Materials Science and Engineering C 42 (2014) 303–311

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Microstructure and properties of alendronate-loaded calcium phosphate cement

Zhonghua Shen [a], Tao Yu [a,b,*], Jiandong Ye [a,*]

[a] School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, China
[b] Department of Orthopaedics and Traumatology, The University of Hong Kong, Pokfulam, Hong Kong

ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form 3 May 2014
Accepted 18 May 2014
Available online 24 May 2014

Keywords:
Calcium phosphate cement
Alendronate
Rheological property
Injectability

ABSTRACT

Calcium phosphate cement (CPC), as an injectable bone substitute material is significant in bone defect treatment. Drugs and biological molecules are often incorporated into CPC to promote the healing of bone defects and treat some bone diseases. In this work, alendronate (ALN)-loaded CPC was prepared and the influences of the content of ALN on the setting time, microstructure of hydrate porosity, mechanical strength, in vitro drug release, rheological properties and injectability of CPC were systematically investigated. The results showed that the addition of ALN had no effect on the final hydration product of CPC. The setting time of CPC was prolonged, while the prolonging effect became weak when the larger amount of ALN was added. With the increment of ALN content, the hydroxyapatite crystals of cured CPC became smaller, and the hydrated CPC became more compact with lower porosity, which resulted in the improvement of compressive strength of CPC with a drug-loaded amount less than 1 wt%. The injectability was dramatically improved due to the addition of ALN, which was corresponding to the decrease of viscosity. The thixotropy of the CPC slurry was promoted with increasing the ALN content, which could enhance the stability of the slurry. However, it was worth noting that an inverted thixotropic loop appeared when the drug content was higher than 3.0 wt%. During the in vitro drug release, the initial burst release turned up for all formulations and the degree of burst release was different from each other. This work would allow advances in understanding the effect of ALN on the setting process and physical and chemical properties of CPC, and we should think over the appropriate content when adding ALN into CPC.

1. Introduction

Calcium phosphate cement (CPC) was first reported by Brown and Chow in the 1980s [1]. With the advantages of biocompatibility, bioactivity, non-exothermic setting and plasticity [2], it has been widely used in the treatment of bone defects. To satisfy the requirement of minimally invasive surgical techniques, injectable CPC (ICPC) has been developed, which is of great significance to broaden the application of CPC. Also, due to its non-exothermic setting trait, CPC is an ideal candidate for drug delivery application [3]. Drugs and biological molecules are often incorporated into CPC to promote the healing of bone defects and treat some bone diseases [4–6]. Lopez-Heredia and his coworkers [7] have developed a local drug delivery system using a CPC as carrier of paclitaxel (PX). The results indicated that PX released from CPC remained active to influence cell viability and demonstrated that CPC is a feasible delivery vector for chemotherapeutic agents. Li and his coworkers [8] have developed a composite bone substitute with rhBMP-2-loaded gelatin microsphere (GM) and calcium phosphate cement (CPC). After implanted in goats for 140 days, bone ingrowth was observed deeper in the rhBMP-2/GM/CPC composites, and this new graft composite released more loaded factors.

Bisphosphonates (BPs) are widely used in treatment of bone disorders. They were found to be effective on managing the diseases associated with bone loss, such as osteoporosis, bone metastases, fibrous dysplasia and myeloma [9]. Alendronate (ALN) is an often used bisphosphonate. Apart from the common function of BPs, it has been found that ALN has significant and dose-dependent effect on osteoblastogenesis by Gustavo Duque et al. [10]. Oral administration and intravenous injection, the traditional ways of drug delivery, were found to have some adverse problems, such as gastrointestinal disorders, flu-like symptoms and low oral bioavailability [11–13]. Therefore, it is necessary to improve the delivery method of BPs.

Much attention has been given to the calcium phosphate biomaterials as the drug carrier of BPs [14–17]. Due to the affinity of BPs to bone mineral, it is easy to obtain BPs-coated hydroxyapatite (HA) through chemisorption. Then, for the purpose of increasing the amount of drug loading and improving the handling and implantation of the material, CPC was introduced as a drug carrier [18–20]. Alendronate and pamidronate have been successfully loaded in the CPC based on α-tricalcium phosphate (α-TCP) [20]. Peter et al. [21] used calcium phosphate to deliver BP and found it resulted in improving

* Corresponding authors.
E-mail addresses: ytbio@hotmail.com (T. Yu), jdye@scut.edu.cn (J. Ye).

http://dx.doi.org/10.1016/j.msec.2014.05.043
0928-4931/

osteointegration between bone tissue and implant. A study by Wu et al. [22] indicated that zoledronate-impregnated CPC reduced bone turnover rate and restored bone architecture in ovariectomized rats. Nevertheless, due to the side effect of BPs, the conversion rate of α-TCP into Ca-deficient hydroxyapatite (CDHA) was decreased during the setting process of CPC [18]. Furthermore, to acquire suitable setting time and mechanical property, the amount of BPs introduced into the CPC is limited. To optimize the physical and chemical properties of BPs-loaded CPC and enlarge the drug loading capacity of CPC, more work should be done to understand the effect of BPs on the setting process of CPC paste.

The additives or drugs are often added to CPC for the aims of improving the physical and chemical properties and endowing the material with new functions [19,23,24]. However, because the setting reaction of CPC is a dissolution–precipitation process [25], any factors that affect the dissolution–precipitation process may impact the curing performance of CPC. To better understand the effect of ALN on the properties of CPC, it is necessary to study the setting behavior of ALN-loaded CPC. It is not only for the aim of improving the properties of an injectable material, but also for the aim of obtaining a better operability.

