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Preparation and properties of calcium phosphate cements incorporated gelatin microspheres and calcium sulfate dihydrate as controlled local drug delivery system

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Abstract To develop high macroporous and degradable bone cements which can be used as the substitute of bone repairing and drug carriers, cross-linked gelatin microspheres (GMs) and calcium sulfate dihydrate (CSD) powder were incorporated into calcium phosphate bone cement (CPC) to induce macropores, adjust drug release and control setting time of α-TCP–liquid mixtures after degradation of GMs and dissolution of CSD. In this study, CSD was introduced into CPC/10GMs composites to offset the prolonged setting time caused by the incorporation of GMs, and gentamicin sulphate (GS) was chosen as the model drug entrapped within the GMs. The effects of CSD amount on the cement properties, drug release ability and final macroporosity after GMs degradation were studied in comparison with CPC/GMs cements. The resulting cements presented reduced setting time and increased compressive strength as the content of CSD below 5 wt%. Sustained release of GS was obtained on at least 21 days, and release rates were found to be chiefly controlled by the GMs degradation rate. After 4 weeks of degradation study, the resulting composite cements appeared macroporous, degradable and suitable compressive strength, suggesting that they have potential as controlled local drug delivery system and for cancellous bone applications.

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1 Introduction

Calcium phosphate cements (CPCs) were proposed two decades ago by LeGeros et al. [1], Brown and Chow [2] as synthetic bone substitutes. In general, all CPCs are formed by mixing one or more calcium orthophosphate powders with an aqueous solution to form a paste which is then placed into the bone defect and sets in situ [3]. However, their slow resorption in vivo prevents them from being widely used in orthopedics or to treat other conditions because the growth process of new bone tissue may be delayed due to the presence of the CPCs [4, 5]. The resorption of CPCs is reported to depend on a dissolution process associated with a cellular process in vivo[6] that small particles can be phagocytized by multinucleated cells such as macrophages and foreign body giant cells, and larger volumes of material are resorbed by osteoclasts [7, 8]. Since CPCs do not have a macroporous structure, which allow migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularization [9], the bone cement surface area is relatively small, making the resorption rate of the cements quite low [10]. Incorporation of gelatin microspheres (GMs) in calcium phosphate cements has proven to introduce macropores during in situ degradation of the microspheres without unacceptably affecting the handling properties or cement setting [11, 12]. Furthermore, these microspheres can be used as drug delivery vesicles for local therapy which is desired to treat or prevent a feared complication associated with bone surgery. Cross-linked GMs overcame the main drawbacks of a rapid solubilization in aqueous environments, introducing these microspheres into CPCs would create macropores in situ and accelerate the setting reaction, and improve the crystallinity of the hydrated product HA. Whereas, the initial setting time of the composite bone cements was prolonged by GMs incorporation [13]. Meanwhile, a high crystallinity of

hydrated product HA resulting of gelatin inducing nucleation and growth leads to a low dissolution rate as well as a low resorption [14, 15]. Therefore, the CPC/GMs composite cements are still too stable to permit material degradation and bone ingrowth in a limited period of time [16–18]. For this reason, new attempts should be made to accelerate the setting time and improve the degradation behavior of CPCs/GMs. Durucan and Brown investigated the effects of additives [$CaHPO_4$(DCP), $CaHPO_4{\cdot}2H_2O$(DCPD), $CaCO_3$ and $CaSO_4{\cdot}1/2H_2O$(CSH)] on the reactivity of α-tricalcium phosphate (α-TCP) in forming hydroxyapatite (HA) at 37°C, and found that all the additives delayed HA formation as determined by the isothermal calorimetric analyses [19]. Their retarding effects in decreasing order are $CaCO_3$, $CaSO_4{\cdot}1/2H_2O$, DCPD, DCP. Only the $CaSO_4{\cdot}1/2H_2O$ containing mixture exhibited slightly higher strength averaging 8.36 ± 0.9 MPa. However, Bohner reports that a controlled setting time of α-TCP–water mixtures can be obtained by using calcium sulfate dehydrate (CSD) instead of $CaSO_4{\cdot}1/2H_2O$ (CSH), without markedly modifying the compressive strength of the resulting hardened cement [20]. Enrique et al. [21] also reported that small additions of CSD powder strongly reduced the cement setting time, but the fraction of unreacted α-TCP powder present after 1 day of incubation increased, indicating that the end of the setting reaction was slowed down.

To overcome the limitation of both composite cements CPC/GMs and CPC/CSD, a new composite cement which can be used as the substitute of bone repairing and drug carriers is developed by mixing α-TCP based cement with calcium sulfate dihydrate (CSD), cross-linked GMs loaded gentamicin sulphate (GS) and an aqueous solution. In this paper, based on the previous research, the composite CPC/10 wt% GMs with a high compressive strength is selected as a control [22]. We describe the preparation of CPC/10GMs/CSD composite bone cement with weight percentage of 2.5, 5, 10, 15 and 20 wt% CSD. Properties including the porous structure, porosity, compressive strength, degradation rate and drug release were investigated.

