Silicon-substituted calcium phosphate compounds: Synthesis, characterization, and bioactivity evaluation

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ABSTRACT: Hydroxyapatite (HA), the main component of bone and teeth, can improve its structure by incorporation of traces of silicon (Si). This paper reports a synthesis of Si-substituted calcium phosphate compounds via a chemical precipitation process. The solutions of hydroxyapatite and fumed silica were homogeneously mixed before precipitation using ammonia. The resultant solutions were then filtered and the obtained powders were calcined at various temperatures. It was found that the phase of as-precipitated powders was HA with an average size of 15 nm. However, β-tricalcium phosphate (TCP) was formed at the calcined temperature of 800 °C. From Fourier-transform infrared spectroscopy spectra, the Si-O peaks were observed when the as-precipitated powder was heated. It was concluded that there was a substitution of silicate ions for phosphate ions in the HA or β-TCP structure. To evaluate the effect of silicon on the bioactivity in vitro, pure HA and silicon-substituted HA (Si-HA) powders were uniaxially pressed and the samples were soaked in simulated body fluid at 37 °C for various periods of time. It was found that after 2 weeks of soaking, the surface of the Si-HA sample was covered by apatite layers, whereas that of pure HA remained unchanged even after 4 weeks. These results showed that the presence of silicon can enhance the bioactivity of HA.

KEYWORDS: hydroxyapatite, fumed silica, silicon-substituted hydroxyapatite, simulated body fluid, bone, teeth

INTRODUCTION

Hydroxyapatite (HA) is the principal inorganic constituent of bones and teeth[1–3]. Synthetic HA has excellent biocompatibility with hard tissues and also with skin and muscle tissues and thus has been used in many biomedical applications. Biological apatite differs from synthetic HA in stoichiometry, composition, and crystallinity. Silicon (Si) is one of the important trace elements that are found in natural bones and teeth. It helps provide natural HA with stability, yet with sufficient reactivity to allow the growth of nanocrystallites in vivo[3,4]. From a study in vitro, it was found that the substitution of phosphate ions by silicate into HA enhances osteoblast cell activity, compared to the pure HA phase[5]. For these reasons, there has been interest in synthesizing silicon-substituted hydroxyapatite (Si-HA). Several methods for the synthesis have been reported including sol-gel[6], hydrothermal[7], solid-state reaction[8], and chemical precipitation, and crystallization[9,10]. Chemical precipitation and crystallization are the most commonly used methods because they are simple and cheap.

Fumed silica (FS) is a by-product of ferrosilicon production and it is normally used as a filler in com-posite constructions or as a filler, an extender pigment, or an emulsification agent in paints[11]. Investigating the use of FS as a source of silicon in Si-HA structure can thus increase its value and also improve its utility.

In this work, we investigated the synthesis of nano-sized Si-HA powder by a simple chemical precipitation process starting from HA and low-priced FS. Powder characterization includes phase composition, functional group determination, and particle morphology. To study their bioactivity, Si-HA and pure HA samples were soaked in simulated body fluid.

MATERIALS AND METHODS

Material synthesis and characterization

Hydroxyapatite (HA, 90%, Fluka) and fumed silica (FS, Sika, Thailand) were used as the starting materials. HA was dissolved in nitric acid (65%, Merck KGaA) and FS was dissolved in 1 M sodium hydroxide solution (99%, AnalaR). These solutions were then homogeneously mixed at three different weight percentages (wt%): 1.5, 3.0, and 4.5 wt% of FS in HA using a magnetic stirrer. After 30 min of mixing, ammonia (25%, Merck KGaA) was then added to perform the precipitation. The precipitate obtained was then filtered and then washed using

deionized water and acetone. After drying, the precipitate was calcined at 600, 800 and 1000 °C for 6 h. The uncalcined and calcined powders were characterized using X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). For the comparative study, HA and FS powders were wet-mixed at the same three weight ratios using deionized water as a medium before calcination and characterization.

