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Preparation and in vitro bioactivity of $CaSiO_3$ powders

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Abstract

Fine $CaSiO_3$ powders were prepared by chemical precipitation with ultrasonic stirring using $Ca(NO_3)_2{\cdot}4H_2O$, $Si(OC_2H_5)_4$ as source material and 0.25 mol/l NaOH as a precipitant. The characteristics of the resultant powders were negatively affected by the presence of the Na component. For this reason, the precipitates were washed with distilled water in order to remove the Na component. The washed precipitates were calcined at 500 and 900 °C. The ground and sieved powders were sintered at 1000 and 1200 °C to bulk density values of 1.49 and 2.035 g/cm^3, respectively. The minimum porosity of 5.00% was obtained on pellets pressed at 240 MPa and sintered at 1200 °C. The sintered pellets at 1200 °C were soaked in simulated body fluid (SBF) solution and hydroxyapatite (HAp) layer formed as ball-like particles on the surface of the $CaSiO_3$ samples after soaking for a short time.

Keywords: A. Sintering; $CaSiO_3$ powders; Chemical precipitation; Hydroxyapatite; Simulated body fluid (SBF); Biocompatibility

1. Introduction

During the last four decades, various materials known as "bioactive materials" such as glasses [1,2], sintered hydroxyapatite [3–5], glass ceramics [1,6], composite materials [1,7–9], etc., have been synthesized and developed for medical applications. Recent studies show that some calcium silicate ceramics such as wollastonite ($CaSiO_3$) [10–13], pseudowollastonite (α-$CaSiO_3$) [14] and dicalciumsilicate (Ca_2SiO_4) powders [15] also have excellent bioactivity and so, $CaSiO_3$ ceramics will be a candidate material for artificial bone and dental root.

A common feature of all bioactive materials is that they can bind to living bone through a hydroxyapatite layer when in contact with simulated body fluids. Hydroxyapatite [(HAp: $Ca_{10}(PO_4)_6(OH)_2$] has received significant attention over the past two decades as an implant material due to its excellent biocompatibility. HAp is a bioactive material chiefly because of its calcium-to-phosphorus ratio being similar to that of natural bone and teeth. A significant characteristic of bioactive materials is also their ability to bond with living bone through the formation of a hydroxyapatite interface layer.

Some researchers reported that the same HAp layer is formed on the surfaces of bioactive materials after soaking in biomimetic solutions, which has ion concentrations similar to human blood plasma [10–12,15–21]. The dissolution of calcium ions from the bioactive materials in the biomimetic solutions and the formation of a silica-rich interlayer on the material surfaces occurred simultaneously [21].

It is reported in literature that compounds including CaO-SiO_2, bio-glasses and ceramics show good biocompatibility [21,22]. Kokubo et al. observed the rapid hydroxyapatite (HAp) formation on glass in simulated body fluid [23]. For this reason, examination of HAp formation on $CaSiO_3$ ceramics in SBF solution and its biocompatibility seemed to be necessary. Due to the low sinterability of $MSiO_3$ powders, homogenous powders need to be prepared in appropriate sizes. The precipitation by NH_4OH solution is reported to be the most suitable method among all the others [24,25]. Hayashi et al. produced $CaSiO_3$ from calcium nitrate and tetraethyl orthosilicate by using NH_4OH precipitant [26]. Siniphannon et al. prepared a fine $CaSiO_3$ powder by the coprecipitation method using 0.33 mol/l NaOH solution as a precipitant and suggested that this gave very good biocompatibility of the $CaSiO_3$ ceramics [20].

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In this study, fine $CaSiO_3$ powders were prepared by chemical precipitation with ultrasonic stirring using Ca $(NO_3)_2 \cdot 4H_2O$, $Si(OC_2H_5)_4$ as source material and 0.25 mol/l NaOH as a precipitant. The sintering behaviour of $CaSiO_3$ powders was investigated and biocompatibility of precipitated $CaSiO_3$ powders was evaluated from behaviour of HAp formation in SBF solution.

