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In vitro bioactivity and dissolution of $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers

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Abstract

In this paper, needlelike and dispersible hillebrandite ($Ca_2(SiO_3)(OH)_2$) and β-dicalcium silicate (β-Ca_2SiO_4) fibers, 1.0–1.5 μm in diameter and 15–25 μm in length were prepared by a modified hydrothermal method. In vitro bioactivity and dissolution of the two types of fibers were evaluated by soaking in the simulated body fluid (SBF) for various periods. Hydroxyapatite-like aggregates appeared on the fibers after soaking for 7 days, and uniform lathlike agglomerates with typical morphology of hydroxyapatite (HAp) formed after 14 days. It was observed that the fibers after soaking still kept stability in fibrous morphology with an aspect ratio in the range of 6–9. The $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers dissolved about 30.91 and 30.42%, respectively, within 14 days. The two types of fibers exhibited certain desirable characteristics including bioactivity, degradability and stability in morphology, and they are promising candidates as reinforcement reagents to develop novel bioactive composites for biomedical applications.

Keywords: Fibres; $Ca_2SiO_3(OH)_2$; Ca_2SiO_4; Bioactivity; Hydroxyapatite; Dissolution

1. Introduction

It is generally accepted that fibers possess many excellent mechanical characteristics. Likewise, fiber-reinforced composites exhibit high tensile, flexural and fracture properties. Thus the approach of fiber-reinforcement is considerably interesting in improving the mechanical properties of bioactive ceramics and polymer composites. During the past two decades some attempts have been made in many studies to develop fiber-reinforced composites in order to obtain desirable stiffness, flexural strength, fatigue life, and other properties for biomedical applications.[1–6] For example, carbon fiber-reinforced polyethylene composites have been used in total knee replacements due to their low rigidity similar to that of human tissue.[7] It is known that HAp possesses excellent bioactivity and osteoconductivity, thus short HAp fiber-reinforced composites have been prepared and used in many applications.[8–12] In addition, there has been an increased interest in glass fiber-reinforced composites in the recent years attributing to their high tensile and flexural properties.[13,14]

However, in vivo studies have shown that carbon fibers cannot form chemical bonds with the host tissue and may release excessive carbon during the healing stage.[15,16] HAp fibers synthesized by hydrothermal and precipitation route suffer from nonstoichiometry or have low thermal stability, and is easy to transfer into lager grains during sintering process.[8–11] Therefore, there is considerably significant to develop novel fibers with certain desirable characteristics, such as bioactivity, degradability and stability in morphology, to satisfy the requisite of reinforcement.

Previous studies have demonstrated that various biomaterials containing CaO-SiO_2 component such as Bioglass,[17,18] $CaSiO_3$ ceramics[19] and β-Ca_2SiO_4 coatings[20,21] were bioactive and could induce the formation of the bonelike HAp in the simulated body environment. Likewise, we have also demonstrated that β-, and γ-Ca_2SiO_4 powders possessed excellent bioactivity when soaked in SBF solution.[22] Thus, fibers of these bioactive materials may be potential candidate as reinforcement reagents for preparation of bioactive composites. In this study, needlelike, highly dispersible and pure $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers were synthesized by a hydrothermal process. In vitro bioactivity and dissolution of the fibers were investigated by

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soaking fibers in SBF to assess the HAp formation and determine ion release profiles of the fibers in SBF.