2 Materials and methods

2.1 Calcium phosphate bone cement reagents

α-TCP was prepared by mixing analytical reagents $CaHPO_4{\cdot}2H_2O$ (Kermel Co., China) and $CaCO_3$ (Bodi Co., China) in a planetary ball mill, then heated in a furnace at 1350°C for 3.5 h and followed by quenching in air. The α-TCP was powdered by milling in a 500 ml agate jar at a rotational speed of 700 rpm and sieved, giving particle sizes less than 50 μm. The plain cement consisted of 86 wt% α-TCP, 5 wt% $CaCO_3$, 5 wt% $Ca(H_2PO_4)_2{\cdot}H_2O$ and 4 wt% HAp, which showed an average setting time of 15 min and compressive strength of 26.8 ± 1.9 MPa as reported by our previous research [23]. Calcium sulfate dihydrate (CSD) was purchased from General Hospital of Tianjin Medical University. The liquid phase applied in this experiment was an aqueous solution of 1 wt% disodium hydrogen phosphate (Na_2HPO_4), added to accelerate the setting reaction of the cement.

2.2 Preparation of gentamicin sulphate loaded gelatin microspheres

GMs were prepared by using W/O emulsion chemically crosslinking method, which has been reported in the literature with some modifications [24, 25]. Briefly, 3 g gelatin was dissolved in 30 ml distilled water at 40°C, then 900 mg GS was added into the aqueous gelatin solution. The resulting solution dripped slowly into 300 ml vegetable oil, which was preheated to 50°C. The biphasic system (vegetable oil and gelatin solution) was thoroughly mixed to form a W/O emulsion using a propeller at 450 rpm for about 60 min. Subsequently, stirring was continued and the flask was put in an ice bath so that the temperature of oil phase was kept at 4°C. After 60 min, microspheres were formed in the aqueous phase, collected by filtration and cross-linked for 2 h using 8 wt% genipin aqueous ethanol solution, then rinsed in an ethanol several times to remove the remaining oil and crosslinking agent on their surfaces. Finally, the dried GMs were sieved with the diameters of 50–150 μm and stored for further use. The degree of crosslinking was determined by ninhydrin assay [24]. Our studies [22] showed that GMs with a 45% degree of crosslinking could be almost totally degraded after 4 weeks immersion in stimulated body fluid (SBF). Ion concentrations of this SBF [26, 27] are 142.0 mM Na^+, 5.0 mM K^+, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 147.8 mM Cl^-, 4.2 mM HCO_3^{2-}, 1.0 mM HPO_4^{2-} and 0.5 mM SO_4^{2-}.

2.3 Preparation of CPC/GMs/CSD composite cements

According to our previous study results, the plain cements incorporated 10 wt% GMs had high macroporosity and compressive strength; therefore, 10 wt% GMs was choose to prepare macroporous cements and marked as CPC/10GMs. The composite cement powders, consisted of the plain cement and variable amounts of CSD as addition, were mixed with GS loaded GMs to prepare composite cements. The cement CPC/10GMs without CSD was used as a control; the addition of CSD is 2.5, 5, 10, 15 and 20 wt% respectively. Samples were coded as CPC/GMs/2.5CSD, CPC/GMs/5CSD, CPC/GMs/10CSD, CPC/GMs/15CSD and CPC/GMs/20CSD, respectively. The composite cement specimens were prepared by using a liquid-to-powder (L/P) ratio

of 0.4 ml/g. After mixing, the pastes were placed into cylindrical stainless steel molds to form specimens with dimensions of 6 mm in diameter and 12 mm in height. About 60 s later, the cylindrical specimens were removed from the mold to an atmosphere of 100% relative humidity at 37°C for 30 min. The samples were then soaked in 20 ml of stimulated body fluid (SBF) and incubated at 37°C in a water bath on a shaker table (70 rpm) for different time.

2.4 Characterization of composite cements

The setting time of CPC/10GMs and CPC/10GMs/CSD cements was tested using Gillmore needle at various interval. The setting time was recorded when the needle failed to create an indentation of 1 mm in depth in three separate areas. Each specimen was repeated five times and the average value was calculated. The porosity of the resulting cement specimens after 4 weeks soaking in SBF was measured by a mercury intrusion method (Autopore II, 9220, USA). Four specimens were measured for each group. The compressive strength of the porous bodies was measured using an Instron-1195 universal testing machine with a crosshead speed of 1 mm/min on specimens with a size of about ϕ6 mm × 12 mm. The cross-sectional area of the sample and the maximum failure load were used to calculate the compressive strength. More than five samples were tested to obtain the average values along with its standard deviation. Sample weight was measured after removal from phosphate buffered saline liquid (PBS) and freeze-drying over night. Mass loss with time (days) was determined using Eq. 1 as described by Chung [10].

$$R_L = \frac{m_0 - m_n}{m_0} \quad (1)$$

where R_L is the mass loss of the sample (%) after soaking for n days; m_0 the mass of the sample (g); and m_n is the mass of the sample (g) after n days soaking.

