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Study on injectable and degradable cement of calcium sulphate and calcium phosphate for bone repair

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Abstract Injectable calcium sulphate/phosphate cement (CSPC) with degradable characteristic was developed by introduction of calcium sulphate (CS) into calcium phosphate cement (CPC). The setting time, compressive strength, composition, degradation, cells and tissue responses to the CSPC were investigated. The results show that the injectable CSPC with optimum L/P ratio exhibited good injectability, and had suitable setting time and mechanical properties. Furthermore, the CSPC had good degradability and its degradation significantly faster than that of CPC in Tris–HCl solution. Cell culture results indicate that CSPC was biocompatible and could support MG63 cell attachment and proliferation. To investigate the in vivo biocompatibility and osteogenesis, the CSPC were implanted in the bone defects of rabbits. Histological evaluation shows that the introduction of CS into CPC enhanced the efficiency of new bone formation, and CSPC exhibited good biocompatibility, degradability and osteoconductivity with host bone in vivo. It can be concluded that the injectable CSPC had a significant clinical advantage over CPC, and might have potential to be applied in orthopedic, reconstructive and maxillofacial surgery, especially for minimally invasive techniques.

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1 Introduction

The search for an osteoconductive, injectable biomaterial has been the quest of many researchers and surgeons interested in accelerating healing of bone fracture or in reconstructed bone defects [1]. Calcium phosphate cements (CPC) have become a subject of much interest in dental and bony biomedical material researches because of their excellent biocompatibility and bioactivity [2]. Many different CPC formulas have been studied, but most of them form hydroxyapatite (HA) as final product [3]. CPC shows good biocompatibility and adequate mechanical properties but has slow resorption in vivo [4]. The CPC, now available on the market, are too stable to permit material degradation and bone ingrowth in a limited period of time, at least for the first years [5]. Biomaterials that could be replaced by living bone are preferred in reconstructive surgery of today because their use avoid complications for the patient such as inflammation, stiffness, pain and, later, bacterial seeding. To assure new bone tissue to grow into the defect, and in order to accelerate bone tissue colonization and resorption of the cement implant, degradability is necessary.

Calcium sulphate (CS) has been applied as bone substitute for over 100 years, and has been proved to be safe and biocompatible [6]. Coetzee treated 110 patients with osseous defects in skull and facial bone in 1980, and he concluded that CS was an outstanding bone graft substitute that produced results comparable with autogenous bone graft [7]. Later, CS has been criticized for its rapid resorption, before the bone tissue has had the time to grow into the defect, and for that reason been replaced by apatite as a filling material for bone defects [8, 9]. Ideal bone substitute should have the same speed of degradation as formation of new bone tissue, and no stimulation to

surrounding tissue. The resorption of CS is faster than formation of new bone in vivo, which is harmful to the reconstruction of bone defect.

To overcome the limitation of both CPC and CS, The aim of this study is to develop an injectable material containing two phases, by mixing CPC with calcium sulphate hemihydrate (CSH) and an aqueous solution. Both CPC and CSH would react with the solution creating: an apatitic phase, that is, hydroxyapatite, and a resorbable phase consisting of calcium sulphate dehydrate (CSD). The CSD phase will be absorbable in the body, creating micropores in the implanted material, and therefore ensure ingrowth of new bone tissue. The apatitic phase (from CPC) will ensure bone conductivity in the defect. It was chosen to work with these materials because both show excellent biocompatibility and have been used in body for many years.

2 Materials and methods

2.1 Preparation and characterization of CSPC cement

The preparation method of CPC powder used in this experiment can be obtained from the relevant literature [10]. The CPC powder was composed of TTCP and DCPA in anequivalent molar ratio. Calcium sulphate hemihydrate ($CaSO_4 \cdot 1/2H_2O$, CSH) was prepared from calcinations of calcium sulphate dihydrate ($CaSO_4 \cdot 2H_2O$, CSD) at 160°C. CPC served as basis for all experiments, and 40 and 60 wt% CSH were added into the CPC powder to form calcium sulphate/phosphate cement (CSPC) powders. The injectable CSPC pastes were made by mixing CSPC powder with cement liquid (water). The prepared injectable cement pastes were individually loaded into a stainless-steel mold and stored at 37°C in a 100% humidity box for setting. After 48 h, the composition of the CSPC samples was characterized by X-ray diffraction (XRD; Rigaku Co., Japan). The fractured surface of the cement samples was examined with scanning electron microscopy (SEM; JSM6360, JEOL, Japan).

The samples were tested at various intervals using a Vicat apparatus, and the time when the needle could only penetrate less than 1 mm into the sample was taken as the setting time [10]. Each experiment was performed in triplicates (n = 3), and the average value was calculated and expressed as means ± standard deviation (mean ± SD). The compressive strength of the hardened specimens ($10 \times 10 \times 10$ mm^3) was measured at a loading rate of 1 mm/min using a universal testing machine (AG-2000A, Shimadzu Autograph, Shimadzu Co., Ltd, Japan). Three replicates were carried out for each group ($n = 3$) and the results were expressed as mean ± SD.