2. Experimental

2.1. Preparation of the $CaSiO_3$ materials

The fine $CaSiO_3$ powders were synthesized by the chemical precipitation method, using $Ca(NO_3)_2 \cdot 4H_2O$ and $Si(OC_2H_5)_4$ (TEOS) as raw materials. These reagents were separately dissolved in ethanol solution and the concentration was adjusted to 0.2 mol/l. $Si(OC_2H_5)_4$ solution was dropped into $Ca(NO_3)_2$ solution under the ultrasonic stirring. Precipitates were obtained by the addition of 0.15, 0.25, 0.35, 0.40, 0.50 and 0.70 mol/l NaOH solutions into the main solution. The resultant powders were designated as Na1, Na2, Na3, Na4, Na5 and Na6, respectively. After the precipitation was completed, the obtained precipitates were washed once, twice and three times (Na2W1, Na2W2, Na3W2, Na3W3, Na5W3) with distilled water, filtered, dried at 100 °C for 12 h and finally calcined at 500 °C (amorphous phase) and 900 °C (crystalline phase) for 2 h. The calcined powders were ground and sieved. Then, the specific surface areas of the powders were measured by the BET method (Autosorb-1, Quantachorome Co., USA) and the densities of the powders were calculated by Archimedes' method. Each powder was pressed into disks of 10 mm in diameter and 2 mm thickness at 120 and 240 MPa sintered at 1000 and 1200 °C for 2 h with a heating rate of 5 °C/min under air atmosphere in order to form bulk materials for in vitro test. The phase compositions of the powders were identified using X-ray diffractometer (XRD; Rigaku Rint Dmax 1000).

2.2. Soaking in SBF

In order to evaluate the bioactivity of the $CaSiO_3$ materials, the compact disks were put into the SBF. The ion concentrations of the SBF solution were adjusted to be similar to those in human blood plasma (Table 1). The simulated body fluid (SBF) was prepared by dissolving reagent-grade $CaCl_2$, NaCl, KCl, $MgCl_2 \cdot 6H_2O$, $K_2HPO_4 \cdot 3H_2O$, $NaHCO_3$ and Na_2SO_4 in distilled water [20,27]. The SBF solution was buffered at pH 7.25 with tris-(hydroxymethyl)-aminomethane $[(CH_2OH)_3CNH_2)]$ and hydrochloric acid (HCl). The sintered $CaSiO_3$ disks were soaked in the SBF solution using the sterile polyethylene containers for 1,3, 5, 7, 10 and 30 days at 36.5 °C. After being soaked, the disks were rinsed with distilled water and dried at 36.5 °C.

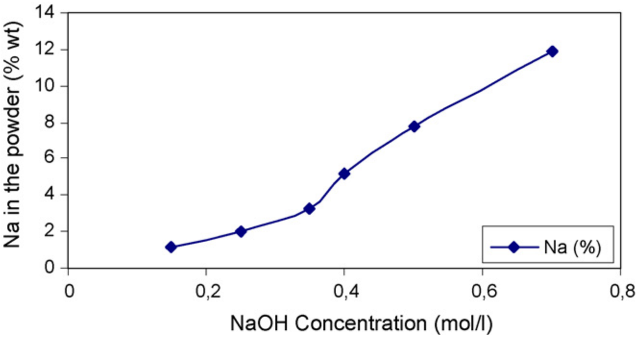

Fig. 1. Change of Na content with increasing concentration of NaOH in non-washed $CaSiO_3$ powder (sample Na2).

The surfaces of the immersed $CaSiO_3$ disks were investigated by XRD and Scanning Electron Microscopy (SEM; Jeol JSM-5410LV) with various soaking times.

3. Results and discussion

3.1. Characterization of $CaSiO_3$ powders

Fig. 1 shows the Na content of the resultant powders obtained directly from the precipitated powder without washing (sample Na2) at different NaOH concentrations. Na content in these non-washed $CaSiO_3$ powders was increased with the increasing concentration of NaOH precipitant (Fig. 1). Since the characteristics of the resultant powders were negatively affected by the high Na content in the powders (Fig. 2), the precipitated powders were washed with distilled water. The changes of Na quantities in $CaSiO_3$ powders with the number of the washing treatment are shown in Table 2. The Na2W2 powder had the lowest Na content (Table 2).

The different ultrasonic stirring time did not change the Na quantities in the powder (Fig. 3) but decreased the particle size of powders. The average particle size of the $CaSiO_3$ powders was obtained 1.15 μm. The XRD patterns of the non-washed (Na2 and Na3) and twice washed (Na2W2) powders calcined at 900 °C were detected β-$CaSiO_3$ and $Na_4Ca_8Si_5O_{20}$ phases in the Na2, Na3 (non-washed) and Na2W2 powders (twice washed) calcined at 900 °C for 2 h (Fig. 2a–c).

It can be seen that Na residue could crystallize together with Ca and Si components to form sodium calcium silicate compounds. Thus, β-$CaSiO_3$ phase was contaminated with $Na_4Ca_8Si_5O_{20}$ compound, especially in the powders synthesized using a high concentration of NaOH solution (Fig. 2c).