Bioactivity evaluation

Simulated body fluid (SBF) was prepared as described by Kokubo[12]. Briefly, the appropriate quantities of chemical reagents: NaCl, NaHCO$_3$, KCl, K$_2$HPO$_4 \cdot$ 3 H$_2$O, MgCl$_2 \cdot$ 6 H$_2$O, CaCl$_2$, and Na$_2$SO$_4$ were dissolved in ion-exchanged and distilled water. The solution was buffered at the physiological pH of 7.4 using Tris(hydroxymethyl) aminomethane (Tris) and hydrochloric acid (1 M). The prepared SBF was kept at 5 °C in a refrigerator and was used within 30 days of preparation.

HA and Si-HA powders were uniaxially pressed into a disc shape with a diameter of 1 cm and a thickness of about 2 mm. The disc samples were soaked in SBF solution and kept in a 37 °C incubator. The SBF solution was changed twice a week and the samples were taken out every week over a period of 4 weeks. The samples were gently washed with distilled water, dried in a desiccator and later sputter-coated. The surfaces of the samples were then studied using SEM and EDX.

RESULTS AND DISCUSSION

Phase analysis

Phases of all uncalcined and calcined powders were studied using XRD. In the case of the chemical precipitation process it was found that, for every wt% of FS, the phase of as-precipitate powders (or uncalcined powders) was that of HA (JCPDS file no. 09-0432). When they were calcined at 600 °C, their phases were still HA, but when the calcined temperature reached 800 °C, the phases changed to β-tricalcium phosphate (TCP) structure (JCPDS file no. 09-0169). This phase transformation temperature is much lower than the normal temperature of 1000 °C. According to the result of the EDX study, the starting powder was a non-stoichiometric HA with a Ca/P ratio of 1.27. Therefore, a reaction might have occurred as described by the following equation[3]: Ca$_{10-x}$H$_{2x}$(PO$_4$)$_6$(OH)$_2$

$$\xrightarrow{\text{800 °C}} \text{β-Ca}_3(\text{PO}_4)_2 + \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2.$$

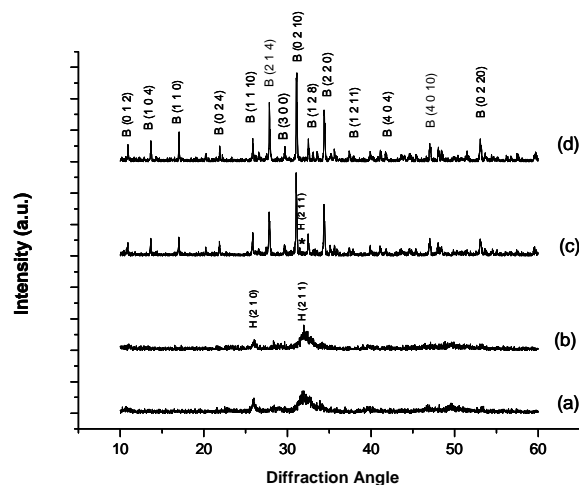From XRD patterns, it was found that there was

Fig. 1 XRD pattern of powders prepared from HA with 3 wt% of FS via chemical precipitation process: (a) uncalcined powders; calcined powders at (b) 600 °C, (c) 800 °C, (d) 1000 °C for 6 h. (B = β-TCP, H = HA).

no observable change of the peak intensities and widths with increasing silicon content for both powders prepared from chemical precipitation and wet-mix processes. This might be due to the low level of FS used in this work. Since all the samples with different wt% of FS gave similar results, XRD patterns of the samples with 3 wt% FS were chosen as the representative result, as shown in Fig. 1. The patterns of uncalcined and 600 °C calcined powders were similar to that of natural apatite with broad peaks at the 2θ angles of HA, indicating a superfine grain of apatite crystallite. Although FS was added into the systems, the peak of silica could not be detected. XRD patterns showed only the HA phase at room temperature and 600 °C, and the TCP phase at 800 and 1000 °C. Since an incorporation of silicate groups does not alter the host structure[13], we could assume that the added silica were now in HA or TCP structures. This assumption was proved by the FT-IR study as described later in this paper.

