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# Bioactivity of $CaSiO_3$/poly-lactic acid (PLA) composites prepared by various surface loading methods of $CaSiO_3$ powder

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**Abstract** Mixing bioactive ceramic powders with polymers is an effective method for generating bioactivity to the polymer-matrix composites but it is necessary to incorporate up to 40 vol% of bioactive ceramic powder. However, such a high mixing ratio offsets the advantages of the flexibility and formability of polymer matrix and it would be highly advantageous to lower the mixing ratio. Since surface loading of ceramic powders in the polymer is thought to be an effective way of reducing the mixing ratio of the ceramic powder while maintaining bioactive activity, $CaSiO_3$/poly-lactic acid (PLA) composites were prepared by three methods; (1) casting, (2) spin coating and (3) hot pressing. In methods (1) and (2), a suspension was prepared by dissolving PLA in chloroform and dispersing $CaSiO_3$ powder in it. The suspension was cast and dried to form a film in the case of method (1) while it was spin-coated on a PLA substrate in method (2). In method (3), $CaSiO_3$ powder was surface loaded on to a PLA substrate by hot pressing. The bioactivity of these samples was investigated in vitro using simulated body fluid (SBF). Apatite formation was not observed in the samples prepared by method (1) but some apatite formation was achieved by mixing polyethylene glycol (PEG) with the PLA, producing a porous polymer matrix. In method (2), apatite was clearly observed after soaking for 7 days. Enhanced apatite formation was observed in method (3), the thickness of the resulting apatite layers becoming about 20 μm after soaking for 14 days. Since the amount of $CaSiO_3$ powder used in these samples was only ≤0.4 vol%, it is concluded that this preparation method is very effective in generating bioactivity in polymer-matrix composites by loading with only very small amounts of ceramic powder.

## Introduction

Although ceramics such as hydroxyapatite (HAp) [1], bioglass [2], apatite/wollastonite glass-ceramics (A-W-GC) [3], $CaSiO_3$ [4], etc show bioactivity, their mechanical properties, especially their brittleness and Young's modulus, are unsatisfactory for biomedical applications. By contrast, polymers have the advantages of flexibility and ease of shaping but they are bioinert and not bioactive. Therefore, composites of bioactive ceramic powders with polymers are thought to be an effective way of producing bioactive materials with suitable mechanical and shaping properties.

Such bioactive ceramic/polymer composites were first reported by Bonfield et al [5], who prepared high-density polyethylene (HDPE) polymer matrix composites with dispersed HAp powder (HAPEX$^{\text{TM}}$). Similar composites were also prepared from Bioglass$^{®}$/HDPE [6] and A-W-GC/HDPE [7]. It is, however, necessary to add a high content (about 40 vol%) of ceramic powder to generate the bioactive function in these composites, determined by *in vitro* experiments using simulated body fluid (SBF) [8].

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Mixing such a high content of ceramic powder with polymer causes drastic changes in the properties of the resulting composites by comparison with HDPE polymer. In the case of Bioglass®/HDPE composites [6], Wang et al. reported a reduction of tensile strength from 17.9 to 10.2 MPa and fracture strain from 360 to 8.5 % but increased Young's modulus (from 0.65 to 2.54 GPa) and hardness (from 7.1 to 8.8 VHN) with increasing content of bioglass powder from 0 to 40 vol%. Although the composites can be prepared by extrusion [6] and injection methods [9], a drastic decrease of the fracture strain reflects the strong degradation of the properties due to the presence of the ceramic powders.

To suppress this disappointing degradation of the properties of composites by the introduction of high contents of ceramic powder into the polymer, it is important to reduce the loading of ceramic powder. The following two approaches have been considered for this purpose; (1) to use a ceramic powder with greater bioactivity than the above-mentioned ceramic powders and (2) to prepare composites with only surface loading of the ceramic powder. For the first approach, $CaSiO_3$ powder is a good candidate because it is known to show very fast bone-like apatite formation in SBF [10]. The maximum formation rate of apatite by $CaSiO_3$ ceramics in SBF is up to about 12 μm/day [11]. Such fast apatite formation by $CaSiO_3$ was also reported by Li and Chang [12] in porous polymer matrix composites containing 40 vol% $CaSiO_3$ powder, i.e. 10 μm/7 days in $CaSiO_3$/poly-lactic acid (PLA). This formation rate is higher than those of similar composites (10 μm/21 days in Bioglass®/PLA and 1 μm/21 days in HAp/polymer [12]). We also investigated the bioactivity of $CaSiO_3$/PLA composites with 10–40 vol% of uniformly dispersed $CaSiO_3$ powder prepared by twin-roll mixing, and found that apatite formation occurred even in a composite containing only 10 vol% of $CaSiO_3$ powder after surface abrading the samples [13].

