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Study on physicochemical properties and in vitro bioactivity of tricalcium silicate–calcium carbonate composite bone cement

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Abstract In this article, a novel bone cement composed of tricalcium silicate (Ca_3SiO_5; C_3S) and calcium carbonate ($CaCO_3$) was prepared with the weight percent of $CaCO_3$ in the range of 0, 10, 20, 30, and 40%. The initial setting time was dramatically reduced from 90 to 45 min as the content of $CaCO_3$ increased from 0 to 40%, and the workable paste with a liquid/powder (L/P) ratio of 0.8 ml/g could be injected between 2 and 20 min (nozzle diameter 2.0 mm). The composite cement showed higher mechanical strength (24–27 MPa) than that of the pure Ca_3SiO_5 paste (14–16 MPa). Furthermore, the composite cement could induce apatite formation and degrade in the phosphate buffered saline. The results indicated that the Ca_3SiO_5–$CaCO_3$ paste had better hydraulic properties than pure Ca_3SiO_5 paste, and also the composite cement was bioactive and degradable. The novel bone cement could be a potential candidate as a bone substitute.

1 Introduction

Materials with self-setting properties have been exploited to augment human bone tissues and have drawn more attention in the recent years. Calcium phosphate cement (CPC) has been receiving much interest for its good plastic properties, biocompatibility and osteoconductivity [1–3]. However, most of the CPCs have hydroxyapatite precipitates during the setting and the resorbability of CPC under physiological conditions is limited by the low-degradation property of hydroxyapatite [4, 5]. Therefore, research into other more resorbable materials, such as brushite cement [6] and calcium carbonate cement [7], has been ongoing. In our previous studies, the properties of tricalcium silicate (Ca_3SiO_5, C_3S) cement as injectable bone substitute have been investigated, and the results indicated that Ca_3SiO_5 paste possessed good injectablility, bioactivity, and moderate in vitro degradability [8]. However, the application of Ca_3SiO_5 as bone cement has been hampered by its low setting rate and, to a lesser extent, low short-term mechanical strength [8, 9].

In the Portland cement industry, calcium carbonates are often used as hydration accelerator and filler component within cement paste, which reduce the setting time and promote the mechanical strength of tricalcium silicate paste [10, 11]. In biomedical applications, calcium carbonates of biological origin (nacre and coral) and their derivatives have been used as biocompatible and absorbale bone substitutes in the form of powder, porous ceramic and hydraulic cement [8, 12, 13]. To combine the advantages of the Ca_3SiO_5 and $CaCO_3$, we present here a Ca_3SiO_5–$CaCO_3$ composite cement. Since Ca_3SiO_5 paste is bioactive and $CaCO_3$ is resorbable, the composite materials are expected to form a biphasic bone cement system with good bioactivity and moderate degradability, and the self-setting properties could also be improved.

In this study, the setting times, injectability and mechanical strength of Ca_3SiO_5–$CaCO_3$ composite cement were investigated. Moreover, the in vitro bioactivity and degradability of the composite cement was also evaluated.

2 Materials and methods

2.1 Material preparation and characterization

Tricalcium silicate powders were prepared by sol–gel method as previously described [14]. The resultant powders

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were ground and sieved to 300-mesh (52 μm) for further experiments. Reagent-grade calcium carbonate (calcite, CC) powders with a particle size around 10 μm were purchased from Sinopharm Chemical Reagent Co., Ltd. The obtained Ca_3SiO_5 and $CaCO_3$ powders were mixed with $CaCO_3$ content in the range of 0–40% (weight ratio). To prepare the pastes, the obtained composite cement powder was mixed with deionized water with a liquid to powder (L/P) ratio of 0.8 ml/g. The mixtures were stirred to form homogeneous pastes within 40 s, transferred to stainless steel moulds with a diameter of 6 mm, and then stored in a 37°C, 100% humidity water bath for various time periods.

