate of 2 °C/min. The crystalline phase transformed to α-$CaSiO_3$ (the high temperature phase) after sintering. The bulk density and water absorption were evaluated by Archimedes' method. The crystalline phases in the samples were identified by powder X-ray diffractometer (XRD; Lab-X XRD-6100, Shimadzu, Kyoto, Japan) using monochromated Cu K_α radiation. In the measurements, pellet samples were mounted in a through-hole type Al sample holder using glue. The microstructure of the ceramics was observed by scanning electron microscope (SEM; JSM-5310, Jeol, Tokyo, Japan) at an accelerated voltage at 20 kV.

2.2. Soaking in SBF

Simulated body fluid (SBF) solution was prepared based on the recipe reported by Kokubo [3–5]. The ion concentrations of SBF are shown in Table I together with those in human blood plasma. The SBF solution has the same cation concentrations as blood plasma but with different concentrations of Cl^- and HCO_3^-. The pH of the SBF solution was 7.25 and buffered with 1 ml/L of HCl and tris-(hydroxymethyl)-aminomethane ($(CH_2OH)_3CNH_2$).

The surfaces of the as-prepared α-$CaSiO_3$ ceramics were polished using 4 μm diamond paste. The ceramics were then soaked in SBF solution at 36.5 °C for various times using three different sample/SBF (S/S) ratios, i.e., 1.0 (250 mg/250 ml), 2.5 (250 mg/100 ml) and 8.3 mg/ml (250 mg/30 ml). After the predetermined soaking time, the ceramics were removed from the SBF solution, gently rinsed with distilled water and dried at room temperature.

TABLE I Concentrations of various ions (mM) of simulated body fluid (SBF) and human blood plasma

	Ca^{2+}	Mg^{2+}	K^+	Na^+	HPO_4^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
SBF	2.5	1.5	5.0	142.0	1.0	4.2	147.8	0.5
Blood	2.5	1.5	5.0	142.0	1.0	27.0	103.0	0.5

The surfaces of the soaked ceramics were investigated by XRD and SEM. The chemical compositions of the formed apatite particles were analyzed with an energy dispersed X-ray (EDX) detector attached to the SEM (JSM-T200, Jeol, Tokyo, Japan). Diffuse reflectance infrared Fourier transform (DRIFT) spectra of the soaked ceramics were measured on KBr disks using a commercial diffuse reflectance accessory (FTIR-8600PC, Shimadzu, Kyoto, Japan). Changes of the Ca, Si and P concentrations in the SBF after soaking the $CaSiO_3$ ceramics were determined by inductively coupled plasma atomic emission spectroscopy (ICP; SPS1500VR, Seiko Instruments, Tokyo, Japan).

3. Results

3.1. Sample

The average particle size of the as-fired powder was 5.7 μm. Since it is considered to be preferable to prepare not fully densified ceramics to enhance their reactivity with SBF, the as-fired powder was sintered without grinding treatment. The bulk density of the resultant ceramic fired at 1400 °C was 2.14 g/cm^3 and the water absorption was 7.9%. The open porosity calculated from these data is 17% and relatively large pores >10 μm are present in the sintered bodies. This $CaSiO_3$ ceramic is apparently more porous than the $CaSiO_3$ ceramics prepared from ground powders [11–13]. The XRD of this sample showed formation of α-$CaSiO_3$ with a small amount of cristobalite indicating a partial crystallization of glassy phase.

3.2. Soaking experiment in SBF

Fig. 1 shows the changes of Ca, Si and P, and the pH of the soaked SBF as a function of soaking time for three soaking experiments at S/S ratios of 1.0, 2.5 and 8.3 mg/ml. The Ca concentrations in the SBF increased after soaking the ceramics in all three experiments because of dissolution of the $CaSiO_3$ ceramics. The rates were fast up to 5 days of soaking but became

