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Calcium–phosphate–silicate composite bone cement: self-setting properties and in vitro bioactivity

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Abstract In this study, a novel low temperature setting calcium phosphate–silicate cement was obtained by mixing $CaHPO_4 \cdot 2H_2O$ (DCPD) and Ca_3SiO_5 (C_3S) with 0.75 M sodium phosphate buffers (pH = 7.0) as liquid phase. The self-setting properties of the obtained DCPD/C_3S paste with liquid to powder ratio (L/P) of 0.6 ml/g, such as setting times, injectability, degradability and compressive strength were investigated and compared with that of DCPD/CaO cement system. The results indicated that, with the weight ratio of C_3S varied from 20% to 40%, the workable DCPD/C_3S pastes could set within 20 min, and the hydrated cement showed significantly higher compressive strength (around 34.0 MPa after 24 h) than that of the DCPD/CaO cement system (approximately 10.0 MPa). Furthermore, the in vitro pH value of the cements was investigated by soaking in simulated body fluid (SBF) for 12 h, and the result indicated that the DCPD/C_3S did not induce significant increase or decrease of pH value in SBF. Additionally, the composite cement possesses better ability to support and stimulate cell proliferation than the DCPD/CaO cement. With good hydraulic properties, improved biocompatibility and moderate degradability, the novel DCPD/C_3S bone cement may be a potential candidate as bone substitute.

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1 Introduction

Recently, with the advent of minimally invasive surgery techniques, materials with self-setting properties have been exploited to augment human bone tissues and have drawn more attention [1–3]. Since the study by Chow and Brown [4], calcium phosphate cements (CPCs) have attracted much interest because of their chemical similarity to the mineral phase of bone tissue [1] and good osteoconductivity for bone reconstruction [5]. In clinical application, calcium phosphate cements can be easily manipulated and shaped, provide intimate adaptation to the contours of defect surfaces, and set in situ in the bone cavity to form a solid restoration [6].

Numerous CPC formulations have been proposed and systematically investigated in the last 25 years, and presently CPCs can be mainly divided into two classes according to their possible end products after hydration, that is, brushite CPC and apatite cement such as hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) or calcium-deficient hydroxyapatite (CDHA, $Ca_9(HPO_4)(PO_4)_5OH$) cement [7]. Previous study described that by mixing a powder consisting of dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$) and calcium oxide (CaO) with a sodium phosphate (NaP) buffer as liquid phase, an apatite CPC with fast-setting ability and good rheological properties was obtained [8]. However, as an apatite cement, the DCPD/CaO cement was essentially non-degradable, and with a relative low mechanical strength (around 10 MPa as compressive strength) and in vitro release of OH^- ions, the material is limited to be used as a root canal filling cement with antimicrobial activity [8, 9].

As one of the most important components of the Portland cement, tricalcium silicate ($3CaO \cdot SiO_2$, C_3S) has been previously used as injectable bone cement, which

showed excellent in vitro bioactivity and moderate degradability [10]. However, the application of C_3S as bone cement has been hampered by its low setting rate and, to a lesser extent, low short-term mechanical strength [10, 11]. In our study, based on the DCPD/CaO cement system, tricalcium silicate was selected to substitute for CaO to form a novel DCPD/C_3S bone cement system. When reacted with water, tricalcium silicate can spontaneously develop strength (spontaneous consolidation) by hydration reaction according to Eq. 1 [12].

$$3CaO \cdot SiO_2 + zH_2O \rightarrow Ca_xSi(OH)_y \cdot nH_2O(C-S-H) + (3-x)Ca(OH)_2 \quad (1)$$

Furthermore, the basic hydration product $Ca(OH)_2$ may react with the neutral DCPD to form hydroxyapatite, which was similar in setting mechanism to some known CPC formulations [6, 8]. Therefore, with self-setting tricalcium silicate as a component, the obtained DCPD/C_3S cement may possess superior mechanical strength as compared to that of the DCPD/CaO cement, and as the hydration product of tricalcium silicate cement is originally degradable [10], the DCPD/C_3S cement may show higher degradability than the DCPD/CaO apatite cement.

In this work, the setting times, mechanical strength and in vitro pH of DCPD/C_3S composite cement were investigated and compared with those of DCPD/CaO cement. Moreover, the in vitro bioactivity and degradability of the composite cement was also evaluated.

