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Comparative study of apatite formation on $CaSiO_3$ ceramics in simulated body fluids with different carbonate concentrations

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Apatite formation on $CaSiO_3$ ceramics was investigated using two different simulated body fluids (SBF) proposed by Kokubo (1990) and Tas (2000) and three sample/SBF (S/S) ratios (1.0, 2.5 and 8.3 mg/ml) at 36.5 °C for 1–25 days. The $CaSiO_3$ ceramic was prepared by firing coprecipitated gel with Ca/Si = 0.91 at 1400 °C. The bulk density was 2.14 g/cm^3 and the relative density about 76%. The two SBF solutions contain different concentrations of HCO_3^- and Cl^- ions, the concentrations of which are closer to human blood plasma in the Tas SBF formulation than in the Kokubo formulation. The pH values in the former solution are also more realistic. The $CaSiO_3$ ceramics show apatite formation in SBF (Kokubo) after soaking for only 1 day at all S/S ratios whereas different phases were formed at each S/S ratio in SBF (Tas). The crystalline phases formed were mainly apatite at S/S = 1.0 mg/ml, carbonate-type apatite at 2.5 mg/ml and calcite at 8.3 mg/ml. At higher S/S ratios the increase in the Ca concentration became higher while the P concentration became lower in the reacted SBF. These changes in SBF concentrations and increasing pH occurred at higher S/S ratios, producing more favorable conditions in the SBF for the formation of carbonate bearing phases, finally leading to the formation of calcite instead of apatite in the higher HCO_3^- ion concentration SBF (Tas). Apatite is, however, formed in the lower HCO_3^- ion concentration SBF (Kokubo) even though the Ca and P concentrations change in a similar manner to SBF (Tas).

1. Introduction

The development by Kokubo [1] of simulated body fluid (SBF) with ion concentrations similar to human blood plasma has enabled the bioactivity of various materials to be evaluated under *in vitro* conditions. This greatly enhanced studies of bioceramics because it is much easier and faster to perform *in vitro* experiments than *in vivo* experiments for the screening of bioactive materials.

Of the various bioceramics reported [2–5], the common components are Ca and Si rather than Ca and P. Therefore, samples based on the $CaSiO_3$ composition were prepared by sintering coprecipitated powder [6]. Soaking experiments of these $CaSiO_3$ ceramics in SBF showed very fast apatite formation (within 1 day) and also a much higher growth rate of the apatite layer than data reported for various other bioceramics [7]. Formation of amorphous SiO_2 was detected by XPS surface analysis [7] preceding apatite formation on the surfaces of $CaSiO_3$ ceramics soaked in SBF, suggesting that the excellent bioactivity of $CaSiO_3$ ceramics is due to the enhancing effect of the amorphous SiO_2, in which the presence of silanol groups accelerates the nucleation and growth of apatite by the mechanism first pointed out by Kokubo [8]. The amount of amorphous SiO_2 was further evaluated in the various samples formed after soaking in SBF and found a relationship between the formation rate of amorphous SiO_2 and the growth rate of the apatite layer. The growth rate of the apatite layer increases with increasing formation rates of amorphous SiO_2 but decreases with further increase of the SiO_2 formation rate [9]. The formation rate of amorphous SiO_2 varies not only with the preparation conditions of the raw powders and ceramics, but also with the SBF reaction conditions. The influence of the sample/SBF (S/S) ratios on apatite formation has already

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been examined using the conventional SBF developed by Kokubo [10]. In the present study, apatite formation on $CaSiO_3$ ceramics was compared using two different SBF solutions, i.e., conventional SBF developed by Kokubo [1] having a lower HCO_3^- ion concentration and higher Cl^- ion concentration than human blood plasma, and a new SBF developed by Tas [11] with HCO_3^- and Cl^- ion concentrations closer to those of blood plasma.

2. Experimental

2.1. Sample preparation and characterization

The $CaSiO_3$ powders were prepared by coprecipitation using NH_4OH as the precipitant. The starting materials used were $Ca(NO_3)_2{\cdot}4H_2O$ and $Si(OC_2H_5)_4$ (TEOS). The former reagent (0.0455 mol) was dissolved in 100 ml of ethanol 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 water added to the former solution sets the total H_2O/TEOS $\cong 6$ for the hydrolysis of TEOS. These 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 whole sample (precipitate and solution) was dried by evaporating at 60 °C and further oven dried at 110 °C overnight. The dried powder was calcined at 600 °C for 2 h to remove residual NH_4-salt, nitrate and ethanol then further fired at 1000 °C for 2 h to obtain a crystalline β-$CaSiO_3$ (wollastonite; the low temperature phase) powder.