Similarly, since all the samples with different wt% of FS gave similar results, the representative XRD patterns of the powders prepared from 3 wt% of FS in HA via the wet-mix process are shown in Fig. 2. It was found that at room temperature, the phase is that of HA. At 600 °C, a trace of β-TCP was detected. The shape peaks of HA show that they are well-formed crystalline powders. As the powder was heated up to 800 °C, β-TCP was formed, with only a small amount of HA remaining. Finally, when the temperature was increased to 1000 °C, only β-TCP was found, without
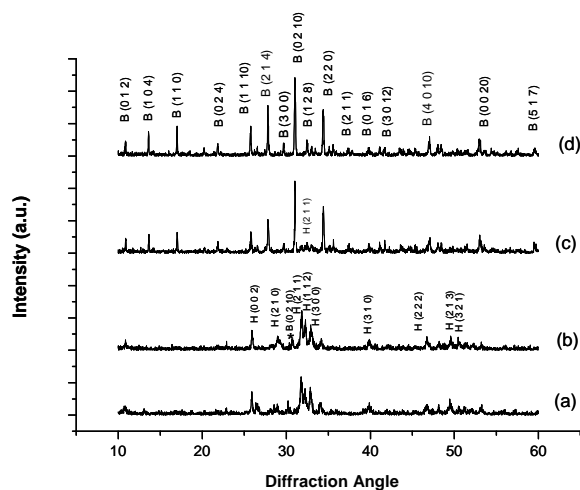
Fig. 2 XRD pattern of powders prepared from HA with 3 wt% of FS via wet-mix process: (a) uncalcined powders; calcined powders at (b) 600 °C, (c) 800 °C,(d) 1000 °C for 6 h. (B = β-TCP, H = HA).

any second phase. Thus in order to determine the existence of silica or silicate groups in the structure, FT-IR study was performed.

Particle size calculation

Particle sizes of the powders can be calculated using Scherrer's equation[14]:

$$D = \frac{k\lambda}{\beta\cos\theta},\qquad(1)$$

where k is the shape coefficient for the reciprocal lattice point ($k = 1$ was chosen, considering that the shape of this point is spherical), β is the full width at half maximum of the peak, and θ is the Bragg angle. It was found that the as-precipitated powders were very fine powders with an average size of 15 nm (Table 1). As the temperature increased, the powder sizes also increased. The amount of FS did not have any affect on the sizes of the powders. In the case of wet-mix process, the uncalcined powders were bigger than the precipitated ones and about the size of starting HA (about 52 nm). As the temperature increased, the powder sizes also increased, but at a lower rate than that of the precipitated ones. This might be due to the smaller size of the precipitated powders and the higher surface energy which led to their better sintering ability. In contrast, for wet-mix powders, the presence of FS in the HA system could be seen as the second-phase inclusion which could inhibit grain growth[15]. The average sizes of all the samples are summarized in Table 1.

Table 1 The particle sizes (in nm) of synthesized powders at various temperatures.

Preparation method	Wt% of FS	Calcined temperature			
		Room T	600 °C	800 °C	1000 °C
Chemical precipitation	1.5 wt%	15	18	85	91
	3.0 wt%	16	18	104	127
	4.5 wt%	15	18	93	109
Wet-mix	1.5 wt%	42	47	61	84
	3.0 wt%	53	65	78	80
	4.5 wt%	49	59	54	79

