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Synthesis and in vitro bioactivity of dicalcium silicate powders

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Abstract

In the present investigation, β-dicalcium silicate (β-Ca_2SiO_4) and γ-dicalcium silicate (γ-Ca_2SiO_4) powders were synthesized by sol-gel process and hydrothermal synthesis, respectively. The in vitro bioactivity of both β-, and γ-Ca_2SiO_4 was investigated by soaking the powders in simulated body fluid (SBF) for various time periods to analyze the nucleation and growth of hydroxyapatite (HAp) on the surface of the powders. After soaked in SBF for 6 h, HAp appeared on the surface of β-, and γ-Ca_2SiO_4 particles, and uniform lathlike aggregates with typical morphology of HAp crystals formed after 5 days. The β-Ca_2SiO_4 showed strong hydration when soaked in SBF and the hydration was favorable for formation of carbonate-containing hydroxyapatite on the surface of the materials. The γ-Ca_2SiO_4 was not hydrated in SBF solution and showed a slower formation of carbonate-containing hydroxyapatite on the surface when compared with β-Ca_2SiO_4. In addition, the Si concentration in the SBF increased quickly up to 5 days and remained invariable with increased soaking time. The results obtained indicate that hydroxyapatite nuclei can form and grow on the β-, and γ-Ca_2SiO_4 particles, and these two dicalcium silicates are potential candidates as biomaterials for hard tissue repair.

Keywords: Bioactivity; Hydroxyapatite; Powders; Silicates; Simulated body fluid

1. Introduction

Since the discovery of Bioglass© by Hench and co-workers in the early 1970s,[1] various types of silicate glasses and glass-ceramics such as bioactive glasses,[2–5] and AW glass-ceramics,[6–10] have been investigated as biomaterials for hard tissue repair and replacement. The results showed that biomaterials containing CaO–SiO_2 appeared to have excellent bioactivity and were found to bond to living bone and soft tissue. Recently, new studies showed that some calcium silicate ceramics such as wollastonite and pseudowollastonite [11–19] also have excellent bioactivity and are potential candidates of new biomaterials for hard tissue repair. Furthermore, Lamy et al. [20] and Liu et al. [21] have shown that dicalcium silicate (Ca_2SiO_4) as plasma sprayed coating on titanium alloys substrates exhibited very good bioactivity. However, there was no detailed report about synthesis and bioactivity of Ca_2SiO_4 powders with different phases.

The polymorphism of Ca_2SiO_4 has been well studied because of the considerable importance of this compound in the Portland cement, [22–26] refractory materials,[27] and corrosion-resistance coating materials. [28,29] It has been established that β-Ca_2SiO_4 itself is a hydraulic cement as it will set and develop strength, and β-Ca_2SiO_4 is not stable at room temperature and easy to transform into γ-Ca_2SiO_4. So some strategies such as extraordinary synthetic methods, adding stabilizers, etc. must be used to stabilize the β form of Ca_2SiO_4.[21, 30–32] The goal of this study was to synthesize pure dicalcium silicate powders and to investigate the in vitro bioactivity of β-, and γ-Ca_2SiO_4. In the present study, a modified sol-gel process without stabilizers was used to synthesize β-Ca_2SiO_4, and γ-Ca_2SiO_4 was prepared by hydrothermal process. Bioactivities and dissolution characteristics of the two Ca_2SiO_4 powders were evaluated by soaking samples in SBF solutions for various time periods.

2. Experimental

2.1. Preparation of β- and γ-Ca_2SiO_4 powders

β-Ca_2SiO_4 powders were prepared by sol-gel process using reagent-grade calcium nitrate tetrahydrate

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$(Ca(NO_3)_2{\cdot}4H_2O)$ and colloidal SiO_2 with an initial CaO/SiO_2 molar ratio of 2.0. The materials were mixed under vigorous stirring for 6 h at 60 °C, and aged at room temperature overnight. The obtained wet gel was further dried at 80° for 48 h. The prepared dry gel was, subsequently, transferred into a corundum crucible and calcined at 800° for 3 h and then cooled to room temperature in the furnace. The resultant powders were ground and sifted through a 325-mesh sieve before soaking experiments.