Table 1
Ion concentrations of SBF solution and human blood plasma

	Ion Concentration (mmol/l)							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Blood plasma[28]	142.0	5.0	1.5	2.5	103	4.2	1.0	0.5
SBF[27]	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

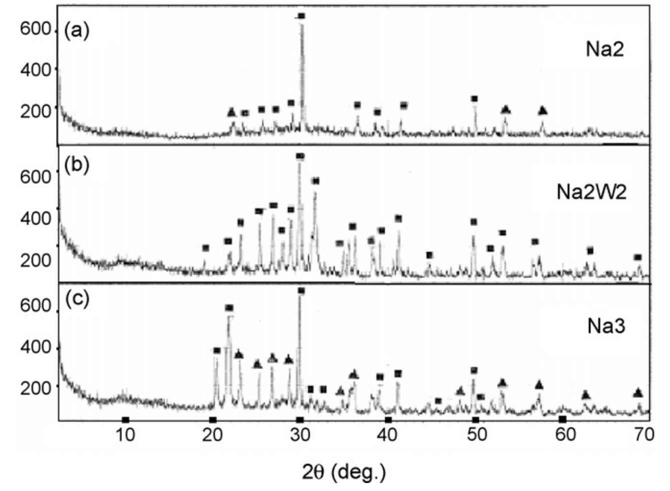

Fig. 2. XRD patterns of Na2, Na3 (non-washed) and Na2W2 powders (two times washed) calcined at 900 °C for 2 h, ((■) β-$CaSiO_3$, (▲) $Na_4Ca_8Si_5O_{20}$).

However, it was difficult to remove the Na component in the Na5 powder even after repeating the washing treatment three times because of the high initial amount of Na component (Table 2).

Na residue negatively affected the characteristics of the resultant powders. This effect was decreased by the washing treatments. It was approximately removed the 0.90 mass% Na by one washing treatment in the Na2 and Na3 powders. In this study, the lowest Na quantity was obtained by using 0.25 mol/l of NaOH as a precipitant under the ultrasonic stirring.

3.2. Characterization of sintered $CaSiO_3$ powders

Na2W2 powders were chosen for sintering treatments since they had the lowest Na content (Table 2). The values of bulk density and porosity percent of sintered Na2W2 powders at 1000 and 1200 °C were compared with the results of non-washed Na2 and Na3 powders. Table 3 shows the changes of the bulk density and porosity percent of the calcined disks at 1000 and 1200 °C, respectively. The average particle size of these powders was obtained 1.15 μm. The obtained average particle size was a lower than that reported for $CaSiO_3$ ceramics sintered from powder prepared by chemical precipitation using NaOH as a precipitant [20]. This result was attributed to ultrasonic stirring during the precipitation process. The bulk densities of the Na2 and Na2W2 which were sintered at the same conditions (pressing loads: 120 and 240 MPa, sintering temperature: 1000 °C) showed close values. Na2 powders melted at 1200 °C during the sintering process however Na2W2 powders reached the highest bulk density (2.04 g/cm^3). When the theoretical density of α-$CaSiO_3$ was assumed to be 2.90 g/cm^3 [20], 2.04 g/cm^3 value for Na2W2 sample corresponds to around 70% of theoretical density. The highest bulk density obtained was lower than that reported for $CaSiO_3$ ceramics sintered from powder prepared by coprecipitation using NaOH [20] and NH_4OH [26] as a precipitant. These differences were attributed to the difference of impurity in the samples used for sintering. Because the presence of Na components in the powder, the sinterability of $CaSiO_3$ powder was decreased.

The effect of the sintering temperature and forming pressure on the porosity of the samples is shown in Table 3. The porosity of the twice washed samples changed from 31.40 to 26.30% at

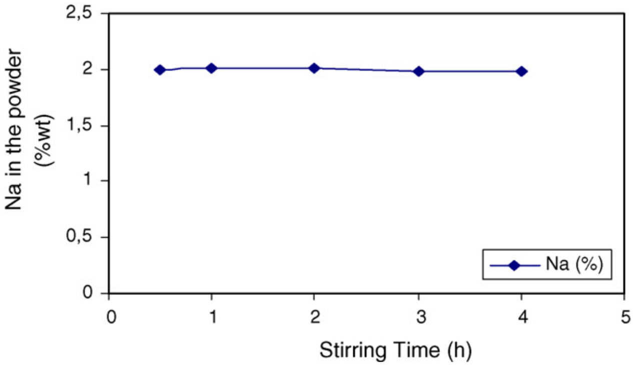

Fig. 3. Change of Na content in the $CaSiO_3$ powder with various stirring time.