The as-fired powder was pressed uniaxially to form green pellets (0.25 g) 10 mm in diameter and fired at 1400 °C for 2 h at a heating rate of 2 °C/min. The bulk density and water absorption were evaluated using Archimedes' method. 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 microscopy (SEM; JSM-5310, Jeol, Tokyo, Japan) with an accelerating voltage of 20 kV.

2.2. Soaking in SBF

Two SBF solutions were prepared based on the recipes reported by Kokubo [1] and Tas [11]. They have been designated as C-SBF and T-SBF, respectively. The ion concentrations and pH values of C-SBF and T-SBF are shown in Table I together with those in human blood plasma. Both SBF solutions have the same cation concentrations but different anion concentrations, especially Cl^- and HCO_3^- ions. The concentrations of these anions in T-SBF are closer to those in human blood plasma than in C-SBF.

The surfaces of the as-prepared α-$CaSiO_3$ ceramics were polished using 4 μm diamond paste, rinsed by distilled water and dried at 110 °C in an oven. The ceramics were then soaked in each SBF solution at 36.5 °C for various times by using three different sample/SBF ratios, i.e., 1.0, 2.5 and 8.3 mg/ml. After the predetermined soaking time, the ceramics were removed from the SBF solution, gently rinsed with distilled water and dried at room temperature.

2.3. Characterization of the formed phases

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

3. Results

3.1. Characterization of $CaSiO_3$ sample

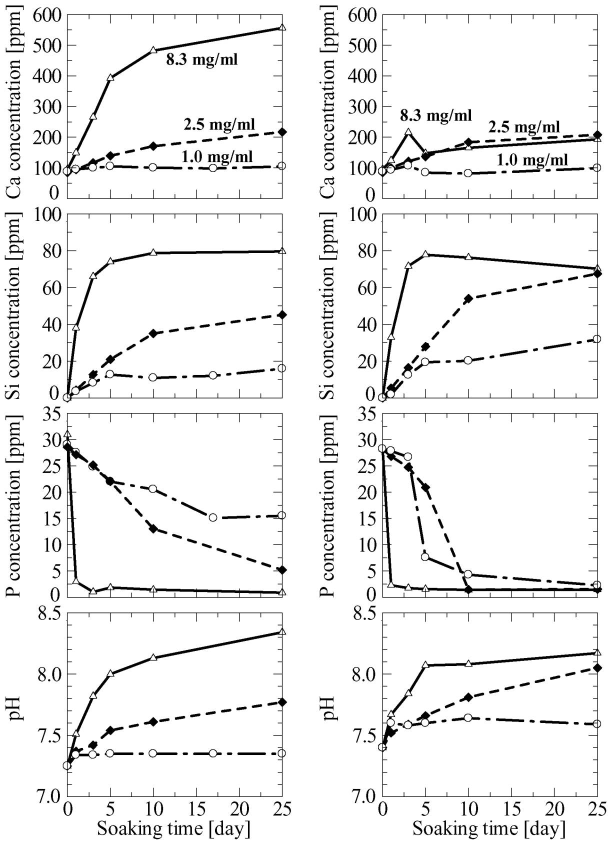
The bulk density of the ceramic fired at 1400 °C was 2.14 g/cm^3 and its water absorption was 7.9%. The open porosity calculated from these data was 17% and relatively large pores >10 μm were present in the fired bodies. The relative density of this sample was calculated to be about 76%. This $CaSiO_3$ ceramic was apparently more porous than the $CaSiO_3$ ceramics prepared from ground powders having relative densities of 80–90% [11–13]. The XRD of this sample showed the formation of α-$CaSiO_3$ (pseudowollastonite; the high temperature phase) with a small amount of cristobalite indicating the partial crystallization of a glassy phase during the cooling process. The crystalline phase was found to transform to α-$CaSiO_3$ from β-$CaSiO_3$ by sintering.