Identification of the crystalline phases of the hydrated products was carried out by X-ray diffraction analysis (XRD; Model D/MAX-2500, Rigaku Co., Japan). The diffraction patterns were collected with a scanning angle 2θ range from 10° to 60° in step-scan intervals of 0.02° at a scanning speed of 4° min^{-1} with Cu Kα radiation, and the X-ray diffractometer operated at 40 kV and 25 mA. The microstructure and Ca/P ratios of the deposited phases were characterized using environmental scanning electron microscopy (ESEM, Philips, XL30) in conjunction with energy dispersive spectroscopy (EDS). The crystal size and shape were characterized by transmission electron microscopy (TEM, Philips, Tecnai G2 F20) and energy spectrums were also recorded.

2.5 In vitro gentamicin sulphate release

2.5.1 Standard curve

GS release measurements were carried out using UV–Vis spectroscopy (UV–visible 8500 spectrophotometer, China) by Zhang's method [28]. Briefly, 1 ml GS solution, 1 ml isopropanol and 1 ml ophthaldialdehyde reagent were reacted for 25 min at room temperature. The absorbance, which corresponds to the GS concentration, was then measured at 332 nm, at which the GS-phthaldialdehyde complex shows an absorbance maximum. Furthermore, Calibration curve (correlation coefficient No. 99) was made for each set of measurements and determined by taking absorbance versus GS concentration between 1 and 100 μg/ml as parameters.

2.5.2 In vitro release assay

The test specimens (cylinders) were immersed in 150 ml of PBS at 37°C and stirred at 50 rpm. In each of the studies 22 samples of 3 ml were withdrawn by pipette at pre-established intervals and filtered with a Whatman filter. The amount of GS in the PBS was determined using spectrophotometric analysis [29].

3 Results

3.1 Setting time

As seen in Table 1, the setting time of CPC paste was 15 min, which was concordant with previous studies done [23]. When incorporated 10 wt% of cross-linked GMs, the setting time of CPC/GMs increased to 34 min, much longer than those of CPC. In contrast, for all the CPC/GMs/CSD composite pastes, the setting time was shorter than those of CPC/GMs paste, and as the content of CSD increased to 20 wt%, the setting time decreased from 34 to 18 min.

3.2 Phase composition

Figure 1 shows the XRD patterns of CPC, CPC/GMs and CPC/GMs/CSD cements with different content of CSD

Table 1 Setting time of CPC/GMs cement pastes with different contents of calcium sulfate dihydrate (CSD) (L/P = 0.4 ml/g)

CSD (wt%)	CPC	0	2.5	5	10	15	20
Setting time (min)	15 ± 1.2	34 ± 2.2	28 ± 2.4	23 ± 1.6	16 ± 1.3	17 ± 1.2	18 ± 1.5

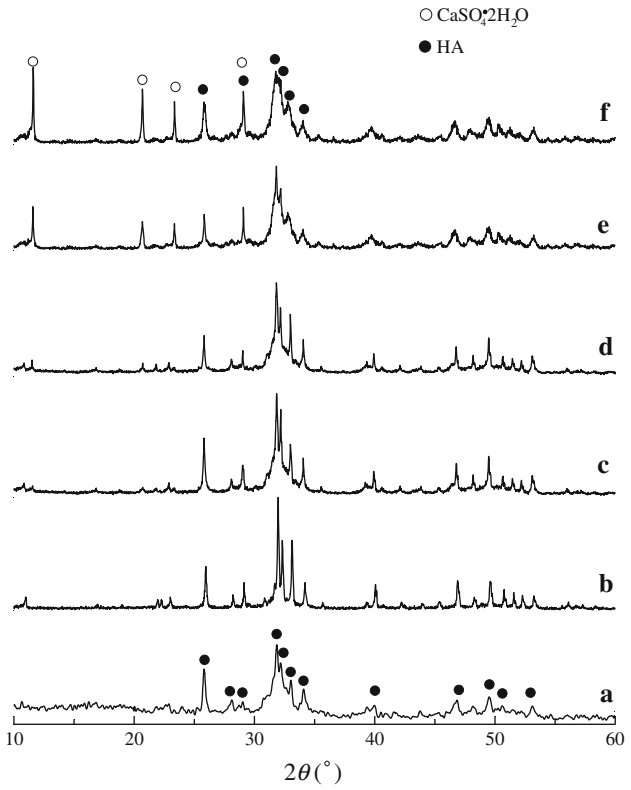

Fig. 1 XRD patterns of CPC (*a*), CPC/GMs (*b*) and CPC/GMs/CSD cements incorporated 2.5 wt% (*c*), 5.0 wt% (*d*), 10 wt% (*e*) and 20 wt% (*f*) of CSD after soaking for 4 weeks in SBF solution

after soaking for 4 weeks in SBF solution. According to the XRD patterns, the immersed cement CPC can be seen to contain only HA crystals which diffraction peaks are broad, revealing that it is composed of poorly crystalline calcium phosphate. Whereas in the pattern for CPC/GMs, the diffraction peaks corresponding to HA are narrow and intense which suggests that the crystallinity of hydrated products HA is higher than that for the CPC, and no α-TCP can be detected, indicating that the presence of gelatin accelerates the setting reaction of the cement into apatite. Figure 1c–f show the effects of CSD on the hydration reaction and the crystallinity of HA. Broadening diffraction peaks corresponding to HA can be observed in all patterns for CPC/GMs/CSD cements, and some CSD was detected in the samples when the content of CSD is up to 10 wt%. Moreover, the relative intensities of the characteristic diffraction peaks of CSD increase with the increment of CSD content.