γ-Ca_2SiO_4 powders were synthesized by hydrothermal technique. Stoichiometric mixtures of calcium oxide (CaO) and reagent-grade quartz were mixed by adding distilled water with a liquid/solid mass ratio of 10. The suspension was heated at 100 °C under vigorous stirring for 8 h. Then, the resulting solution was cooled, filtrated and calcined at 1450 °C for 2 h. The obtained powders were sifted through a 325-mesh sieve before soaking experiments.

2.2. Soaking in simulated body fluid

The β- and γ-Ca_2SiO_4 powders were placed in polystyrene vials containing simulated body fluid, with ion concentrations nearly equal to human blood plasma as shown in Table 1.[8] The SBF solutions were buffered at pH 7.40 with trishydroxymethylateaminomethane $[(CH_2OH)_3CNH_2]$ and appropriate amount of 1.0 M hydrochloric acid (HCl). The vials with the SBF solutions and samples were maintained at 37.0 °C in a shaking bath for predetermined intervals of 6 h, and 1, 5, 10 and 15 days at a liquid/solid ratio of 1.50 ml/mg without refreshing the soaking medium. After various soaking periods the samples were filtrated and gently rinsed with deionized water to remove SBF solutions, and followed by drying at room temperature for further characterization. The SBF solutions after soaking were collected for determination of ion concentration and pH changes.

2.3. Analysis of hydroxyapatite (HAp) formation

The dried β- and γ-Ca_2SiO_4 powders were crushed to fine powders. Then, the surface structure and morphology of the specimens before and after soaking in the SBF solutions were characterized by X-ray diffraction (XRD, Geigerflex, Rigaku Co., Japan) using monochromated CuK_α radiation, Fourier transitioned-infrared spectroscopy (FTIR; Nicolet Co., USA) and scanning electron microscopy (SEM; JSM-6700F, Jeol, Tokyo, Japan). The concentrations of Ca, P, and Si of the SBF solutions after soaking were determined by inductively coupled plasma atomic emission spectroscopy (ICP–AES; Varian Co., USA), and changes in pH of soaking solutions were also measured by an electrolyte-type pH meter (PHS-2C, Jingke Leici Co., Shanghai, China).

Table 1
Ion concentrations of SBF and human blood plasma

Types	Ion concentrations (mM)						
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^2
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0

3. Results and discussion

3.1. Characterization of β- and γ-Ca_2SiO_4 powders

Fig. 1(a) shows XRD patterns of the two Ca_2SiO_4 powders prepared by different techniques. It is clear to see the numerous sharp peaks and low backgrounds in the XRD patterns indicating that the powders were composed of pure crystalline β- and γ-Ca_2SiO_4. It is thought that β-Ca_2SiO_4 is instable and can undergo transformation to a more stable γ-phase. Therefore, chemical stabilizers were often used to prevent the morphological transformation to γ phase during cooling.[33] In this study using a modified sol-gel process we demonstrated that the desirable β morphology of Ca_2SiO_4 could be preserved by reaction at low temperature without use of chemical stabilizers, and there were no impurities in the prepared powders such as silicon dioxide (SiO_2), calcium oxide (CaO) and other silicates. The grain size and surface morphologies of both β-, and γ-Ca_2SiO_4 powders are shown by the SEM micrographs [Fig. 1(b)], which indicates that particle size of β-Ca_2SiO_4 powders was smaller than γ-Ca_2SiO_4 powders. The high magnification of SEM micrographs shows that the β-Ca_2SiO_4 powders were composed of crystals with 100 nm in diameter and formed aggregates, while γ-Ca_2SiO_4 powders consisted of larger crystals around 10 μm with some fine fibrous crystals of 100 nm in diameter on the surface.

