Materials Science and Engineering C 33 (2013) 1048–1053

Contents lists available at SciVerse ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Improved workability of injectable calcium sulfate bone cement by regulation of self-setting properties

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ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form 27 September 2012
Accepted 13 November 2012
Available online 21 November 2012

Keywords:
Injectable
Calcium sulfate
Bone cement
Self-setting property
Regulation

ABSTRACT

Calcium sulfate hemihydrate (CSH) powder as an injectable bone cement was prepared by hydrothermal synthesis of calcium sulfate dihydrate (CSD). The prepared materials showed X-ray diffraction peaks corresponding to the CSH structure without any secondary phases, implying complete conversion from CSD phase to CSH phase. Thermogravimetric (TG) analyses showed the crystal water content of CSH was about 6.0% (wt.), which is near to the theoretic crystal water value of CSH. From scanning electron microscopy (SEM) micrographs, sheet crystal structure of CSD was observed to transform into rod-like crystal structure of CSH. Most interesting and important of all, CSD as setting accelerator was also introduced into CSH powder to regulate self-setting properties of injectable CSH paste, and thus the self-setting time of CSH paste can be regulated from near 30 min to less than 5 min by adding various amounts of setting accelerator. Because CSD is not only the reactant of preparing CSH but also the final solidified product of CSH, the setting accelerator has no significant effect on the other properties of materials, such as mechanical properties. In vitro biocompatibility and in vivo histology studies have demonstrated that the materials have good biocompatibility and good efficacy in bone regeneration. All these will further improve the workability of CSH in clinic applications.

1. Introduction

Calcium sulfate hemihydrate (CSH, $CaSO_4 \cdot 0.5H_2O$) has long been used for filling bone defects because of its excellent biocompatibility and capability for bone repair [1–5]. In particular, when mixed with water, CSH powder is converted into calcium sulfate dihydrate (CSD, $CaSO_4 \cdot 2H_2O$), which continues to be the object of research and interest as one of the most successful bone cements [6–8], because it has the ability to undergo in situ setting after filling the defects, has a good biocompatibility without inducing an inflammatory response, and promotes bone healing [9].

However, the present orthopedic implants of CSH have a major disadvantage in that they exist in solid-preformed block shape. The surgeon has to fit the surgical site around the implant due to a lack of available implants of the desired shape, which can lead to increased bone loss and a longer time for the surgery [10]. In the recent years, injectable bone cements that can undergo in situ setting after filling the defects have been exploited to augment human bone tissue and have drawn increasing attention for its good handling characteristics [11–13]. Bone cement can be easily molded and is self-setting in vivo, which would minimize damage effects of large muscle retraction, reduce the sizes of the scars, lessen postoperative pain, and achieve rapid recovery [11].

CSH powder mixed with water (or water solutions) can form a paste state which can be injected into bone defects by the clinician and which is left to harden completely in situ to the dihydrate form [14,15]. So CSH can be an injectable bone cement. For injectable bone cement, appropriate self-setting property is an important factor for clinical applications, however, so far less research on the regulation of self-setting properties of CSH has been reported except that many studies concerning using CSH as repairing fillers for the treatment of bone defection are reported now and then. In fact, the unalterable self-setting time will greatly limit the clinical applications of injectable bone cement. Here, we will elaborate the regulation of self-setting properties of CSH as bone cement in order to improve its workability.

2. Materials and methods

2.1. Materials

Calcium sulfate dihydrate (CSD), medicine grade, was purchased from Merck & Co., Inc. (Germany), and sodium citrate (analytical reagent) and aluminum sulfate (analytical reagent) were from Sinopharm

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http://dx.doi.org/10.1016/j.msec.2012.11.019

Chemical Reagent Co., Ltd. (China). Culture medium and reagents (fetal bovine serum, penicillin, streptomycin and methylthiazol tetrazolium) were purchased from Genom Biomedical Technology. Inc. (China).

2.2. Material synthesis

2.2.1. Preparation of α-calcium sulfate hemihydrate

α-Calcium sulfate hemihydrate (CSH) suspension was prepared by heating and stirring calcium sulfate dihydrate (200 g) dispersed in deionized water (1134 ml) in a sealed reaction pot at 120 °C with a stirring velocity of 400 r/min for 6 h. Sodium citrate (0.5 g) and aluminum sulfate (0.5 g) were also added into the reaction pot as a crystal modifier. After the reaction finished, CSH powder was obtained by immediately filtering the suspension and washing the filtration residue 5 times with boiling water to remove the crystal modifier. Finally, the resultant powder was stored in the oven at 105 °C for a night, and then ground and sieved to 100-mesh.

2.2.2. Preparation of calcium sulfate bone cement

CSH powder with setting accelerator (CSD) at various contents (0%, 5%, 10%, 15% and 20%, weight ratio) was mixed with deionized water with liquid to solid (L/S) ratios of 0.4–0.8 ml/g at 0.1 ml/g interval. The mixture was stirred to form homogeneous paste immediately, and then it was injected into Teflon molds with a diameter of 6 mm and stored in 100% humidity at 37 °C to set. The resultant samples were used to carry out the mechanical experiment.