Functional group determination

Fourier-transformed infrared spectroscopy (FT-IR) was used in the wavenumber range of 4000–400 cm^{-1}. The FT-IR spectra of uncalcined powders are shown in Fig. 3. The IR bands at 3571 and 633 cm^{-1} belong to the vibration of the hydroxyl group, the bands at 1092, 1035, and 960 cm^{-1} belong to the phosphate symmetric stretching vibration, and the bands recorded at 602, 565, and 470 cm^{-1} are due to the phosphate bending vibration. The carbonate ions peaks were observed at 1410 and 876 cm^{-1}. From the XRD and FT-IR studies, it can be concluded that the starting material and the as-precipitated powder are carbonated hydroxyapatite[1–3]. The wet-mixed powder had the same FT-IR spectrum as the starting hydroxyapatite, suggesting that there is no chemical reaction at this stage. However, these carbonate and hydroxyl groups were easily eliminated during the calcination process, as can be clearly seen in Fig. 4.
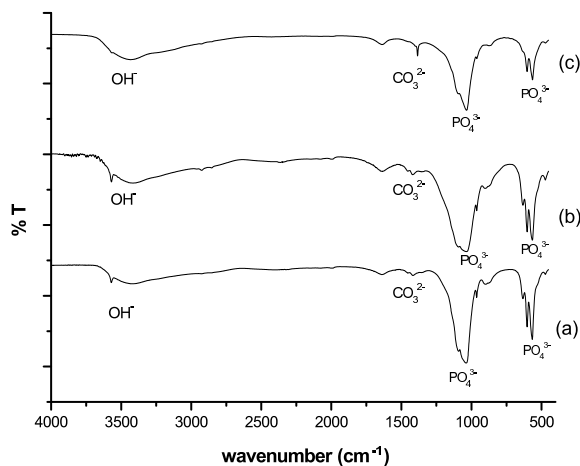

Fig. 3 FT-IR spectra of uncalcined powders: (a) starting HA, (b) wet-mixed powder at 3wt% FS (c) precipitated powder at 3 wt% FS.
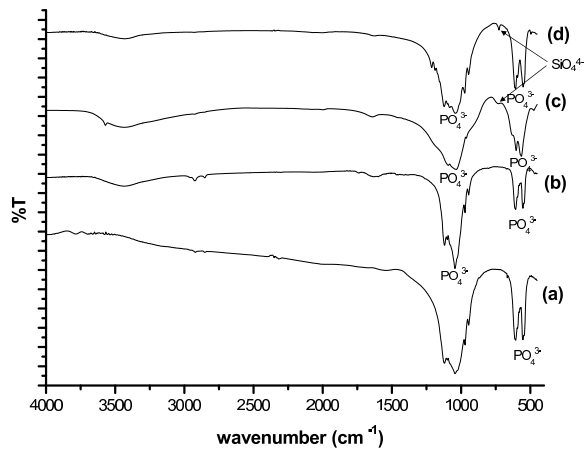
Fig. 4 FT-IR spectra of (a) starting HA powder calcined at 1000 °C (b) wet-mixed powder calcined at 1000 °C (c) precipitated powder calcined at 600 °C (d) precipitated powder calcined at 1000 °C. All the synthesized powders were prepared at 3 wt% FS. Arrows indicate existence of silicate groups in structures.

After calcination at 1000 °C, the starting HA powder (Fig. 4a) and wet-mixed powder (Fig. 4b) gave similar results. The 6 main peaks at 1100–900 cm^{-1} are due to asymmetric vibration of phosphate groups in the β-TCP structure, but the carbonate group is no longer observed. This is in agreement with the XRD results where only the β-TCP phase was observed when the powders were calcined at 1000 °C. Figs. 4c and 4d show the FT-IR spectra of precipitated powders (3 wt% FS) calcined at 600 and 1000 °C, respectively. From Fig. 4c, it can be clearly seen that the phosphate peaks are those of symmetric vibration in the HA structure, which is also in agreement with the XRD result. In the case of Fig. 4d, 6 peaks of phosphate asymmetric vibration were observed, revealing its β-TCP structure. Moreover, for both Figs. 4c and 4d, the additional peak at 756 cm^{-1} can be observed. In addition, the intensities of the peaks corresponding to phosphate groups decreased compare to the ones in Figs. 4a and 4b. This peak corresponds to a replacement of phosphate with silicate group in the HA structure[9, 13]. A mechanism of this replacement can be described by the following equation[9]: $10\,\mathrm{Ca}^{2+} + (6-x)\,\mathrm{PO}_4^{3-} + x\,\mathrm{SiO}_4^{4-} + (2-x)\,\mathrm{OH}^- \longrightarrow \mathrm{Ca}_{10}(\mathrm{PO}_4)_{6-x}(\mathrm{SiO}_4)_x(\mathrm{OH})_{2-x}$.