As CPCs are generally formed by a combination of two or more calcium orthophosphate components, the effects of BPs on setting process of different system of CPCs should be different from each other. Furthermore, the past studies of ALN-loaded materials mainly focus on drug release and biological assessment, and less research is about the effect of drugs on operability and physical and chemical properties of drug-loaded materials. In this work, we used partially crystallized calcium phosphate (PCCP)-based bone cement to load ALN in different concentrations. The effects of ALN on operability and physical and chemical properties of the CPC were systematically studied. The relationship between the changes of properties and microstructure was also discussed.

2. Materials and methods

2.1. Materials and preparation

The bone cement used in this study consisted of two components [26]: the partially crystallized calcium phosphate (PCCP, with the chemical formula of $Ca_3(PO_4)_2$) and dicalcium phosphate anhydrous ($CaHPO_4$, DCPA). PCCP was synthesized from an aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$ (0.36 mol/L) and $(NH_4)_2HPO_4 \cdot 12H_2O$ (0.15 mol/L) by chemical precipitation method in our laboratory. The detailed synthetic method of PCCP has been described in our previous reports [26,27]. DCPA was purchased from Shanghai No.4 Reagent & H.V. Chemical Co. Ltd. (Shanghai, China). Alendronate was commercially obtained from Tianfeng Fine Chemicals Co. Ltd. (Henan, China).

The amounts of ALN (0.02, 0.1, 0.2, 0.4 and 0.6 g, respectively) were mixed with 10 g of DCPA in the medium of ethanol (20 mL) and stirred for 8 h with magnetic stirrer to make them uniform distribution. The ethanol was then removed by drying at 60 °C for 24 h. The ALN-loaded CPC powder was obtained by mixing the DCPA with PCCP at a mass ratio of 1:1 (10 g PCCP was added to the ALN-DCPA mixture, obtained before). The ALN-loaded CPCs were contained 0.1, 0.5, 1.0, 2.0 and 3.0 wt% of ALN based on the CPC powder, respectively. To further study the change of setting time and injectability, 0.3 wt% of ALN content group was added in the corresponding tests; 4.0 wt% of ALN content group was also studied in setting time test and rheological analysis.

The ALN-loaded CPC and unloaded CPC (control group) powders were mixed with deionized water at a liquid to powder ratio of 0.4 mL/g to obtain the pastes. Then, the pastes were ready for setting time and injectability examinations. Cylindrical samples were prepared by pouring the pastes into steel cylindrical molds, which had an inner diameter of 6 mm and a height of 12 mm. After curing and demoulding, the samples were stored in an incubator at 37 °C and 97% humidity. After 3d, the specimens were ready for the phase analysis, microstructure observation and mechanical testing. The CPC slurry, used in rheological tests, was prepared at a liquid to powder ratio of 0.5 mL/g to obtain enough flowability.

2.2. Phase analysis and microstructure observation

The hydrated CPC samples were respectively milled into powder and tested by using XRD (X'Pert PRO, PANalytical, the Netherlands) with Cu target and K_α radiation at a testing voltage of 40 kV. Data were collected for 2θ from 10° to 80° with a scanning step size of 0.017. The morphology and microstructure of the specimens were observed with a scanning electron microscope (SEM, Nova NanoSEM 430, FEI, the Netherlands) on gold-coated samples at an accelerating voltage of 5 kV.

2.3. Compressive strength

During the process of sample preparation for mechanical testing, a stress of about 700 kPa was applied to the samples with a plunger for 5 s to remove the big bubbles in the pastes, after pouring the cement paste into steel molds. The compressive strength of the cylindrical samples was measured using a universal material testing machine (Instron 5567, Instron, Britain) at a crosshead speed of 0.5 mm/min according to the international standard ISO 13779-1, Implants for Surgery—Hydroxyapatite—Part 1: Ceramic Hydroxyapatite. Each measurement was repeated six times and the average value was calculated.

2.4. Porosity determination

The Archimedes technique was used to determine the porosity of the ALN-loaded CPC samples. Ethanol was used as displacement liquid. The weight of a dried sample was firstly measured and recorded as G_s. Second, the weight of a specific gravity bottle full of ethanol was recorded as G_1. Then the sample was placed in the bottle and evacuate under vacuum for 0.5 h to remove the air in open pores of the sample. The bottle full of ethanol and with the sample in it was weighed and recorded as G_2. After that, the sample, saturated with ethanol, was taken out from the bottle and weighed promptly. The weight was recorded as G_3. Finally, the porosity (P) was calculated using the following equation:

$$P = \frac{G_3 - G_S}{G_3 + G_1 - G_2} \times 100\% \quad (1)$$

The porosity of the sample was the average of six replicates.

N_2-gas adsorption–desorption method was used to measure the specific surface area and the pore size distribution of ALN-CPCs and the control according to the international standards ISO 9277:2010, Determination of the Specific Surface Area of Solids by Gas Adsorption—BET Method, and ISO 15901-2: 2006, Pore size Distribution and Porosity of Solid Materials by Mercury Porosimetry and Gas Adsorption—Part 2: Analysis of Mesopores and Macropores by Gas Adsorption, using a specific surface area and pore size analyzer (Nova 4200E, Quantachrome, USA). All samples were degassed for 6 h at 150 °C under high vacuum. In addition, the measurement was carried out at 77 K.

2.5. In vitro test of drug release

In vitro ALN release trials were performed in a shaking incubator at 60 rpm under 37 °C. The as-prepared cylindrical samples were soaked in phosphate-buffered solution (PBS, pH 7.4) at superficial area to volume of 0.1 cm^2/mL. At regular time intervals, 5 mL of the sample media was collected and replenished. The collected solutions were measured by using the quantitative method described in the literature [28]. An iron (III) chloride/perchloric acid solution was used to extract ALN into the aqueous phase. The solution was evaluated using UV spectrophotometer.