3.3 SEM observation

SEM images of fracture surface of the samples after soaking in SBF for 4 weeks are shown in Fig. 2. All the composite cements present similar interconnective macropore structure formed by in-situ degradation of GMs, whereas the significantly differences of the micropore structure and crystal morphology were observed in the cement matrix. The SEM image of CPC/GMs was chosen as the typical macropore structure shown in Fig. 2a. The macropore size ranged from 150 to 500 μm and some connected pores can also be observed between macropores. For the CPC/10GMs, as showing in Fig. 2b the microstructure of the matrix area with large magnification shows a network structure constituted by large flake-like crystals, this result is consisted with Bigi's reports [30]. With the addition of 2.5 wt% CSD, the composite cement is composed of network structure built by short rod-like crystals and compact particle-like structure as showing in Fig. 2c. Increasing the contents of CSD, more large rod-like crystals could be seen in the CPC/GMs/CSD cements as exhibited in Fig. 2d, e for samples with 5 and 10 wt% of CSD incorporation, respectively. When the content of CSD reached to 20 wt%, large flake-like crystals and high porosity could be observed in this cement matrix showing in Fig. 2f. From above results, it can be concluded that CSD significantly affects the shape and size of the hydration products.

3.4 TEM observation of CPC/MGs/CSD cement powder

Figure 3 exhibited the corresponding TEM image of the ground powder of CPC/GMs/20CSD cement after soaking for 4 weeks. It can be seen that the specimen (Fig. 3a) consisted of needle-like crystals and irregular nano-particles. Energy spectrum analysis in Fig. 3b shows that the needle-like crystals consisted of Ca, S, O elements, which are typical of CSD, confirming that some CSD was still remained in the cement after 4 weeks immersion. Whereas Fig. 3c shows that the small and irregular particles have a ratio of Ca/P 1.56, further proving that the hydration product was calcium deficient hydroxyapatite (CDHA).

3.5 Compressive strength of immersed cements

GMs dissolved almost completely after 4 weeks soaking in SBF, the porosities and the compressive strengths of scaffolds with different content of CSD are plotted in Fig. 4. It indicated that the porosities of the composite cements slowly increased with increasing CSD content and were slightly higher than that of CPC/10GMs (about 63%), and the composite cement with 20 wt% CSD had the highest porosity of about ~72%. Figure 4 also showed that the compressive strengths of the composite cements increased with increasing CSD content when the content of CSD was below 5 wt%, and the composite cements with 5 wt% CSD showing the highest compressive strength (6.9 ± 0.7 MPa) after 4 weeks immersion. Then, the