3.2. Formation of HAp on the surface of Ca_2SiO_4 powders

Figs. 2(a) and 3(a) show XRD patterns of the two Ca_2SiO_4 powders before and after soaking in SBF solutions for various time periods. It is obvious that the characteristic peaks of β-Ca_2SiO_4 decreased after 6 h and disappeared after 5 days of soaking. New peaks for $Ca_3Si_2O_7{\cdot}2H_2O$ were apparent after 6 h and disappeared again after 5 days of soaking, while the characteristic peaks for HAp appeared after 1 day and increased up to 15 days. Compared with the obvious changes of XRD patterns of β-Ca_2SiO_4 in the early days of soaking [Fig. 2(a)], no evident changes appeared in the XRD patterns of γ-Ca_2SiO_4 before 1 day of soaking as seen in Fig. 3(a). With prolonged soaking from 1 day to 15 days, the intensities of HAp peaks increased indicating the formation of HAp on the surface of the γ-Ca_2SiO_4 powders. In addition, if we compare the XRD patterns of γ-Ca_2SiO_4 with the patterns of β-Ca_2SiO_4, the intensities of the HAp peaks for the γ-Ca_2SiO_4 samples are lower than that for the β-Ca_2SiO_4 towards the end of the soaking period.

The FTIR spectra of the two Ca_2SiO_4 powders were shown in Fig. 2(b) and 3(b). When the soaking time increased, the intensity of silicate absorption bands at 472 cm^{-1} decreased. Simultaneously, new absorption bands at 566 and 603 cm^{-1} could be recognized after 1 day of soaking, which are split from the P–O bending vibration (ν_4) in a PO_4 tetrahedron around 598 cm^{-1}. The P–O stretching vibration (ν_3) of the PO_4 unit occurs at range 1030–1090 cm^{-1}. Because this is superimposed with the strong vibration of the Si–O bond (ν_3 mode in SiO_4 unit), it is difficult to distinguish the P–O vibration from the Si–O vibration at the early stage of the reaction.

According to the report of infrared correlation charts,[34] the bands at 566, 603 and 1030–1090 cm^{-1} were all the characteristic of HAp crystals, which indicated the formation of HAp on the surface layer of Ca_2SiO_4 powders after soaking in SBF for 1 day. Furthermore, the C–O stretching of CO_3^{2-} groups at 1415 and 1490 cm^{-1} were observed when two Ca_2SiO_4

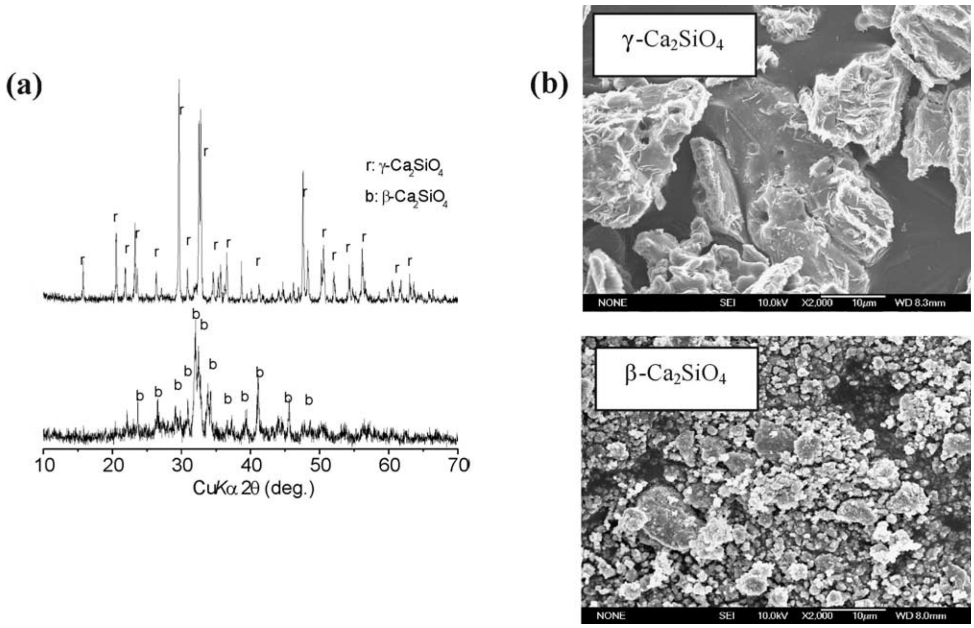

Fig. 1. XRD patterns (a) and SEM micrographs (b) of β-, and γ-Ca_2SiO_4 powders.