2. Experimental procedure

2.1. Synthesis and characterization of the fibers

$Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers were prepared by a modified hydrothermal method described previously[23] using calcium oxide (CaO) and finely dispersed aerosil 200 (amorphous SiO_2) as starting materials. Briefly, CaO was freshly prepared by heating reagent-grade $CaCO_3$ at 1050 °C for 1 h. CaO and aerosil were mixed in an initial CaO/SiO_2 molar ratio of 2:1 under vigorous shaking on a roller for 6 h. Then, deionized water was added to make a solid/liquid ratio of 1:10 (w/w). The suspension was further shaken for 4 h and then transferred into a stainless steel autoclave. The autoclave was, subsequently, heated to 210 °C with a heating speed of 10 °C/h and maintained at 210 °C for 36 h, followed by cooling to room temperature naturally. After this treatment, the obtained suspension was filtrated and washed with anhydrous ethanol twice, and the resultant powders were dried at 80 °C overnight. For preparation of β-Ca_2SiO_4 fibers, the powders were calcined at 900 °C for 2 h in a muffle furnace. The fibers before and after calcining were characterized using X-ray diffraction (XRD; Geigerflex, Rigaku Co., Japan) with monochromated Cu Kα radiation according to JCPDS 42-0438 and 83-0461. Free CaO content in the obtained fibers were determined by the glycol–ethanol method.[24] The morphology of the fibers was observed by scanning electron microscopy (SEM; JSM-6700F, JEOL, Japan).

2.2. Soaking in simulated body fluid

The samples were placed in polystyrene bottles containing simulated body fluid (SBF) with ion concentrations nearly equal to human blood plasma.[25] The bottles with the samples and SBF were maintained at 37.0 °C in a shaking water bath for 1, 7 and 14 days, respectively, at a solid/liquid ratio of 1.50 mg/ml without refreshing the soaking medium. After various soaking periods, the samples were filtrated and gently rinsed twice with deionized water to remove SBF followed by drying in vacuum at 80 °C.

The formation of HAp on the surface of the fibers was characterized by XRD and Fourier transform infrared spectroscopy (FTIR; Nicolet Co., USA), and the morphology of the samples was observed using SEM. The concentrations of Ca, P and Si in the SBF solutions after soaking were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Varian Co., USA), and changes in pH of the solutions were measured using a pH meter (pH; Jingke Leici Co., China). Based on the fact that there is no Si in SBF before soaking, the dissolution ratio (S) of the two types of fibers in different time periods was calculated by the following equation: $S = (c_{\mathrm{Si}} \times v_{\mathrm{S}})/m_{\mathrm{Si}} \times 100\%$, where c_{Si}, v_{S}, and m_{Si} were the Si concentration in SBF (mg ml^{-1}), volume of SBF (ml) and Si content (mg) of the samples soaked in SBF, respectively.

3. Results and discussion

3.1. Characterizations of the as-prepared fibers

Fig. 1 shows XRD patterns of $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers synthesized by the hydrothermal method. It can be seen that there were numerous sharp peaks and low background in the XRD pattern of the $Ca_2(SiO_3)(OH)_2$ fibers (Fig. 1a). Meanwhile, the XRD pattern of the sample did not reveal any other phase than $Ca_2(SiO_3)(OH)_2$. In addition, chemical analysis using glycol–ethanol method showed that the free CaO in the prepared fibers was only 0.12%, which suggested a high purity of the product. Fig. 1b shows the XRD patterns of the prepared β-Ca_2SiO_4 fibers. It can be seen that the sharp peaks with minor diffuse background represented crystalline β-Ca_2SiO_4, and the chemical analysis showed that the free CaO content in the β-Ca_2SiO_4 fibers was 0.15%. Singh et al.[23] have studied synthesis of β-Ca_2SiO_4 fibers by a similar method, in which the $Ca_2(SiO_3)(OH)_2$ fibers were prepared by a hydrothermal reaction at 210 °C for 24 h, and β-Ca_2SiO_4 fibers were prepared by calcination of the $Ca_2(SiO_3)(OH)_2$ for 1 h at 900 °C. They results showed that the content of free CaO in their preparation was 0.8–1.0%, which was higher than that in our preparation. In our study, we modified the method by controlling the heat treatment conditions, in particular the hydrothermal and calcination times. Our results showed that heat treatment time could affect the purity of the products, and a longer hydrothermal reaction time, from 24 to 36 h, and calcinations time, from 1 to 2 h, could result in a lower content of free CaO in the prepared $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers.