3.2. Concentration change of SBF by soaking

Changes in Ca, Si and P concentrations in the two SBF solutions are shown in Fig. 1 as a function of soaking time. In C-SBF, the Ca^{2+} ion concentrations increased

TABLE I Concentrations of various ions (mM) and pH values of C-SBF, T-SBF and human blood plasma

	Ca^{2+}	Mg^{2+}	K^+	Na^+	HPO_4^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	pH
C-SBF	2.5	1.5	5.0	142.0	1.0	4.2	147.8	0.5	7.25
T-SBF	2.5	1.5	5.0	142.0	1.0	27.0	125.0	0.5	7.40
Blood plasma	2.5	1.5	5.0	142.0	1.0	27.0	103.0	0.5	7.2–7.4



(a) C-SBF **(b) T-SBF**

Figure 1 Changes of Ca, Si and P components and pH of C-SBF (a) and T-SBF (b) reacted at S/S = 1.0, 2.5 and 8.3 mg/ml as a function of soaking time.

steeply up to 5 days soaking and changed less steeply with longer soaking times. The increase in the Ca^{2+} ion concentration was observed to become greater with higher S/S ratios. In T-SBF, the changes in the Ca^{2+} ion concentrations were smaller and more complicated than those in C-SBF, increasing up to 3 days soaking but decreasing from 3 to 5 days soaking at S/S = 1.0 and 8.3 mg/ml. By contrast, the Ca^{2+} ion concentration change at S/S = 2.5 mg/ml showed a monotonic increase up to 10 days soaking and became almost constant at longer soaking times. This trend in the changes of the Ca^{2+} ion concentration at S/S = 2.5 mg/ml was similar to that observed in C-SBF though the changes were very different at S/S = 1.0 and 8.3 mg/ml, especially at 8.3 mg/ml.

The Si concentrations in the two SBF solutions during all the soaking experiments, showed a similarly steep increase at the initial stage of soaking but the increase became more gradual with longer soaking times. The Si concentrations after soaking for 25 days were low in C-SBF than in T-SBF, with increasing S/S ratio. The differences in the Si concentrations of the two SBF solutions were much smaller than those of the Ca^{2+} ion concentrations. Since the Si concentrations after 25 days soaking show a good linear relationship with the pH values, the Si component is thought to be almost saturated in the reacted SBF solutions. Thus, partial precipitation of the Si component is considered to occur from the reacted SBF solutions by dissolution of $CaSiO_3$ and precipitation of amorphous SiO_2 due to its lower solubility than the Ca component.

The P concentrations in the reacted SBF solutions showed a very steep decrease within 1 day of soaking at S/S = 8.3 mg/ml. By contrast, the decrease of the P concentrations at S/S = 1.0 and 2.5 mg/ml differed slighttly between the two SBF solutions. In C-SBF, the P concentration decreased gradually and was still present after soaking for 25 days, while in T-SBF the P concentration decreased steeply up to 10 days soaking and little P was present after soaking for 25 days. Thus, differences in the behavior of the P concentration were apparent between the two SBF solutions.

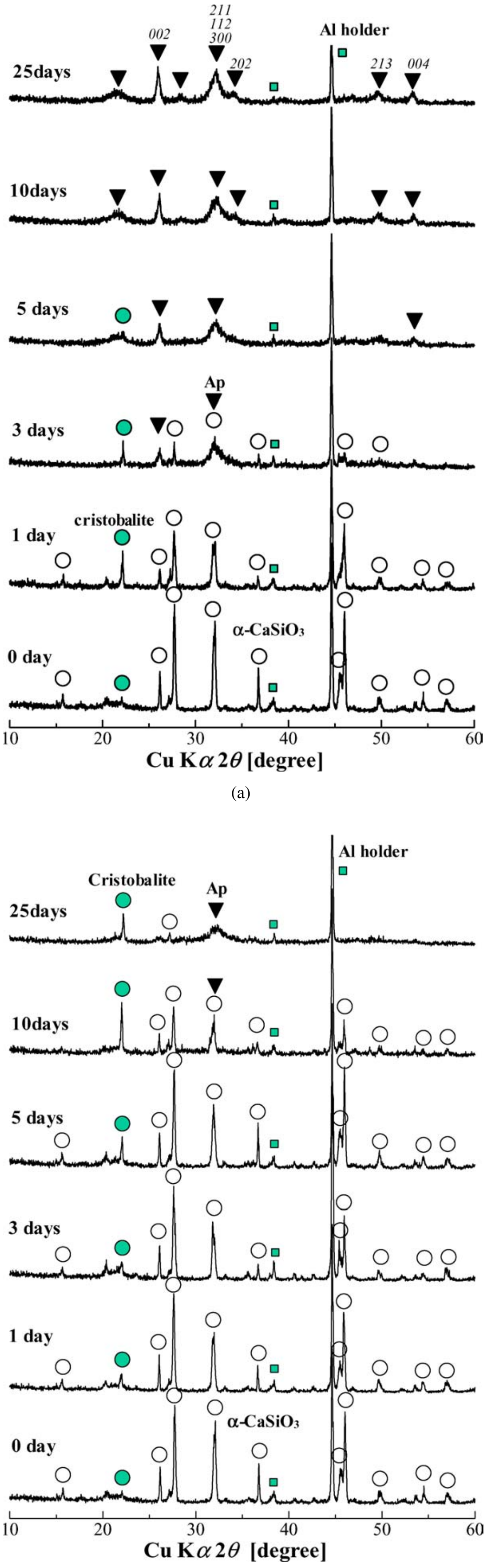
In all the soaking experiments, the pH values increased and the increments became greater with higher S/S ratios in the two SBF solutions. The changes were more pronounced in C-SBF than in T-SBF and were highest at S/S = 8.3 mg/ml (pH = 8.34 after 25 days of soaking). The changes in the pH were greater at the initial stage, but became less severe at longer soaking times in both SBF solutions, as was also found with the changes in the Ca and Si components.