In this paper, the influence of surface loading of $CaSiO_3$ powder in reducing the required amount of ceramic powder was examined for $CaSiO_3$/PLA composites prepared by three methods, i.e. (1) casting, (2) spin coating and (3) hot pressing. The bioactivities were evaluated under in vitro conditions using SBF.

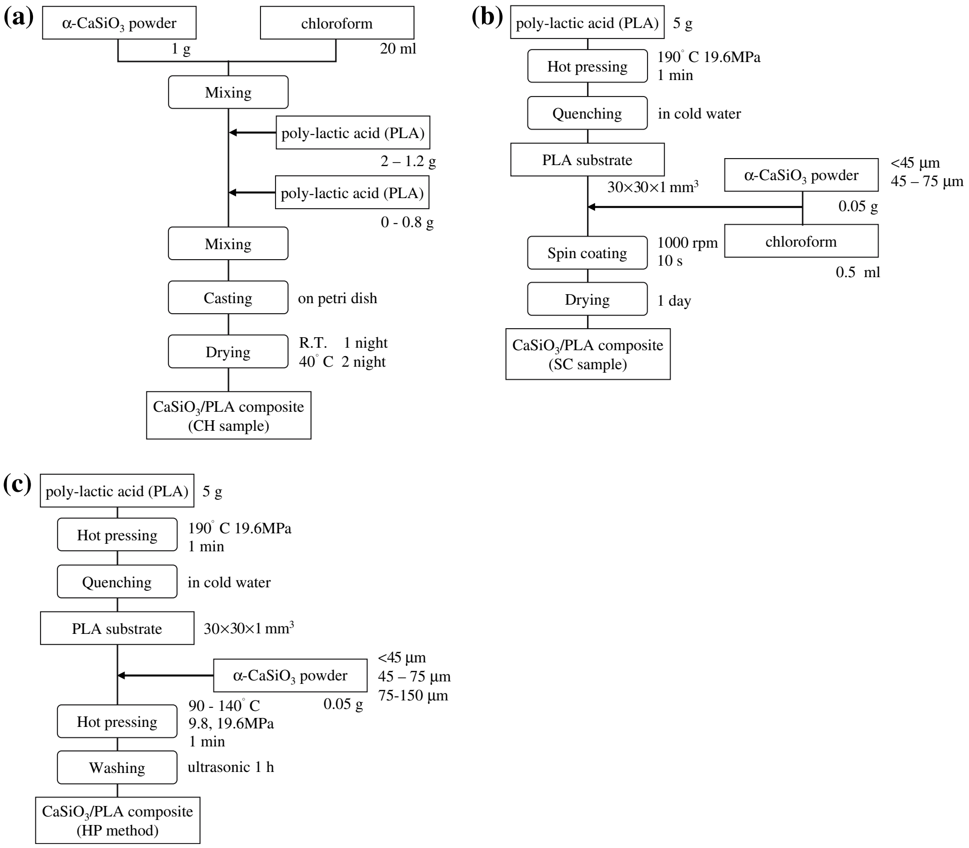


**Fig. 1** Experimental flowchart for the three sample preparation methods: (**a**) casting method; (**b**) spin coating method; and (**c**) hot pressing method