Fig. 2 shows SEM micrographs of the as-prepared (Fig. 2a and b) $Ca_2(SiO_3)(OH)_2$ and (Fig. 2c and d) β-Ca_2SiO_4

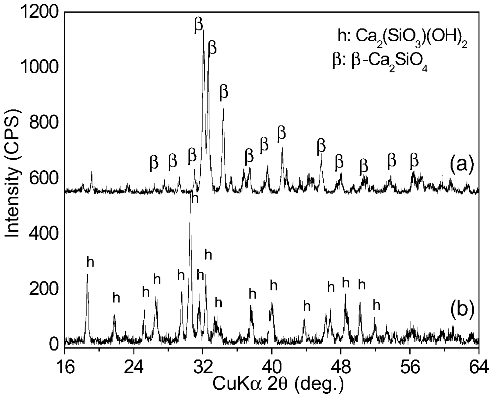

Fig. 1. XRD patterns of the as-prepared fibers. (a) $Ca(SiO_3)(OH)_2$; (b) β-Ca_2SiO_4.

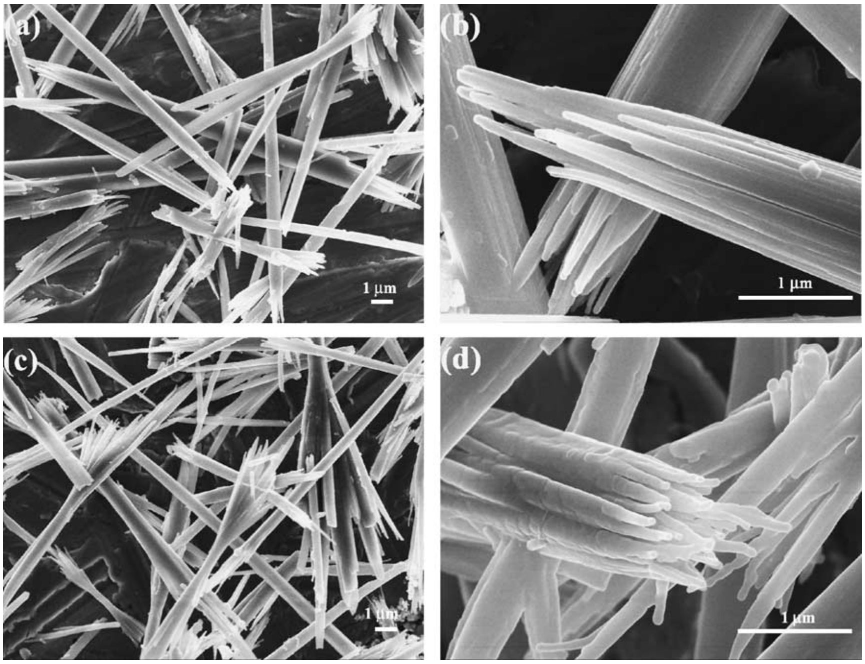

Fig. 2. SEM micrographs of the as-prepared fibers. (a and b) $Ca_2(SiO_3)(OH)_2$; (c and d) β-Ca_2SiO_4.

fibers. It can be seen that $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers both were in a typical needlelike morphology, 1.0–1.5 μm in diameter and 15–25 μm in length, with smooth surface. The high magnification SEM showed that each fiber was an aggregate of several microfibers with a diameter of 100–200 nm and appeared as a lath having spreading ends (Fig. 2b and d). In general, the two types of fibers exhibited high level of dispersibility with minor quantities of aggregation, which was favorable to fabricate composites as reinforcement reagent. According to the previous studies by Sasaki et al.,[26] β-Ca_2SiO_4 fibers obtained from $Ca_2(SiO_3)(OH)_2$ maintained needle morphology when the heat-treatment temperature increased from 600 to 1100 °C. Our results confirmed this observation and suggested that β-Ca_2SiO_4 fibers possessed excellent morphological stability during the heating process.