3.3. Formed phases during soaking experiments

The surfaces of the reacted pellet samples were examined by XRD. Fig. 2 shows the XRD patterns of the samples soaked in C-SBF (a) and T-SBF (b) at S/S = 2.5 mg/ml for various times. In C-SBF (Fig. 2(a)), the α-$CaSiO_3$ peaks became weaker after 1 day of soaking and disappeared completely after 5 days soaking. This reflects the formation of the precipitated phase on the surfaces of the reacted samples, with new peaks beginning to be observed at $2\theta \cong 26$ and 32° after 3 days of soaking. After longer soaking times these new peaks increased in intensity and more new peaks were detected. The product phase was identified as apatite on the basis of the JCPDS data (hydroxylapatite: card No. 9-432).

In T-SBF (Fig. 2(b)), the changes in the XRD patterns are slower than in C-SBF. The XRD peaks assigned to α-$CaSiO_3$ showed a slight change up to 5 days but clearly decreased with longer soaking time. The XRD peak intensity of cristobalite became stronger with extended soaking time, especially from 5 days soaking. These changes indicate dissolution of α-$CaSiO_3$ and relative condensation of cristobalite in the surfaces of the reacted samples. Clear formation of apatite was detected only on the sample soaked for 25 days together with cristobalite and trace of α-$CaSiO_3$. In this way, the starting of apatite formation detected by XRD is apparently slower in T-SBF than in C-SBF. The amount of product apatite is seemed to be lower in T-SBF than in C-SBF.

Fig. 3 shows field maps of the product phases by soaking in C-SBF (a) and T-SBF (b) as functions of soaking time and S/S ratio. In C-SBF, all the product phase was apatite as shown in Fig. 3(a). By contrast, the product phases were different according to soaking time and S/S ratio in T-SBF. No crystalline product was observed by 3–5 days soaking. With higher S/S ratio and longer soaking time, the product phase was calcite and not apatite. The formation region of apatite is, thus, more limited in T-SBF than in C-SBF.



(a)

(b)

Figure 2 XRD patterns of the $CaSiO_3$ pellet samples soaked at S/S = 2.5 mg/ml for various days in (a) C-SBF and (b) T-SBF.

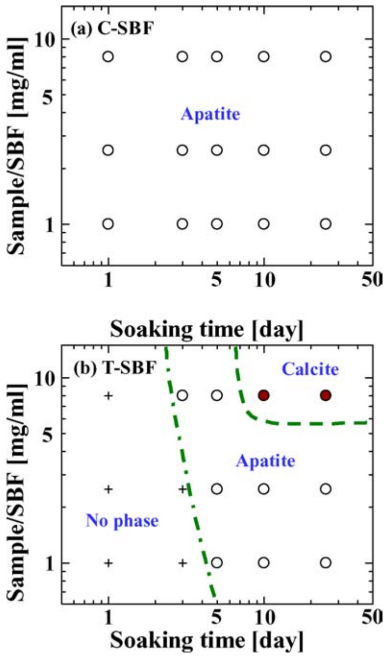


Figure 3 Field maps of the products in C-SBF (a) and T-SBF (b) as functions of soaking time and S/S ratio.