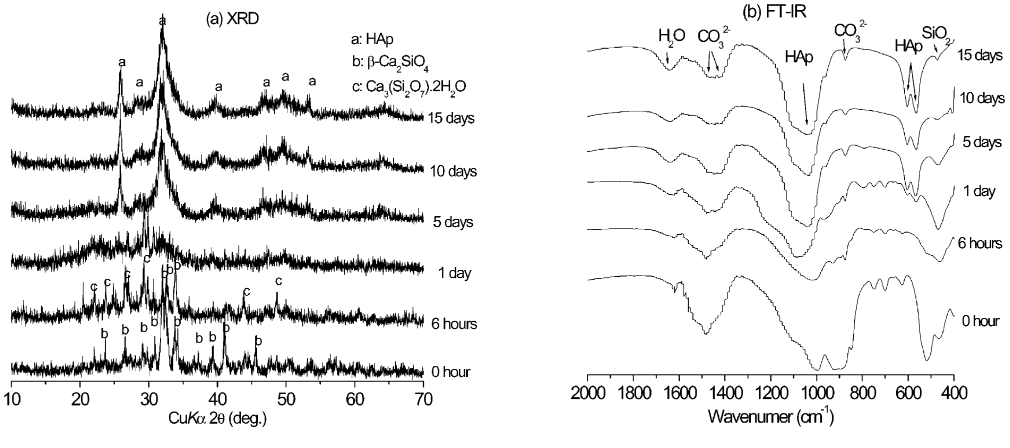

Fig. 2. XRD patterns (a) and FTIR spectra (b) of β-Ca_2SiO_4 powders soaked in SBF solutions for various periods.

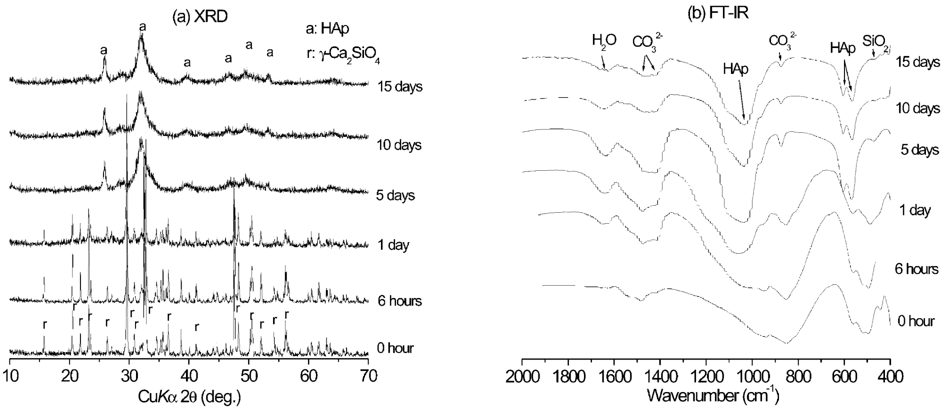

Fig. 3. XRD patterns (a) and FTIR spectra (b) of γ-Ca_2SiO_4 powders soaked in SBF solution for various periods.

powders were soaked for 6 h, and the C–O stretching of CO_3^{2-} groups at 872 cm^{-1} appeared after soaking for 1 day.