3.2. *Formation of HAp on the fiber surface*

Fig. 3 shows XRD patterns of $Ca_2(SiO_3)(OH)_2$ (Fig. 3a) and β-Ca_2SiO_4 (Fig. 3b) fibers after soaking in SBF for various periods. From Fig. 3a, it can be seen that the characteristic peaks of $Ca_2(SiO_3)(OH)_2$ disappeared and a new calcium silicate hydrate, $Ca_3Si_2O_7{\cdot}2H_2O$, were apparent after soaking for 1 day. After prolonged soaking for 7 and 14 days, HAp was detected in the XRD patterns and peaks of the calcium silicate hydrate disappeared ultimately. Similarly, the β-Ca_2SiO_4 fibers transformed into the identical calcium silicate hydrate ($Ca_3Si_2O_7{\cdot}2H_2O$) after soaking for 1 day (Fig. 3b), and the characteristic peaks of HAp formed at day 7.

Fig. 4 shows the FTIR spectra of the samples soaking in SBF for various periods. In spectra for $Ca_2(SiO_3)(OH)_2$

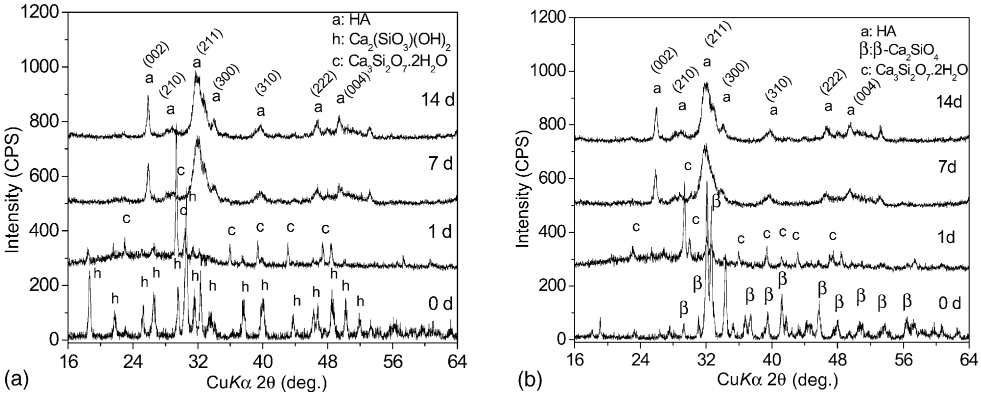

Fig. 3. XRD patterns of fibers soaked in SBF for various periods. (a) $Ca_2(SiO_3)(OH)_2$; (b) β-Ca_2SiO_4.

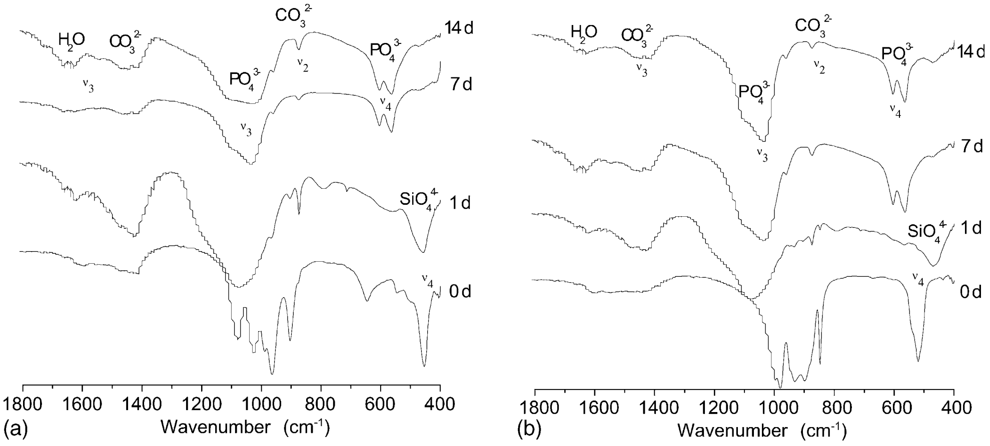

Fig. 4. FTIR spectra of fibers soaked in SBF for various periods. (a) $Ca_2(SiO_3)(OH)_2$; (b) β-Ca_2SiO_4.