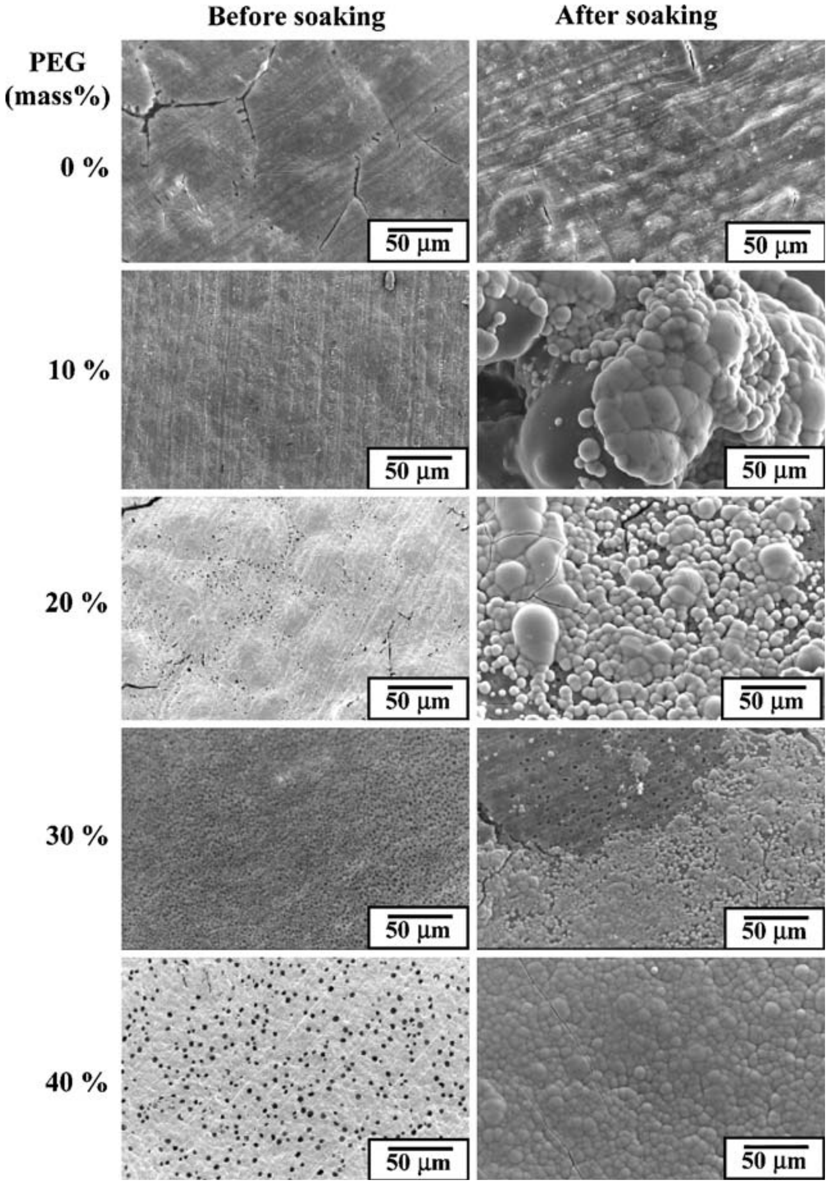


**Fig. 2** Microstructures of the CH samples with various polyethylene glycol contents before and after soaking in SBF. The soaking time is 7 days except for the PEG = 0% sample (5 days)

## Experimental

### Preparation and characterization of $CaSiO_3$ powder

The $CaSiO_3$ powders were synthesized by coprecipitation using NaOH as the precipitant. The starting materials were $Ca(NO_3){\cdot}4H_2O$ and $Si(OC_2H_5)_4$ (TEOS). 0.03 mol of each was separately dissolved in ethanol (150 ml) and the solutions were mixed by stirring for 2 h. A precipitate was obtained by rapidly adding 180 ml of NaOH (0.06 mol) to the solution. The precipitate was filtered, washed twice with 90 ml of distilled water and dried in an oven at 110°C overnight. The dried powder was calcined at 700°C for 6 h to remove residual nitrate and ethanol then fired at 1300°C for 2 h to obtain crystalline $\alpha$-$CaSiO_3$ powder (pseudowollastonite; the high temperature phase). This powder was ground in an agate mortar and pestle and sieved to <100 mesh.

The chemical composition of the $\alpha$-$CaSiO_3$ powder was determined by X-ray fluorescence (RINT-2000, Rigaku, Tokyo, Japan). The crystalline phases in the samples were identified by powder X-ray diffraction (Lab-X XRD-6100, Shimadzu, Kyoto, Japan) using monochromated Cu K$\alpha$ radiation.

### Preparation of $CaSiO_3$/PLA composites

PLA (Lacty®, Shimadzu, Kyoto, Japan) of particle size 3–4 mm was used. This had a ratio of mass average molecular weight/number average molecular weight of 1.7.

The flowchart for the preparation of composite samples by the casting method is shown in Fig. 1a. $\alpha$-CaSiO$_3$ powder (1 g) was dispersed in 20 ml of chloroform and added to PLA (2 g) with stirring. The suspension was then cast in a petri dish and dried at room temperature overnight, then at 40°C for 2 days. The $\alpha$-CaSiO$_3$ powder in the composite was calculated to be about 18 vol%. Since the resulting composites were not soft and flexible, 10–40 mass% of polyethylene glycol (PEG) was mixed with the PLA at a constant total amount of polymer. These composite samples are designated CH.

The flowchart for the preparation of composite samples by the spin coating method is shown in Fig. 1b. PLA sheets were prepared by hot pressing the PLA powder at 190°C and 19.6 MPa for 1 min. A suspension of $\alpha$-CaSiO$_3$ powder in chloroform was spin coated on the PLA substrate at 1000 rpm for 10 s. Two $\alpha$-CaSiO$_3$ powders with particle sizes of $\le$45 and 45–75 μm were used for the preparation of these composites. The amount of $\alpha$-CaSiO$_3$ powder in these composites is calculated to be about 0.4 vol%, considerably less than in the cast samples (18 vol%) and also in other reported composites (40 vol%) [5–7, 9, 12]. These samples are designated SC.

