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Antibiotic-loaded Sr-doped porous calcium phosphate granules as multifunctional bone grafts

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Abstract

Multifunctional porous calcium phosphate granules intended as osseous fillers and drug carriers were designed. Strontium- and magnesium-co-substituted biphasic hydroxyapatite (HA)/β-tricalcium phosphate powders (TCP) with compositions close to the mineral part of human bone [(Ca+Sr+Mg)/P=1.62] were prepared by precipitation, heat treated and deagglomerated, and characterized by XRD and SEM. Highly concentrated aqueous suspensions (up to 60 vol%) were prepared from the powders heat treated at 1000 °C. Starch was added as pore forming agent and the suspensions were dripped into a setting sodium alginate solution to obtain spherical granules. Drying and sintering enabled obtaining porous granules that were impregnated with an antibiotic solution (levofloxacin), frozen and then lyophilized. The drug release profile was then assessed *in vitro* by UV spectrophotometry, with the best release profile being obtained for the Sr-doped granules. The granules' osteocompatibility was evaluated using a pre-osteoblastic cell line. The Sr-doped granules exhibited the highest proliferation yields and efficiency in osteoblastic maturation, including acquisition of a differentiated morphology and high levels of secreted alkaline phosphatase. The microstructural features and the *in vitro* performance of the granules make them promising multifunctional materials for applications in tissue engineering as antibiotic-loaded bone grafts.

Keywords: Porous granules; Biphasic calcium phosphates; Ionic substitutions; Drug delivery; Levofloxacin

1. Introduction

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 [1,2]. The inability to maintain high antibiotic concentrations at the site of infection is the major failure in the treatment of this disease [3,4]. A rational approach for osteomyelitis treatment often combines local delivery of antimicrobials following surgery. Such an approach offers several advantages compared to oral or systemic treatment, including higher concentrations of drug availability at the target site with reduced adverse effects. However, the bone defect resulting from the surgical procedure still persists as a problem [5]. A 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 [6]. Ceramic-based local drug delivery systems have been suggested as potential materials for the treatment of osteomyelitis [7–10]. In this regard, hydroxyapatite (HA) received particular attention due to its biocompatibility, bioactivity, osteoconductivity and osteophilic nature [11,12], but its inability to be resorbed is a weak point [10]. Therefore, the most recent studies gave special emphasis to biodegradable systems that do not require surgical removal [13–15]. Biphasic calcium phosphates (HA+β-TCP) possess a set of attractive characteristics including: (a) different dissolution rates (HA, non-resorbable and β-TCP, resorbable); (b) rapid bone formation around the implant site; and (c) similarity to the inorganic component of

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bones [16–18], conferring them higher potential as bone replacement materials [19,20] and drug delivery systems [21,22].

Numerous studies dealing with CaPs bone grafts put the focus on ionic substitutions in crystal lattices of pure phases or biphasic mixtures [23–25]. The main motivations behind were the trace amounts of certain elements in bone composition (other than Ca and P) and some experimental evidences that these play essential roles in biological processes after implantation. For example, strontium increases osteoclast apoptosis and enhances pre-osteoblastic cell proliferation and collagen synthesis, and consequently depresses bone resorption, maintaining bone formation [24]. Therefore, Sr-substituted CaPs are expected to produce enhanced biological and chemical responses in the body [26,27]. Magnesium is closely associated with mineralization of calcified tissues, and indirectly influences mineral metabolism [28]. From the materials science perspective, Mg confers interesting features in terms of structural stability even after heat treatment at high temperatures [29,30].

Although different methods have been proposed to fabricate porous materials (polymeric sponge replication, foaming, incorporation of fugitive inclusions, etc.) producing ceramics with tailored pore structure is still challenging [31]. The present study aimed at preparing porous biphasic Mg- and Sr-doped CaPs that can be loaded with drugs to obtain multifunctional bone grafts. Mixtures of the CaPs powders and starch (pore former) were dispersed in an aqueous solution of sodium alginate (gelation agent) and the suspensions were dropped into a setting $CaCl_2$ solution to obtain spherical granules [32,33]. The selected drug to be tested was levofloxacin (LEV) is a suitable antibiotic for osteomyelitis due to its activity towards susceptible pathogens generally found in bone and joint infections, as *Staphylococcus aureus*, and its adequate penetration into osteoarticular tissues [34,35]. The heat-treated granules were impregnated with LEV and its releasing profiles were evaluated. The *in vitro* cellular viability, alkaline phosphatase activity and morphology of osteoblasts were also assessed and reported.