fibers before soaking (Fig. 4a, 0 h), the absorption bands of silicate group were evident. The most intense band at 980 cm^{-1} was assigned to the Si–O–Si asymmetric stretch, the band at 902 cm^{-1} to the Si–O symmetric stretch, and the band at 472 cm^{-1} to the Si–O–Si vibrational mode of bending. After soaking for 7 days, the FTIR spectra were analogous to that of the carbonate-containing HAp, where the bands at 1035, 601 and 568 cm^{-1} could be assigned to the phosphate group (PO_4^{3-}), and those at 1453, 1419 and 872 cm^{-1} to the carbonate group (CO_3^{2-}). In spectra for the β-Ca_2SiO_4 fibers before soaking (Fig. 4b, 0 h), bands for the silicate were also evident and bands at 512, 860, 902 and 980 cm^{-1} were assigned to the Si–O–Si and Si–O vibrational modes. After 7 days, the characteristic spectra for the carbonate-containing HAp at 1453, 1419, 1035, 872, 601 and 568 cm^{-1} appeared, which were similar to that in the spectra for $Ca_2(SiO_3)(OH)_2$ fibers after soaking in SBF.

Figs. 5 and 6 show the SEM micrographs of the $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers after soaking in SBF for 7 and 14 days, respectively. From Fig. 5a and b, it was obvious that a layer of microsized lathlike network with a fine structure of HAp overgrew on the surface of the $Ca_2(SiO_3)(OH)_2$ fibers at day 7. After soaking for 14 days, clusters of HAp agglomerates grew as a result of increasing immersion time (Fig. 5c and d). For the β-Ca_2SiO_4 specimens (Fig. 6), lathlike HAp precipitated on the fibers after soaking for 7 days. Increasing immersion time up to 14 days, there was no longer appreciable difference in morphology between β-Ca_2SiO_4 and $Ca_2(SiO_3)(OH)_2$ specimens, with a similar fiber aspect ratio in the range of 6–9. This unique

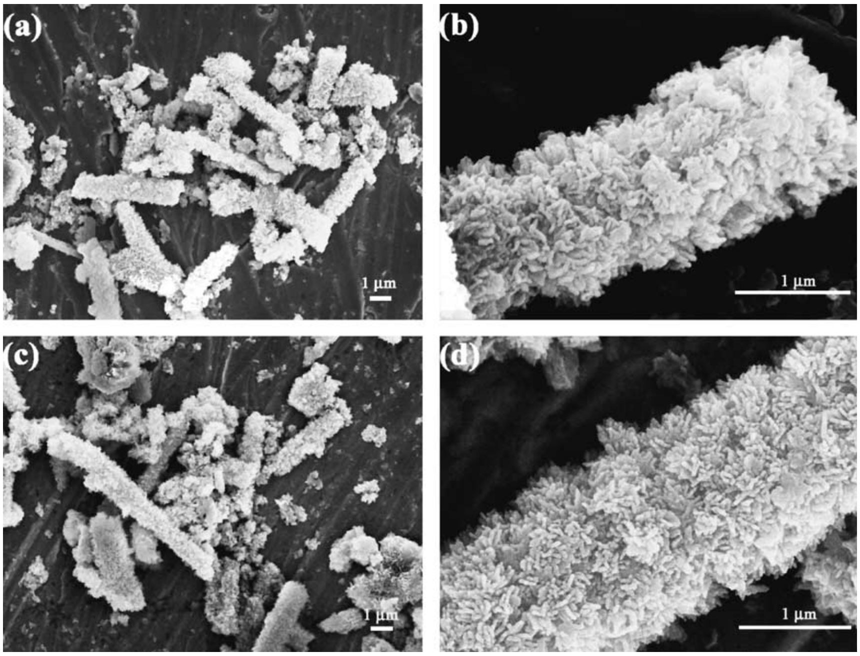

Fig. 5. SEM micrographs of $Ca_2(SiO_3)(OH)_2$ fibers soaked in SBF for various periods. (a and b) 7 days; (c and d) 14 days.

