Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Design and properties of novel gallium-doped injectable apatitic cements

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ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 6 May 2015
Accepted 22 May 2015
Available online xxxx

Keywords:
Calcium phosphate cements
Gallium
Design
Handling properties
Biocompatibility

ABSTRACT

Different possible options were investigated to combine an apatitic calcium phosphate cement with gallium ions, known as bone resorption inhibitors. Gallium can be either chemisorbed onto calcium-deficient apatite or inserted in the structure of β-tricalcium phosphate, and addition of these gallium-doped components into the cement formulation did not significantly affect the main properties of the biomaterial, in terms of injectability and setting time. Under *in vitro* conditions, the amount of gallium released from the resulting cement pellets was found to be low, but increased in the presence of osteoclastic cells. When implanted in rabbit bone critical defects, a remodeling process of the gallium-doped implant started and an excellent bone interface was observed.

Statement of Significance

The integration of drugs and materials is a growing force in the medical industry. The incorporation of pharmaceutical products not only promises to expand the therapeutic scope of biomaterials technology but to design a new generation of true combination products whose therapeutic value stem equally from both the structural attributes of the material and the intrinsic therapy of the drug.
In this context, for the first time an injectable calcium phosphate cement containing gallium was designed with properties suitable for practical application as a local delivery system, implantable by minimally invasive surgery. This important and original paper reports the design and in-depth chemical and physical characterization of this groundbreaking technology.

1. Introduction

The use of synthetic calcium phosphate-based bone substitutes in human bone surgery is continuously expanding [1–6]. These bioactive implants have the unique capacity to be resorbed *in vivo* according to bone remodeling kinetics and replaced by natural bone. While first generation products were dominated by porous ceramics, injectable calcium phosphate cements (CPCs) are currently under intense investigation [7–13] since they offer additional advantages, including primary mechanical properties similar to those of cancellous bone, along with an injectability suitable for implantation of the composite under minimally invasive surgery and adapted to any shape of defects.

In addition, the development of calcium phosphate cements opened up new applications in the field of drug delivery systems. Indeed, when implanted, CPCs combined with drugs may not only act, as their main primary function, as mechanically resistant sacrificial calcium phosphate source for bone reconstruction, but are also well-suited to address bone-related diseases or infections [14]. Therefore, CPCs have been considered as carriers for local and controlled supply of antibiotics, anti-inflammatory or anti-cancer agents [15–24], thus potentially providing a reliable strategy for producing efficient pharmacological effects only to specifically intended target sites.

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http://dx.doi.org/10.1016/j.actbio.2015.05.027
1742-7061/

In this context, bisphosphonate antiresorptive drugs (BP), one of the most conventional treatments of osteoporosis worldwide, have been successfully combined to deficient calcium apatite. Pre-clinical experiments using a large animal osteoporosis model (femoroplasty surgical approach) have thus shown both resorbability of the implanted calcium phosphate material and replacement by newly formed bone, with a therapeutic value stemming from both the attributes of the calcium phosphate matrix and the intrinsic biological activity of the bisphosphonate [25]. Then, a novel injectable BP-loaded calcium phosphate cement was designed and shown to be suitable in terms of (i) rheology, in order to be worth considering for implantation using minimally invasive surgery, and (ii) setting time and strength for reinforcing fragile bone sites [26]. In addition, this CPC-BP combined device provided cement-driven primary mechanical properties (bone augmentation) and offered a better bioavailability of the drug locally [7,27].

More recently we have explored the potential of gallium-doped calcium phosphate materials. Indeed, many studies have shown that gallium(III) ions inhibit bone resorption [28–34], and for example gallium nitrate has been approved by the FDA for the treatment of cancer-related hypercalcemia and Paget's disease [35–38]. Therefore, we have investigated the *in vitro* effects of gallium nitrate, using well-established osteoclastic and osteoblastic models [39,40]. It was shown that gallium reduced the resorption activity, differentiation and formation of osteoclasts by non-cytotoxic mechanisms, in a dose-dependent (0–100 μM) manner. In addition, gallium did not induce any adverse effect on osteoblastic bone forming cells. These results suggested that gallium may offer a promising option for regulating the excessive osteoclastic activity taking place in osteoporosis or in some osteolytic bone tumours [41]. Interestingly, other groups in the literature have also reported that Ga^{3+} ions show antimicrobial properties as well as a capacity for reducing arthritis-related pain [42–46] and inflammation [47]. Finally, it is interesting to note that ^{67}Ga citrate is used in nuclear medicine for tumor imaging by scintigraphy, especially for lymphoma [48–51].

However, the bioavailability of gallium nitrate is very low and thus requires a long and continuous IV administration. For this reason, we have investigated the possibility to combine gallium with injectable apatitic cements for the development of a local delivery system of this ion in osteoporotic sites. In the present paper, we describe the development of a suitable protocol for this purpose while preserving the textural, mechanical and setting properties of the cement. The ability of the gallium-doped cements to release gallium ions was investigated under *in vitro* conditions, and *in vivo* implantation of these cements was also performed in bone critical defects on a rabbit animal model.