2 Materials and methods

2.1 Preparation of the DCPD/CaO cement

To prepare the DCPD/CaO cement, all chemicals of analytical reagent grade (DCPD, $CaCO_3$, NaH_2PO_4, $Na_2HPO_4 \cdot 10H_2O$) were purchased from Sinopharm Chemical Reagent Co. To obtain CaO powders, commercial $CaCO_3$ was heated at 900°C for 6 h to remove H_2O and CO_2 and stored in a vacuum desiccator. The DCPD/CaO cement powders were prepared by weighing appropriate amounts of the two components to obtain the desired calcium-to-phosphate molar ratio of 1.67. The liquid phase was 0.75 M sodium phosphate (NaP) buffer (pH = 7) prepared from NaH_2PO_4 and $Na_2HPO_4 \cdot 10H_2O$, and when the cement powders were incorporated in the liquid phase, the liquid-to-powder (L/P) ratio was set to 0.6 ml/g. The parameters mentioned above were selected according to the previous study [8], in which the DCPD/CaO cement system showed preferred injectability and mechanical properties under these conditions.

2.2 Preparation of the DCPD/C_3S cement

Tricalcium silicate powders were prepared by sol–gel method as previously described [13], and the resultant powders were ground and sieved to 300-mesh. To prepare the DCPD/C_3S cement powders, DCPD and C_3S powders were weighted and mixed to form mixtures, and the weight ratio of C_3S within the composite was 20, 30 and 40%.

For the sake of clarity, the liquid phase for DCPD/C_3S cement was 0.75 M sodium phosphate buffers (pH = 7) prepared from NaH_2PO_4 and $Na_2HPO_4 \cdot 10H_2O$, and the L/P ratio was set to 0.6 ml/g.

2.3 Characterization of the paste samples

After mixing for 2 min, the DCPD/CaO and DCPD/C_3S pastes were moved into molds (6 mm diameter × 2 mm high), clamped and stored at 37°C and 100% relative humidity (RH) for 7 days. Then, the paste samples were immersed in ethanol for 2 h to stop hydration and air-dried. The phase composition of the samples was characterized by X-ray diffraction (XRD; Geigerflex, Rigaku Co., Japan) using monochromated CuK_α radiation in a continuous scan mode. The 2θ range was from 10° to 80° at a scanning speed of 10°/min. The cross-section of the samples was observed by scanning electron microscopy (SEM; JSM-6700F, JEOL, Tokyo, Japan) equipped with an energy dispersive X-ray detector (EDX, INCA Energy, Oxiford Instruments, UK), 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.

2.4 Setting time and injectability of the cement paste

The setting times of the DCPD/CaO and DCPD/C_3S pastes were measured with the Vicat needle 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 ± 1 mm 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 repeated 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 [14], 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. Two 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. 2 [14]. Each test was repeated at least three times and the average value was calculated.

$$\text{Inj}\ (\%) = \frac{\text{Pasteweightexpelledfromthesyringe}}{\text{Total paste weight before injecting}} \quad (2)$$

2.5 Measurement of the mechanical strength

For the compressive mechanical testing, the as-prepared slurries were poured into a cylindrical polytetrafluoroethylene mold (6 mm diameter and 8 mm height). Air bubbles entrapped in the paste during mixing were allowed to escape by gently shaking the mold. After aging at 37°C in 100% RH for pre-set periods (1, 3, 7 and 14 days), the cement samples were immediately removed from the molds and the wet compressive strength 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).

2.6 In vitro pH measurement

To test the in vitro pH variation of the cement paste in simulated body environment, the cement slurries were directly poured into simulated body fluid (SBF) with the paste/saline volume ratio of 1:3 and stored in a 37°C, 100% humidity water bath [15, 16]. After 1 h of immersion, the suspension was collected and the pH value was recorded using an electrolyte-type pH meter (pHS-2C, Jingke Leici Co., Shanghai, China), and then the SBF solution for immersion was renewed. The procedure was repeated every hour over a period of 12 h.

2.7 In vitro bioactivity and degradability

The SBF was prepared according to the procedure described by Kokubo [15, 17]. The ion concentrations of the SBF are similar to those in human blood plasma, and the pH value of the SBF was exactly adjusted to 7.40 at 37°C [17]. The 24-h-set paste disks (6 mm in diameter and 2 mm in height) were soaked in the SBF solution at 37°C in a shaking water bath for 7 days with a surface area-to-volume ratio of 0.1 cm^{-1} [15, 17]. After the pre-selected soaking time, the disks were gently rinsed with deionized water to remove SBF solutions followed by drying at room temperature. The samples were characterized by X-ray diffraction (XRD, Geigerflex, Rigaku Co., Japan), scanning electron microscopy (SEM; JSM-6700F, JEOL, Tokyo, Japan).