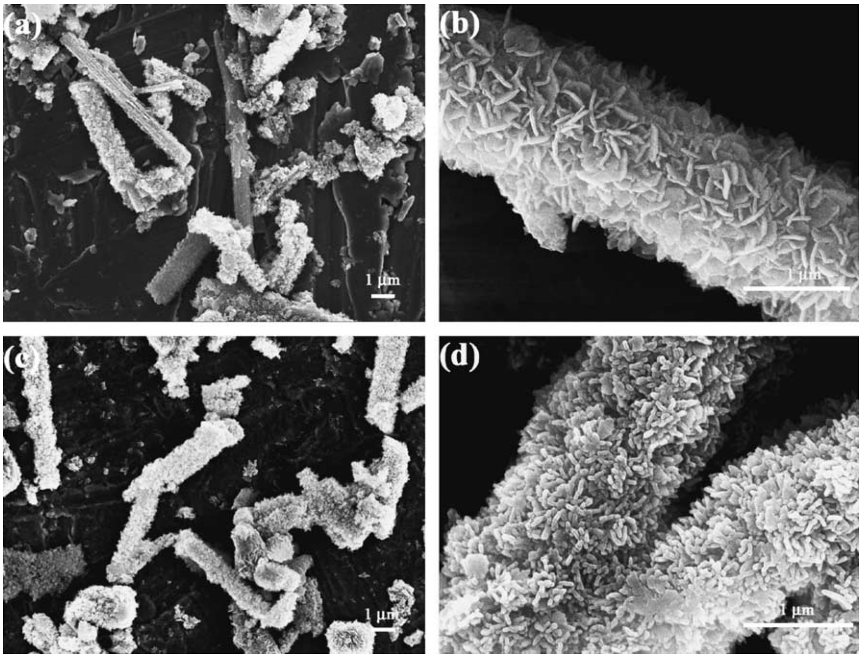

Fig. 6. SEM micrographs of β-Ca_2SiO_4 fibers soaked in SBF for various periods. (a and b) 7 days; (c and d) 14 days.

lathlike texture gave rise to preferred orientation in the XRD patterns manifested as strong intensities of the (2 1 1), (2 2 2), and (0 0 2) HAp reflections (Fig. 3), analogous to the crystals of biological HAp in bone (elongated along the crystallographic *c* axis).[27]

It is obvious from the results described above that β-Ca_2SiO_4 fibers, similar to the β-Ca_2SiO_4 powders,[22] could also develop bonelike carbonate-containing HAp layers on its surface when exposed to SBF. It is a common notion that carbonate-containing HAp, which is a synthetic calcium phosphate that resembles bone mineral, plays an essential role in the formation, growth and maintenance of the tissue-biomaterial interface.[28] Therefore, the present study suggests that β-Ca_2SiO_4 fibers possess good bioactivity, and may be used for preparation of bioactive materials.

To date, $Ca_2(SiO_3)(OH)_2$ is mainly paid attention to prepare β-Ca_2SiO_4 as the precursor in cement research due to their identical Ca/Si molar ratio of 2, and the $Ca_2(SiO_3)(OH)_2$ powders prepared by hydrothermal process are often needle-shaped and mainly lie on (0 0 1), extending along the *b* axis, with a single-chain structure.[29,30] In our study the XRD pattern (Fig. 3a) indicated that the $Ca_2(SiO_3)(OH)_2$ fibers first transformed into another calcium silicate hydrate ($Ca_3Si_2O_7{\cdot}2H_2O$), like the β-Ca_2SiO_4,[31] in SBF. There was then a hydrated silica layer formation on the surface of the fibers, which provided favorable sites for HAp nucleation similar to the β-Ca_2SiO_4 fibers. Although the detailed mechanism of the transformation of $Ca_2(SiO_3)(OH)_2$ into $Ca_3Si_2O_7{\cdot}2H_2O$ is still unknown, our results indicate that $Ca_2(SiO_3)(OH)_2$ fibers possess good bioactivity and stability in morphology in SBF similar to the β-Ca_2SiO_4.