The flowchart for the preparation of composite samples by hot pressing is shown in Fig. 1c. PLA slabs were prepared as in the spin coating method. In this case, the $\alpha$-CaSiO$_3$ powder was placed on a hot pressing plate, overlaid with the PLA slab and hot pressed at 90, 120 and 140°C at pressures of 9.8 and 19.6 MPa for 1 min. The resulting samples were ultrasonically washed for 1 h. Three $\alpha$-CaSiO$_3$ powders with particle sizes of $\le$45, 45–75 and 75–150 μm were used to prepare these composites. The amount of $\alpha$-CaSiO$_3$ powder in these samples was 0.4 vol% before washing, decreasing to <0.4 vol% after washing. These samples are designated HP.

### Soaking in SBF

Simulated body fluid (c-SBF) was prepared using the recipe reported by Kokubo [7]. The composite samples were cut to $10 \times 20\ \text{mm}^2$ (for cast samples) and $30 \times 30\ \text{mm}^2$ (for the other samples), and immersed in the SBF solution at 36.5°C for various times. The volume of SBF solution was adjusted to a sample/SBF ratio of 2.5 mg/ml. After the predetermined soaking time, the samples were removed from the SBF solution, gently rinsed with distilled water and dried at room temperature.

The surfaces of the soaked and unsoaked samples were investigated using XRD and SEM (JSM-T200, JEOL, Tokyo, Japan). Changes in the Ca, P and Si concentrations of the SBF solution after soaking the samples were determined using inductively coupled plasma atomic emission spectroscopy (ICP-OES; Prodigy, JEOL Datum, Tokyo, Japan).

## Results and discussion

### Cast samples

The microstructures of as-prepared and soaked CH samples (5 days in the case of the PEG = 0% sample and 7 days in the case of the PEG-containing samples) are shown in Fig. 2. The surface of the as-prepared CH