2.2 Degradation determination

In vitro degradation of the injectable CSPC was investigated by immersing the pre-hardened body samples in Tris–HCl solution and the degradation rate of the cement was characterized by its residual weight ratio. After setting for 48 h at 37°C and 100% humidity, samples ($\phi 10 \times 3$ mm^3) were dried at 60°C for 24 h. The sample with initial weight W_0 was immersed into the Tris–HCl solution at 37°C with a weight-to-volume ratio of 0.2 g/ml. The solution was continuously shaken at a rate of 100 r/min in a water bath. After every 7 days, the sample was removed from the solution, cleaned with deionized water, dried at 60°C for 2 h and its new weight W_t was recorded. It was then re-immersed into a fresh Tris–HCl solution at the same weight-to-volume ratio. The residual weight ratio of each sample was calculated according to the equation: residual weight ratio (%) = $W_t/W_0 \times 100\%$. Three samples were tested for each kind of cement and the results were expressed as means ($n = 3$). The porosity of the 40 wt% CSPC after soaking in Tris–HCl solution for different time was measured in distilled water by the Archimedes method. The average porosity was calculated based on five samples. At some time point, the pH of the Tris–HCl solution was determined using an electrolyte-type pH meter.

2.3 Cell proliferation and morphology

After setting for 48 h, samples ($\phi 5 \times 2$ mm^3) of 40 wt% CSPC (CPC and 60 wt% CSPC as control) were sterilized by autoclaving at 120°C for 20 min. The proliferation of MG-63 cells cultured on the cement samples was assessed quantitatively using MTT assay. Cement samples were first put in each well of the 96-well plate. MG_{63} cells were then seeded onto the cement samples at a density of 5×10^4 cells/sample, followed by incubation at 37°C and 100% humidity with 5% CO_2 in a DMEM-BFS medium. The medium was changed every 2 days. After culturing for 1, 3 and 5 days, 100 μL methyl thiazoly tetrazolium (MTT) solution was added into each well in the plate. The plate was then incubated for further 4 h. The supernatant of each well was then removed and 200 ml dimethyl sulfoxide (DMSO) added. After shaking for 10 min, the optical density (OD) at 490 nm was measured with an enzyme-linked immunoadsorbent assay plate reader. Sample-cell constructs were washed twice with PBS solution and fixed with 4% formalin in PBS (pH = 7.4) for 20 min. They were subsequently washed twice with PBS solution and dehydrated in a graded ethanol series (50, 60, 70, 80, 90, and 100% v/v) for 3 min at each concentration. Samples were air-dried in a desiccator overnight, glued onto copper specimen stubs and sputter-coated with gold palladium for SEM observation.

2.4 Biocompatibility and osteogenesis in vivo

The healthy New Zealand white rabbits weighting about 3.0 kg each were used for the implantation of the injectable CSPC cement with 40 wt% CS content. Under general anesthesia and sterile conditions, the left femur of each rabbit was exposed and one defect (ϕ6 mm) was drilled in the distal part of the femur. The bone cavities were carefully washed to eliminate bone debris and dried with gauze. Cylindrical preset samples of CSPC with the size of $\phi 6 \times 5$ mm^3 were implanted into the defects in the rabbit femora. Rabbits from each group were sacrificed by an overdose abdominal injection of pentobarbital sodium at 1, 2, 3 and 6 months after implantation. The bone specimens were harvested immediately after sacrifice. For histological evaluation, the samples together with surrounding tissues were excised, fixed in 10% neutral buffered formalin. The fixed samples were dehydrated in a series ethanol and embedded in methyl methacrylate (MMA). Thin un-decalcified sections (20 μm) were made with a diamond saw (KDG 95, IsoTis, B.V., The Netherlands). At least five sections were made from each implant and stained with methylene blue and basic fuchsin for histological observation.

2.5 Statistical analysis

Statistical analysis was performed using one-way ANOVA with post hoc tests. All results are expressed as the mean ± SD. Differences were considered statistically significant at $P < 0.05$.

3 Results

3.1 Setting time

Figure 1a exhibits the influence of CS content in CPC on the setting time of the injectable CSPC at optimum L/P ratios of 0.3 ml/g. It can be seen that the setting time decreased slightly with the increase of CS content in CPC, the results show that adding 40 and 60 wt% CS into CPC had not obviously effect on the setting time of CSPC. Figure 1b exhibits the influence of L/P ratio on the setting time of the CSPC. It can be drawn that the setting time for 40 and 60 wt% CSPC increased with the increase of L/P ratio. When the L/P ratio less than 0.25 ml/g, the mixture of powder and solution was difficult to be handled and could not form cement dough; when the L/P ratio was more than 0.35 ml/g, the CSPC showed a longer setting time. The optional setting time for 40 and 60 wt% CSPC was obtained at 15 and 11 min when L/P ratio was 0.3, respectively. The results reveal that L/P ratio had significantly effect on the setting time of CSPC, and there was no obviously difference on setting time between 40 and 60 wt% CSPC.