It is obvious from the results described above that the β-, and γ-Ca_2SiO_4 powders could develop a carbonate-containing hydroxyapatite layer on the surface (Figs. 2 and 3). It is considered that a bonelike carbonate-containing HAp layer, which precipitates on the surface of a bioactive material in human body, plays an essential role in forming the chemical bond of the bioactive material to the living bone. This carbonate-containing HAp layer can be reproduced in vitro in simulated body fluid. Work done by Ohtsuki et al.[35] suggests that CaO–SiO_2 glass free of P_2O_5 also can form HAp layer on the surface, and that the rate of the HAp nucleation on the surface of the CaO–SiO_2-based glass is much higher than on the CaO–P_2O_5-based glass in the early stage of soaking. Hench [36] proposed that a sequence of five reactions involved in the formation of HAp layer on the surface of bioactive glasses containing CaO–SiO_2 components and the hydrated silica could provide specific favorable sites for the HAp nucleation on these bioactive glasses. Kokubo et al.[9] has shown that HAp formation was also much sensitive to the concentration of OH^- ions in the surrounding solution, and an increase in pH of the solution would favor the deposition of HAp on the surface of AW glass-ceramics. It is known that β-Ca_2SiO_4 can hydrate in aqueous solution, while γ-Ca_2SiO_4 has no hydration property. The hydration kinetics of β-Ca_2SiO_4 has been well studied by Brunauer et al.[37] and their study suggests that when β-Ca_2SiO_4 powders are dissolved into water, the isolated SiO_4^{4-} tetrahedrons may be condensed into $Si_2O_7^{6-}$ dimeric ions, and the pH of the solution will increase. The hydration reaction can be expressed as: $2SiO_4^{4-} + H_2O = Si_2O_7^{6-} + 2OH^-$. With prolonged soaking time, the concentration of $Si_2O_7^{6-}$ increases which may result in an increase of pH. Our results confirmed the hydration of β-Ca_2SiO_4 in SBF solution [Figs. 2(a) and 6], and the hydration process resulted in an extensive increase in pH at the early stage of soaking, which could lead to a faster precipitation of HAp.

3.3. Microstructure of the formed HAp

Fig. 4 shows SEM micrographs of the β-Ca_2SiO_4 powders before and after soaking in the SBF solutions for various time periods. In comparison with the particles before soaking (Fig. 4, 0 hr), tiny sediments were observed on the surface of β-Ca_2SiO_4 powders soaked for 6 h and 1 day. After soaking for 5 days, numerous uniform lathlike crystallites were evident. The sizes of the crystallites were 200–300 nanometers in length and many of crystallites formed agglomerates, which showed a typical morphology of HAp.[38,39] After prolonged soaking for up to 15 days, clusters of agglomerated HAp particles increased and the surface structure of the samples became more compact.

From the SEM micrographs of the γ-Ca_2SiO_4 powders (Fig. 5), circular flake-like agglomerated particles thought to be HAp were observed on the surface of the sample soaked for 6 hours, while the fibrous crystallites originally located on the surface of the unsoaked γ-Ca_2SiO_4 powders were disappeared. After soaking for 1 day, the amounts of agglomerated HAp particles increased accompanied by the increase of the particle size. With increased soaking time (5, 10 and 15days), layers of lathlike crystallites formed a network with a fine structure overgrow on the sample surface (Fig. 5).

3.4. Concentration changes of the SBF solution

Fig. 6 shows changes of the pH and concentrations of Ca, P and Si of SBF solutions measured by ICP after soaking for various periods. It is obvious that the ion concentrations of SBF solutions changed markedly after

1 day of soaking. The Ca and Si concentrations in SBF increased rapidly while P concentration gradually decreased at the early stage of soaking. After prolonged soaking, the Si concentration of the SBF solution containing β-Ca_2SiO_4 still increased after 5 days, and reached 9.9 mM up to 15 days due to the continuous hydration reaction, whereas the Si concentration of the SBF solution containing γ-Ca_2SiO_4 reached 8.6 mM after 5 days and remained constant towards the end of the soaking periods. Simultaneously, the Ca concentrations continued to increase in both solutions, although the Ca concentration in the SBF solution containing