Table 2
Change of Na content in the $CaSiO_3$ powder with the number of washing treatments

Sample no.	Number of washing treatment	NaOH concentration (mol/l)	Na content in powder (%wt.)
Na2W1	Once	0.25	0.55
Na2W2[a]	Twice	0.25	0.12
Na3W2	Twice	0.35	1.47
Na3W3	Three times	0.35	0.82
Na5W3	Three times	0.50	2.85

[a] Contained the lowest Na content after twice washing treatment.

Table 3
Properties of $CaSiO_3$ ceramic powder subjected to different pressing loads and sintering temperatures

Sample no.	Forming pressure (MPa)	Density (g/cm^3) (sintering at 1000 °C)	Density (g/cm^3) (sintering at 1200 °C)	Porosity (%) (sintering at 1000 °C)	Porosity (%) (sintering at 1200 °C)
Na2 (non-washed)	120	1.29	Melting	58.30	–
Na2 (non-washed)	240	1.58	Melting	8.10	–
Na2W2 (twice washed)	120	1.27	0.90	31.40	46.60
Na2W2 (twice washed)	240	1.49	2.04	26.30	5.00

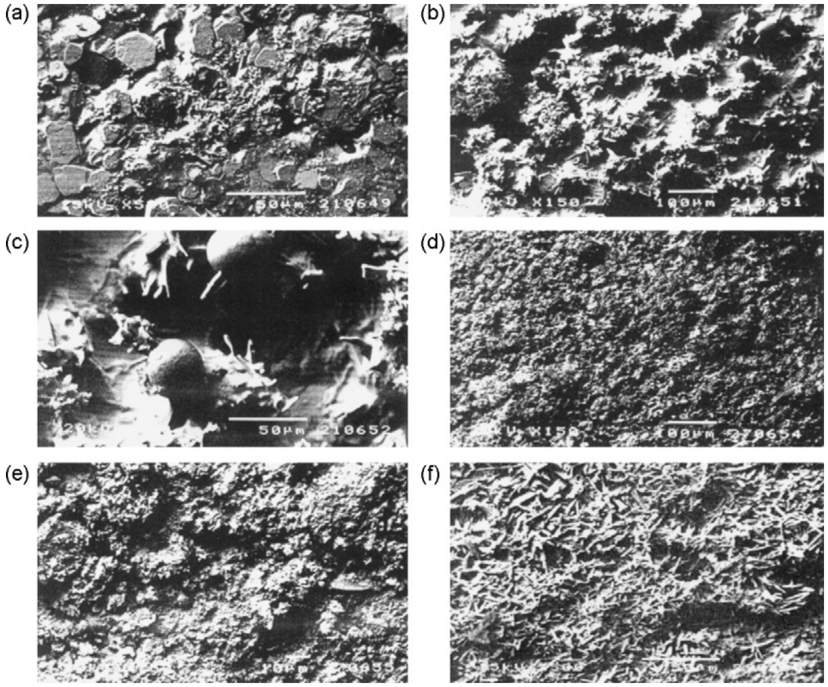

Fig. 4. SEM micrographs of the surfaces of β-$CaSiO_3$ after soaking in the SBF solution: (a) 1, (b) 3, (c) 5, (d) 7, (e) 10 and (f) 30 days (thermally etched at 600 °C).

1000 °C and from 46.60 to 5.00% at 1200 °C while the forming pressure increased from 120 to 240 MPa, respectively.

3.3. Characteristics of the formed HAp

The bioactivity behaviour of the $CaSiO_3$ pellets sintered at 1200 °C from the Na2W2 powder was evaluated in the SBF solution. Fig. 4 shows the surface micrographs of the $CaSiO_3$ ceramics after immersion of 1, 3, 5, 7, 10 and 30 days, respectively. The surface microstructure of the $CaSiO_3$ ceramic soaked for 1 day was dissolved by the SBF solution (Fig. 4a). The HAp formed on the surfaces of the $CaSiO_3$ disks soaked in SBF during the 3 and 5 days was observed to be ball-like (Fig. 4b and c).The formation of HA phase gradually increased with increasing of soaking time after soaking 5 days (Fig. 4c–f) and HAp peaks became the main component in the XRD patterns with increasing soaking time (Fig. 5a and b). The appearance of ball-like HAp particles exhibits an initial deposition of an amorphous phase. When the $CaSiO_3$ ceramic gets in contact with SBF, a partial dissolution occurs in the material network and this partial dissolution produces an ionic exchange between Ca^{2+}, PO_4^{3-} and OH^- ions and silanol groups on the surface of the ceramic material. These ion exchanges enhance crystallization of nuclei for the Ca–P and the nucleation and growth of the HAp layer proceeds by reaction of ions from the SBF. After prolonged soaking, the amorphous layer grows and crystallizes to HAp with incorporation of PO_4^{3-} and OH^- ions.