After the pastes were set for given intervals, they were soaked in acetone for 2 h to stop hydration and then air-dried. The phase composition of the paste after setting was characterized by X-ray diffraction (XRD, Geigerflex, Rigaku Co., Japan) using monochromated CuK_α radiation ($\lambda = 1.5418$ Å, 100 mA, 40 kV) in a continuous scan mode. The 2θ range was from 10° to 80° at a scanning speed of 10°/min. To cross section the materials, the as-prepared paste samples were carefully cleaved by hand and no further polishing procedure was conducted on the samples. Then the obtained cross section of the material was gold coated using a sputter coater at 30 mA for 150 s. The cross section of the samples was observed by scanning electron microscopy (SEM, JXA-8100, JEOL, Tokyo, Japan), and the SEM data were collected using the apparatus operating at a voltage of 20.0 kV and a working distance (WD) of 11 mm.

2.2 Setting time and injectability of the cement paste

The setting times of the composite pastes were measured by the Vicat needle method according to ISO9597–1989E. The initial setting time is defined as the time necessary so that the light needle (280 g, Ø1.13 mm) plunges into the paste and has a span of 5 ± 1mm to the tube bottom. The final setting time is defined as the time necessary so that the heavy needle (350 g, Ø2.0 mm) no longer leaves a visible print on the surface of the paste. Each specimen was measured five times and the average value was calculated.

Injectability of the composite paste was evaluated by extruding a certain amount of paste through a disposable syringe by hand according to a modified method described previously [15, 16], which suggested that injection by hand was acceptable in practice since the standard deviations for injection by hand was even slightly lower than that for injection by machine with preset load. The syringes have a capacity of 2.5 ml with an opening nozzle diameter of 2.0 mm. Six grams of as-prepared paste were added into the syringe and after restored in a water bath at 37°C for pre-set time periods, the paste was gently extruded from the syringe by hand until it was completely unable to be injected. Then the weight of the paste expelled from the syringe was measured and the injectability was calculated using Eq. 1 [15, 16]. Each test was repeated at least three times and the average value was calculated.

$$\text{Inj}(\%) = \frac{\text{Paste weight expelled from the syringe}}{\text{Total paste weight before injecting}} \quad (1)$$

2.3 Mechanical test of the cement paste

After stored in a 100% humidity water bath at 37°C for 2, 7, 10, 14, 21 and 28 days, the hardened cement samples were removed from the steel moulds (6 mm diameter × 10 mm high). The compressive strength of the samples was measured at a loading rate of 0.5 mm min^{-1} using a universal testing machine (Instron-1195, USA) according to ASTM D695-91. Six replicates were carried out for each group and the results were expressed as mean ± standard deviation (mean ± SD).

2.4 In vitro bioactivity

The SBF solution was prepared according to the procedure described by Kokubo [17]. The ion concentrations of the SBF are similar to those in the human blood plasma. The 7-day-set paste disks (6 mm in diameter and 2 mm in height) were soaked in the SBF solution at 37.0 °C in a shaking water bath for 4 days with a surface area-to-volume ratio of 0.1 cm^{-1} [17, 18]. At each time point, the disks were gently rinsed with deionized water to remove SBF solutions followed by drying at room temperature. The samples were characterized by XRD (Geigerflex, Rigaku Co., Japan) and SEM (JSM-6700F, JEOL, Tokyo, Japan).

2.5 In vitro degradation of the paste

For evaluation of degradation, the 7-day-set paste disks were soaked in phosphate buffered saline (PBS; pH = 7.3–7.4) at 37°C in shaking water bath for 2, 4, 7, 10, 14 and 21 days with a surface area-to-volume ratio of 0.1 cm^{-1} [17, 19]. The solution was refreshed every day. After the set soaking time, the disks were dried at 60°C for 24 h and the final weight of each sample was measured accurately. The degradation rate was calculated by dividing the weight loss by its initial weight.