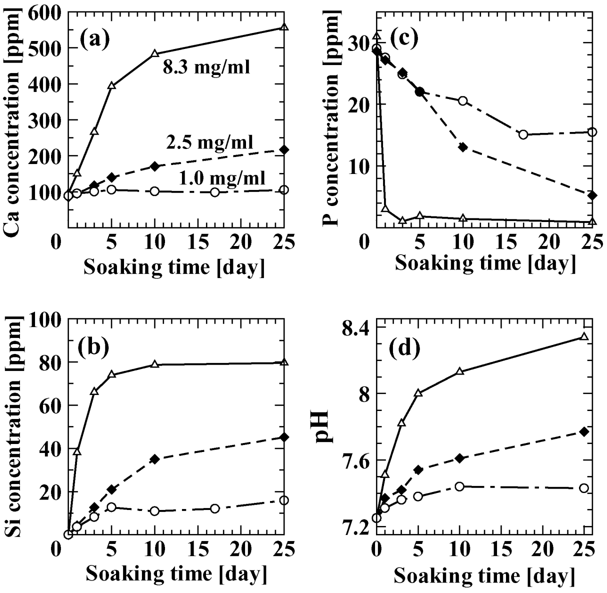


Figure 1 Changes of Ca, Si and P concentrations and pH of the SBF as a function of soaking time.

slower with longer soaking time. The Ca concentrations after soaking for 25 days differed largely with different S/S ratios, reaching 556 ppm (13.9 mM) at $S/S = 8.3$ mg/ml, 217 ppm (5.42 mM) at 2.5 mg/ml but only 105 ppm (2.62 mM) at 1.0 mg/ml. The increase of Ca^{2+} in the respective SBF solutions calculated from the concentration change after 25 days soaking was 0.345, 0.330 and 0.106 mmol respectively and only that at 1.0 mg/ml was lower than the others. Correspondingly, the Si concentrations increased similarly to the Ca concentrations. The Si concentrations reached 80 ppm (2.84 mM) at $S/S = 8.3$ mg/ml, 45.2 ppm (1.61 mM) at 2.5 mg/ml but only 15.9 ppm (0.57 mM) at 1.0 mg/ml. In contrast with the Ca and Si concentrations, the P concentrations decreased with soaking due to adsorption by the $CaSiO_3$ ceramics. The decrease in the P concentration was very steep at $S/S = 8.3$ mg/ml and the initial P concentration of 31 ppm (1.00 mM) decreased to 3.0 ppm (0.10 mM) after soaking for only 1 day. The decrease of the P concentrations at the other two S/S ratios was, however, much slower, their concentrations decreasing to 5.2 ppm (0.17 mM) at 2.5 mg/ml and 15.5 ppm (0.50 mM) at 1.0 mg/ml only after 25 days soaking. The pH values of the soaked SBF solutions showed an initial steep increase, becoming gentler at longer soaking time at all the three S/S ratios. These changes in pH are caused by changes in the concentration of SBF by dissolution of the $CaSiO_3$ ceramics and precipitation of new phases. The changes are therefore correspond to the Ca concentration changes, having largest ($7.25 \rightarrow 8.34$) at 8.3 mg/ml and smallest ($7.25 \rightarrow 7.35$) at 1.0 mg/ml.