3.2 Compressive strength

The relationship between CS content and the compressive strength of CSPC at optimum L/P ratios of 0.3 ml/g is shown in Fig. 2a. It can be obtained that the compressive strength of CSPC decreased with the increase of CS content, the compressive strength was 42, 35 and 31 MPa as CSPC with 0, 40 and 60 wt% CS content, respectively. The results reveal that the CS content had an obvious effect on the compressive strength of CSPC.

Figure 2b shows the effect of L/P ratio on the compressive strength of the CSPC. It can be seen that the compressive strength of the CSPC decreased with the increase of L/P ratio, the compressive strength decreased from 41 to 32 MPa with the increase of L/P ratio from 0.3 to 0.35 ml/g after setting for 48 h. In addition, we found that the injectability of the CSPC paste improved as the P/L ratio decrease. With the P/L ratio less than 0.25 ml/g, the CSPC paste was difficult to be injected. As the L/P ratio decreased to more than 0.30 ml/g, the injectability of the cement paste improved. The results show that improving the injectability with the increase L/P ratio but decrease the compressive strength of the CSPC.

3.3 XRD analysis

Figure 3 shows the XRD patterns of CSPC after setting for 48 h in 100% relative humidity at 37°C. According to the XRD spectra, it can be seen that the hardened CSPC sample contained a mixture of CSD and hydroxyapatite [HA]. The formation of CSD appeared due to the hydration of CSH, and the presence of HA could be attributed to the hydration of CPC. No significant effects for both CPC and CSH on their hydration were found.

3.4 SEM analysis

Figure 4 illustrates SEM micrographs for the fracture surfaces of CPC and 40 wt% CSPC samples, respectively, after hardening for 48 h at 37°C in 100% relative humidity. It can be seen that CSPC formed a powder-like structure with one crack while CPC formed particle-like structure with a little micropores.

3.5 Degradation of cements

Figure 5 shows the weight loss ratios of (a) CPC, (b) 40 wt% CSPC (c) and 60 wt% CSPC after immersing in Tris–HCl solution for various time periods. The degradation

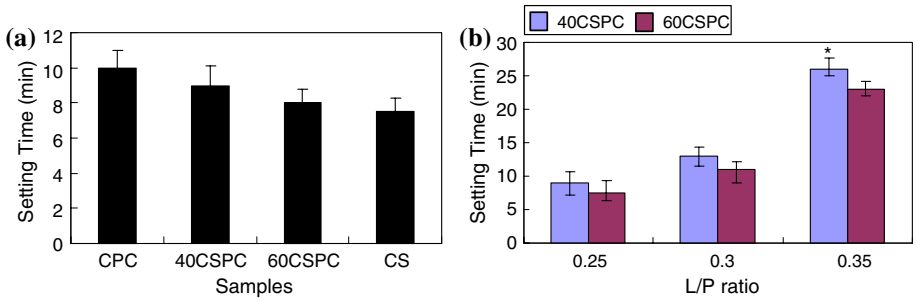

Fig. 1 Effect of **a** CS content in CPC and **b** L/P ratio on setting time of cement samples after setting for 48 h

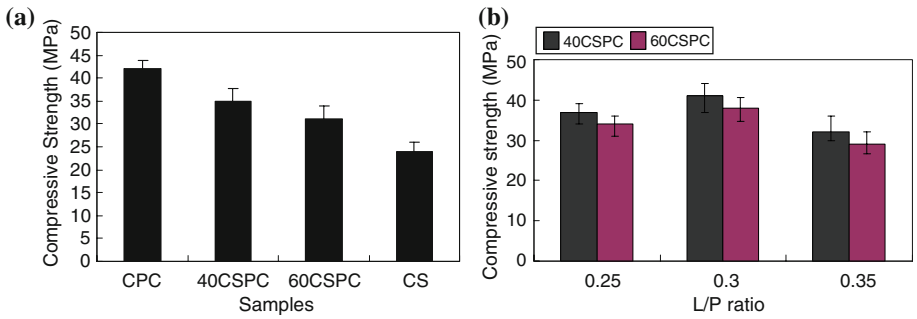

Fig. 2 Effect of **a** CS content in CPC and **b** L/P ratio on compressive strength of cement samples after setting for 48 h