3 Results

3.1 Characterization of the cement paste

Figure 1 shows the XRD patterns of Ca_3SiO_5–$CaCO_3$ composite paste with *L/P* ratio of 0.8 ml/g after setting for 7 days at 37°C and 100% relative humidity. For pure

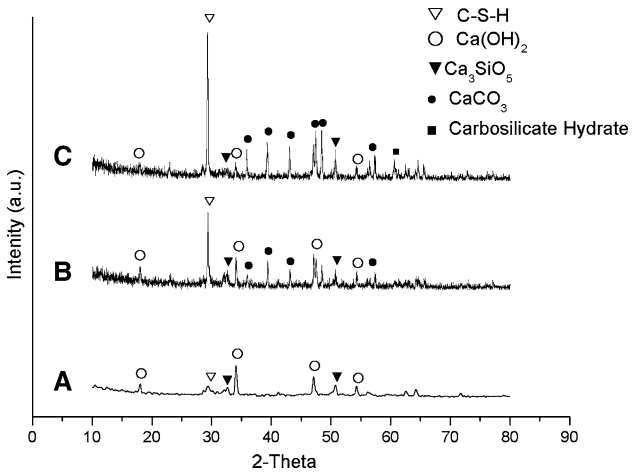

Fig. 1 XRD patterns of the paste samples with *L*/*P* ratio of 0.8 ml/g after setting for 7 days, (a) the pure tricalcium silicate paste; (b) the composite paste with 10% $CaCO_3$; (c) the composite paste with 30% $CaCO_3$

Ca_3SiO_5 paste, three phases were identified in XRD patterns. Calcium hydroxide [$Ca(OH)_2$] and calcium silicate hydrate (C–S–H) appeared due to the hydration of Ca_3SiO_5. In addition, unhydrated tricalcium silicate was also observed, while for the composite samples, two additional phases (carbosilicate hydrate and $CaCO_3$ as seen in Fig. 1b and c) were observed within the pastes as compared with those in the pure Ca_3SiO_5 paste [10]. It was also noticed that with the increase of $CaCO_3$ content in the composite cement, the intensity of the peaks for C–S–H and carbosilicate hydrate increased.

Figure 2 shows SEM micrographs of the cross section of the Ca_3SiO_5–$CaCO_3$ composite paste with *L*/*P* ratio of 0.8 ml/g after setting for 7 days. No significant structural differences were observed among composite samples with different weight ratio of $CaCO_3$. Therefore, SEM micrographs of the composite with 30% $CaCO_3$ were taken as representation for micro-structural characteristics of composite pastes. Comparison of composites and pure tricalcium silicate pastes indicated that $CaCO_3$ crystals were uniformly located on the surface of the hydrate particles of the Ca_3SiO_5/$CaCO_3$ cement (Fig. 2c and d).

3.2 Workability and mechanical strength of the cement paste

Figure 3 shows the initial and final setting time of the composite pastes with different amount of calcium carbonate. The result indicated that $CaCO_3$ had an accelerating effect on the setting rate of the tricalcium silicate paste and the setting time decreased with the increase of the weight ratio of $CaCO_3$. The composite with 40% $CaCO_3$ showed the shortest initial (45 min) and final (85 min) setting time.

Figure 4 shows the injectability of the paste with different contents of $CaCO_3$. It indicated that as the content of $CaCO_3$ increased up to 20%, the paste still had an excellent injectability similar to that of the tricalcium silicate paste.