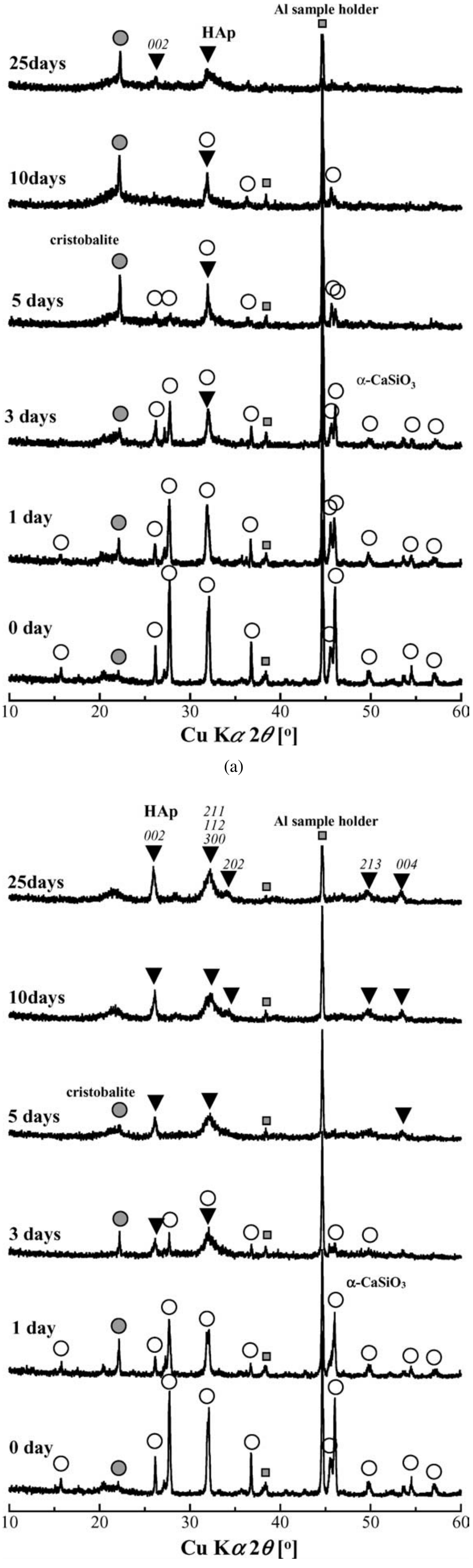
Changes of phases formed on the surface of the $CaSiO_3$ ceramics soaked in SBF were examined by XRD. Fig. 2 shows the XRD patterns of the samples before and after soaking for various times. Here, a strong peak at $2\theta \cong 44^\circ$ and a small peak at $2\theta \cong 38^\circ$ are due to the Al sample holder used to mount the samples. For the samples soaked at $S/S = 8.3$ mg/ml, the XRD peak intensities of α-$CaSiO_3$ decreased gradually whereas new peaks due to apatite appeared and gradually increased in intensity with longer soaking time. The XRD peak intensity of cristobalite also became stronger with soaking ≥ 5 days. This may indicate selective leaching of α-$CaSiO_3$ in SBF and the increase in relative concentration of cristobalite. For samples soaked at $S/S = 2.5$ and 1.0 mg/ml, the XRD peaks of apatite were clearly observed after 3 days soaking and increased in intensity with longer soaking time. Compared with the JCPDS data for hydroxylapatite (card No. 9-432), the relative intensities of 002 ($2\theta \cong 26^\circ$) and 004 ($2\theta \cong 53^\circ$) were stronger in the present XRD patterns, especially in the sample soaked for 25 days at $S/S = 1.0$ mg/ml. This indicates preferred orientation to (00*l*) of apatite crystals on the pellet samples. The XRD patterns soaked for a long time showed only apatite peaks and a halo at $2\theta \cong 20^\circ$ corresponding to amorphous SiO_2. From the XRD patterns of the samples soaked at three S/S ratios, the apatite is seen to increase and become more highly oriented in the (00*l*) plane as the S/S ratio is decreased.

The surface microstructures of the samples soaked for various times were observed by SEM. Fig. 3 shows SEM micrographs of the samples before and after soaking for 1, 3 and 10 days at the three S/S ratios. Spherical particles of apatite were observed in the 1 day soaked samples. The observed spherical particles are known to consist of agglomerated very fine platy apatite particles [3–5, 11]. Comparing the three 1 day samples soaked at different S/S ratios, the agglomerated particle sizes of apatite are different; the particle sizes formed at $S/S = 8.3$ mg/ml are smaller than in the other two samples. In the 3 days soaked samples, the surfaces of the $CaSiO_3$ ceramics are found to be completely covered by the product apatite particles. There are still differences in the particle sizes, which are smaller in the $S/S = 8.3$ mg/ml sample. In the 10 days soaked samples, the apatite particles formed at $S/S = 8.3$ mg/ml are still small, $<$several μm in diameter, but the sizes of agglomerated spherical particles formed at $S/S = 2.5$ and 1.0 mg/ml show a further increase, most being >10 μm. The packing of the spherical apatite particles appears to become denser with longer soaking time.

4. Discussion

Apatite formation in SBF solution using $CaSiO_3$ ceramics with relatively high porosity prepared by sintering NH_4OH coprecipitated powder ($Ca/Si = 0.91$) was examined by soaking at three solid/solution (S/S) ratios. Formation of apatite was recognized with all the S/S ratios after a short soaking time but there was a difference in the amounts and particle sizes of the apatite formed.