SEM micrographs of the $CaSiO_3$ ceramic surfaces immersed in the SBF for various times showed a dissolved structure of $CaSiO_3$ in the SBF solution. This derives from the crystalline $CaSiO_3$ grains dissolving easier than the glassy phase at the grain boundaries [20]. The HAp was observed as tiny ball-like shapes on the surface for samples immersed for 2 days. The ball-like HAp particles were completely covered on the $CaSiO_3$ surface after 3 days soaking.

The formation of HAp was rapidly increased with increasing soaking time in SBF solution (Fig. 4a–f and Fig. 5). The sample soaked in SBF for 30 days was completely covered by needle-like deposits after thermally etched at 600 °C (Fig. 4f). Kokubo et al. observed needle-like deposits at the surface of glasses under SEM after soaking in SBF [29].

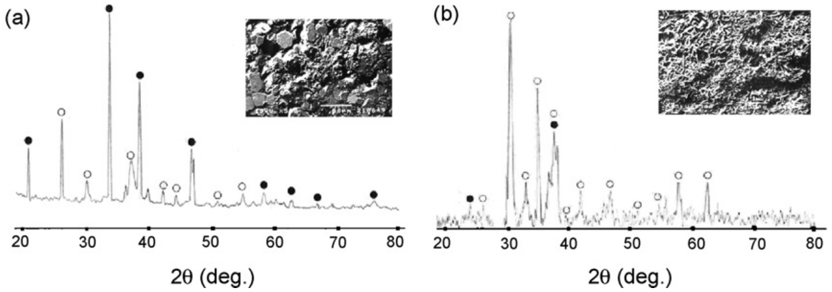

Fig. 5. XRD patterns of $CaSiO_3$ ceramics soaked in SBF solution for (a) 1 day and (b) 30 days, ((●) β-$CaSiO_3$, (○) Apatite).

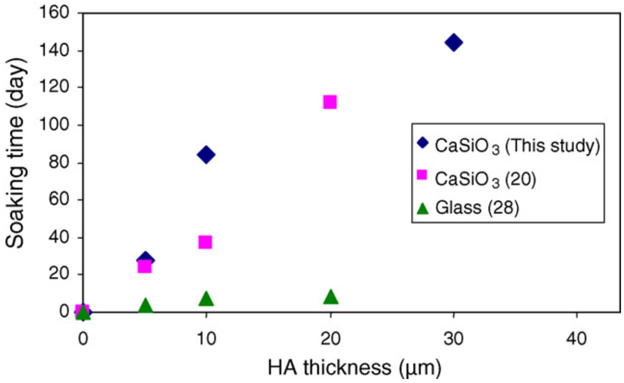

Fig. 6. Change of thickness of hydroxyapatite layer on the $CaSiO_3$ disks with soaking time.

Fig. 6 shows the change of HAp layer thickness as a function of soaking time. HAp layer thickness on the $CaSiO_3$ surface was examined by SEM from the cross section of the samples. The thickness reached about 144 μm in 30 days. Biocompatibility studies [10–15,18–21,23,27] were done for various $CaSiO_3$ ceramics and these reported that HAp layer was formed on $CaSiO_3$ surface. Work done by Kokubo suggested that CaO-SiO_2 glass free of P_2O_5 could also form HAp layer on the surface in the early stage of soaking [30]. Previous studies [10–15,17–21,23,27–31] showed that calcium silicate based ceramics are bioactive and can induce the formation of HAp on their surfaces when soaked in SBF. The results also showed that $CaSiO_3$ has an excellent in vitro bioactivity.

4. Conclusions

Fine $CaSiO_3$ powders were synthesized by the chemical precipitation using $Ca(NO_3)_2{\cdot}4H_2O$, $Si(OC_2H_5)_4$ as source material and 0.25 mol/l NaOH as a precipitant under ultrasonic stirring and kept in oven to obtain compact $CaSiO_3$ ceramics. The bioactivity of the $CaSiO_3$ ceramics was evaluated by soaking them in SBF solution.

Na content in the precipitated powder increased with increasing NaOH concentration. Na residue negatively affected the characteristics of the resultant powders. However, Na residues were removed by the washing treatments. The powders washed two times contained the lowest Na content and ultrasonic stirring provided homogenous particle size. The ground $CaSiO_3$ powder had an average particle size of 1.15 μm and was sintered to around 70% of theoretical density by firing at 1200 °C for 2 h. In vitro studies showed that the sintered CaSiO3 ceramics could induce the formation of HAp after soaking in SBF solution.

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