Acta Biomaterialia 31 (2016) 326–338

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

A simple and effective approach to prepare injectable macroporous calcium phosphate cement for bone repair: Syringe-foaming using a viscous hydrophilic polymeric solution

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ARTICLE INFO

Article history:
Received 20 August 2015
Received in revised form 28 October 2015
Accepted 25 November 2015
Available online 26 November 2015

Keywords:
Calcium phosphate cements
Si-HPMC hydrogel
Silated polymer
Macropores
Bone regeneration

ABSTRACT

In this study, we propose a simple and effective strategy to prepare injectable macroporous calcium phosphate cements (CPCs) by syringe-foaming via hydrophilic viscous polymeric solution, such as using silanized-hydroxypropyl methylcellulose (Si-HPMC) as a foaming agent. The Si-HPMC foamed CPCs demonstrate excellent handling properties such as injectability and cohesion. After hardening the foamed CPCs possess hierarchical macropores and their mechanical properties (Young's modulus and compressive strength) are comparable to those of cancellous bone. Moreover, a preliminary *in vivo* study in the distal femoral sites of rabbits was conducted to evaluate the biofunctionality of this injectable macroporous CPC. The evidence of newly formed bone in the central zone of implantation site indicates the feasibility and effectiveness of this foaming strategy that will have to be optimized by further extensive animal experiments.

Statement of significance

A major challenge in the design of biomaterial-based injectable bone substitutes is the development of cohesive, macroporous and self-setting calcium phosphate cement (CPC) that enables rapid cell invasion with adequate initial mechanical properties without the use of complex processing and additives. Thus, we propose a simple and effective strategy to prepare injectable macroporous CPCs through syringe-foaming using a hydrophilic viscous polymeric solution (silanized-hydroxypropyl methylcellulose, Si-HPMC) as a foaming agent, that simultaneously meets all the aforementioned aims. Evidence from our *in vivo* studies shows the existence of newly formed bone within the implantation site, indicating the feasibility and effectiveness of this foaming strategy, which could be used in various CPC systems using other hydrophilic viscous polymeric solutions.

1. Introduction

Calcium phosphate cements (CPCs) are attractive as bone substitutes due to their injectability and self-setting ability in physiological conditions, as well as to their higher similarity to biological apatites and higher reactivity than that of a ceramic hydroxyapatite [1–5]. Moreover, from the biological point of view, CPCs have the following excellent properties: on the one hand, they have proved to be biocompatible, resorbable and osteoconductive [6–11] and, on the other hand, they contain intrinsic micropores [12] allowing nutrients and metabolic wastes to flow and be transported throughout the implant site, which is beneficial to bone regeneration.

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http://dx.doi.org/10.1016/j.actbio.2015.11.055
1742-7061/

However, not only micropores but also macropores[2] seem necessary to accelerate resorption of CPCs and bone ingrowth. The micropores are too small to ensure the deep migration and proliferation of bone cells as well as the angiogenesis [13]. As a result, CPCs without macropores, especially apatitic cements given their low physicochemical solubility, present a slow rate of resorption and can only be slowly replaced by new bone from periphery of the defect [13–15]. Therefore, it appears imperative to introduce macropores in CPCs in order to achieve a fast resorption of CPCs and facilitate bone ingrowth not only from the periphery but also throughout the implant.

One of the challenges of introducing macropores in CPCs is to create macropores and simultaneously keep the *in situ* setting reaction of CPCs without disintegration in the presence of a liquid medium. In this sense, the techniques used to prepare macroporous CPCs are completely different from the ones used for porous ceramics [16–20] in which macropores are obtained by eliminating porogenic substances during sintering and sometimes by using toxic materials. The ideal methods for preparing macroporous CPCs should fulfill the following features: first of all, the macroporous CPCs should not lose the self-setting and injectable characteristics of cement-type materials. Second, the pastes of macroporous CPCs should present a good cohesion until full setting, without disintegration in contact with blood or other physiological solutions. In addition, the hardened macroporous CPCs should have a certain mechanical strength to support the structure of tissue layer. Last but not least, the additives used in macroporous CPCs should be nontoxic and biocompatible.

To date, a number of different approaches have been tried to prepare macroporous CPCs, including adding soluble porogenic agents [21–23], gas-generating compounds [24,25], surfactant [26], and resorbable polymers [27–31], even using three-dimensional printing [32] and solid free fabrication process [33]. However, adding a large amount of porogenic agents to insure the interconnectivity of the porosity could compromise the workability and biocompatibility of CPCs. Besides, some of these routes could only be applied on pre-set cements, and therefore could not be used in minimally invasive surgery; in addition, some of the routes presented required complex processes of preparation.