From these results, it can be concluded that silicon-substituted HA and silicon-substituted TCP can be synthesized via a chemical precipitation process. This silicon-substituted HA (Si-HA) and its bioactivity was studied in comparison to pure HA in
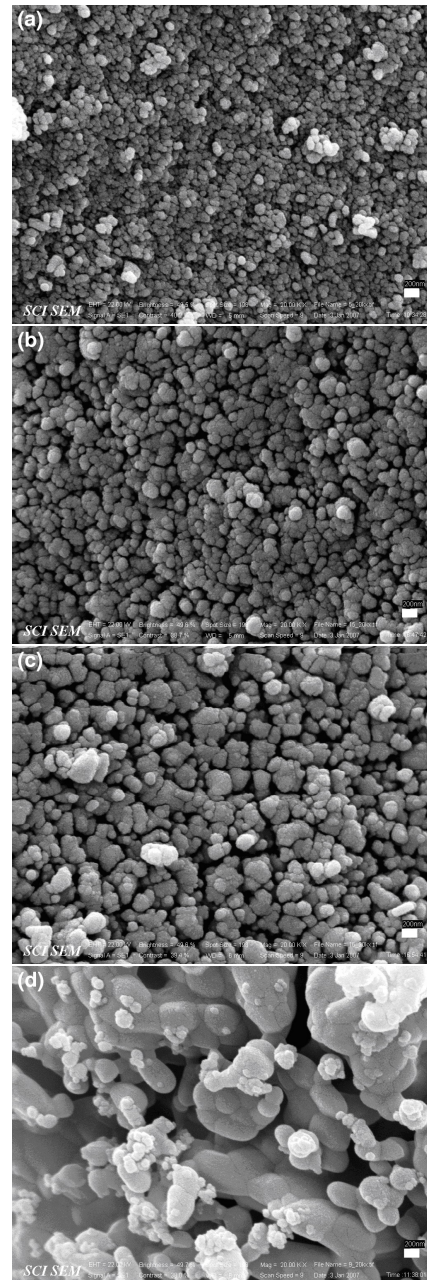

Fig. 5 SEM micrographs of precipitated powders (3 wt% FS): (a) uncalcined; calcined at (b) 600 °C (c) 800 °C (d) 1000 °C (scale bar: 200 nm).

SBF for up to 4 weeks.

Morphology study

The microstructural characteristics of the synthesized powders investigated by SEM are shown in Fig. 5 and Fig. 6. Since the powders prepared from every weight percentage of FS gave similar results, representative
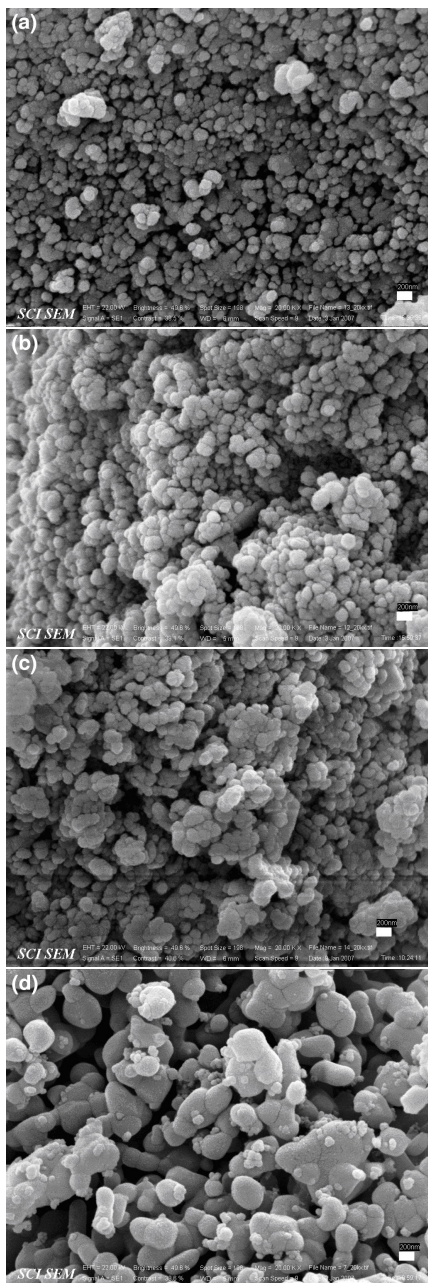
Fig. 6 SEM micrographs of wet-mix powders (3 wt% FS): (a) uncalcined; calcined at (b) 600 °C (c) 800 °C (d) 1000 °C (scale bar: 200 nm).