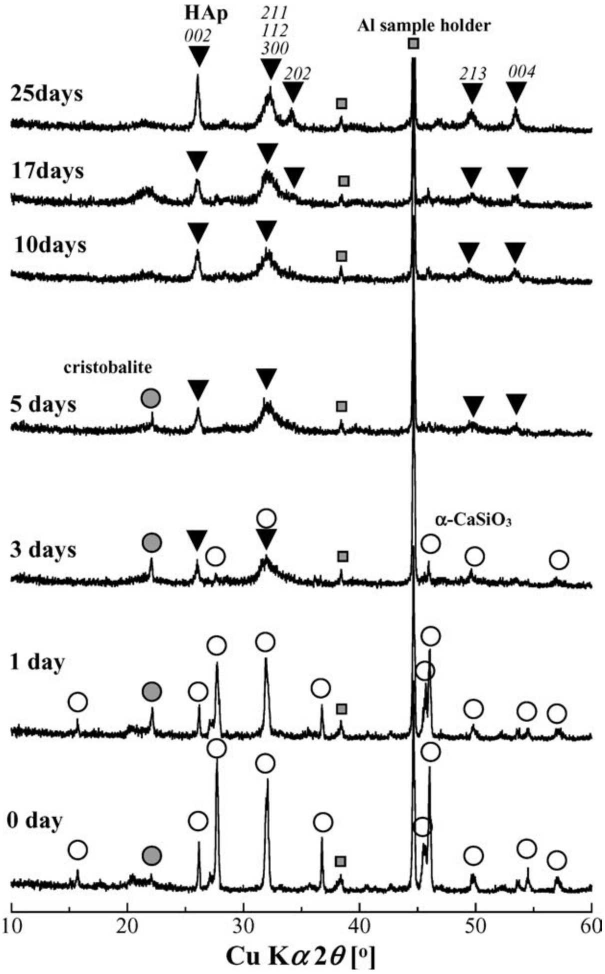
Apatite formation in SBF is believed to depend on the presence of silanol groups (Si—OH) in the amorphous SiO_2 phase, since these react preferentially with Ca^{2+} under alkaline pH conditions, causing enhanced adsorption of phosphate ion to crystalline apatite [3–5]. In the reaction of $CaSiO_3$ ceramics with SBF, formation of an amorphous SiO_2 surface layer was recognized by XPS surface analysis after soaking for 1 day preceding apatite formation [11, 14]. After this stage, the surface chemical composition changed very steeply with an increase of Ca and P, and a decrease of Si. With longer soaking time, the surface eventually contains only Ca and P with a Ca/P ratio determined by XPS to be constant (about 1.3) [11, 14]. This Ca/P ratio is lower than the ideal ratio of apatite ($Ca/P = 1.67$). This may mean that the apatite formed in SBF is calcium deficient. The chemical analysis by SEM-EDX in this study showed that the apatite product is not pure hydroxylapatite but contains considerable amounts of Na and Cl ions. Incorporation of Na ion is the reason for the low Ca/P ratio in this apatite. Incorporation of CO_3^{2-} in the apatite is also a possibility but its presence was not detected in the FTIR spectra. Since we have data for the Ca, P and Si concentrations in the soaked SBF, the amount of amorphous SiO_2 can be evaluated by assuming congruent dissolution of $CaSiO_3$ with formation of amorphous SiO_2 and Ca phosphate (average $Ca/P = 1.3$). The calculated results are shown in Fig. 4 as a function of soaking time. The results clearly indicate the formation of amorphous SiO_2 from the early



(a)

(b)

Figure 2 XRD patterns of $CaSiO_3$ ceramics before and after soaking. The sample/SBF (S/S) ratios are 8.3 mg/ml in (a), 2.5 mg/ml in (b) and 1.0 mg/ml in (c). (Continued.)



(c)

Figure 2 (Continued).

stage of soaking at all the S/S ratios. The presence of the amorphous SiO_2 is also indicated by the halo observed at $2\theta \cong 20^\circ$ in the XRD patterns. The formation of apatite after a short soaking time (1 day) at all the S/S ratios in the present experiments may therefore be attributed to the fast formation of amorphous SiO_2 on the surfaces of the soaked samples.