4. Discussion

As mentioned before, the formation conditions of apatite are found to differ considerably in C-SBF and T-SBF, which have different HCO_3^- ion concentrations as listed in Table I. Since the difference in the apatite formation has to be attributed to the difference in their HCO_3^- ion concentrations, the differences in the formation rates of apatite and product phases are discussed.

As already reported by Kokubo [1, 8] and also by our research group [7, 9], fast apatite formation in SBF was found in the presence of an amorphous SiO_2 layer on the surface of the reacted samples by dissolution of $CaSiO_3$. The mechanism for this fast apatite formation is thought to occur by selective adsorption of Ca^{2+} to Si—OH on the surface of amorphous SiO_2 and subsequent adsorption of phosphate ions to form apatite. During apatite formation, it has been found that an appropriate formation rate of amorphous SiO_2 derived from an appropriate dissolution rate of α-$CaSiO_3$ is important for fast formation and growth of apatite [9]. The formation rates of the amorphous SiO_2 before calcite formation (up to 3 days) are evaluated to be 1–2 mg/day for six samples. These values are within the range appropriate for fast apatite formation in C-SBF [9]. However, apatite formation is actually observed within 1 day soaking at all three S/S ratios in C-SBF. However, apatite does not form up to 3–5 days soaking in T-SBF. This difference in the apatite formation rate is attributed to the different HCO_3^- ion concentrations in the two SBF solutions. A higher HCO_3^- ion concentration is found to suppress apatite formation in SBF. Since adsorption of phosphate ions subsequent to Ca^{2+} ion adsorption is considered to be necessary for fast formation of apatite as mentioned, a higher concentration of HCO_3^- ion in SBF is suggested to suppress phosphate adsorption to the surface of $CaSiO_3$ ceramics because of the competitive adsorption of HCO_3^- to Ca^{2+} ions.

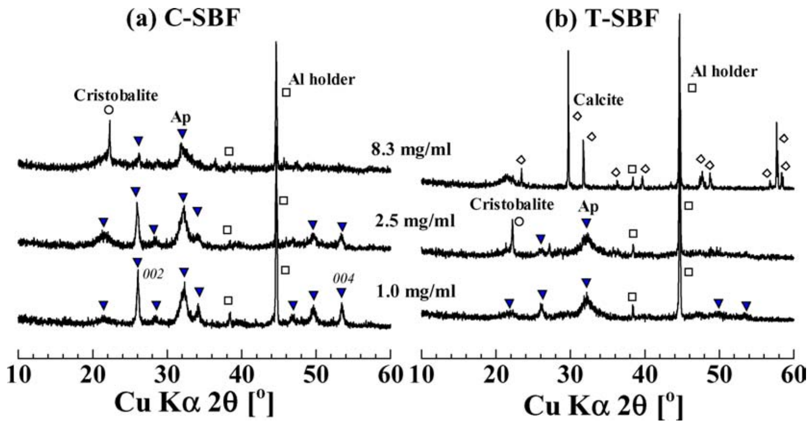


Figure 4 XRD patterns of the CaSiO$_3$ pellet samples soaked for 25 days at S/S = 1.0, 2.5 and 8.3 mg/ml in C-SBF (a) and T-SBF (b).

The products obtained after soaking for 25 days are characterized by XRD, SEM and FTIR. Fig. 4 shows the XRD patterns of the pellet samples. In C-SBF, the amounts of resultant apatite are higher on the samples soaked at S/S = 1.0 and 2.5 mg/ml than that at 8.3 mg/ml. The XRD patterns of apatite formed at S/S = 1.0 and 2.5 mg/ml show more preferred orientation to the (00*l*) plane by the strengthening of the intensities of the 002 and 004 reflections. In T-SBF, formation of apatite is observed on the samples soaked at S/S = 1.0 and 2.5 mg/ml but not on that at 8.3 mg/ml. The amounts of apatite formed in the two samples are found to be lower than those in C-SBF by comparison of the XRD patterns.