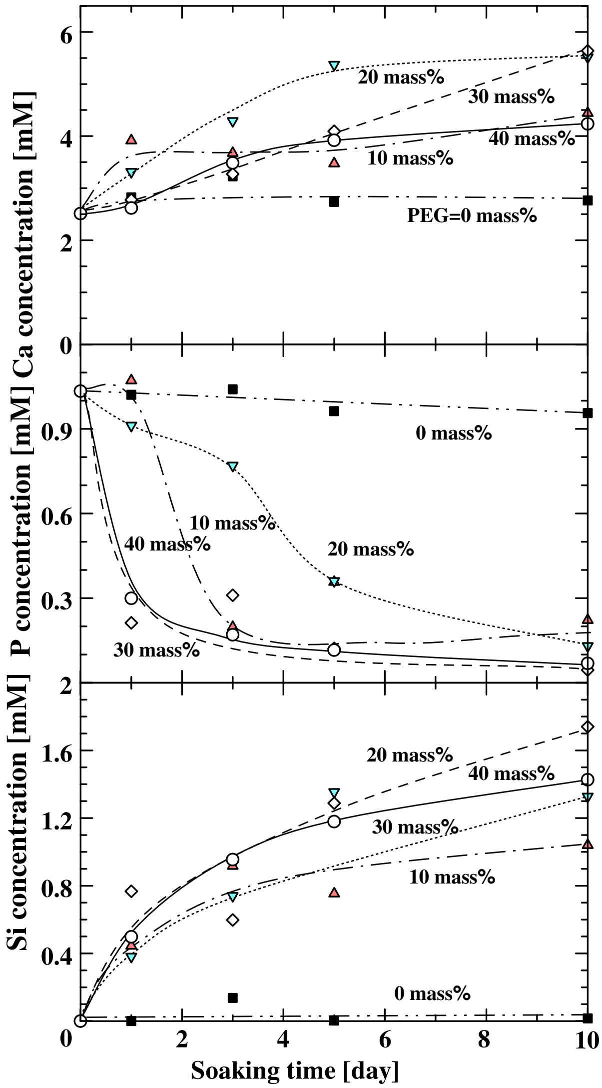


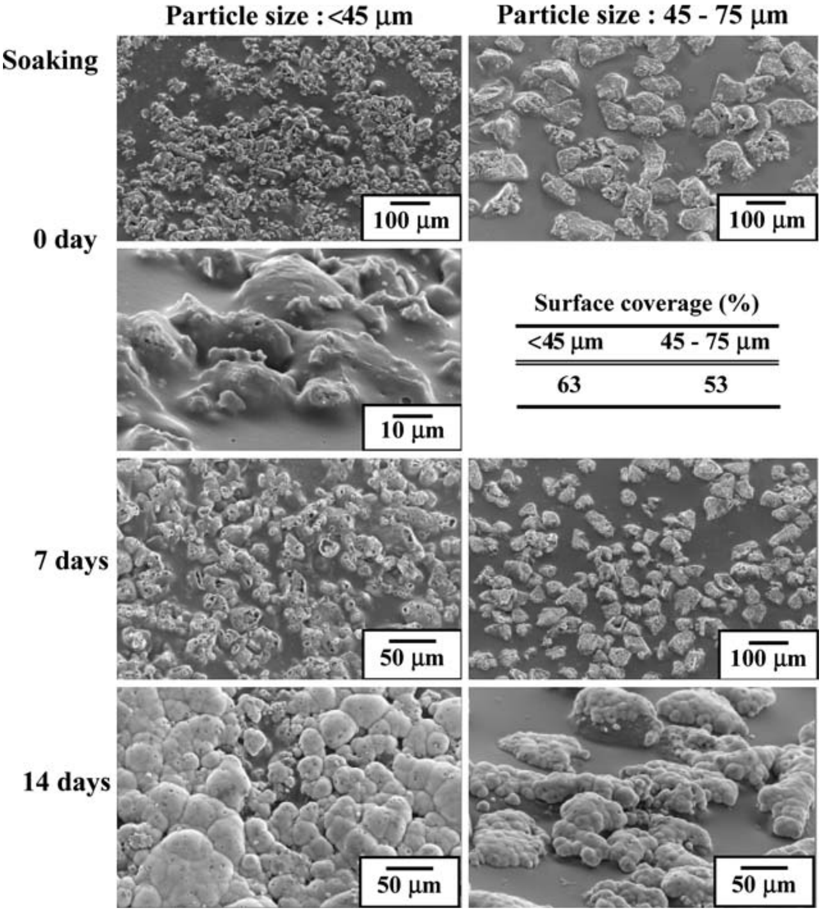
**Fig. 3** Change in the Ca, P and Si concentrations of SBF in the CH samples as a function of soaking time

sample (PEG = 0%) was relatively smooth but showed many cracks, thought to be formed during the evaporation of chloroform upon drying. This suggests that the composite is not flexible enough to recover the formed spaces due to the rather rigid polymer matrix. The XRD pattern of this sample clearly showed many peaks of crystalline PLA and $\alpha$-$CaSiO_3$. The crystallization of PLA may be the reason for the rigid state of this sample. After soaking in SBF, the surfaces became uneven due to hydrolysis of the PLA, but no apatite was formed after soaking for 10 days. Changes in the concentrations of Ca, P and Si in the SBF soaking solution are shown in Fig. 3 as a function of soaking time. Almost no change occurred in the concentrations after soaking. Although we expected relatively fast dissolution of PLA in SBF, the degradation rate of PLA was found to be rather slow and the $\alpha$-$CaSiO_3$ powder contained in the composites was not exposed in the surface of the CH sample.

To improve the flexibility of sample CH, PEG was mixed with PLA as a plasticizer. With increasing content of PEG, the composites became soft and flexible. The microstructures of the unsoaked CH samples show the evolution of small pores in the surfaces of the composites containing PEG. The number and size of the pores increased with increasing content of PEG. After soaking for 7 days, these composites clearly showed apatite formation (Fig. 2). The pores in these composites are thought to be formed by phase separation, producing a porous polymer matrix. As a result, the $\alpha$-$CaSiO_3$ particles in the composites have greater contact with the SBF. In these CH samples, the Ca and Si concentrations in the SBF soaking solution increased while the P concentration decreased with increasing soaking time (Fig. 3). These concentration changes are caused by selective leaching of $Ca^{2+}$ from $\alpha$-$CaSiO_3$ and the formation of amorphous $SiO_2$, which then forms apatite by reaction with sorbed $Ca^{2+}$ and $HPO_4^{2-}$ ($+H_2PO_4^-$) from the SBF [7].

### Spin coated samples

As described above, bioactivity can be achieved by mixing 18 vol% of $\alpha$-$CaSiO_3$ powder with PLA/PEG to form polymer matrix composites. To further decrease the amount of $\alpha$-$CaSiO_3$ powder required, the composites were prepared by spin-coating the suspension



Surface coverage (%)

| <45 μm | 45 - 75 μm |
|---|---|
| 63 | 53 |

**Fig. 4** Microstructures of the SC samples containing ≤45 and 45–75 μm particles before and after soaking in SBF (7 and 14 days)

used in the casting method on a PLA substrate. In this case, the amount of α-$CaSiO_3$ powder in the composites was found to be only 0.4 vol%. The microstructures of the as-prepared and soaked composites (SC samples) are shown in Fig. 4. The surfaces were covered with many α-$CaSiO_3$ particles in an island structure. The surface coverage ratios (%) evaluated from the SEM photographs are 63 and 53% in the SC samples loaded with ≤45 and 45–75 μm particles, respectively. The particles are seen to be covered with a thin PLA polymer film and are not directly exposed on the surface. After soaking for 7 days, a small amount of apatite is formed on the surface of the SC sample loaded with ≤45 μm particles, by hydrolysis of the surface PLA film. By contrast, no apatite formation occurred in the sample loaded with 45–75 μm particles due to slower hydrolysis and/or fewer α-$CaSiO_3$ particles. After soaking for 14 days, the former sample showed almost complete surface coverage by apatite while only the surfaces of the α-$CaSiO_3$ particles were covered by apatite in the latter sample. The surface covering by the PLA film is the main reason for slower apatite formation in the SC samples compared with the PEG-containing CH samples.