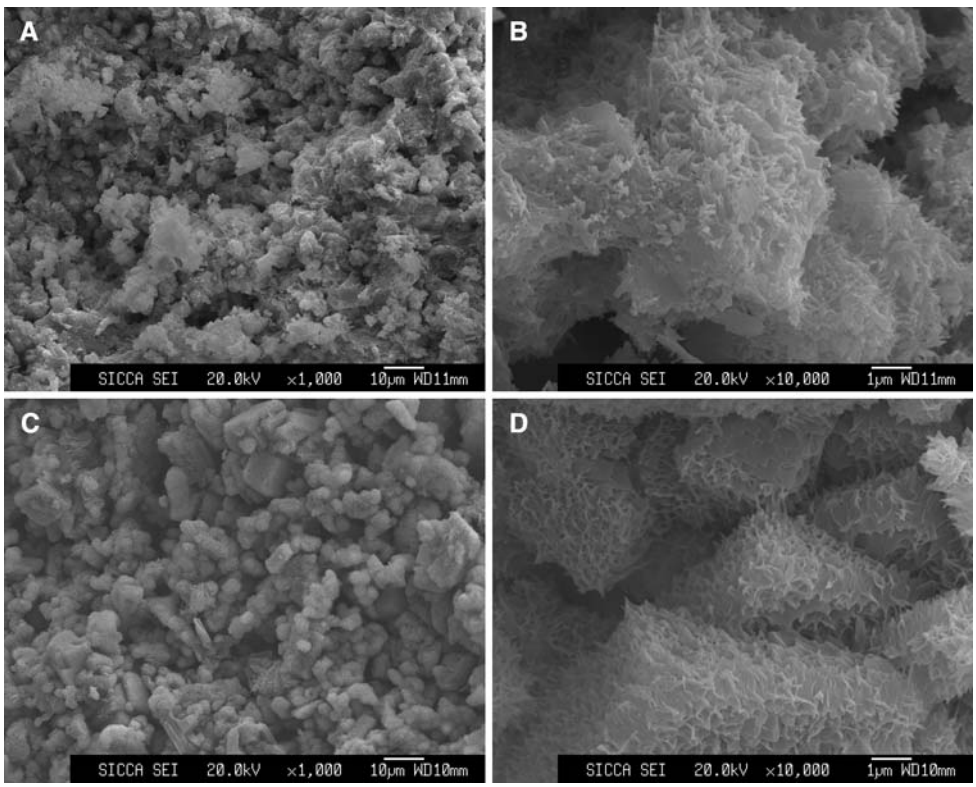

Fig. 2 SEM micrographs of the pure Ca_3SiO_5 paste (**a**, **b**) and the Ca_3SiO_5–$CaCO_3$ composite paste with 30% $CaCO_3$ (**c**, **d**) after setting for 7 days (**a**, **c** ×1,000; **b**,**d** ×10,000)

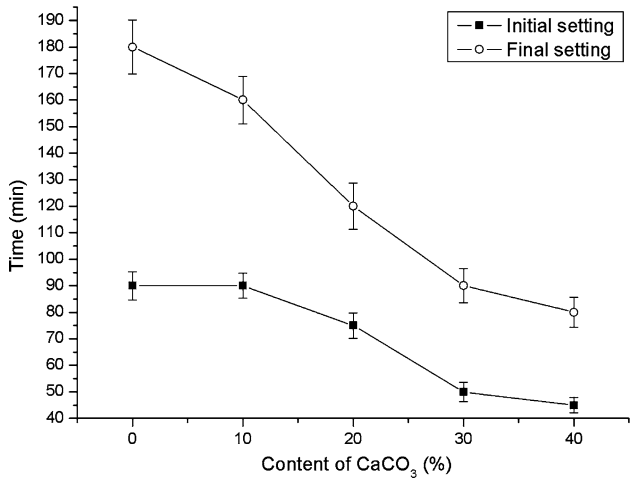

Fig. 3 The initial setting and final setting time of the paste samples ($L/P = 0.8$ ml/g)