The microstructures of the samples are shown in Fig. 5. In C-SBF, the microstructures of apatite formed at S/S = 8.3 mg/ml are different in particle size compared with the other two samples but those at S/S = 1.0 and 2.5 mg/ml are similar to each other. The difference in the particle size of apatite, which is formed by agglomeration of very fine platy apatite crystals [7, 8] is attributed to the differences in the degree of supersaturation of the reacted SBF solutions (Fig. 1). In T-SBF, the samples reacted at each S/S ratio show very different microstructures. The apatite particles on the sample reacted at S/S = 1.0 mg/ml are spherical. The particle size on the top surface is several μm in size but is >10 μm in the lower surface. These particles are similar to those formed in C-SBF. By contrast, the apatite particles on the sample reacted at S/S = 2.5 mg/ml are much smaller (<1 μm) than the apatite formed on the other samples and different in particle shape, i.e. confetti-like. The product in the sample reacted at S/S = 8.3 mg/ml was calcite and the particles developed into prismatic shapes and consisted of twinning crystals elongated to >100 μm in size.

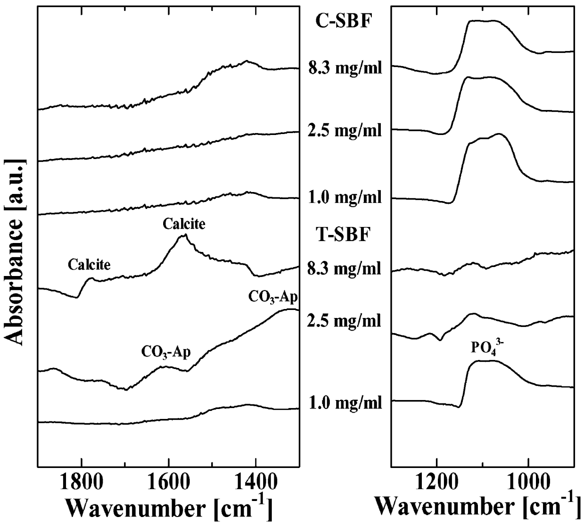


Figure 5 SEM photographs of the products soaked for 25 days at S/S = 1.0, 2.5 and 8.3 mg/ml in C-SBF and T-SBF.

Since the apatite formed in the sample reacted at S/S = 2.5 mg/ml in T-SBF is different in particle size and shape compared to the other product apatites, further characterization of the samples was performed using FTIR. Their DRIFT spectra are shown in Fig. 6. The spectra of three samples soaked in C-SBF show a broad doublet absorption band at 1050–1150 cm^{-1} assigned as overlapped absorption bands created by PO$_4$ tetrahedra of apatite [12]. The spectrum of the sample soaked at S/S = 1.0 mg/ml in T-SBF shows a similar absorption band. By contrast, the spectrum at S/S = 2.5 mg/ml in T-SBF shows only a weak absorption band at about 1120 cm^{-1} and not in the vicinity of 1050 cm^{-1} as is the case of the above mentioned four spectra. This sample shows broad absorption bands at about 1370 and 1620 cm^{-1}. These bands as well as those at 1000–1150 cm^{-1} for this sample are resemblant of those reported in CO$_3$-type apatite [13]. From the results of DRIFT spectra and SEM observation, the apatite formed in the sample soaked at S/S = 2.5 mg/ml in T-SBF is considered to be CO$_3$-type apatite and different to the apatite formed on the other samples.

The chemical composition of the apatite formed on the four samples soaked at S/S = 1.0 and 2.5 mg/ml were analyzed by SEM-EDX. All the apatites showed the presence of small amounts (several atm%) of Na and Cl components besides Ca and P components. They are considered to substitute Ca^{2+} and OH$^-$ ions in the apatite, respectively. The Ca/P ratios of the apatite are about 1.3 and are apparently lower than an ideal value of 1.67. Owing to the incorporation of these ions, the apatite formed in SBF is Ca-deficient and contains Cl$^-$ and CO$_3^{2-}$ ions. This may be one of the reasons for low crystallinity and very fine particle size of apatite formed in SBF.