For evaluation of degradation, the 7-day-set paste disks were soaked in phosphate buffered saline (PBS) solution at 37°C in shaking water bath for 4, 7, 10, 14 and 21 days with a surface area-to-volume ratio of 0.1 cm^{-1} [15, 18], and the 7-day-set paste disks were selected because the cements were assumed to be fully hydrated after the period [8, 9]. 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 accurately measured. The degradation was calculated by dividing the weight loss by its initial weight.

2.8 Cytotoxicity test for the composite cements

The cell proliferation assay was performed by extraction method with the mice bone marrow stromal cells (mBMSC) according to the method reported in ISO 10993-5 [19]. The 24-h-set DCPD/CaO paste and the composite paste with 40% C_3S were crushed to powders and sieved through a 300-mesh (50 μm) for further experiments. The dissolution extracts were prepared by adding the cement powders to Roswell Park Memorial Institute 1640 (RPMI 1640; Gibco, USA) cell culture medium for 1 day at 37°C in a humidified atmosphere of 5% CO_2 and 95% air without agitation. Ratios between the powder weight (mg) and the medium volume (ml) were 12.5, 25, 50, 100 and 200 mg ml^{-1}. After incubation, the mixture was centrifuged and the supernatant was collected.

The cell suspension was adjusted to a density of 1×10^4 cell ml^{-1}, and 100 μl cell suspension was added to each well of a 96-well plate and incubated for 24 h. The culture medium in each well was then removed and replaced by 50 μl of extracts and 50 μl of RPMI 1640 medium supplemented with 20% fetal calf serum (FCS) every second day. The number of viable cells was quantitatively assessed by MTT test [20]. MTT (Sigma, USA) (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) is a yellow tetrazolium salt, which can be enzymatically converted by living cells to a purple formazan product. The intensity of the color produced is therefore directly proportional to the number of viable cells in culture, and thus to their proliferation in vitro. The absorbance of the color observed can be measured at 590 nm (A_{590}). In brief, after incubating at 37°C and 5% CO_2 for 5 days, 100 μl of 0.5 mg ml^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added to extract/cell constructs and cultured for 4 h at 37°C. Then 100 μl dimethyl sulfoxide (DMSO) was added to each well, the plate was shaken for 5 min, and the optical density (OD) at 590 nm was measured with an enzyme-linked immunoadsorbent assay (ELISA) plate reader (ELX800, Bio-TEK, USA).

The medium supplemented with 10% FCS without addition of extracts was used as a control. Six samples per group were tested in the experiment and results were reported as OD units.

2.9 Statistical methods

The experimental values were analyzed using standard analysis of Student's *t*-test and expressed as mean ± standard deviation (SD). A P-value < 0.05 was considered statistically significant.

3 Results

3.1 Characterization of the paste samples

Figure 1 shows the XRD patterns of the DCPD/CaO and DCPD/C_3S (20%, 40%) pastes after setting at 37°C and 100% RH for 7 days. For the DCPD/CaO paste, mainly peaks for calcium-deficient hydroxyapatite (CDHA) were observed. With the substitution of C_3S for CaO, it was noticed that the diffraction peaks of CDHA for the hydrated DCPD/C_3S pastes were not obviously altered, and in the XRD patterns of the composite paste with 20% C_3S, peaks for unreacted DCPD were detected. Furthermore, the presence of calcium silicate hydrate (C–S–H) (the hydration product of C_3S) within DCPD/C_3S pastes was also confirmed by XRD patterns.

Figure 2 shows the SEM micrographs of the cross section of the DCPD/CaO and DCPD/C_3S (20%, 40%) composite paste after setting for 7 days. It was observed

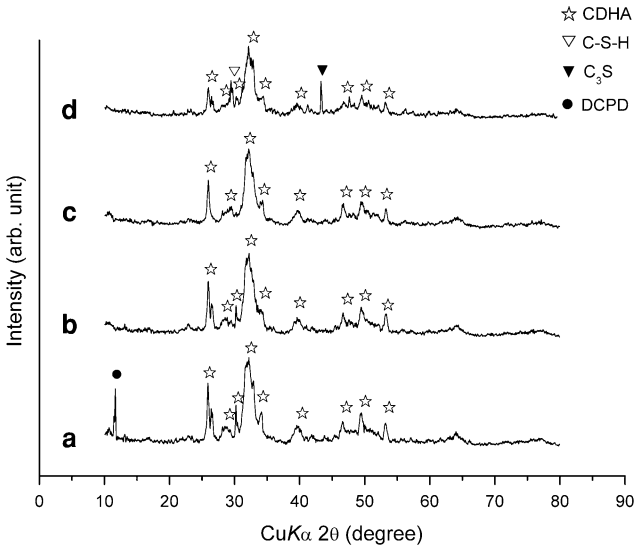

Fig. 1 XRD patterns of the paste samples after setting for 7 days, (a) DCPD/CaO paste; (b) the DCPD/20%C_3S paste; (c) the DCPD/30%C_3S paste; (d) the DCPD/40%C_3S paste