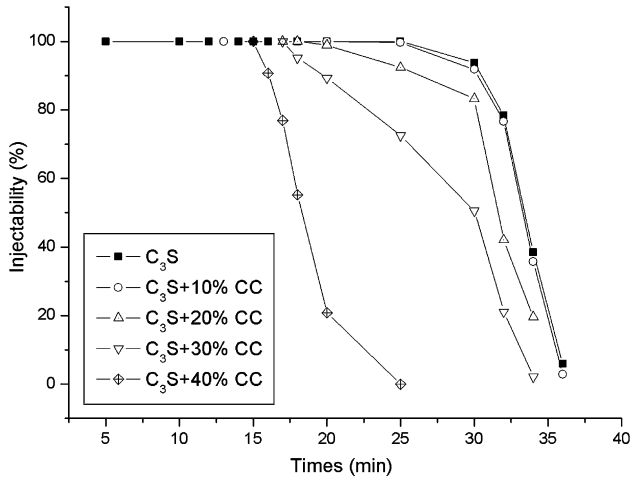

Fig. 4 The injectablity of the paste samples with various contents of $CaCO_3$ ($L/P = 0.8$ ml/g)

However, a significant decrease in the injectability was observed, when the content of $CaCO_3$ exceeded 30%.

Figure 5 shows the compressive strength of the paste with L/P ratio of 0.8 ml/g after setting for various time periods. The results indicated that the compressive strength of all the paste samples increased gradually with time proceeding. At each setting period, the compressive strength of the composite paste increased with the increase of $CaCO_3$ contents, reached the maximal value at 30% $CaCO_3$ addition, and then decreased dramatically with further increase of $CaCO_3$ to 40%.

3.3 In vitro bioactivity

To determine the bioactivity of the composite pastes, the paste disks with different amount of $CaCO_3$ were soaked in

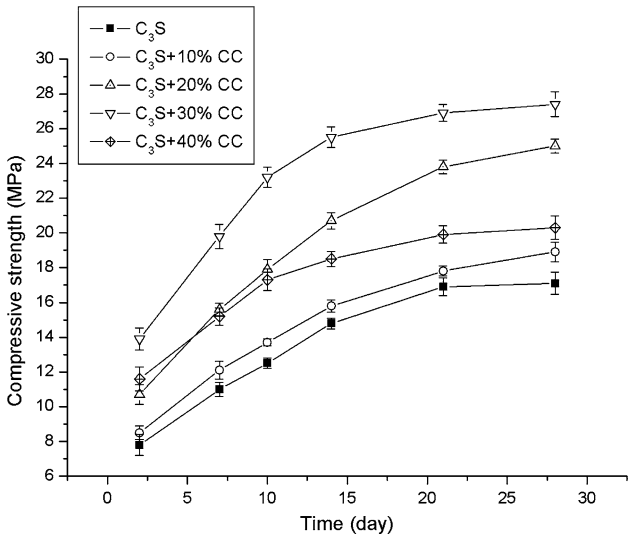

Fig. 5 The compressive strength of the paste samples with various contents of $CaCO_3$ versus setting times ($L/P = 0.8$ ml/g)

SBF, and it is observed that for all the composite samples in the present study, apatite layers were formed on the surface after soaking. Taken the composite with 30% $CaCO_3$ for example, XRD and SEM patterns of the samples after soaking in SBF are shown in Figs. 6 and 7. It was clear to see from Fig. 6 that characteristic peaks of $Ca(OH)_2$ disappeared and characteristic peaks for apatite at $2\theta = 26.04°$ and 32.72° appeared after 7 days of soaking [8, 17, 18]. Figure 7 shows SEM micrographs of the surfaces of composite paste with 30% $CaCO_3$ after soaking in SBF for 7 days which showed that a bone-like apatite layer was formed on the composite paste surface [18]. The higher magnification SEM micrograph (Fig. 7b) showed that the lath-like particles of bone-like apatite formed agglomerates.