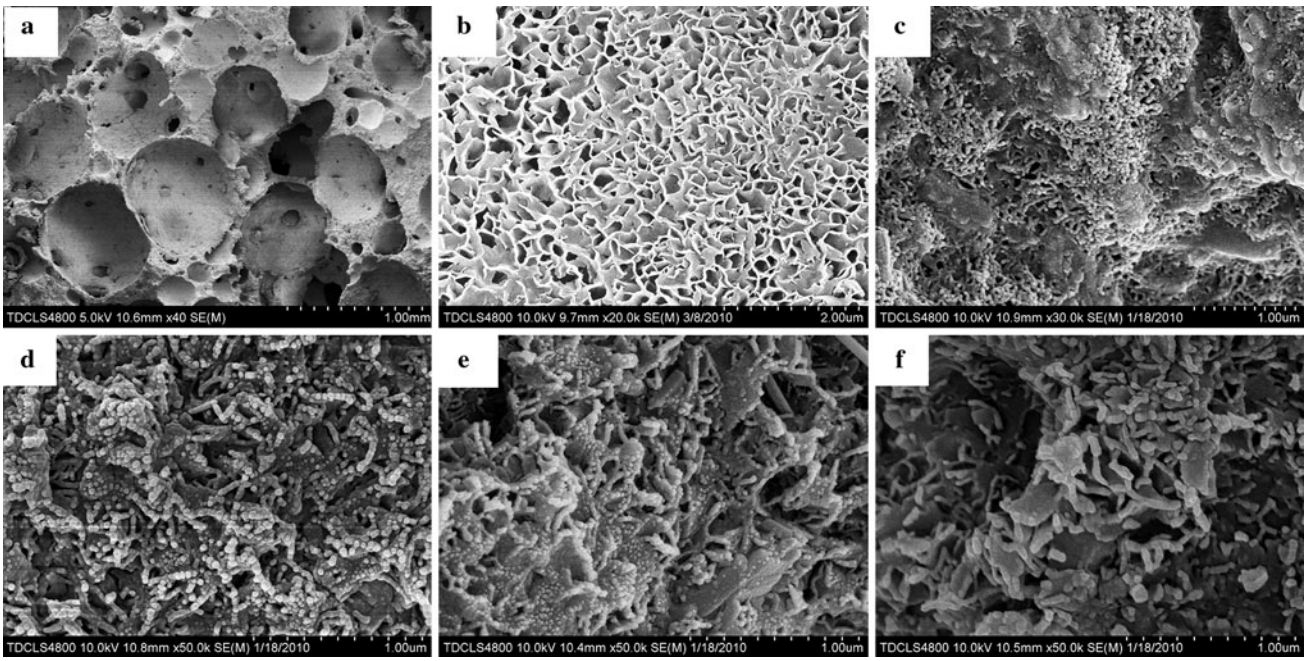

Fig. 2 SEM images of fracture surface of the samples with 0, 2.5, 5.0, 10, and 20 wt% of CSD incorporation after 4 weeks soaking in SBF

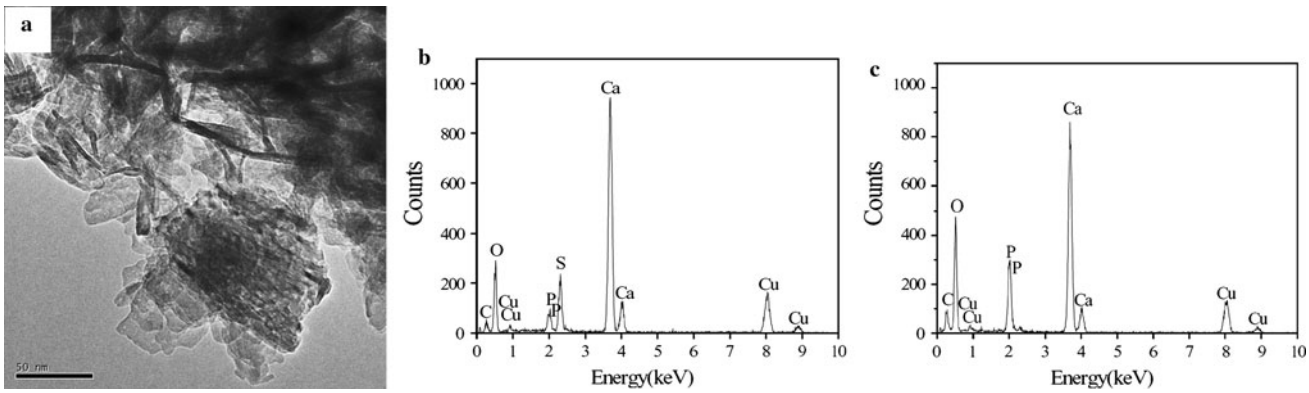

Fig. 3 TEM image of the ground powder of CPC/GMs/20CSD composite cement after 4 weeks immersion (**a**) and energy spectrums of the needle-like crystals (**b**), and nano-particles (**c**)

compressive strength of the composite cements slowly decreased when CSD content was more than 5 wt%.

3.6 In vitro degradation behavior

Figure 5 shows the degradation of CPC/MGs and CPC/MGs/CSD composite cements with different contents of CSD after soaking in PBS for various time periods. It was found that the degradation rate of all the CPC/MGs/CSD samples was slightly higher than that of the CPC/GMs samples during the immersing period, and the degradation rate increased with the increase of the CSD amount. While in the soaking period of 21–24 days a significant weight loss can be observed in all samples, especially for those samples incorporated more than 10 wt% of CSD content, the weight loss reached to 10%.

3.7 Gentamicin sulphate release from cross-linked gelatin microspheres

Figure 6 displays the cumulative GS release from cylinders according to CSD ratio in the composites. It can be observed that GS from all samples showed a similar release behavior, which presented an initial burst of about 42% of the total amount of GS in the initial 24 h, followed by a slow and sustained drug release up to 21 days, and then a fast release rate occurred in the period of 21–24 days. With the increase of CSD content, difference in GS release

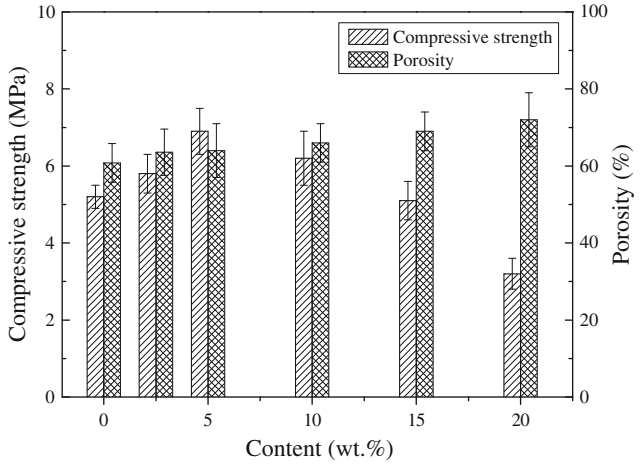

Fig. 4 Effects of the CSD content on the compressive strength and porosity of the cement incorporated 10 wt% GMs (CPC/GMs) after 4 weeks of soaking