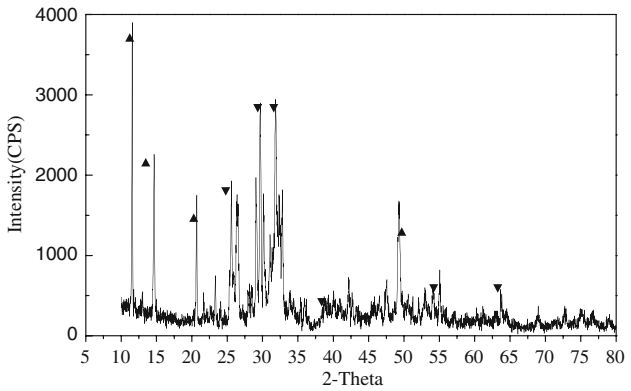

Fig. 3 XRD of 40 wt% CS in CPC after harden for 48 h, ▲ represents CSD and ▼ represents apatite

rates of the samples were characterized by their weight loss ratios in Tris–HCl solution. It is obverted that 60 wt% CSPC degraded most rapidly, while CPC degraded least during the immersion period. The degradation rate of CSPC was higher than that of CPC due to the presence of CS. Comparison of the CSPC samples containing different amounts of CS suggested that the higher the percentage of CS, the higher the degradation rate.

Figure 6 shows the SEM micrographs of the surface morphologies of the 40 wt% CSPC samples after immersing in SBF for 4 and 8 weeks, respectively. It can be clearly seen that the degradation of CSPC had occurred, and many micropores appeared with the increase of time.

In addition, the change of pH value of Tris–HCl solution after CSPS immersed for 14 days is also shown in Table 1. The results reveal that the pH of Tris–HCl solution decreased slightly up to 7 days (from 7.4 to 7.23), and then maintained nearly 7.24 up to 14 days. Furthermore, no obvious changes of pH value for CPC were found up to 14 days (from 7.4 to 7.35).

3.6 Cell proliferation and cell morphology

The results of proliferation of cells cultured on the (a) CPC, (b) 40 wt% CSPC and (c) 60 wt% CSPC samples are shown in Fig. 7. The OD values from MTT assay provide

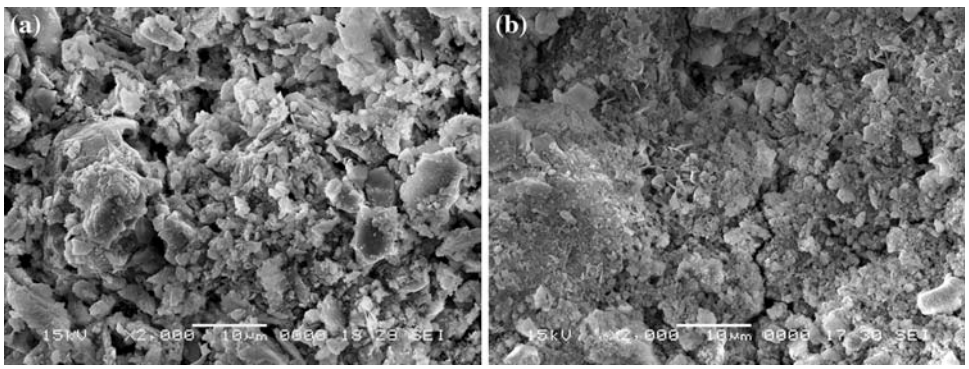

Fig. 4 SEM micrographs for the fracture surfaces of **a** CPC and **b** 40 wt% CSPC, respectively, after hardening for 48 h

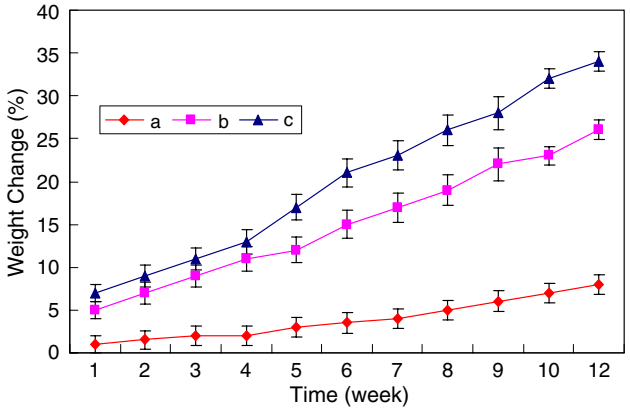

Fig. 5 Weight changes of (*a*) CPC, (*b*) 40 wt% and (*c*) 60 wt% CSPC immersed in Tris–HCl solution

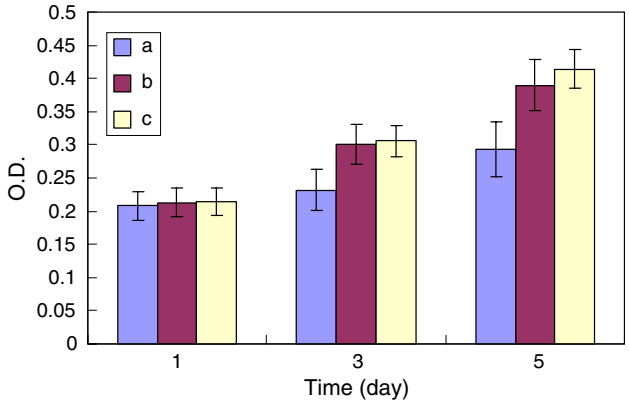

Fig. 7 Effects of (*a*) CPC, (*b*) 40 wt% CSPC and (*c*) 60 wt%CSPC on cell proliferation

an indication of cell growth and proliferation on various materials. It can be seen that the cell proliferation on the three kinds of materials increased with culture time. The proliferation of cell for CSPC is obviously higher than CPC after culturing for 3 and 5 days, despite the fact that no significant difference appeared after 1 day's culture. The results show that there was not significantly deference between 40 and 60 wt% CSPC on cell proliferation.