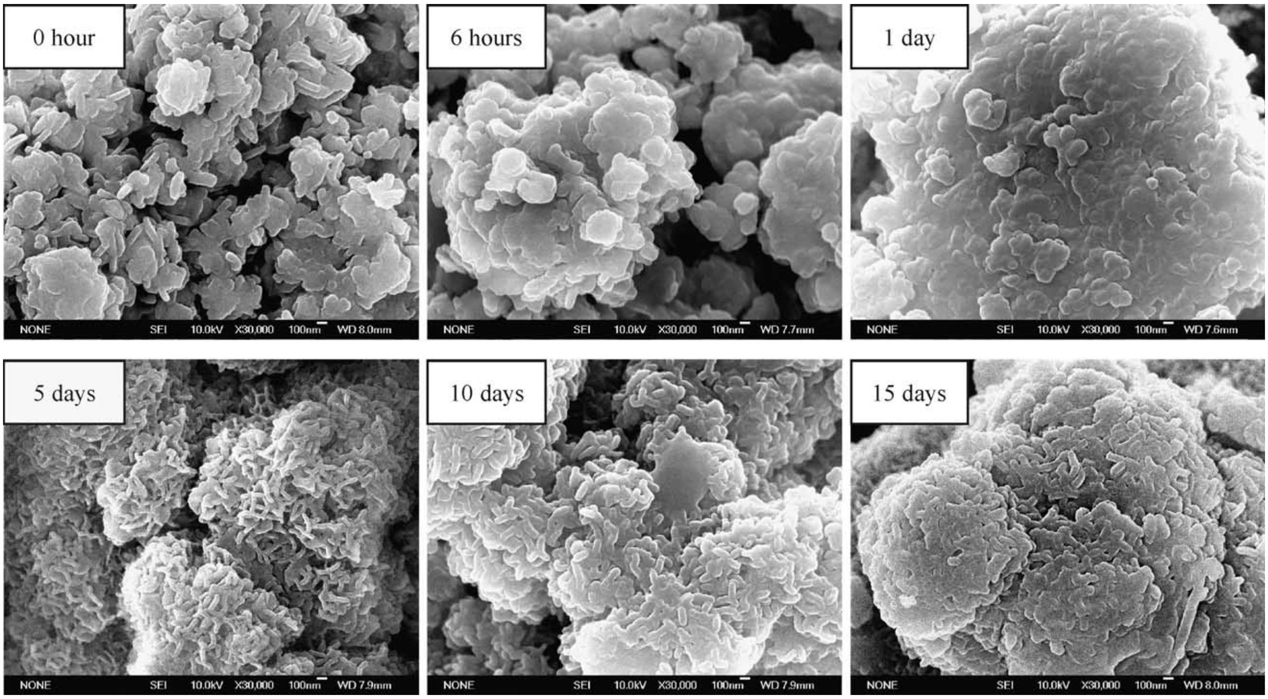

Fig. 4. SEM micrographs of the surfaces of β-Ca_2SiO_4 powders soaked in SBF solutions for various periods.