2. Materials and methods

2.1. Materials

Commercially available Na_2HPO_4 (Fluka), anhydrous dicalcium phosphate (DCPA –Sigma Aldrich, France), gallium nitrate hydrate (Alfa Aesar, Germany), Hydroxypropylmethylcellulose (HPMC, E4 M® – Colorcon-Dow chemical (Bougival, France)) were used as received. CDA was prepared by alkaline hydrolysis of DCPD using aqueous ammonia, as previously described [52].

α-TCP (alpha tricalcium phosphate) was prepared by calcination of a 2:1 M mixture of $CaHPO_4$ and $CaCO_3$ at 1350 °C for at least 4 h, and subsequent rapid cooling to room temperature. The obtained reaction product contained less than 5% of β-TCP.

Gallium-doped β-TCP ($Ca_{10.5-1.5x}Ga_x(PO_4)_7$, $x = 0.5$) was prepared by solid state reaction as previously reported [53].

Gallium-doped CDA. In a typical procedure, a mixture of gallium nitrate hydrate and calcium nitrate tetrahydrate was dissolved in a beaker containing 125 mL of ultrapure water, with a (Ca + Ga)/P molar ratio of 1.515 and a Ga/Ca molar ratio in the 0–0.08 range. The pH of the solution was adjusted in the 9–9.5 range by means of a concentrated solution of ammonia. The reaction mixture was then introduced in a three-neck angled round bottom flask placed in an oil bath and equipped with a dropping funnel. The temperature of the reaction mixture was raised to 50 °C and 1.089 g of diammonium hydrogen phosphate (8.25 mmol) dissolved in 125 mL of ultrapure water was added dropwise over a 5–10 min period. The mixture turned white and the pH was adjusted in the 7.5–8 range by means of a concentrated solution of ammonia. After 30 min, the obtained suspension (pH was neutral) was filtered off while hot and washed with 250 mL of ultrapure water. After repeating this procedure four times, the white waxy product was dried in an oven at 80 °C for 24 h. The gallium content of the collected aqueous fractions was measured by atomic absorption spectroscopy, to determine the amount of gallium incorporated in the isolated solid phase. Two samples were prepared containing 1.5 and 3 wt.% of gallium, respectively.

Preparation of the cement samples. The composition for the solid phase of the commercially available apatitic cement reference (Graftys® QUICKSET noted as QS-CPC) was the following: α-TCP ($Ca_3(PO_4)_2$, 78 wt.%), mixed with anhydrous dicalcium phosphate [DCPA] ($CaHPO_4$, 10 wt.%), calcium deficient apatite ($Ca_{10-x}[\ ]_x(HPO_4)y(PO_4)_{6-y}(OH)_{2-z}[\ ]_z$, 10 wt.%), hydroxypropyl methyl cellulose [HPMC] (2 wt.%). The composition of the different cement samples is reported in Table 1. For clarity, the references chosen for the CPC powders doped with gallium (Table 1, left column) indicate their gallium weight content and the nature of the Ga-doped component. Each CPC powder was milled to obtain a similar particle size distribution, and then sterilized by γ-irradiation.

Paste samples were prepared by mixing 6 g of the powdered preparation with 2.7 mL of a 0.5 wt.% Na_2HPO_4 aqueous solution for 2 min (liquid/solid ratio = 0.45 mL g^{-1}).

2.2. Methods

^1H and ^{31}P solid-state magic angle spinning (MAS) NMR experiments were performed on a Bruker Avance 300 spectrometer operating at 7.0 T (^1H and ^{31}P Larmor frequency of 300.0 and 121.5 MHz, respectively) using a 4 mm double-resonance MAS probe. ^1H MAS NMR spectra were recorded at a spinning frequency of 14 kHz using a π/4 flip angle (pulse length of 2.5 μs) and a recycle delay of 3s. All ^{31}P NMR spectra were recorded using a spinning frequency of 14 kHz and ^1H SPINAL-64 decoupling [54] (RF field strength of 70 kHz) was applied during signal acquisition. ^{31}P quantitative MAS spectra were obtained using a π/9 flip angle (pulse length of 0.8 μs) and a recycle delay of 30 s to ensure no saturation. 1D {^1H}–31P CP-MAS and 2D ^1H–31P heteronuclear correlation (HETCOR) spectra were recorded at different contact times (ranging from 0.25 to 12.5 ms) using a recycle delay of 1s. The ^{71}Ga solid-state NMR experiments were performed on Bruker Avance 750 and 850 spectrometers operating at 17.6 T and 20.0 T (71 Ga Larmor frequencies of 228.8 and 259.3 MHz, respectively) using a 1.3 mm double-resonance MAS probe. ^{71}Ga 1D MAS central transition spectra were recorded at high spinning frequencies ranging from 60 to 65 kHz using a Hahn echo sequence with a ^{71}Ga nutation frequency of 62.5 kHz (central transition selective π/2 pulse length of 4 μs). The echo delay was set to two rotor periods and the recycle delay was set to 3s. Under these experimental conditions, ^1H decoupling was not applied since it did not improve the spectral resolution for the studied sample nor reduce the linewidth of the ^{71}Ga resonances. ^1H, ^{31}P and ^{71}Ga chemical shifts were

Please cite this article in press as: C. Mellier et al., Design and properties of novel gallium-doped injectable apatitic cements, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.05.027