Changes in the Ca, P and Si concentrations in the SBF solutions from these two SC samples are shown in Fig. 5 as a function of soaking time. The two samples show a similar trend of decreasing Ca and P concentration and increasing Si concentration. The unusual behavior of the $Ca^{2+}$ concentration (generally increasing) is related to the amount of α-$CaSiO_3$ powder used in the preparation of the composites, i.e. consumption of $Ca^{2+}$ by apatite formation > dissolution of α-$CaSiO_3$ due to the very small amount of α-$CaSiO_3$ powder in the composites. From the microstructural observation, the amount of apatite formed is greater in the sample loaded with smaller particles (≤45 μm) but the concentration changes are larger in the sample loaded with larger particles (45–75 μm). One possible explanation for this result is the formation of an amorphous calcium phosphate phase in addition to apatite in the latter sample because greater leaching of α-$CaSiO_3$ particles may result from the higher Si concentration in the soaked SBF. More detailed investigation is, however, necessary to fully understand this unexpected result.

### Hot pressed samples

The spin coating method has the advantage of being able to reduce the loading of α-$CaSiO_3$ powder but the thin PLA film formed on the surfaces of the α-$CaSiO_3$ particles suppressed apatite formation on exposure to SBF. It is thus found that direct exposure of α-$CaSiO_3$ powder on the surface of the composites is vitally important. This led to the third method, i.e. hot pressing of α-$CaSiO_3$ powder on a PLA substrate to load the surface with α-$CaSiO_3$. The microstructures of the three HP samples (pressed at 90°C and 9.8 MPa) loaded with ceramic powder of different particle sizes are shown in Fig. 6 for the as-prepared sample (a), the sample after ultrasonic washing (b), and soaked for 7 days (c) and 14 days (d). The surface coverage ratios of the three HP samples were higher than in samples prepared by the other methods, and increased from 73 to 86% with smaller ceramic particle size. Since these particles were only pressed into the surface of the samples, the less-firmly fixed particles were washed away by the ultrasonic treatment. After this washing

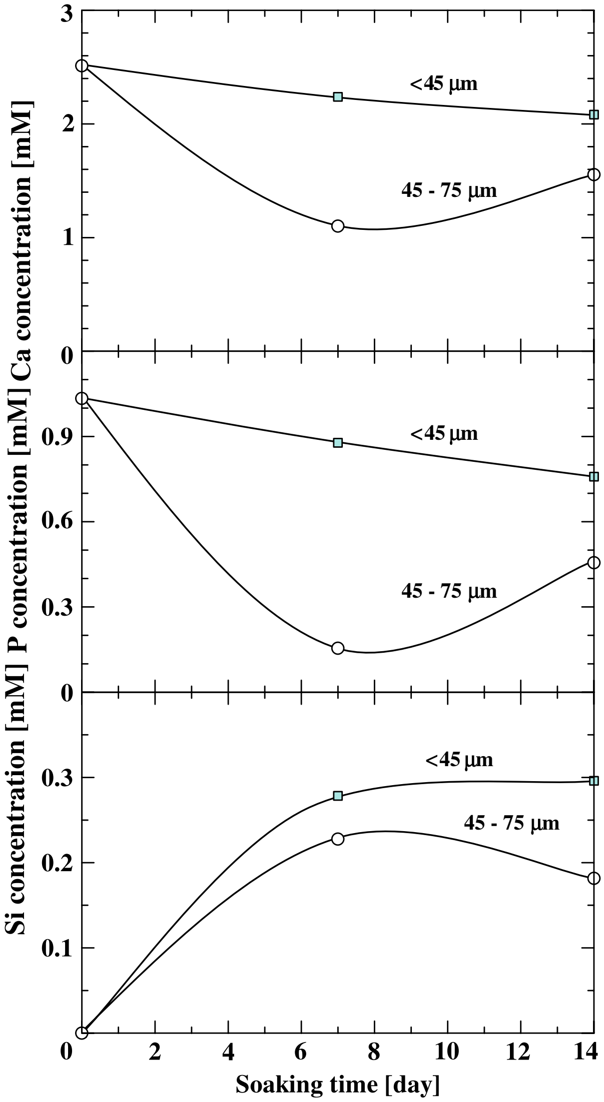


**Fig. 5** Change in the Ca, P and Si concentrations of SBF in the SC samples as a function of soaking time

treatment (Fig. 6b), the sample loaded with ≤45 μm particles showed a greater degree of powder attachment than the other two samples. Although we also prepared HP samples at higher pressing temperatures (120 and 140°C) and/or higher pressures (19.6 MPa), the attachment of the α-$CaSiO_3$ particles was lower in these than in the samples pressed at 90°C and 9.6 MPa.