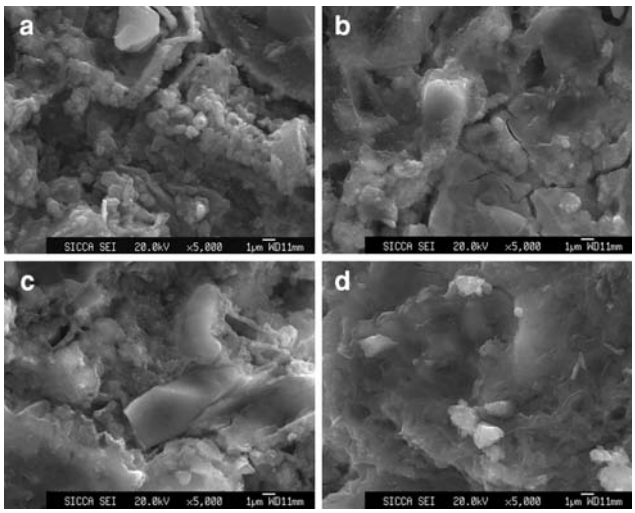

Fig. 2 SEM micrographs of the cross-sections of the paste samples, **a** the DCPD/CaO paste; **b** the DCPD/20%C_3S paste; **c** the DCPD/30%C_3S paste; **d** the DCPD/40%C_3S paste

from the SEM micrographs that the DCPD/C_3S pastes samples showed a more compact microstructure than the DCPD/CaO paste sample. Furthermore, as the content of C_3S increased, the microstructure of the hydrated composite cement became more compact.

3.2 Setting time and injectability of the cement paste

As seen in Table 1, the initial and final setting times of DCPD/CaO paste were 9 min and 13 min, respectively, which were similar to the result of published work by Briak et al. [8] on that cement system. In contrast, for all the DCPD/C_3S composite pastes, the setting times were longer than those of the DCPD/CaO paste, and as the content of C_3S increased from 20% to 40%, the initial and final setting times increased from 17 min and 32 min to 36 min and 60 min, respectively.

Figure 3 represents the injectability of the DCPD/CaO and DCPD/C_3S composite pastes. The result indicated that the injectability DCPD/C_3S composite paste was significantly improved as compared with that of the DCPD/CaO paste, and meanwhile, the DCPD/C_3S composite paste did not give any demixing or filter pressing during extrusion from the syringe as described in other papers [14, 21].

Table 1 The initial and final setting time of the cement pastes (L/P = 0.6 ml g^{-1})

	DCPD/CaO	DCPD/20%C_3S	DCPD/30%C_3S	DCPD/40%C_3S
Initial setting time (min)	9 ± 0.8	17 ± 1.3	19 ± 1.1	32 ± 1.6
Final setting time (min)	13 ± 0.9	36 ± 2.2	41 ± 2.8	60 ± 3.5

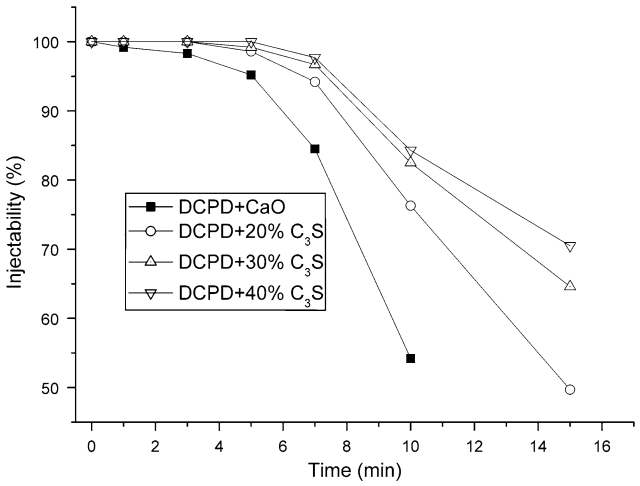

Fig. 3 The injectability of the cement pastes versus setting time (L/P = 0.6 ml g^{-1})