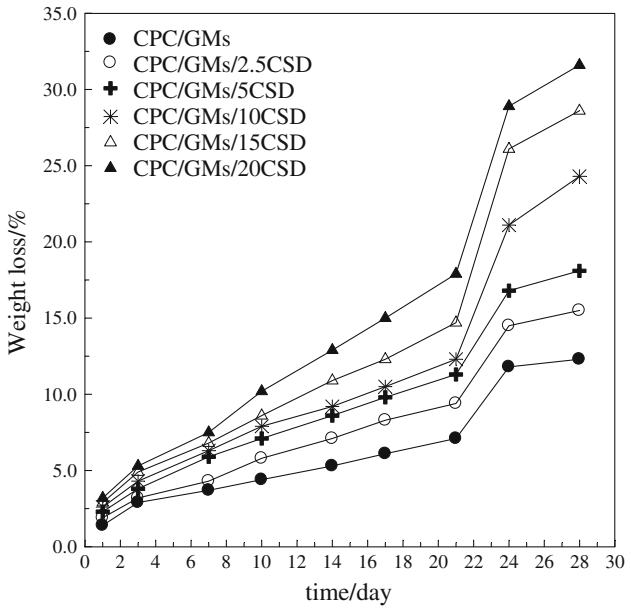

Fig. 5 Evolution of the weight loss of the cement CPC/GMs as a function of CSD content after various time of soaking

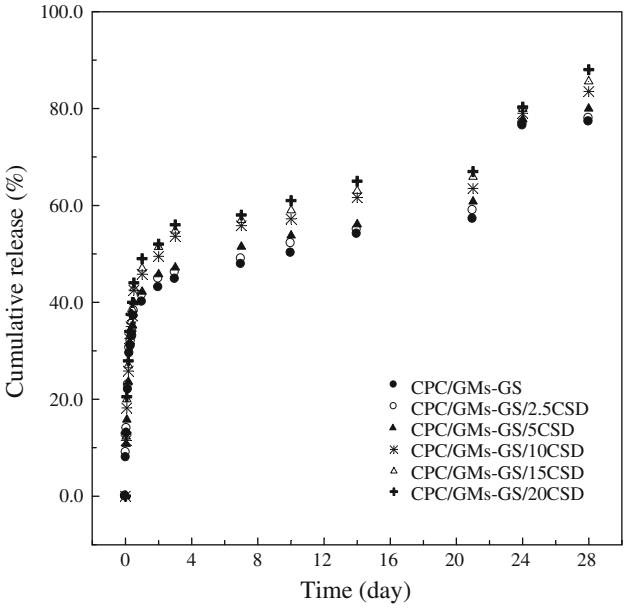

Fig. 6 In vitro release of GS from composite cements CPC/GMs with different CSD contents

became more apparent as time progressed towards the end of the in vitro study, the cumulative GS release of the CPC/GMs and CPC/GMs/20CSD samples on the day 28 is nearly 80 and 90% of the total amount, respectively, showing that the CSD content slightly affected the drug release.

4 Discussion

From the perspective of the clinical uses, the initial setting time of a CPC cement paste is of paramount importance in many applications like in situ fracture fixation in orthopaedics, filling root canals and sealing furcation in endodontics and vertebroplasty. Therefore, the CPC must have an optimal setting time in order to prevent migration of cement to undesirable sites and to obtain an acceptable mechanical strength after hardening.

When 10 wt% of cross-linked GMs were incorporated to CPC, the setting time (34 ± 2.2 min) of CPC/GMs composite cement is much longer than that of CPC (15 ± 1.2 min). This finding is inconsistent with the reports by Li et al. [13] that the presence of 5 wt% GMs affected the setting time slightly. The great distinction may be related to the difference of the powder composition and the degree of crosslinking of GMs. Bigi et al. [30] and Shie et al. [31] also confirmed that CPC containing uncross-linked-gelatin took a long time to harden. The reason might be that gelatin may produce polyanions when dissolving, and the excessive amount of polyanions could destroy the balance of the CPC formulation, leading to a very slow setting process or no setting at all. The degradation of GMs is related to the extent of gelatin crosslinking [32]. In comparison to the degree of the cross-linked gelatin reported by Shie MY and Bigi, the GMs in our study has a low degree of crosslinking (~45%), which produced a relatively high quantity of polyanion in the process of dissolving. Therefore, the CPC/GMs took a remarkably long time to harden. In our studies, GMs with nearly 4 weeks degradation period were used to be the macroporosity agent and the carrier of drug.