Figure 8 shows SEM micrographs of morphologies of cells cultured on 40 wt% CSPC surfaces. After 4 days' culture (Fig. 8a), the cells firmly attached and spread well on the sample surfaces. In Fig. 8b, after culturing for 7 days, cells formed a confluent layer with intimate contact to the sample surface, while maintaining physical contact with each other. These results suggest no adverse cellular response by the CSPC samples.

3.7 Histological evaluation

The histological evaluation of the CSPC samples implanted in the bone defects of rabbit femora are shown in Fig. 9. After 1 month's implantation (Fig. 9a), the CSPC implant was encapsulated by bone tissue, the interface between CSPC implant and the host bone was clearly visible, and the CSPC sample started to degrade from the edge of the implant. After 2 months, as shown in Fig. 9b, some new bone tissues formed and grew into the pores of the sample. Cement resorption at the bone-cement interface was prominent and the new bone was in direct contact with the surface of the CSPC implant. After 3 months' implantation (Fig. 9c), the resorption of CSPC continued and paralleled the new bone formation in many areas of the implant. The boundary between the cement and host bone was unclear due

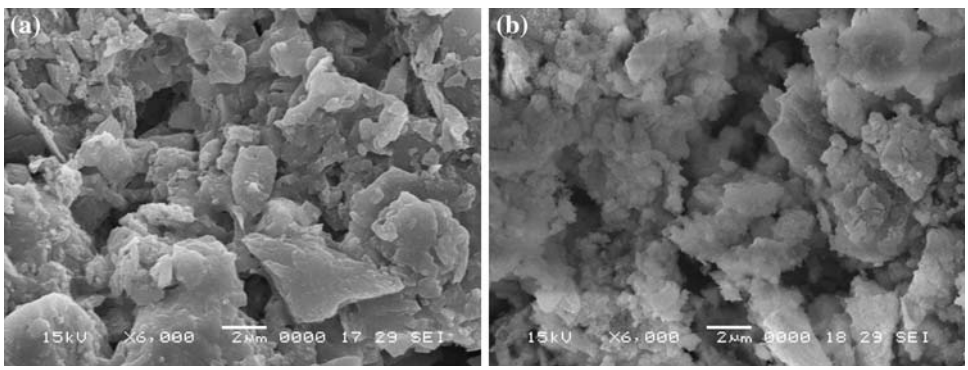

Fig. 6 SEM micrographs of 40 wt% CSPC immersed in Tris–HCl solution for 4 (**a**) and 8 (**b**) weeks

Table 1 Changes of pH of the Tris–HCl solution after CSPS soaking over time

Time (day)	0	1	3	5	7	14
pH (CSPC)	7.4 ± 0.02	7.36 ± 0.02	7.31 ± 0.01	7.26 ± 0.01	7.23 ± 0.02	7.24 ± 0.01
pH (CPC)	7.4 ± 0.02	7.37 ± 0.01	7.35 ± 0.01	7.35 ± 0.01	7.33 ± 0.02	7.35 ± 0.01

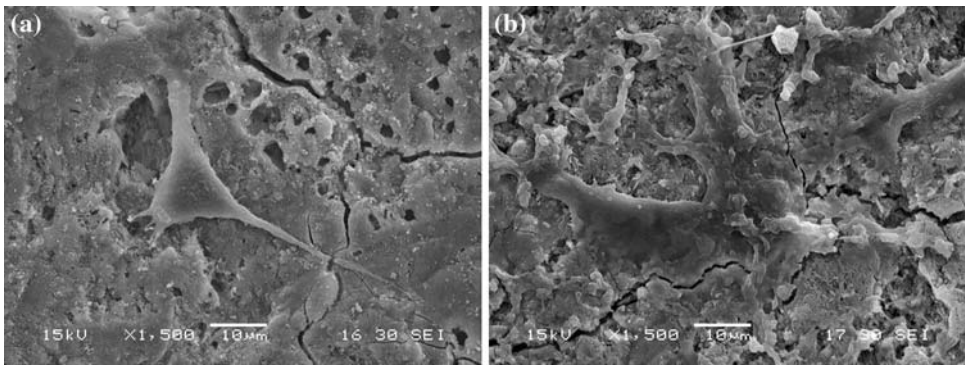

Fig. 8 Scanning electron micrographs of MG_{63} cells cultured on 40 wt% CSPC surfaces for **a** 4 and **b** 7 days

to the sufficient formation of mature bone tissues which had grown into the pores of the cement and bonded tightly with the material. After 6 months (Fig. 9d), most of the original CSPC implant was replaced with the new bone. The interface between the cement and the host bone was hardly detectable and a close union without gap was formed.