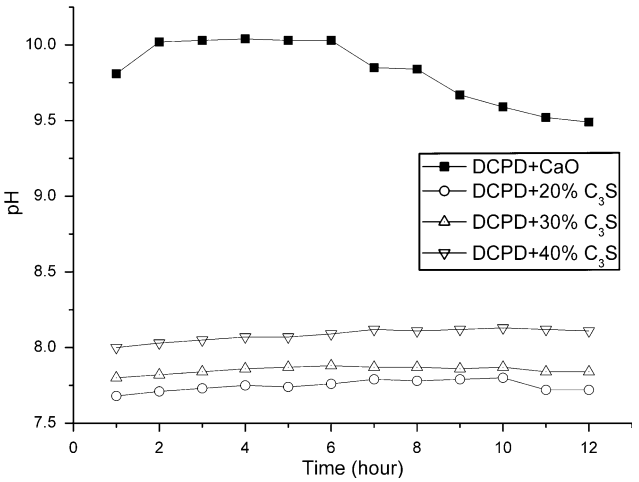

Fig. 5 Changes in pH value of SBF soaked with DCPD/CaO and DCPD/C_3S composite pastes

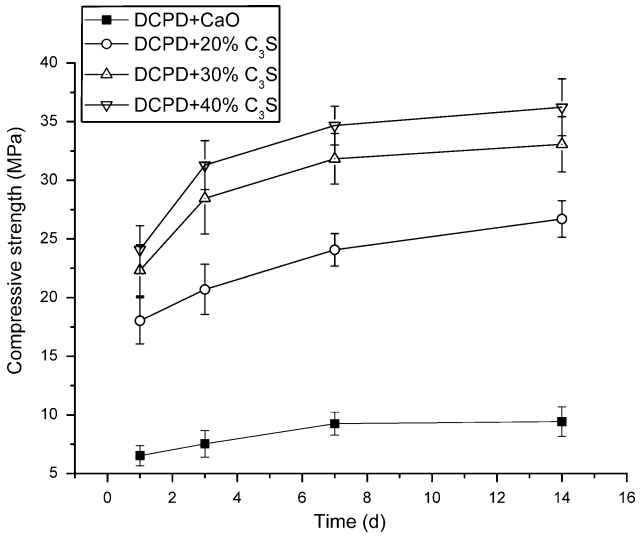

Fig. 4 The compressive strength of the DCPD/CaO and DPCD/C_3S composite pastes after aging for 1, 3, 7 and 14 days (L/P = 0.6 ml g^{-1})

3.3 Mechanical strength of the paste samples

Figure 4 shows the compressive strength of DCPD/CaO and DCPD/C_3S pastes after aging at 37°C and 100% RH for 1, 3, 7 and 14 days. The results indicated that the compressive strength of the DCPD/CaO stabilized around 9.0 MPa during the storage period in 37°C and 100% RH (up to 14 days). In contrast, the compressive strength of the DCPD/C_3S composite paste increased with the increase of the aging time. After preset aging time, the compressive strength of the DCPD/C_3S composite paste increased with the increase of C_3S content, and the composite with 40% C_3S showed the highest compressive strength after a prolonged aging time (36.2 MPa).

3.4 In vitro pH measurement

Figure 5 shows the changes in pH values of SBF caused by the cement pastes. The results showed that, at the beginning of the immersion in SBF solution, the pH value for the DCPD/CaO paste increased rapidly, reached maximum at 10.2 after 3 h, and then decreased gradually to 9.5 after 12 h. Whereas for all the DCPD/C_3S composite paste, the pH increased slowly form 7.35–7.60 to 8.20–8.65 through out the testing period without an initial rapid change.

3.5 The bone-like apatite formation of the paste disks in SBF

Since the main hydration product of both DCPD/CaO and DCPD/C_3S cement systems was CDHA, it is difficult to clarify the ability of the materials to induce the deposition of apatite in SBF. However, since formed apatite grains and layers have characteristic features, the apatite formation can be estimated by SEM [17]. Therefore, to determine the bioactivity of the composite pastes, the paste disks were soaked in SBF and the SEM micrographs of surface of the samples before and after soaking in SBF for 7 days are shown in Fig. 6. It was observed that the DCPD/CaO paste present a porous microstructure consist of large crystals after soaking in SBF. Whereas deposition of tiny crystals with characteristic of bone-like apatite was observed on the DCPD/C_3S sample after soaking in SBF, which has been considered as an important feature of bioactive materials such as Bioglass and calcium silicate ceramics [22, 23]. Furthermore, such apatite layers grew more densely with higher content of C_3S in the cement system.