When the CSD was incorporated to CPC/10GMs, the setting time of the composite cements was modulated by

the CSD amount. At a low CSD (<10 wt%) content, a large decrease of the cement setting time was observed at all samples. The dissolution of CSD and α-TCP, and subsequent crystallization to form CDHA is generally accepted as a setting mechanism for CPC/CSD cements [21]. According to our experiments, it can be considered that the introduction of CSD particles accelerates the second step of the mechanism. Calcium sulfate dehydrate (CSD) is a relatively soluble calcium-rich phase and is more soluble than α-TCP and CDHA at neutral and slightly basic pH values, therefore, the presence of CSD crystals in the cement should lead to a rapid increase of the concentration of dissolved calcium ions in the mixing liquid, and react with the phosphate ions from the liquid phase (1 wt% Na_2HPO_4) to form CDHA precipitation, consequently accelerating the initial setting rate and decreasing the setting time as proposed also by Bohner [20]. Cleary, this effect on the cement setting time depends on the dissolution rate of CSD and the phosphate concentration. Simultaneously, the fast dissolving of CSD increases the saturation of calcium and phosphate ions in the mixing liquid that would slow down α-TCP dissolution and α-TCP transformation into CDHA. Therefore, an increase of CSD amount should lead to an increase of the setting time. It can be explained that the setting time increased when CSD content is more than 10 wt%. However, no significant diffraction peaks corresponding to α-TCP can be observed in the patterns of the composite cements incorporated different content of CSD after soaking in SBF for 4 weeks. This implies that the setting time depends on the initial setting rate of the cement, whereas the setting rate of the cement during the soaking is not a constant value, it varies with time. Although the conversion reaction of α-TCP into CDHA is prevented by the fast dissolution of CSD in the initial setting stages when CSD content is above 10 wt%, a slow degradation of GMs in the soaking periods can accelerate the crystallization of CDHA through fast ionic interaction between Ca^{2+} with –COO– in gelatin [33] and keep the setting reaction lasting for a long time. Therefore, no unreacted α-TCP was detected in the patterns of XRD for all composite cements even with high contents of CSD at the end of the experiment.

Higher porosity is required to offer sufficient space for tissue growth and to increase the volume of the invasion of surrounding tissue; an interconnecting pore network is also essential for tissue ingrowth, vascularization and diffusion of nutrients [34, 35]. In addition, it has been reported that macropore size of at least 100 μm and a porosity of more than 70% is beneficial in facilitating cell infiltration, bone ingrowth and internal mineralized bone formation [36].

The incorporation of GMs into CPCs has been reported to be an effective method to develop macroporous CPCs by the degradation of GMs [17, 18]. Therefore, GMs used in the present study had a diameter ranging from 50 to 100 μm. Our finding demonstrated that after 4 weeks of SBF soaking, interconnective porosity could be obtained in CPC/GMs/CSD cements. The macropore diameter ranged from 150 to 500 μm, much larger than those of incorporated GMs, which is attributed to the swelling of GMs in SBF soaking. Increasing CSD content, no significantly difference of the macropore structure was observed in the cement matrix, while the incorporation of CSD significantly affects the shape and size of the hydration products, and the microstructure changed from irregular pore network built by plate-like particles to uniformly micropore structure consists of a dense conglomerate of nanospheres. Apparently, the incorporation of CSD results in a high density of the apatite nuclei, preventing subsequently growth of the crystals. This suggests that the fast dissolution of CSD in the initial setting stage accelerated the nucleation of CDHA crystals. The result is consistent with Bohner's [20] report that CSD had an effect on the growth of CDHA crystals in the CPC/CSD composite cement. It is indeed well known that a finer microstructure leads to larger mechanical properties, therefore, the change in microstructure and the smaller crystal size of the setting cements due to the addition of CSD improved the compressive strength (Fig. 4). However, the degradation of CSD in the SBF will produce a higher porosity which has a negative effect on the compressive strength. After 4 weeks of soaking, the maximum of the compressive strength was obtained by the addition of 5 wt% CSD, higher than that of CPC/GMs, as shown in Fig. 4. It could be explained that the positive effect on the compressive strength due to entanglement of the fine CDHA crystals exceeded the negative effect of CSD dissolution which increases the porosity of the cement itself. Above 5 wt%, we found that the compressive strength of CPC/GMs/CSD decreased and was less than that of the control CPC/GMs. Therefore, the slightly higher porosity might be one of the factors decreasing the compressive strength. In addition, for sample CPC/GMs/10CSD, the degradation result demonstrates that the obtained composite cement with a Ca/P ratio of 1.56 loses 24.3% of its initial weight after soaking for 4 weeks in PBS, larger than the total weight of the incorporated GMs (CS) and CSD. XRD pattern shown in Fig. 1 indicates that CSD is not dissolved completely after soaking 4 weeks, further confirming that the CDHA scaffold is degradable. In contrast, the cement prepared from CPC/GMs with a Ca/P ratio of 1.66 shows a weight loss of 12.2% (as shown in Fig. 5) of its initial weight by the end of the degradation experiment, a little higher than the weight of incorporated GMs. When CSD content reached to 10 wt%, the weight loss after immersed in PBS for 4 weeks is 24.2%, much larger than the total weight of GMs and CSD. The large weight loss was occured at the