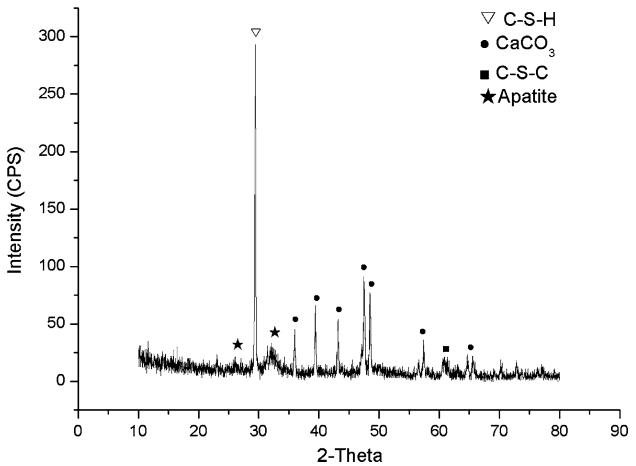

Fig. 6 XRD patterns of the composite paste with 30% $CaCO_3$ after soaking in SBF for 7 days

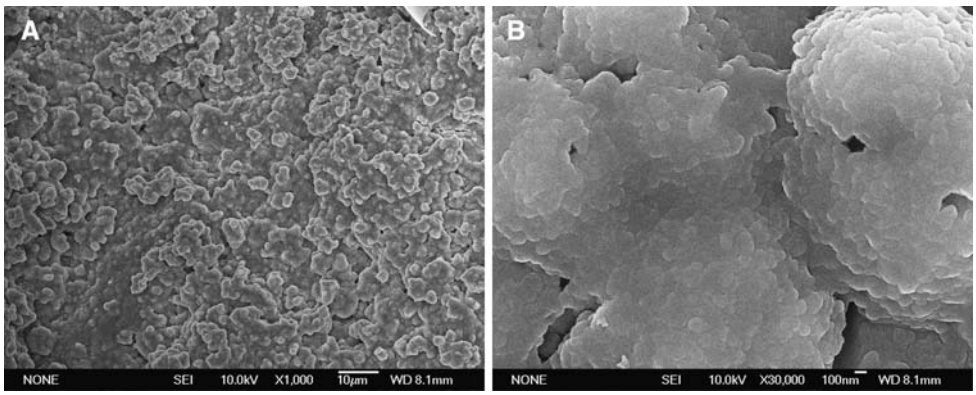

Fig. 7 SEM micrographs of the surfaces of the composite paste with 30% $CaCO_3$ after soaking in SBF for 7 days (**a** ×1000, **b** ×10,000)

3.4 In vitro degradation

Figure 8 shows the weight loss of the composite paste with different contents of calcium carbonate after soaking in PBS for various time periods. It was clear to see that, with the addition of calcium carbonate, the degradation rate of the composite paste was significantly higher than that of pure Ca_3SiO_5 paste and the degradation rate of the composite increased with the increase of $CaCO_3$ contents.

4 Discussion

The applicability of injectable self-setting biomaterials is largely dependent on its self-setting characteristics. Although with good injectability, the long setting time of Ca_3SiO_5 paste due to its low hydration rate greatly limited its clinical application. In the present study, the results indicated that the addition of $CaCO_3$ could significantly reduce the initial and final setting time of Ca_3SiO_5 paste,

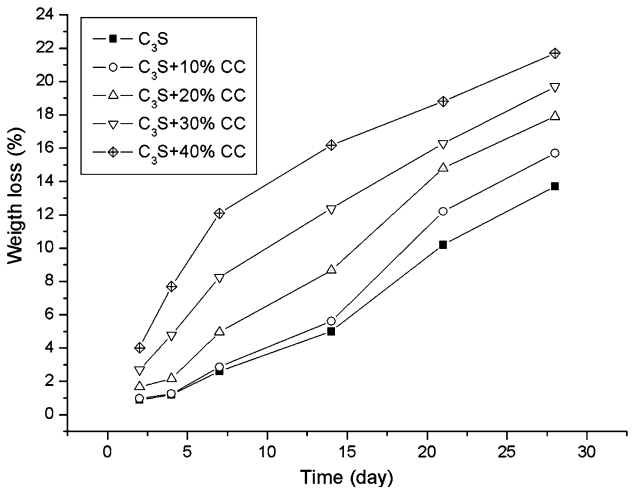

Fig. 8 Degradation of the paste samples with various contents of $CaCO_3$ after soaking in PBS for various times (L/P = 0.8 ml/g)