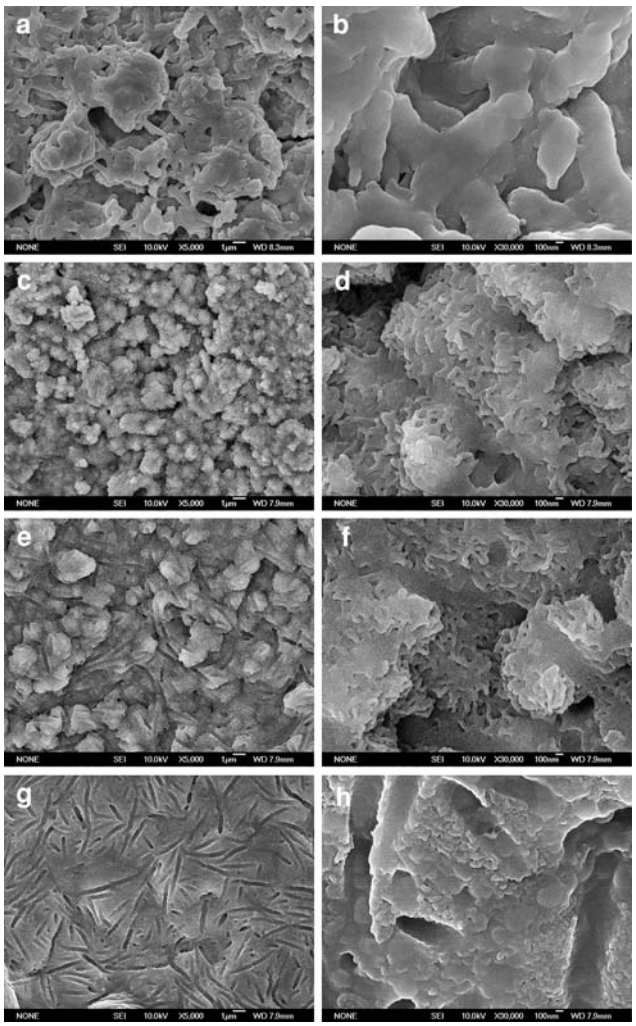

Fig. 6 SEM micrograph of the paste samples after soaking in SBF for 7 days, **a**, **b** the DCPD/CaO paste; **c**, **d** the DCPD/20%C_3S paste; **e**, **f** the DCPD/30%C_3S paste; **g**, **h** the DCPD/40%C_3S paste

3.6 In vitro degradation

Figure 7 shows the degradation of the DCPD/CaO and DCPD/C_3S composite paste with different contents of tricalcium silicate after soaking in PBS solution for various time periods. It was found that the degradation rate of all the DCPD/C_3S paste samples was slightly higher than that of the DCPD/CaO paste samples, and comparison of the DCPD/C_3S composites with different amounts of C_3S indicated that the degradation rate increased with the increase of the C_3S amount.

3.7 Cytotoxicity test

Figure 8 shows the result of cytotoxicity test on the extracts of both DCPD/CaO and DCPD/C_3S composite pastes against the mBMSC after incubation for 5 days. It was observed that the extract of DCPD/CaO paste showed

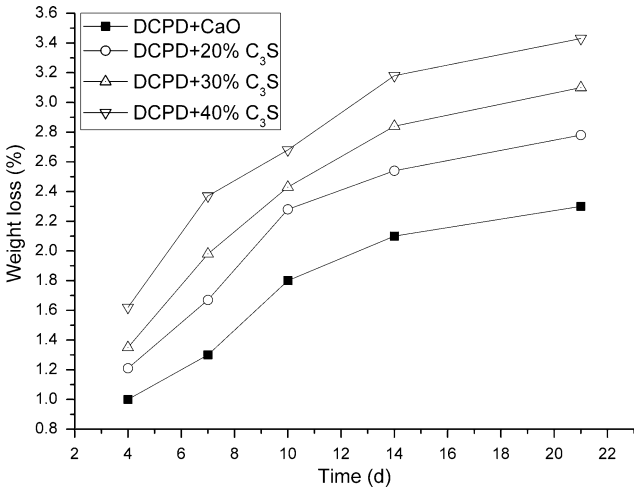

Fig. 7 Weight loss of the DCPD/CaO and DCPD/C_3S composite paste samples after soaking in PBS for various times