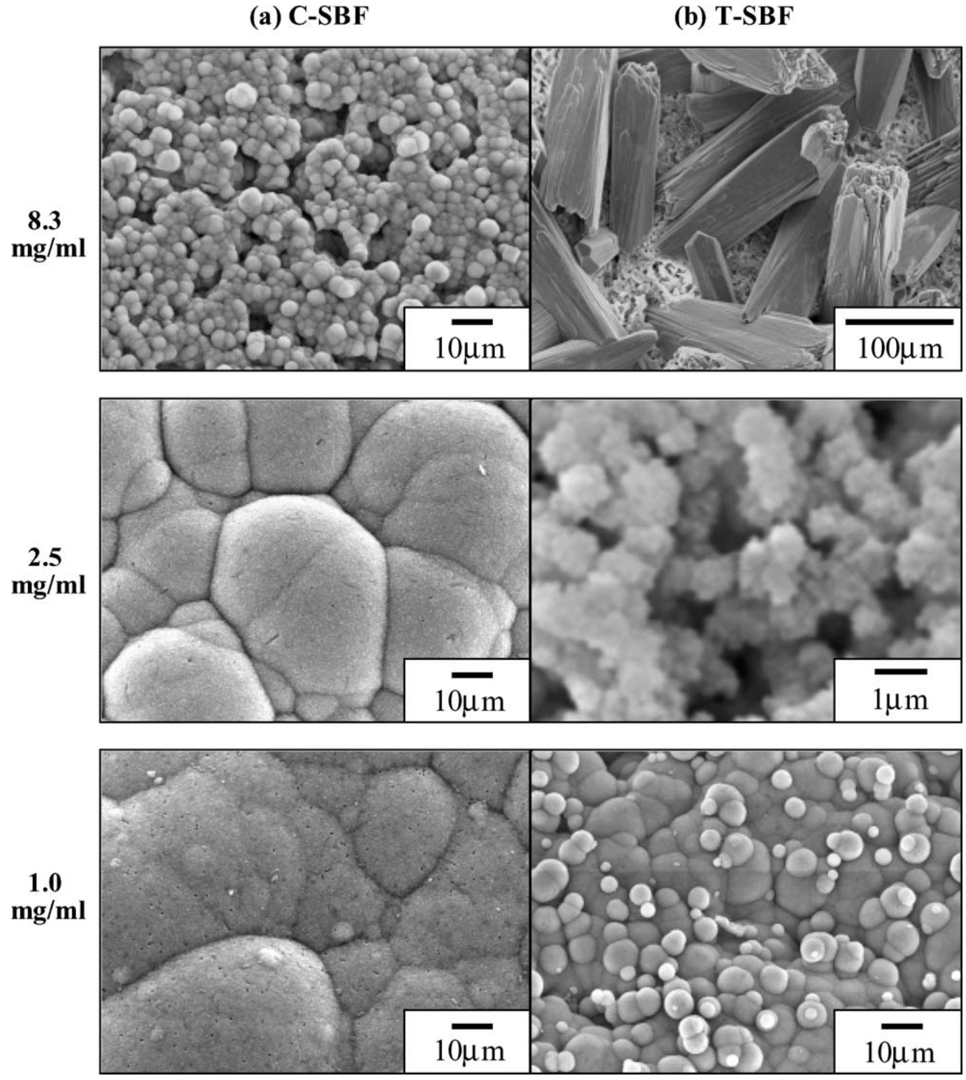


Figure 6 DRIFT spectra of the $CaSiO_3$ pellet samples soaked for 25 days at S/S = 1.0, 2.5 and 8.3 mg/ml in (a) C-SBF and (b) T-SBF.

5. Conclusion

Apatite formation on $CaSiO_3$ ceramics was investigated using two SBF solutions with different HCO_3^- and Cl^- ion concentrations at three sample/SBF (S/S) ratios to elucidate the effect of carbonate concentration. The following results were obtained:

(1) By reacting with C-SBF which has a lower HCO_3^- ion concentration than blood plasma, the $CaSiO_3$ ceramic shows apatite formation within 1 day at all three S/S ratios. The amount of apatite formed is lower in the sample at S/S = 8.3 mg/ml than in those at S/S = 1.0 and 2.5 mg/ml. This is attributed to the rapid uptake of P component in SBF by the $CaSiO_3$ ceramic due to the high S/S ratio.

(2) By reacting with T-SBF which has the same HCO_3^- ion concentration as blood plasma, the $CaSiO_3$ ceramic shows the formation of different products at three S/S ratios. With higher S/S ratio, the products change from apatite to CO_3-type apatite and further to calcite.

(3) Apatite formation is suppressed to limited conditions in T-SBF. This is suggested to be due to the competitive reaction of carbonate ions with phosphate ions in reaction with Ca^{2+} to form apatite.

(4) The formed apatite was found to be a Ca-deficient-type and contained Na^+, Cl^- and CO_3^{2-} ions. This is considered to be the reason for low crystallinity and fine particle size.

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