which could be attributed to accelerative effect of $CaCO_3$ on the hydration of Ca_3SiO_5 [20, 21], resulting in acceleration of the setting process of Ca_3SiO_5 paste [9–11, 20]. Moreover, the formation of insoluble phase as carbosilicate hydrate (probably scawtite, $Ca_7(Si_6O_{18})(CO_3) \cdot 2H_2O$ [10]) between $CaCO_3$ and calcium silicate hydrate can promote the setting rate of the composite paste [10, 11]. This improved setting process could result in an enhanced mechanical strength of the cement paste at the early stage of the setting as compared with the pure tricalcium silicate cement.

It is well accepted that the addition of fine $CaCO_3$ particles into Portland cement with Ca_3SiO_5 as a main component can lead to superior mechanical strength as compared to that of Portland cement, which is attributed to its filler effect and accelerative effect on the hydration of the cement [10, 11, 20, 22]. Now, our results confirmed this phenomenon with pure Ca_3SiO_5. In addition, our study also indicated that the effect of $CaCO_3$ particles on the mechanical strength of Ca_3SiO_5 cement was dose dependent, and as the addition of $CaCO_3$ exceeded a certain weight ratio (as 30% in present study), the mechanical strength began to decrease. The above results suggested that there was an optimal incorporation ratio of $CaCO_3$ into Ca_3SiO_5–$CaCO_3$ bone cement system as presented here, and similar results have been claimed by researches on other $CaCO_3$-contained cement systems [20, 22].

Some reviews are available on the developing trends of self-setting biomaterials, indicating the fact that the bioactive and degradable materials exhibit extensive prospects [23, 24]. Ideally, the materials should be bioactive and be replaced ultimately by natural tissue while it stimulates tissue regeneration. The results of XRD and SEM analysis (Figs. 6 and 7) in the present study verified that Ca_3SiO_5–$CaCO_3$ composite paste could induce the formation of bone-like apatite layer on its surface in SBF regardless of the ratio of the incorporated $CaCO_3$ within the range of 0–40%. In addition, the composite paste showed a significantly higher degradation rate than that of the pure

Ca_3SiO_5 paste, and the degradation rate of the composite paste increased with the increased amount of $CaCO_3$. Considering that the degradability is primarily governed by the chemical composition and physical characteristics of the material, it is assumed that $CaCO_3$ has a higher dissolution rate as compared with that of calcium silicate hydrate [7, 25], which resulted in a higher degradation rate of the composite paste as compared with that of pure Ca_3SiO_5 paste. Our results also suggested that the degradation rate of the composite paste could be adjusted by the addition of $CaCO_3$. However, further studies need to be conducted to confirm the in vivo degradation properties of the composite paste.

5 Conclusions

In this article, a biphasic bioactive bone cement made of tricalcium silicate and calcium carbonate was studied. The Ca_3SiO_5–$CaCO_3$ composite cement showed dramatically reduced setting time and enhanced mechanical strength as compared with those of pure tricalcium silicate cement. In addition, the composite cement with various compositions showed good bioactivity, and the degradation rate of the composite cement could be adjusted by the modulation of $CaCO_3$ content for specific clinical application. With the combination of enhanced self-setting properties, good bioactivity and adjustable degradation rate, the Ca_3SiO_5–$CaCO_3$ composite cement might be a potential candidate as bioactive self-setting biomaterial for applications as injectable implant material for bone regeneration.

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