After soaking for 7 days (Fig. 6c), all three HP samples showed apatite formation. The amount of apatite was greater in the samples containing ceramic powder of smaller particle size. This is related to the increased surface coverage of the HP samples. The apatite almost covered the surface of the HP sample loaded with the smallest α-$CaSiO_3$ particles (≤45 μm), but small, isolated apatite particles were formed on the surfaces of the α-$CaSiO_3$ particles in the samples prepared with the largest α-$CaSiO_3$ particles (75–150 μm). After 14 days soaking (Fig. 6d), the apatite formed a continuous layer about 20 μm thick on the sample loaded with the smallest α-$CaSiO_3$ particles. This apatite formation rate is similar to that reported for porous $CaSiO_3$/PLA composites containing 40 vol% $CaSiO_3$ [12]. It is thus evident that the HP loading method is very effective in generating bioactive functionality in composites with very small amounts of α-$CaSiO_3$ loading. By contrast, a decreased amount of apatite was formed in the HP sample loaded with larger α-$CaSiO_3$ particles. These results suggest that the particle size of α-$CaSiO_3$ on the surface of the composites is an important factor in the formation of apatite, and better results are obtained by loading with smaller α-$CaSiO_3$ particles.

Changes in the Ca, P and Si concentrations in the SBF solution after soaking the three HP samples are shown in Fig. 7 as a function of soaking time. Although there were apparent differences in the

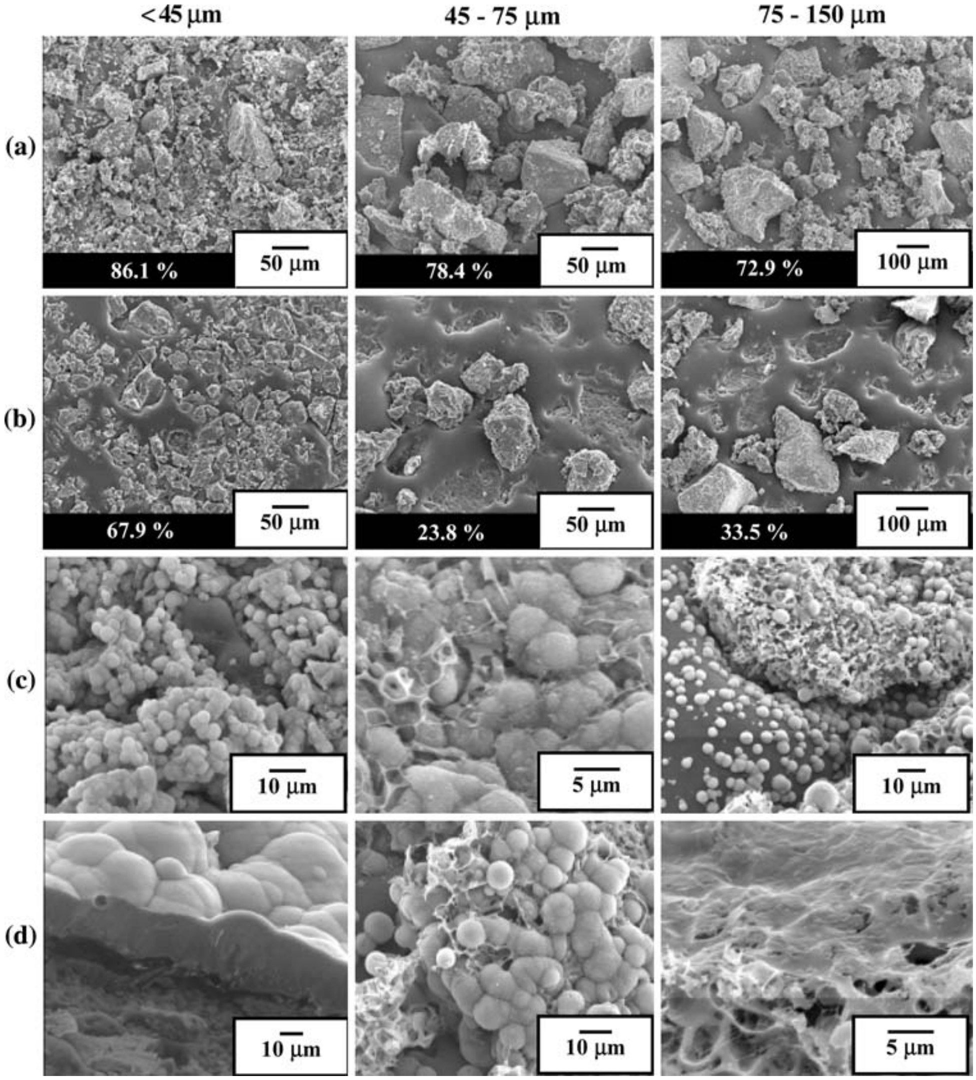


**Fig. 6** Microstructures of the HP samples containing ≤45, 45–75 and 75–150 μm particles before and after soaking in SBF: (**a**) as-prepared; (**b**) after ultrasonic washing; (**c**) after 7 days soaking; and (**d**) after 14 days soaking