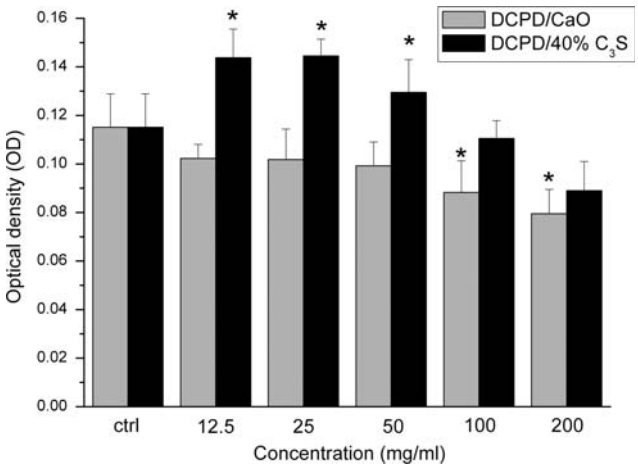

Fig. 8 mBMSC proliferation in the presence of the dissolution extracts of the powders of the DCPD/CaO and DCPD/C_3S composite pastes with 40%C_3S after culturing for 5 days. The asterisk (*) indicates the cell proliferation of experimental group was significantly different from that of negative control ($P \leq 0.05$)

cytotoxicity against osteoblasts when the concentration of the extracts was higher than 100 mg ml^{-1} ($P \leq 0.05$). In contrast, the extracts of the DCPD/C_3S composite paste did not show significant cytotoxicity against osteoblasts in the whole tested concentration range (12.5–200 mg ml^{-1}), which indicated the superior biocompatibility of the DCPD/C_3S paste as compared with that of the DCPD/CaO paste. Furthermore, it was found that, after incubating for 5 days, the mBMSC proliferation in the extract of the DCPD/C_3S paste was significantly higher than in the negative control ($P \leq 0.05$) when the concentration of the extracts was in the range from 12.5 to 50 mg ml^{-1}, while no enhanced proliferation of the cells was observed in the extracts of the DCPD/CaO paste.

4 Discussion

The applicability of injectable self-setting biomaterials is largely dependent on its self-setting characteristic, such as injectability and setting times. The DCPD/CaO in present study possessed rapid-setting property that makes it suitable for the application as root canal sealing material [8]. However, when used as a bone cement in other bone-related biomedical application, the DCPD/CaO may show a poor mouldability due to the rapid-setting properties that leave little time to the clinicians for operation [24]. As compared with the DCPD/CaO paste, the homogeneous DCPD/C_3S composite pastes showed a retarded setting time (17 ~ 32 min), which is attributed to the low hydration rate of tricalcium silicate. However, taking into account that in clinical applications the cement must be applied before the initial setting time, the extra time obtained from the DCPD/C_3S pastes results in an obvious advantage for surgeons or dentists who will have a comfortable time to work with the paste before it sets.

A critical problem that limits wider clinical application of the calcium phosphate cements is their mechanical properties, and multiple researches have been focused on improvement of the mechanical strength of the cements [25–28]. For instance, Wang et al. found that addition of dicalcium silicate (β-Ca_2SiO_4) improved the mechanical strength of amorphous calcium phosphate (ACP) cement [29]. However, such enhancement was mainly attributed to the filler effect of calcium silicate hydrated (C–S–H) phase, which was one of the main hydration products of dicalcium silicate, and no chemical interaction between the CPC matrix and dicalcium silicate particles was identified. In present study, the DCPD/C_3S cements showed significantly higher compressive strength (36.2 MPa) than the DCPD/CaO cement (9.0 MPa), which can also be partially attributed to the filler effect of calcium silicate hydrate phase. In addition, the presence of CDHA within the hydrated DCPD/C_3S cement confirmed that C_3S may also act as an indirect reactive component within the cement system, and similar to the reaction between DCPD and CaO as found previously [8, 9], the formation of CDHA in hydrated DCPD/C_3S pastes could be ascribed to the following two-step reactions: (a) $3CaO \cdot SiO_2 + H_2O \rightarrow C–S–H + Ca(OH)_2$; and (b) $CaHPO_4 \cdot 2H_2O + Ca(OH)_2 \rightarrow CDHA$. The indirect reaction between DCPD and C_3S, together with the filler effect of calcium silicate hydrate phase, could result in a more compact and homogeneous microstructure of the hydrated DCPD/C_3S cement with consequently superior mechanical strength as compared to that of the DCPD/CaO cement. However, further work is needed to illuminate the exact reaction mechanism between DCPD/C_3S.