micrographs of 3 wt% FS precipitated and wet-mix powders are shown. The powders are composed of nano-sized round grains. As the calcined temperature increased, the grain size increased and grain-growth behaviour can be observed. The results are in agreement with the particle size determination from Scherrer's equation (1).
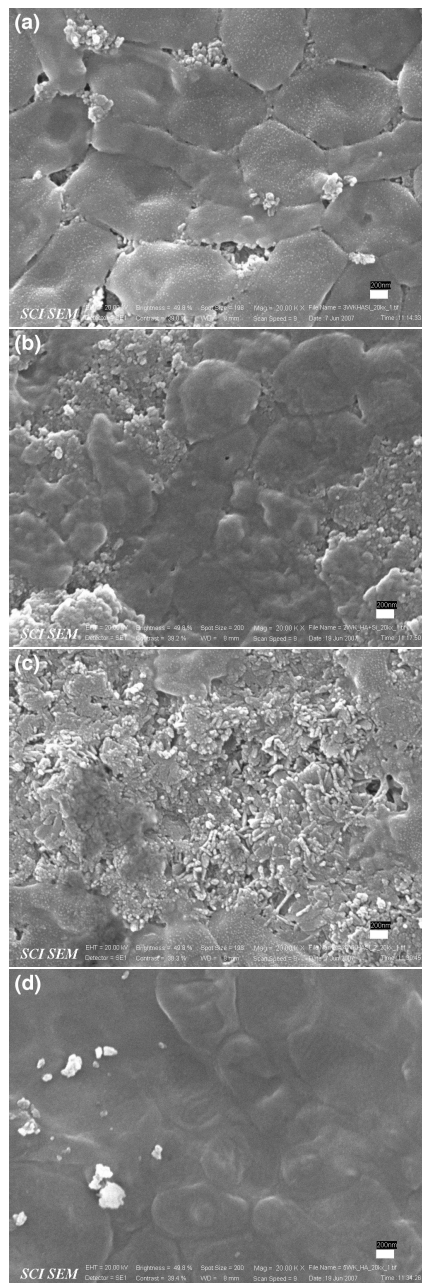

Fig. 7 SEM micrographs of the surfaces of Si-HA samples after soaking in SBF for (a) 2 weeks (b) 3 weeks (c) 4 weeks; (d) HA sample after soaking for 4 weeks (scale bar: 200 nm).

Bioactivity study

The samples of pure HA and Si-HA (3 wt% FS) calcined at 600 °C were soaked in SBF for up to 4 weeks and their surfaces were examined using SEM. From Fig. 7 it can be seen that the surfaces of Si-HA samples appear to be covered by a new material with

acicular-like morphology since the second week. In contrast, the surface of the pure HA sample remains unchanged even though it had been soaked in SBF for 4 weeks. The chemical composition of this new material was studied using EDX and it was found to consist of Ca, P, C, and O (data not shown). As the apatite phase can be formed on the surface of bioactive materials [1–3], it is safe to assume that this material is a carbonated apatite.

We did not examine the bioactivity Si-HA samples with 1.5 and 4.5 wt% FS. However, from the literature where the study on the growth of human osteoblast cells on the Si-HA deposited substrates with various amounts of Si (0, 0.8, 1.2, and 1.6 wt%) was performed [16], it was found that the number of such cells increased with the amount of Si during the in vitro culture. On the other hand, a study by Hing et al [17] showed that the Si-HA scaffold with 0.8 wt% Si had the best response for both bone forming and bone resorbing cells, compared to the ones with 0, 0.2, 0.4, and 1.5 wt% Si.

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