4 Discussions

Injectable and degradable bone cement consists of calcium sulphate and calcium phosphate cement was prepared, and the CSPC cements can be handled as paste and easily injectable, which is different from the some traditional calcium phosphate cements in setting time, degradability, bioactivity and composition etc. Two hydration processes are thought to proceed simultaneously when CSPC powder comes in contact with the cement liquid (water). One process is the hydration of CS; the other is the hydration process of CPC. Traditional CPC, which has a slowly degradability due to the end product is hydroxyapatite [11]. Therefore, the bioactive and degradable cement of CSPC prepared in this experiment may have better properties and superior to other some conventional calcium phosphate cement biomaterials, and has a huge potential for repair or substitute of bone fracture and defects.

As with any cement, the setting time is very important in clinic use [12]. In this study, the results reveal that the L/P ratio plays an important role on the setting time of the cement and significantly affects the setting time of CSPC. If the L/P ratio increased beyond the optimum range, the powders of the cement may be too excessive to be completely wet by the little liquid, and thereafter the obtained cement pastes would fail to reach a workable state.

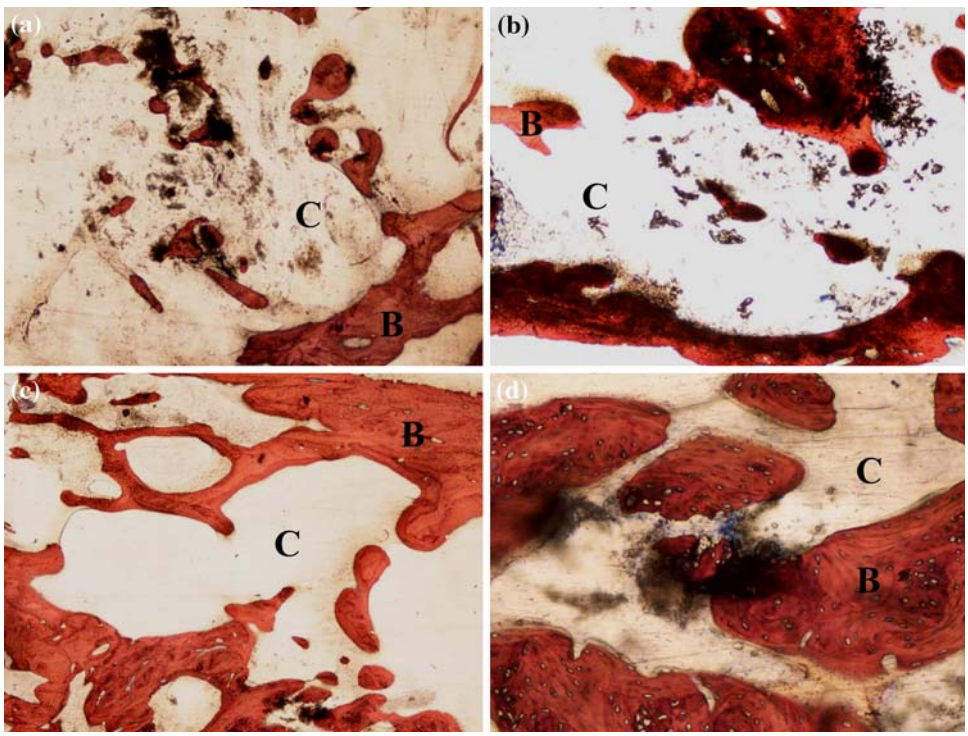

Fig. 9 Hematoxylin/eosin-stained sections of 40 wt% CSPC samples implantation in vivo for 1 (**a** ×20), 2 (**b** ×20), 3 (**c** ×20) and 3(**d** ×40) months. In the photos, *B* denotes newly formed bone tissue, while *C* denotes cement

On the other hand, too low L/P ratio may produce high levels of porosity which would result in poor mechanical properties of hardened cement. On the basis of our preliminary results, the L/P ratio of 0.3 ml/g was selected for CSPC samples because this ratio yielded cement mixtures with the desired consistency and workability. In addition, no obviously difference on setting time between 40 and 60 wt% CSPC was found, and the CSPC cements with 40 and 60 wt% CS content sets within 11–15 min in this study, which are very suitable for clinical application. Moreover, the results also reveal that the setting time decreased slightly with the increase of CS content in CPC, and adding 40 and 60 wt% CS into CPC have not obviously effect on the setting time of CSPC.