Recently, a new strategy was proposed, namely mixing the cement paste or the powder phase of cement with a foam which was obtained by using a foaming agent (such as albumen [34], polysorbate 80 [35,36], gelatine [37], soybean hydrogel [38] or the mixture of the latter two [39]. This approach operates without affecting the *in situ* setting of CPCs and can produce injectable macroporous CPCs having the possibility to be implanted via minimally invasive surgical techniques. The important point in this approach is the selection of the foaming agent, which must be water soluble, nontoxic and biocompatible, as well as associated with a good foaming ability and foam stability. In view of the works described in the scientific literature, there is still a need for a material combining all the above-mentioned properties, associated to a simple and robust fabrication process which can produce a highly stable and injectable foam.

To this aim, in previous studies [40,41], we found that hydroxypropyl methylcellulose (HPMC) and silanized-hydroxypropyl methylcellulose (Si-HPMC), which are biocompatible hydrophilic polymers, can not only improve the injectability and cohesion of the CPC pastes, but also enhance the resistance of CPCs to cracking. Moreover, we observed increasing amounts of macropores in both HPMC and Si-HPMC composite CPCs owing to the air bubbles unavoidably trapped during the preparation of composite CPC pastes [40,41]. Based on the above work, it could be anticipated that by using these biocompatible viscous hydrophilic polymers as foaming agents to voluntarily entrain air bubbles, it may be possible to prepare macroporous CPCs being injectable, cohesive, self-setting and with improved fracture behavior, which are critical factors for surgical applications of CPCs. Especially, Si-HPMC seems more attractive as the foaming agent because of its self-crosslinking property which endows the CPC pastes with an appealing rheological behavior at the early stage of setting and thus the ability to be used in open bone defects [41,42].

In this study, a simple and effective approach is proposed to prepare α-tricalcium phosphate (α-TCP)-based macroporous apatitic CPCs through an original syringe-foaming process, using Si-HPMC as the foaming agent. The injectability, cohesion, microstructure and mechanical properties of this macroporous CPC are systematically investigated, and a preliminary *in vivo* study of this new biomaterial is carried out in distal femoral sites of rabbits.

2. Materials and methods

2.1. Preparation of Si-HPMC foamed CPCs

All of the reagents used in this study were of analytical grade and were used without further purification. α-TCP powder and Si-HPMC solution are the two main components used to prepare Si-HPMC foamed CPCs. α-TCP powder was synthesized by heating a stoichiometric mixture of dicalcium phosphate anhydrous (CaHPO4; Alfa Aesar, Germany) and calcium carbonate (CaCO3; VWR, BDH, Prolabo) with a molar ratio 2:1 at 1360 °C for 15 h, followed by quenching in air to room temperature. X-ray diffraction (XRD, X'pert pro, PANalytical, Netherlands) was applied to examine the phase purity of the synthesized α-TCP powder and no other phase was observed. The α-TCP powder was further mixed with 2 wt.% of precipitated calcium-deficient hydroxyapatite (CDHA) taken as a seed for subsequent crystallization of apatite to get the solid phase of CPCs, which was milled in a Mortar Grinder (Retsch RM100, Germany) for 1 h to get a fine powder. The mean particle size of the fine powder mixture was 6 μm, measured using laser diffraction granulometry (Beckman Coulter LS230, USA) after dispersion in ethanol in an ultrasonic bath.

The Si-HPMC powder was synthesized by silanizing HPMC (Methocel® E4M, Colorcon-Kent-England) with 3-glycidoxypropyl trimethoxysilane (Aldrich, Germany) according to the method described by Bourges et al. [43]. Fatimi et al. [44] detailed the synthetic procedure of Si-HPMC, including the molecular weight of HPMC used (about 290,000 g/mol determined by light scattering) and the grafted silane percentage (0.59 wt.% determined by inductively coupled plasma atomic emission spectroscopy). The Si-HPMC solution was prepared by dissolving the aforementioned Si-HPMC powder in 0.2 M NaOH solution at 25 °C for 48 h, followed by dialysis against NaOH solution (0.09 M) for 16 h using a 6–8 kDa D-Tube Dialyzer (Spectra/Por®, UK). The pH value of the resulting Si-HPMC solutions was around 12.8.

Si-HPMC solutions are stable in strong basic media (pH > 12.1). When its pH decreases, Si-HPMC solution starts to gel, transforming into a hydrogel consisting of 98 wt.% of water and 2 wt.% of polymer, with a three dimensional network of Si-HPMC chains. The gelation principle of the Si-HPMC has been detailed elsewhere [45]. In this study, a 30 wt% NaH_2PO_4 solution was used to initiate the gelation of the Si-HPMC, reaching a final pH ranging from 7 to

[2] It is worth noting that the terms micro and macropores used in this field is different from those proposed by IUPAC (International Union of Pure and Applied Chemistry) which defines micropores as pores smaller than 2 nm and macropores as pores above 50 nm. As a common sense in the bone substitute research community, micropores are normally defined as pores smaller than a few microns; and the pores, which are larger than 100 μm, are referred to as macropores [16,47]. Moreover, sometimes researchers also define everything smaller than 10 μm as micropores, and mesopores are also mentioned.