As compared with bioactive bone substitution materials such as A-W bioglass and calcium silicate ceramics, the calcium phosphate cements show weaker ability to induce homogeneous bone-like apatite layer on the surface to form chemical bond to bone tissue at the early stage of the implantation [29, 30]. The results of SEM analyses in our study (Fig. 6) suggested that the DCPD/C_3S composite pastes could induce homogeneous apatite deposition on the paste within 7 days, which indicated that the composite paste possessed excellent bioactivity [17]. Such result could be attributed to C_3S as a component of the cement, and the formation of the homogenous apatite layer was dependent on the content of C_3S. As generally accepted, the deposition of apatite on the surface of calcium phosphate cement is largely dependent on supersaturation of Ca^{2+} and PO_4^{3-} ions, and indeed such process proceeds with low rate resulting in the absence of homogeneous apatite layer formation at the early stage of the implantation [17, 31]. With the presence of C_3S in the cement, the $HSiO_3^-$ ions are released during the hydration of the composite paste, which could play an important role in inducing the formation of the apatite in the simulated body environment by providing favorable sites for nucleation of apatite crystals and hence accelerate the deposition of apatite on the surface. It is understandable that when the content of C_3S is low, the concentration of $HSiO_3^-$ ions that act as nucleation site for apatite was not high enough to support the formation of homogeneous apatite layer on the surface of the composite paste. However, when the content of C_3S increased to a certain extent (30% in present study), the concentration of $HSiO_3^-$ ions is high enough for the dispersion of the ions within the composite paste and provides more nucleation sites for apatite crystals, which resulted in the formation of a homogeneous apatite layer on the surface of the whole composite paste. With the significantly improved bioactivity, the DCPD/C_3S composite cement is expected to form a stronger bond with the surrounding bone tissue compared with the DCPD/CaO cement, but this assumption need to be confirmed by further in vivo study.

Considering the fact that the degradability is primarily governed by the chemical composition and the physical characteristics of the material, the reason for the slightly higher degradation rate of the DCPD/C_3S cement could be the higher solubility of the calcium silicate hydrate (C–S–H) formed by the hydration of tricalcium silicate as compared to that of calcium-deficient hydroxyapatite (CDHA). Our results also suggest that the degradation rate of the composite pastes can be adjusted by controlling the DCPD/C_3S ratio of the composite pastes.

It is generally accepted that, for most human cells, a long-term exposure to either an acidic (pH $\leq$ 6.0) or basic

environment (pH $\geq$ 9.0) would have adverse effect on growth and proliferation of cells [32]. Therefore, the basic surrounding pH resulting from the hydration of DCPD/CaO paste may be undesirable clinically. The present study confirmed that DCPD/CaO paste revealed cytotoxicity against mBMSC as the concentration of the paste extracts varied from 100 mg ml^{-1} to 200 mg ml^{-1} (Fig. 8). In contrast, the DCPD/C_3S did not cause significant increase or decrease in surrounding pH value (Fig. 5), which could be attributed to consumption of $Ca(OH)_2$ by formation of CDHA during the hydration of the composite cement. Therefore, the composite cement did not show any cytotoxicity against mBMSC. Moreover, it is observed that the ionic dissolution products of the DCPD/C_3S composite pastes in certain concentration stimulated cell proliferation. According to previous studies, dissolution extracts of bioactive silicate materials such as bioactive glasses and ceramics could stimulate cell proliferation [33, 34]. A recent study also showed that tricalcium silicate paste could stimulate cell proliferation [10]. This stimulatory effect may attribute to the dissolution of silicate ions [33]. The present study indicated that the extracts of the DCPD/C_3S composite paste also stimulated proliferation of mBMSC, suggesting that the composite cement was not only biocompatible and non-cytotoxic, but also possessed excellent bioactivity at cellular level.

Many investigations have been conducted on the improvement of the mechanical strength of the calcium phosphate cements with admirable obtainment [30, 31, 35, 36]. However, most of these studies focused on reducing the porosity of the materials, which always results in compromised bioactivity and degradability of the materials [31]. Our present study suggested that, by the use of bioactive self-setting tricalcium silicate as component in calcium phosphate cement system, calcium–phosphate–silicate composite cements with improved mechanical strength, bioactivity and degradability superior to their non-silicate counterparts could be obtained. Furthermore, since the main hydration product of the DCPD/C_3S is CDHA that is similar to the inorganic composition of natural bone tissue, the novel composite material may have extra benefit when biocompatibility is concerned.

5 Conclusion

In this paper, novel bioactive calcium–phosphate–silicate composite cements were successfully prepared by mixing powders of dicalcium phosphate dihydrate with tricalcium silicate. As compared with DCPD/CaO cement, the mechanical strength of the DCPD/C_3S composite paste was significantly improved. Furthermore, the composite paste showed excellent bioactivity, as indicated by the formation of bone-like apatite in SBF, and the improved degradation rate as compared with that of the DCPD/CaO cement. The cytotoxicity test showed that the DCPD/C_3S composite cement was not only biocompatible and non-cytotoxic, but also stimulatory on cell proliferation. Our study suggests that the use of bioactive tricalcium silicate as reactive component in traditional calcium phosphate cement is a possible approach to obtaining bioactive self-setting composite cements with superior properties for bone regeneration.

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