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Strontium doped injectable bone cement for potential drug delivery applications

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ABSTRACT

Microwave assisted wet precipitation method was used to synthesize calcium deficient strontium doped β-tricalcium phosphate (Sr-βTCP) with a chemical formula of $Ca_{2.96-x}Sr_x(PO_4)_2$. Sr-βTCP was reacted with monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2.H_2O$, MCPM] in presence of water to furnish corresponding Sr containing brushite cement (Sr-Brc). The samples were characterized by using X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR) and field emission scanning electron microscopy (FESEM). Strontium content in the prepared samples was determined by using inductively coupled plasma optical emission spectrometry (ICP-OES). The effect of Sr^{2+} ions on the structural, mechanical, setting properties and drug release of the cement is reported. Incorporation of Sr^{2+} ions improved the injectability, setting time and mechanical properties of the Brc. The release profiles of antibiotics incorporated in Brc and Sr-Brc confirmed that the Sr incorporation into the Brc results in the efficient release of the antibiotics from the cement.

1. Introduction

Calcium phosphate-based cements such as dicalcium phosphate (DCP) has received considerable attention of researchers due to their potential applications in dental, maxillofacial and orthopaedic surgery [1,2]. In addition, DCP is able to harden in situ and present appropriate mechanical properties [3,4]. Adequate mechanical strength, good setting time and the ability to inject the cement into a defect are now considered characteristics of paramount importance for minimally invasive surgery [5,6]. The use of trace elements in a biomaterial is an interesting alternative to growth factors when one wants to favor bone formation. Strontium is known to improve bone formation in osteoporotic patients [7–10], which is naturally present in the mineral phase of bone (1 wt%), especially in regions of high metabolic turnover [11]. The partial replacement of Ca^{2+} ions by Sr^{2+} ions can improve the mechanical properties [12] and the dissolution of the material [13].

Strontium is considered as a bone-seeking element that presents a beneficial effect on bone growth [14]. Its ability to decrease bone resorption and to enhance bone formation in vivo has also been proven [15–17]. Synthetic methods such as solid state calcination, co-precipitation, sol gel process and hydrothermal method [18–20] have been extensively studied for the preparation of Sr-TCP. Sr^{2+} ions have the same physiological and chemical behavior as Ca^{2+}, which can be embedded into the mineral structure of bone by ion substitution in place of Ca^{2+} [21]. Recent studies on Zn- and ZnSr-substituted brushite cements injected into trabecular bone defects in pigs proved that Zn and Sr are good inductors of osteoprogenitor cell proliferation and differentiation [22]. Results have indicated that Sr is a more potent inhibitor of osteoclastic activity than Zn, as much fewer osteoclast-like cells could be found in ZnSr-containing implants. Several studies have also shown that Sr-containing cements are more osteoconductive than Zn-containing cements [11,23,24].

Recently, strontium ranelate, a newly developed drug treating osteoporosis, has shown to have dual effects of stimulating osteoblast differentiation and inhibiting osteoclast activity (bone resorption), which could reduce the incidence of fractures in osteoporotic patients [16]. In addition, the partial substitution of Ca^{2+} by Sr^{2+} can apparently improve the biological properties of calcium phosphate based materials [25]. In vitro studies of SOAS cells treated with 625 μg/mL of Sr-HA ($Sr_{10}(PO_4)_6OH_2$) showed no signs of apoptosis [26]. In addition, Sr doped hydroxyapatite ($Ca_5Sr_5(PO_4)_6(OH)_2$)/chitosan composite scaffolds showed good osteoconductivity [27].

Osteomyelitis is a bone infection usually caused by bacteria, mycobacteria, or fungi. Its treatment mainly involves operative debridement, removal of all foreign bodies and antibiotic therapy [28,29]. The inability to maintain high antibiotic concentrations at the site of infection is the major cause of failure in the treatment of this disease. A

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good approach to overcome this situation is to fill the defect with an antibiotic-loaded bone graft together with the further local administration of the drug whenever required. Different types of biomaterials have been used as local drug delivery systems in resistant cases of osteomyelitis [30]. Ceramic-based local drug delivery systems have been suggested as potential materials for the treatment of osteomyelitis.

Most drug-delivery systems in clinical use are based on ceramic materials such as nanoporous alumina [31], silicon carbide [32] and calcium phosphates [33]. Many studies have investigated combinations of therapeutic agents with different calcium phosphates such as sintered hydroxyapatite (HA) [34], precipitated amorphous calcium phosphate [35] and calcium polyphosphates [36]. The success of this idea was favored by the easy incorporation of pharmaceutical and biological substances into the cement solid or liquid phases, the intimate adaptation of the cement paste to bone defects and the high cement porosity that permits the release of the entrapped substance to the local environment [37]. Furthermore, the low-temperature setting of CPCs allows the incorporation of heat-labile medicaments and substances into the cement matrix during its preparation. Water is part of the setting reaction of brushite cement and enables adjustment of the cement porosity, a determinant factor for the release kinetics of the loaded drug [38]. Investigation of osteotransductive brushite-based materials as carriers for antimicrobial agents [39,40] has been motivated by the difficulty of treating bone infections due to the poor accessibility of antibiotics to the infection site and the formation of antibiotic-resistant bio- films [41–44]. In this study, an array of dicalcium phosphate cement containing Sr^{2+} ions was prepared and the influence of Sr^{2+} doping on the setting time, injectability, compressive strength, porosity and drug release from dicalcium phosphate cement is reported.