On the question of the different particle sizes of the formed apatite, the differences in Ca and P concentrations at the different S/S ratios are thought to be important. As shown in Fig. 1(a), the order of the increase in the Ca concentrations of the soaked SBF is 8.3 mg/ml $\gg$2.5 mg/ml $>$1.0 mg/ml. Since the degree of supersaturation of Ca ion in the soaked SBF corresponds to this order, the number of nuclei is also thought to follow the same order. Thus, the particle size of apatite at S/S = 8.3 mg/ml is much smaller than in the other samples because of its higher Ca concentration.

The thickness of the formed apatite layer in the sample soaked at S/S = 8.3 mg/ml is different from those of the other S/S ratios. Fig. 5 shows the microstructures of the surfaces and cross-sections of the samples soaked for 25 days. The difference in the thickness of the apatite layers is clearly seen from the micrographs of their cross-sections. The apatite layer formed at S/S = 8.3 mg/ml is much thinner and more porous than those at S/S = 2.5 and 1.0 mg/ml and consists of

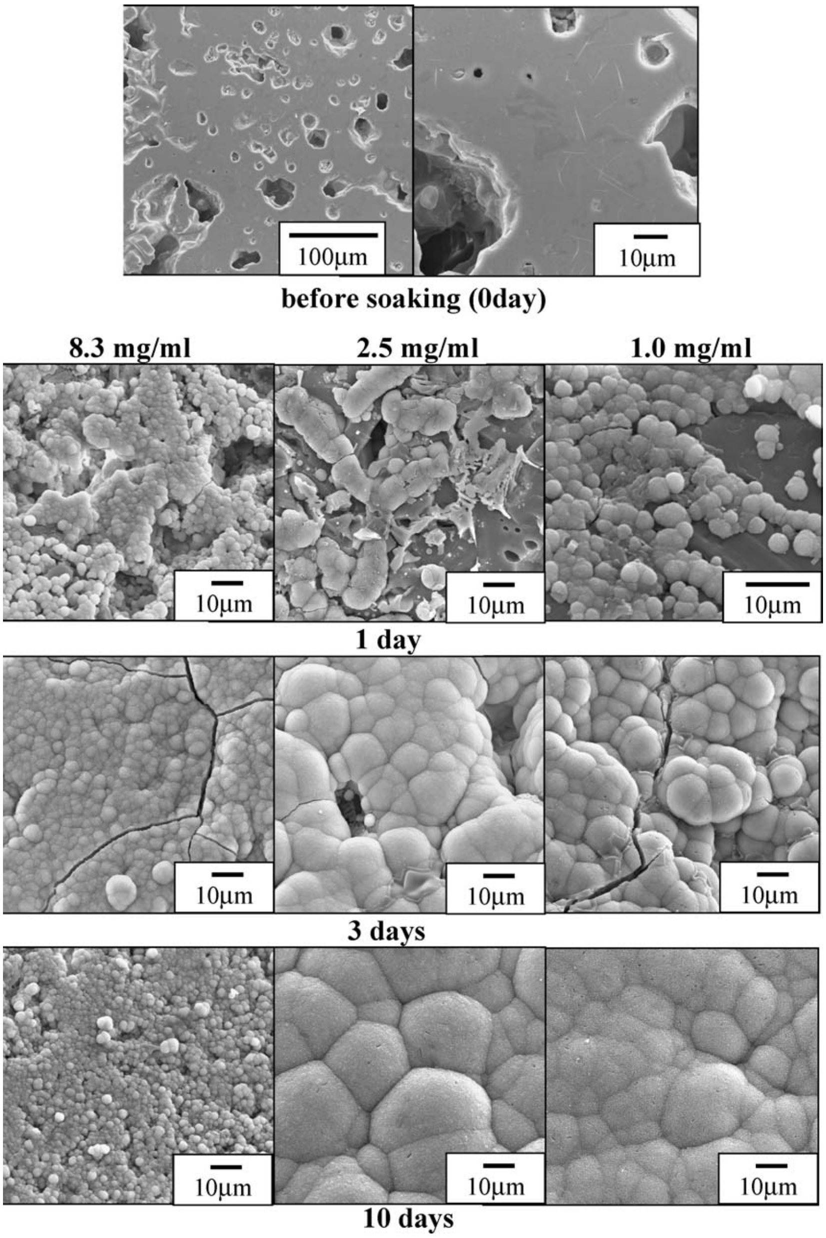


Figure 3 SEM micrographs of the surfaces of $CaSiO_3$ ceramic before and after soaking.