3.3. Concentration and pH changes of the SBF solution

Fig. 7 shows changes of pH and concentrations of Ca, P, and Si of SBF solution measured by ICP after soaking for various periods. It is obvious that the ion concentrations of the solution changed markedly within 1 day of soaking. Calcium ions (Ca^{2+}) and silicate ions ($SiO_4{}^{4-}$) were released from the fibers while P concentration decreased as the uptake of phosphate ions ($PO_4{}^{3-}$) from the solution onto the fibers. There was also a simultaneous rise in pH from 7.25 to 8.55. During the soaking period from 1 to 14 days, Ca and Si concentrations increased slightly slower than that in the early stage but P concentration and pH decreased drastically. Generally, the profile of the changes of ion concentrations in SBF were also confirmed the precipitation of HAp on the fibers, which corresponded to the results of the XRD, FTIR and SEM characterizations above.

Degradability is an important requirement for the biomaterials in tissue engineering applications.[32–34] Corresponding to the Si concentration in SBF after soaking the samples, the quantitative dissolutions of β-Ca_2SiO_4 and $Ca_2(SiO_3)(OH)_2$ fibers were calculated and the results showed in Table 1. It is evident that the two types

Table 1
The dissolution ratio of $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers in SBF for various periods

Sample	Dissolution ratio		
	1 day	7 days	14 days
$Ca_2(SiO_3)(OH)_2$	8.94	24.37	30.90
β-Ca_2SiO_4	9.36	25.90	30.42

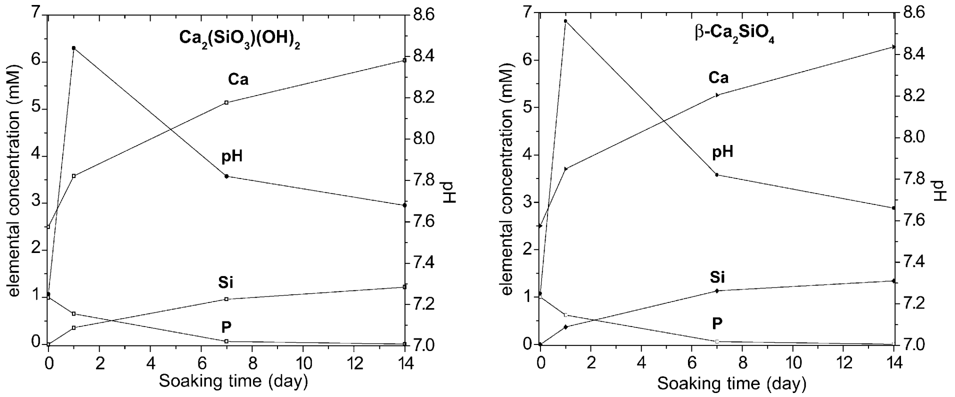

Fig. 7. Changes of Ca, Si and P concentrations and pH value in SBF after soaking $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers for various periods.

of fibers showed a considerable dissolution in the soaking periods. After soaking for 14 days, the $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers both dissolved nearly 31.0% in SBF, which indicated that the two types of fibers also exhibit moderate degradability in SBF and might be suitable for preparation of bioactive and degradable composite scaffolds for tissue engineering applications.

4. Conclusion

Needlelike, highly dispersible and pure $Ca_2(SiO_3)(OH)_2$ and β-Ca_2SiO_4 fibers have been prepared by a hydrothermal process. The β-Ca_2SiO_4 fibers exhibit strong hydration in SBF in the early stage and subsequently can develop a bonelike carbonate-containing HAp layer accompanying with moderate degradability. The $Ca_2(SiO_3)(OH)_2$ fibers as a novel calcium silicate hydrate, can also precipitate carbonate-containing HAp on its surface within several days in SBF with moderate degradability. This study suggests that these fibers possess certain desirable characteristics including bioactivity, degradability and morphological stability in SBF, and both are potential candidates as reinforcement agents in developing novel fibre-reinforced composites for biomedical applications.

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