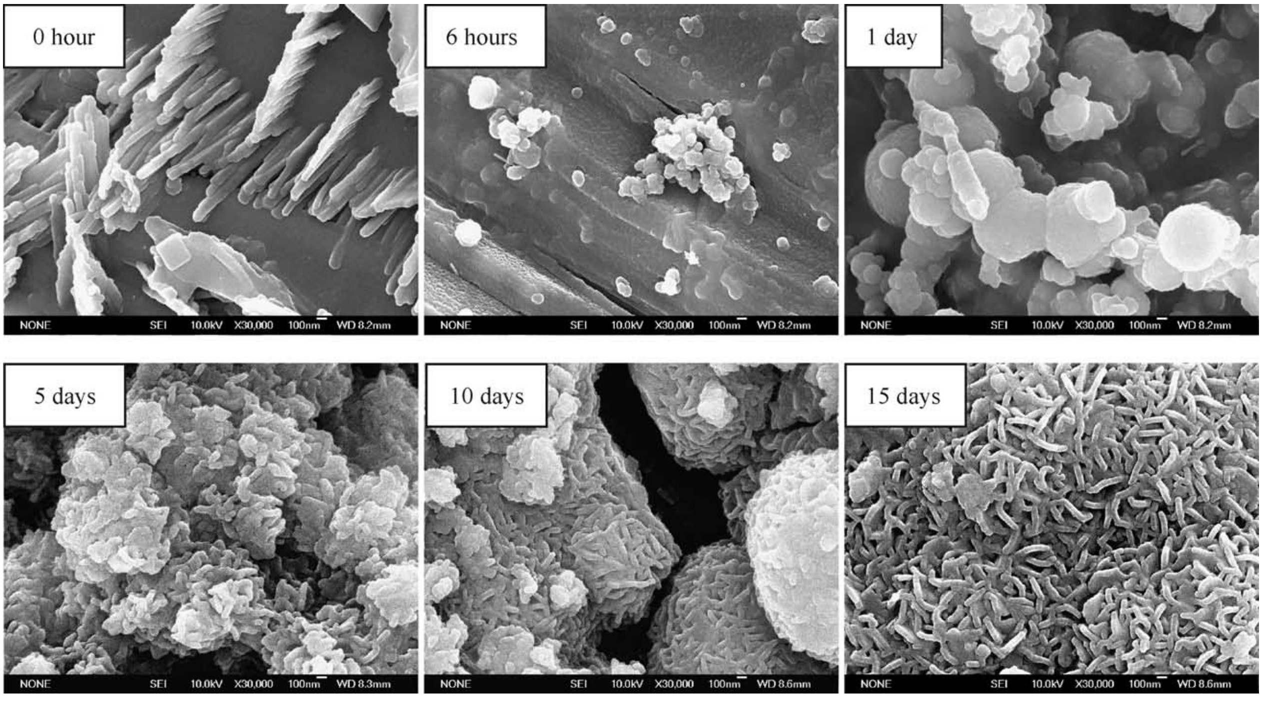

Fig. 5. SEM micrographs of the surfaces of γ-Ca_2SiO_4 powders soaked in SBF solutions for various periods.

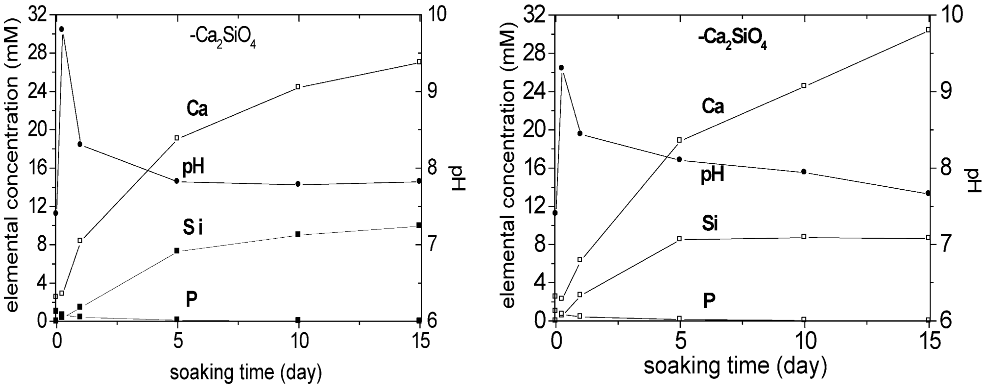

Fig. 6. Changes of Ca, Si, and P concentrations, and pH of the SBF solutions after soaking the Ca_2SiO_4 powders for various periods.

β-Ca_2SiO_4 increased slightly slower than that in the solution containing γ-Ca_2SiO_4 due to a faster consumption of Ca ions during the subsequent formation of HAp on the surface of the powders. In contrast to the increase of Ca and Si, the P concentration of SBF solutions decreased during the soaking periods due to the formation of HAp by consuming P ions from SBF solutions. Accompanied with the ion release at the early stage, the pH of SBF solutions reached a maximum in the first hours for the two samples, and then decreased gradually due to consuming OH^- ions in the formation of HAp. Furthermore, probably due to the hydration reaction, the β-Ca_2SiO_4 resulted in a higher rate of pH increase in the first 6 h of the soaking as compared with the γ-Ca_2SiO_4.

4. Conclusion

Pure β-Ca_2SiO_4 was synthesized using a modified sol-gel process without chemical stabilizers, and γ-Ca_2SiO_4 was synthesized by hydrothermal technique. β-Ca_2SiO_4 showed strong hydration when soaked in SBF solution and the hydration was favorable for formation of carbonate- containing HAp on the surface of the powders. γ-Ca_2SiO_4 was not hydrated in SBF solution and showed a slower formation of carbonate-containing HAp on the surface as compared with β-Ca_2SiO_4. Our results suggest that both β-, and γ-Ca_2SiO_4 have excellent in vitro bioactivity as shown by the formation of HAp in SBF solutions and are potential candidates as tissue repair biomaterials.

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