2.3. Material characterization

The prepared CSH was characterized using X-ray diffraction analyzer (XRD, D/max-2500PC, Rigaku, Japan), thermogravimetric analysis (TG 209 F1, NetZSch, Germany) in the temperature range from 25 to 300 °C at a heating rate of 10 °C min^{-1} and scanning electron microscopy (SEM, JSM6460, JEOL, Japan).

2.4. Injectability and self-setting properties

Injectability of CSH was evaluated by whether the paste can be gently extruded from a disposable syringe by hand. The syringes have a capacity of 5 ml, with an opening nozzle diameter of 2.0 mm. The setting times of CSH cement with 0%, 5%, 10% and 20% contents of setting accelerator were measured under various L/S ratios with a Vicat needle as previously described [16]. Six samples were tested for each group to calculate the mean and standard deviation ($n=6$).

2.5. Mechanical test

The compressive strength and modulus of solidified samples (6 mm diameter × 10 mm high) were measured at a loading rate of 1 mm min^{-1} by a universal testing machine (ZWICKZ005, ZWICK, Germany) after they were removed from the Teflon molds. Six samples were used for each group to calculate the mean and standard deviation ($n=6$).

2.6. Cell behavior on the cement

In order to evaluate the biocompatibility of materials, marrow stromal cells (MSCs) isolated from the tibias of New Zealand white rabbits were cultured on the materials. In brief, after the solidified samples were cut into disks (with 5 mm in diameter and 2 mm in thickness), they were placed into 48-well culture plates. Then the samples were sterilized with 75% ethanol for 4 h, soaked and rinsed five times with phosphate buffer solution (PBS). Finally, the culture medium was added to each well to soak the samples for 30 min in order to facilitate protein adsorption and cell attachment on the materials.

The cells were seeded and cultured on the processed materials at a density of 3×10^4 cells cm^{-2} in Dulbecco's Modified Eagle Medium (DMEM) supplemented containing 10% fetal bovine serum, 50 U ml^{-1} penicillin, and 50 μg ml^{-1} streptomycin at 37 °C with 5% CO_2 in an incubator. The medium was changed every 2 days.

After incubating for 2, 4, and 6 days, the methylthiazol tetrazolium (MTT) colorimetric assay was performed to study the cell behavior on the cement as previously described [17]. Briefly, the samples were washed three times with PBS after the culture medium was removed. About 200 μl serum-free DMEM and 20 μl MTT solution were added to each well. Following incubation for 4 h, the MTT formazan crystal formed. Then the culture medium and remanent MTT solution were removed, and 200 μl dimethylsulfoxide was added to each well to solubilize the MTT crystals for 30 min. Lastly, the solution was moved into a 96-well plate, and the optical absorbance at 490 nm was measured by a microplate reader (Bio-Rad, Model 680, USA). Three samples for each time point of each group ($n=3$) were tested in the experiment.

SEM analyses were also performed after cells were cultured for 2 days. The culture medium was removed after cell seeding for 48 h, and the samples were rinsed two times with PBS. After PBS was removed, 3% glutaraldehyde solution was poured into each well of culture plates and the samples were maintained at room temperature for 2 h to fix the cells on the cement. Then the samples were rinsed twice with PBS again, dehydrated by successive immersion in a graded series of ethanol solutions (50%, 70%, 80%, 90%, 95%, and 100% vol.%) for 10 min each, and freeze-dried in vacuum overnight. The samples were observed by SEM at an accelerating voltage of 10.0 kV after they were sputter coated with gold.

2.7. Implantation and histological analysis

Six Japanese white rabbits weighing about 2.8 kg were intravenously anesthetized with 3% pentobarbital sodium (30 mg/kg body weight). After they were positioned, the hair on rabbit mandibular and neck was depilated by 8% Na_2S. Under aseptic conditions, the skin was incised and mandible buccal side was exposed along the lower edge of rabbit mandibular, and a 13 × 10 mm box-shaped defect model was created on each side of rabbit mandible with a dental handpiece under constant cooling with saline, respectively. CSH cement with 20% content of setting accelerator was injected and implanted into the segmental defect, and the wounds were sutured layer by layer. Then the animals were given 400,000 units of benzylpenicillin potassium each day for 3 days to prevent infection. The animals were sacrificed at 4, 8, and 12 weeks after surgery. Rabbit mandibular tissue in box-shaped defect regions were extracted and fixed in 4% paraformaldehyde. Then they were defatted with chloroform, demineralized with 10% ethylenedimine tetra acetic acid (EDTA), dehydrated in a graded series of ethanol solutions, and embedded in paraffin. Sections (5 μm in thickness) were cut and stained with Masson's trichrome, and they were examined under a light microscope (BX51; Olympus). Histological evaluation which consisted of a morphological description of sections of each implant was carried out.

2.8. Statistics and data analyses

The experimental results were compared by one-way analysis of variance (ANOVA) using Origin 7.5 software. In all evaluations, $p<0.05$ was considered statistically significant.

3. Results

3.1. Material characterization

The conversion of CSD phase to CSH phase by hydrothermal synthesis of CSD was confirmed by conducting XRD analyses (Fig. 1). Only peaks (2θ (intensity), 14.752 (40), 25.576 (30), 29.756 (60),