composite cements with more than 10 wt% of CSD, which is consistent with the significant decrease of compressive strength. It is clear that the degradation of CDHA scaffold from CPC/GMs/CSD is faster than that of HA scaffold from CPC/GMs in PBS, implying that the Ca/P ratio of the hydration product obviously affects the degradation of the apatite scaffolds, which also changes the microstructure of the composite cements and subsequently affects the compressive strength. Though the prepared CDHA scaffold from CPC/GMs/CSD was stronger than other reported macroporous CPCs with similar porosity [37, 38], it still could not be used as a temporary load-bearing device.

GS is an aminoglycosidic antibiotic for bacterial infections caused by staphylococcus which are sensitive to Gram-negative bacteria [39, 40] and of particular interest in orthopaedic surgery where it can be combined with bone graft materials to prevent from infection after surgery. In this study, the GS was chosen and entrapped within GMs, then incorporated into the CPC/CSD cement to obtain a local drug delivery system. The aim of the present study was to investigate the effects of CSD concentration on GS release profiles of the composite cements in vitro. Figure 6 shows the effect of CSD on the drug release profiles corresponding to 0, 2.5, 5, 10, 15 and 20 wt% specimens. The shapes of the release curves were quite similar in all these samples, as shown in the graphs. The initial burst release was observed in all the scaffolds. For CPC/10GMs sample, an initial release in the initial 24 h was around 42% of the total amount of GS. The followed release rate decreased with increasing time, and the cumulative release did not exceed 70% until 21 day soaking, thereafter a little fast release was observed that the amount of released GS reached to nearly 80% as time progressed towards the end of the in vitro study, the fast release might be related to the burst of GS loaded MGs. To develop high macroporous and degradable bone cements which can be used as the substitute of bone repairing and drug carriers, cross-linked GMs with diameters ranged from 50 to 150 μm were adopted. The swelling degree of GMs and the drug release period are controlled by the degree of crosslinking. In this study, GMs were formed in the aqueous phase, and then cross-linked using genipin aqueous ethanol solution. The crosslinking reaction from the surface to the interior of the GMs would result in an inhomogeneous structure. It meant that the internal gelatin in GMs had not enough time to react with genipin. Therefore, after soaking in SBF for 21 days, the internal, uncrosslinked gelatin began to contact with SBF, which resulted in a fast degradation of GMs and exhibiting an unexpected increase of gentamicine releasing. For CPC/MGs/CSD, the release rates also increased in the final soaking period when the CSD content increased to 20 wt%. CSD, therefore, CSD did not significantly influence the release behavior, although the CSD concentration led to an increase in the porosity. From above results, GS release could be attributed to the drug liberation by GMs degradation and the diffusion through pores in the cement matrix. For both kinds of scaffolds, the effect on release resulting of degradation of hydration product CDHA during the soaking period is ignorable. Comparing the curves of the weight loss of the CPC/GMs/CSD cements and their corresponding cumulative releases, the weight loss resulting from CSD dissolution, GMs and CDHA degradation showed a stable increase up to the burst of GMs. Therefore, it is speculated that the release of GS follows a swelling-controlled release mechanism. According to our previously research, GS is physically entrapped in GMs, thus, the main diffusion barrier is gelatin network. On the other hands, for CPC/GMs/CSD, the hydration products formed a compact layer with micro porous structures and acted as a physical barrier that slowed down the release rate. Meanwhile, the fast dissolution of CSD resulted in more micropores in the cement matrix which would increase the delivering processes. The two opposite effects made the CPC/GMs/CSD cements have a similar release behavior as that of CPC/10GMs when the concentration of CSD is below 10 wt%, whereas for specimens incorporated more than 10 wt% of CSD the release rates after burst of the GMs are slightly larger than those of specimens incorporated less CSD content. It might be related to the CSD dissolving and then the forming of a higher porosity.

5 Conclusion

In this study, calcium phosphate cements incorporated with cross-linked GMs loaded GS and calcium sulphate dihydrate were formulated and showed setting times of 16–27 min and porosities of 63–72%. The highest compressive strength of 6.9 MPa was obtained when the content of CSD is 5 wt%. The addition of CSD powder to CPC/GMs strongly decreased their setting time, and offset the prolonged setting time caused by the incorporation of GMs. Sustained release of GS was obtained after an initial bust release, and release rates can be controlled by the degree of crosslinking of GMs, which modulated drug diffusion. With the gradual degradation of the incorporated GMs and the dissolution of CSD, this composite cement evolved gradually into a porous material with about 70% porosity, and interconnectivity between pores could be obtained. Taking into account the potentials of these composite cements for sustained drug delivery and induced macroporosity, CPC/GMs/CSD composites appear to be a promising scaffold material for bone regeneration and bone tissue engineering.

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