Mechanical strength is another important factor for clinical use of bone cements [13]. The compressive strength of the CSPC cement with 40% CS content has a normal value of around 35 MPa after hardening for 48 h, which is suitable for clinical use. The compressive strength of the CSPC cements would decrease with the increase of the content of CS, the results showed that increasing CS content had an obvious effect on the compressive strength of the cement. The decrease of the compressive strength of the CSPC cement is probably because the addition of the CS into CPC damaged the bond among the CPC crystals. In addition, the compressive strength of the CSPC decreased with the increase of L/P ratio, the compressive strength decreased from 41 to 32 MPa with the increase of L/P ratio from 0.3 to 0.35 ml/g after setting for 48 h. The L/P ratio has significantly effect on the compressive strength of the CSPC. It was found that the injectability of the CSPC improves as the L/P ratio increase. However, improving the injectability of the CSPC while decreases the compressive strength.

Degradability of CPC has been investigated for many years. Some studied results revealed that the degradability of the CPC was very slow both in vivo and in vitro [14, 15]. In order to improve the degradability of CPC, adding calcium sulphate into CPC was performed in present study. The results show that the degradation rate of the CSPC in Tris–HCl solution was lower than that of CS but higher than that of CPC. The hydroxyapatite formed by the hydration of CPC had a relatively low dissolution rate, leading to the low degradation rate of CPC [16]. The improved degradation rate of CSPC should be attributed to the rapid dissolution of CS. The results suggest that the higher the percentage of CS in CPC, the higher the degradation rate of CSPC. Moreover, the higher degradable rate of CSPC in Tris–HCl solution was because of first degradation of calcium sulphate, the quickly dissolution of CS on the CSPC surface to form a number of micropores on the cement surface, which increased the contact area of cement with solution. Therefore, the degradation rate of CSPC could be controlled to a certain extent by varying the percentage of CS in CSPC.

Generally, in vitro cell culture experiment is a useful approach to evaluate the biocompatibility of the biomaterials. The MG_{63} cells were able to proliferate on the CSPC, as demonstrated by the MTT assay, suggesting a positive cellular behavior. Furthermore, the proliferation rate was obviously improved on the CSPC as compared with CPC, indicating that the CSPC could promote cellular proliferation superior to CPC. Thus, the CSPC were biocompatible, with no obvious negative effects on cellular viability, or proliferation. The biocompatibility of biomaterials is very closely related to the cell behavior in contact with them and particularly to cell spreading on their surface. The SEM results indicated that the cells firmly attached on the CSPC surfaces after 1 day. In addition, the cells spread well and formed a confluent layer with intimate contact to the samples surface, while maintaining physical contact with each other after 5 days. These results indicated that the CSPC had no negative effects on cell morphology and viability. Having been applied in clinic for many years, CPC is proved to be biocompatible because its individual components [$Ca_4(PO_4)_2O$, $CaHPO_4$] and the hydration product (apatite) are biocompatible, and CS has been proved to be biocompatible both in vitro and in vivo [17, 18]. In present study, the results show that CSPC stimulated cell proliferation better than CPC, and the morphologies of MG_{63} cell in direct contact with the CSPC were normal, indicating that CSPC had good biocompatibility.

Macroscopic evaluation results show that the preset CSPC samples implanted into the bone defects of rabbits exhibited no foreign body reaction, no inflammation and no necrosis with host bone. Histological evaluation studies show that the CSPC samples implanted into the bone defects of rabbits were encapsulated by bone tissues after 1 month. Some new bone tissues were observed to extend along the surface of implant, and the samples had formed direct bonding with host bone without intervention of soft tissue after 2 months of implantation. The contact of bone to the material was intimate and direct, exhibiting better osteoconduction at the interface between material and bone. In present study, degradation process of CSPC was observed through the 6-month implantation. As the implantation time prolonged, new bone regenerated and gradually penetrated into the implant, accompanied by the resorption of the implant. It is possible that the chemical dissolution and resorption of CSPC occurred at the early stage of implantation. The dissolution at initial stage enlarged the microstructure of the implant, which might facilitate cell-mediated resorption later. The area of CSPC implant continued to reduce with the gradually increase of newly formed bone, indicating that a cell-mediated resorption of CSPC occurred. New bone deposited directly

on the CSPC surface with the resorption of CSPC. Histological and macroscopic findings confirmed that CSPC implants exhibited high efficiency of bone regeneration. It is suggested that CSPC present not only good biocompatibility and biodegradability but also faster and more effective osteogenesis.

5 Conclusions

Injectable CSPC was prepared by combining CS with CPC. CSPC cement is a novel Injectable biomaterial, which provides a new way to prepare degradable and bioactive bone repair materials. The results show that addition CS into CPC had not significantly effect on setting time but obviously effect on compressive strength of the prepared CSPC compared with CPC. The degradation rate of the CSPC was higher than CPC in Tris–HCl solution, and accelerated with the increase of CS content in CSPC. The CSPC could support MG_{63} cells attachment and proliferation, the cell proliferation rate on CSPC was significantly higher than that of CPC, indicating that CSPC has good biocompatibility. Macroscopic observation of CSPC implanted into the bone defects of rabbits show that the implants exhibited no foreign body reaction, no inflammation and no necrosis in vivo. Histological evaluation confirm that CSPC implants formed direct bonding with host bone, and exhibited high efficiency of bone regeneration.