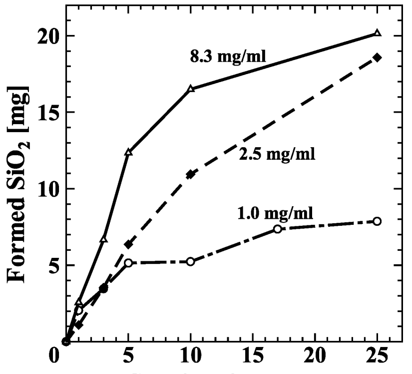


Figure 4 Changes of the estimated amount of amorphous SiO_2 formed on the surfaces of the soaked $CaSiO_3$ ceramics at three S/S ratios as a function of soaking time.

smaller apatite particles. Based on the observations of apatite layer thickness from SEM micrographs, changes of apatite thickness in the samples soaked at three different S/S ratios are plotted in Fig. 6 as a function of soaking time. Also shown is the fastest-growing apatite layer previously reported on a $CaSiO_3$ ceramic [11, 14]. The thickness of apatite layer in the sample at S/S = 8.3 mg/ml increases very slowly compared with a relatively faster growth rate compared with the other two samples. The initial growth rates of apatite layers in the samples soaked at S/S = 2.5 and 1.0 mg/ml are very similar (about 6–7 μm/day). These rates are almost half the fastest rate observed in the $CaSiO_3$ ceramics but are however still faster than the values reported for other bioceramics [11]. Very slow growth rate at a S/S ratio of 8.3 mg/ml may be due to the very quick consumption of P in the soaked SBF (Fig. 1(c)) making it impossible to form further apatite from the soaked SBF by further uptake of P. At this stage, it is possible to increase the layer thickness only by crystallization of the adsorbed amorphous Ca phosphate on the surface of the $CaSiO_3$ ceramic.

The present investigation shows that fast dissolution in SBF of $CaSiO_3$ ceramics with a porous