2. Materials and methods

All chemicals for the synthesis of βTCP were purchased form Qrec (New Zealand) and were of reagent grade, whereas MCPM ($Ca(HPO_4)_2.H_2O$, 99.9%, MW 234.05) was purchased from Sigma-Aldrich (USA). Gentamicin sulfate, Ampicillin, and Amoxicillin trihydrate were sourced from Science Lab (Texas, USA).

Phase purity, lattice parameters and degree of crystallinity were evaluated by using Bruker D8 Advance X-ray diffractometer (XRD), the diffractogram was recorded between 2θ range of 20° - 80° at room temperature with the step size of 0.02° and step time of 1 s. The crystallinity noted by Xc corresponds to the fraction of crystalline βTCP phase in the investigated volume of powdered sample by using $Xc = 1- V_{300/0210} / I_{0210}$, where I_{0210} is the intensity of (0 2 10) reflection of βTCP structure and $V_{300/0210}$ is the intensity of the hollow between the (3 0 0) and (0 2 10) reflections. Lattice parameters were calculated by using Unit Cell software (program UnitCell-method of TJB Holland & SAT Redfern 1995). Morphology and elemental composition were studied by FESEM (Zeiss-LEO 1530) attached with Energy Dispersive X-Ray Analysis (EDX) (Oxford instrument, Swift ED 3000) operated at 20 kV. Readings at 5 different locations were recorded to calculate the average elemental composition. Functional groups were identified by FTIR analysis carried out on FTIR Nicolet iS50 spectrometer by using classic KBr pellet technique. The spectra were recorded in a wavenumber range of 400–4000 cm^{-1} in transmission mode with 32 scans and resolution of 4 cm^{-1}.

2.1. Preparation of Sr doped β-tricalcium phosphate

βTCP and Sr-βTCP were synthesized by a microwave assisted wet precipitation method. Different samples were prepared with a Ca/P and (Ca + Sr)/P molar ratio of 1.48 (Table 1). In a typical reaction, calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$) was dissolved in (100 mL) double distilled water. Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) was added dropwise under constant stirring to the solution of calcium nitrate, pH of the solution was adjusted to 7 by using ammonium hydroxide and the mixture was refluxed in a microwave oven (SHARP, model R-218LS) at 800 W for 5 min. The resulting suspension was filtered, washed with double distilled water, dried at 80 °C for 17 h and calcined at 1000 °C for 2 h to produce βTCP. The synthesis of four different strontium substituted β-$Ca_3(PO_4)_2$ compositions were prepared according to the procedure given above. In brief, calcium-deficient apatites containing Sr^{2+} ions were synthesized via a microwave assisted wet precipitation route by the slow addition of $(NH_4)_2HPO_4$ solution to the continuously stirred (1000 rpm) solution mixture of $Ca(NO_3)_2.4H_2O$ and $Sr(NO_3)_2$. The wt% of Sr in the sample varied between 2.4 and 8.9 (Table 1). The pH of the mixed solution/suspension was maintained at 7.4 by adding the required amounts of 8 M ammonium hydroxide (NH_4OH) solution.

Table 1
Molar quantities of reactants used for the synthesis of βTCP and Sr-βTCP.

Sample ID	$Ca(NO_3)_2.4H_2O$ (mol)	$(NH_4)_2HPO_4$ (mol)	$Sr(NO_3)_2$ (mol)	Sr (wt%)
β-TCP	8.88	6	0.00	–
1Sr-βTCP	8.63	6	0.25	2.4
2Sr-βTCP	8.38	6	0.50	6.6
3Sr-βTCP	8.13	6	0.75	7.4
4Sr-βTCP	7.88	6	1.00	8.9

2.2. Cement preparation

βTCP/Sr-βTCP (1.00 g) and MCPM (1.00 g) were thoroughly mixed and the liquid phase, water (1 mL) was added to the powder. The resulting mixture was mixed until a homogenized paste was achieved (Eq. 1).

$$Ca_3(PO_4)_2 + Ca\,(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4CaHPO_4 \cdot 2H_2O \quad (1)$$

2.3. Setting time

Initial and final setting times of the cements were measured by using Gillmore needle (ASTM C266–89). Powder phase components were mixed in a mortar for about 2 min and then mixed with liquid phase thoroughly to form a homogeneous paste. The cement paste was poured into a split Teflon mold of 6 mm diameter and 12 mm height. A needle of 2.12 mm diameter and 113.4 g was placed on the cement sample [6]. Initial setting time was recorded when the needle could not leave an impression on the surface of the cement paste. Similarly, a needle of 1.06 mm diameter and 453.6 g weight was used to determine the final setting time. The setting time reported is an average of 3 measurements.

2.4. Injectability of dicalcium phosphate cement

The paste of DCP cements was introduced in a commercial syringe with an aperture of 2 mm in diameter (13 mm diameter cartridge with a nominal capacity of 10 mL). A 5 kg compressive load was then mounted vertically on the top of the plunger to start the injection. Injections were carried out until the paste was no longer injectable. The percentage injectability was calculated by applying Eq. 2[45]:

$$Inj\% = (W_F - W_A)/(W_F - W_E) \times 100 \quad (2)$$

Where Inj% is the percentage injectability, W_F is the weight of the syringe full of paste and W_A is the weight of the syringe after the injection and W_E is the weight of the empty syringe.