In summary, CSPC presented not only good biocompatibility and degradability but also faster and more effective osteogenesis in the defect area. The CSPC cement prepared in this experiment has a reasonable setting time, suitable mechanical strength, excellent degradability and bioactivity for bone repair, which can be handled as paste, and easily injected. Such cement could be a good artificial bone material for clinical application and has a promising prospect.

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References

1. Shona PY, Kurisawa M, Gao S, Chung JE, Ying JY. The development of a nanocrystalline apatite reinforced crosslinked hyaluronic acid-tyramine composite as an injectable bone cement. Biomaterials. 2009;30:822–8.
2. Habraken WJEM, De Jonge LT, Wolke JGC, Yubao L, Mikos AG, Jansen JA. Introduction of gelatin microspheres into an injectable calcium phosphate cement. J Biomed Mater Res B. 2008;87:643–55.
3. LeGeros RZ. Calcium phosphate-based osteoinductive materials. Chem Rev. 2008;108:4742–53.
4. Grover LM, Gbureck U, Wright AJ, Tremayne M, Barralet JE. Biologically mediated resorption of brushite cement in vitro. Biomaterials. 2006;27:2178–85.
5. Theiss F, Apelt D, Brand B, Kutter A, Zlinszky K, Bohner M. Biocompatibility and resorption of a brushite calcium phosphate cement. Biomaterials. 2005;26:4383–94.
6. Rauschmann MA, Wichelhaus TA, Stirnal V, Dingeldein E, Zichner L, Schnettler R, et al. Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite carrier material for local delivery of antibiotics in bone infections. Biomaterials. 2005;26:2677–84.
7. Nilsson M, Fernandez E, Sarda S, Lidgren L, Planell JA. Characterization of a novel calcium phosphate/sulphate bone cement. J Biomed Mater Res. 2002;61:600–7.
8. Gitelis S, Piasecki P, Turner T, Haggard W, Charters J, Urban R. Use of a calcium sulfate-based bone graft substitute for benign bone lesions. Orthopedics. 2001;24:162–6.
9. Kelly CM, Wilkins RM, Gitelis S, Hartjen C, Watson JT, Kim PT. The use of a surgical grade calcium sulfate as a bone graft substitute: results of a multicenter trial. Clin Orthop Rel Res. 2001;382:42–50.
10. Liu CS, Shao HF, Chen FY, Zheng HY. Rheological properties of concentrated aqueous injectable calcium phosphate cement slurry. Biomaterials. 2006;27:5003–13.
11. Link DP, Van Den Dolder J, Van Den B, Jeroen JJP, Mikos AG, Jansen JA, et al. Evaluation of an orthotopically implanted calcium phosphate cement containing gelatin microparticles. J Biomed Mater Res A. 2008;87:760–9.
12. Xu Hockin HK, Takagi S, Quinn JB, Chow LC. Fast-setting calcium phosphate scaffolds with tailored macropore formation rates for bone regeneration. J Biomed Mater Res A. 2004;68:725–34.
13. Wang X, Ye J, Li X, Dong H. Production of in-situ macropores in an injectable calcium phosphate cement by introduction of cetyltrimethyl ammonium bromide. J Mater Sci Mater Med. 2008;19:3221–5.
14. Bodde EW, Cammaert CT, Wolke JG, Spauwen PH, Jansen JA. Investigation as to the osteoinductivity of macroporous calcium phosphate cement in goats. J Biomed Mater Res. 2007;83:161–8.
15. del Real RP, Ooms E, Wolke JG, Vallet-Regi M, Jansen JA. In vivo bone response to porous calcium phosphate cement. J Biomed Mater Res. 2003;65:30–6.
16. Damien CJ, Parsons JR, Prewett Annamarie B, Frank Huismans, Shors Edwin C, Holmes Ralph E. Effect of demineralized bone matrix on bone growth within a porous HA material: a histologic and histometric study. J Appl Biomater. 1995;9:275–88.
17. Julien M, Khairoun I, LeGeros RZ, Delplace S, Pilet P, Weiss P, et al. Physico-chemical-mechanical and in vitro biological properties of calcium phosphate cements with doped amorphous calcium phosphates. Biomaterials. 2007;28:956–65.
18. Enrique F, Maria DV, Maria MG, Jose L, Ricardo T, Juan VC, et al. Modulation of porosity inapatitic cements by the use of alpha-tricalcium phosphate-calciumsulphate dihydrate mixture. Biomaterials. 2005;26:3395–404.