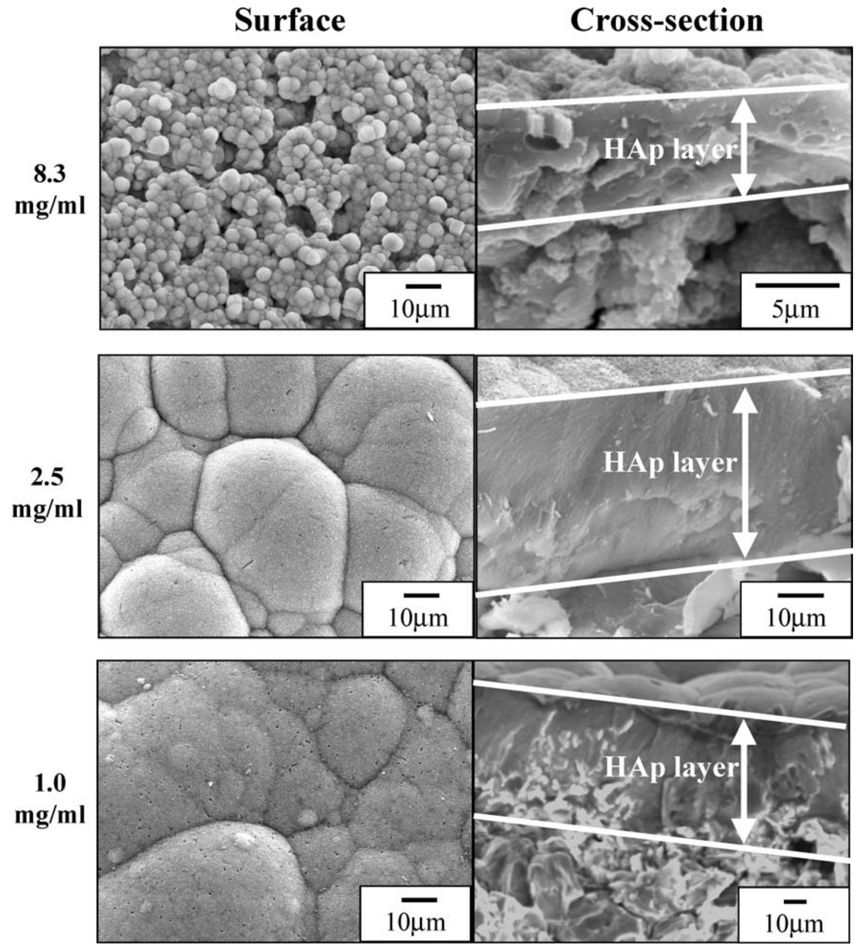


Figure 5 SEM micrographs of the surfaces and cross-sections of 25 days soaked $CaSiO_3$ ceramic.

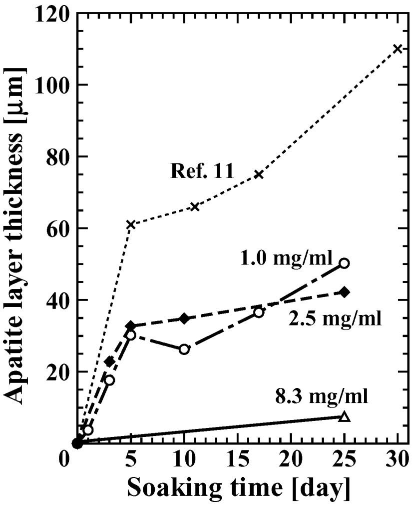


Figure 6 Changes of the apatite layer thickness of $CaSiO_3$ ceramics soaked at three S/S ratios as a function of soaking time, together with data from Ref. [11].

microstructure causes a steep increase in the Ca and Si concentrations and a steep decrease in the P concentration of the SBF at high S/S ratio. These steep changes in the concentrations do not however result in a high growth rate of apatite on the surface of the ceramic. This very rapid uptake of P from the soaked SBF may therefore merely form amorphous Ca phosphate rather than apatite. Moderate dissolution of the $CaSiO_3$ ceramic is preferable to increase the growth rate of the apatite layer, as in the case of $CaSiO_3$ with a higher bulk density (2.44 g/cm^3) [12, 13]. The suppression of apatite formation resulting from too rapid a dissolution rate of the $CaSiO_3$ at a high S/S ratio becomes smaller at lower S/S ratios. Apatite forms relatively fast at lower S/S ratios even using the same $CaSiO_3$ ceramics. This is because sufficient concentration of P remains in the soaked SBF even after 25 days of soaking at these S/S ratios.

5. Conclusion

The effect of the solid/solution (S/S) ratio on apatite formation of $CaSiO_3$ ceramics in simulated body fluid (SBF) was investigated at 36.5 °C using relatively porous $CaSiO_3$ samples with a Ca/Si ratio of 0.91. These were prepared by sintering $CaSiO_3$ powder co-precipitated from ethanol solution of $Ca(NO_3)_2{\cdot}4H_2O$ and $Si(OC_2H_5)_4$ using NH_4OH as precipitant. S/S ratios of 1.0, 2.5 and 8.3 mg/ml were studied, with the following results:

1. Apatite was formed on the $CaSiO_3$ ceramics at all three S/S ratios after soaking for 1 day. This fast apatite formation is attributed to the preceding formation of an amorphous SiO_2 layer on the surface of the $CaSiO_3$ resulting from dissolution and precipitation mechanisms.
2. The amount of apatite obtained at a S/S ratio of 8.3 mg/ml was lower than for the other two S/S ratios. A very steep decrease of P concentration in the soaked

SBF solution resulting from very rapid adsorption by the $CaSiO_3$ rendered the formation of further apatite difficult.

3. Apatite formation behavior at S/S ratios of 1.0 and 2.5 mg/ml is similar in both cases. The apatite layers are formed from agglomerated spherical particles consisting of very fine apatite crystals, which showed a preferred orientation to (00*l*). The growth rate of the apatite layers were about 6–7 μm/day; This is lower than found in denser $CaSiO_3$ ceramics prepared from coprecipitated powder using NaOH as the precipitant [11] but faster than reported for the various other bioceramics.

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