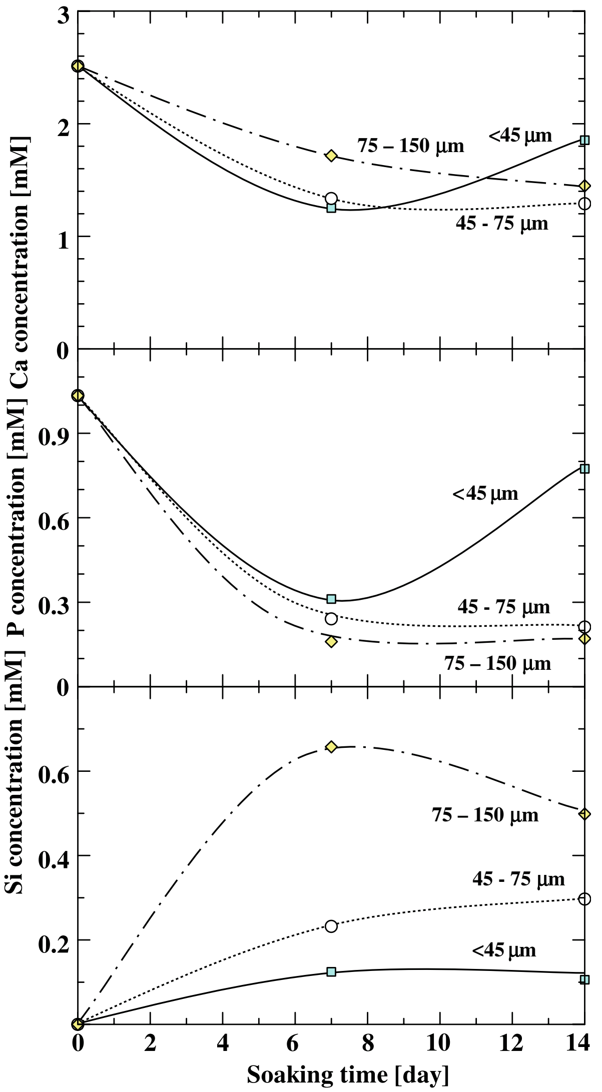


**Fig. 7** Change in the Ca, P and Si concentrations of SBF in the HP samples as a function of soaking time

amount of apatite formed on the HP samples, the changes in Ca and P concentrations are very similar, significant differences being observed only in the Si concentrations, which are higher for the larger α-$CaSiO_3$ particle size. This indicates a greater degree of dissolution of α-$CaSiO_3$ powder and corresponds to a trend towards lower apatite formation. Thus, more amorphous calcium phosphate is formed in the samples with larger α-$CaSiO_3$ particle size. This result is consistent with that obtained for the SP samples. As we have reported elsewhere [11], the rate of apatite formation in SBF largely depends on the formation rate of amorphous $SiO_2$ by selective leaching of α-$CaSiO_3$ particles. We thus consider that the rate of α-$CaSiO_3$ dissolution and precipitation of amorphous $SiO_2$ in SBF is a factor in the enhancement of apatite formation.

## Conclusion

$CaSiO_3$/poly-lactic acid (PLA) composites were prepared using three different methods to produce PLA matrices with lower amounts of $CaSiO_3$ powder and the resulting bioactivities were investigated. The following results were obtained;

(1) CH method: Composites prepared by casting a suspension of PLA and dispersed α-$CaSiO_3$ particles in chloroform showed no apatite formation in SBF. By mixing polyethylene glycol (PEG) with the PLA, the composites began to show apatite formation as a result of the formation of porous polymer matrix.

(2) SC method: Composites prepared by spin coating the above suspension on a PLA substrate showed apatite formation in SBF. Since the composites contained only 0.4 vol% of α-$CaSiO_3$ powder, this method is much better than the casting method. Apatite formation in the composites was enhanced by using smaller α-$CaSiO_3$ particles but the presence of a thin PLA film covering the particles suppressed apatite formation.

(3) HP method: Composites prepared by hot pressing α-$CaSiO_3$ powder onto a PLA substrate showed the fastest apatite formation of the three methods, with the smallest amount of α-$CaSiO_3$ powder (<0.4 vol%). A maximum thickness of apatite was formed on composites containing <45 μm powder and the formation rate was about 20 μm/14 days.

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