2. Material and methods

2.1. Synthesis of different calcium phosphate powders

Calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$], diammonium hydrogen phosphate [$(NH_4)_2HPO_4$], strontium nitrate [$Sr(NO_3)_2$] and magnesium nitrate hexahydrate [$Mg(NO_3)_2 \cdot 6H_2O$] were used as starting chemical precursors for Ca, P, Sr and Mg, respectively. Different Sr- and Mg- co-substituted calcium-deficient apatite compositions were prepared *via* a wet chemical route by slowly adding the P precursor solution to a mixed solution of Ca precursor and doping elements precursors (Sr, Mg) under mechanical stirring (1000 rpm). A constant molar ratio of (Ca+Sr+Mg)/P=1.62 was keep for all compositions. Table 1 presents the number of centi-moles of Ca, Mg and Sr precursors that were combined for each composition and the respective sample code. The pH of the mixed solution/suspension was increased to 9 and maintained at this value by adding the required amounts of 8 M ammonium hydroxide (NH_4OH) solution. The reaction was performed at 90 °C for 2 h under constant stirring conditions (1000 rpm). The precipitated slurries were poured out from the reactor and the solid particles were separated through vacuum filtration and dried at 80 °C overnight. The dried cakes were ground to fine powders, sieved through a 200 μm mesh, and used for characterization studies. The dried powders were calcined at different temperatures, varying within the range of 800–1200 °C in a Thermolab furnace (Pt30%Rh/Pt6%Rh thermocouple) using a heating rate of 5 °C min^{-1} up to the predetermined temperature, followed by a dwelling time of 2 h at that temperature and then cooled to room temperature (RT) at the rate of 5 °C min^{-1}.

Table 1
Number of centi-moles of Ca, Mg and Sr precursors used in the synthesis of the biphasic calcium phosphate samples and their respective codes.

		C	14Sr	14MgSr
(Ca+Sr+Mg)/P		**1.62**	**1.62**	**1.62**
Elements	**Precursor salts**	**Molarity (cmol)**		
Ca	Calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$]	**194**	**180**	**180**
P	Diammonium hydrogen phosphate [$(NH_4)_2HPO_4$]	**120**	**120**	**120**
Sr	Strontium nitrate [$Sr(NO_3)_2$]	**–**	**14**	**7**
Mg	Magnesium nitrate hexahydrate [$Mg(NO_3)_2 \cdot 6H_2O$]	**–**	**–**	**7**

2.2. Powders characterization

The physical characteristics of the powders were evaluated using a particle size analyser (COULTER LS230, UK) with Fraunhofer optical model for measuring the particle size distributions. The morphological features of the powders heat-treated at 1000 °C were analyzed by scanning electronic microscopy (SEM) (Hitachi SU-70, Hitachi High-Technologies Europe, GmbH, Germany), under an acceleration voltage of 25 kV and a beam current of 10 μA. The phase assemblage of both as-prepared and heat-treated powders was studied by X-ray diffraction using a high-resolution Rigaku Geigerflex D/Mac, C Series diffractometer with Cu Ka radiation (k=1.5406 Å) produced at 30 kV and 25 mA, which scanned the diffraction angles (2θ) between 20° and 60° with a step size of 0.02° $2\theta\,s^{-1}$. A spectra-fitting software called MAUD (Materials Analysis Using Diffraction) was utilized to quantify the percentages of crystalline phases for the powders heat-treated at 1200 °C (the selected sintering temperature for the granules). MAUD software requires crystallographic information files (CIFs) to fit and quantify each specific phase within a XRD spectrum. CIFs utilized in the evaluation of the powders were; HA (COD: ICSD-50656), β-TCP (COD: ICSD-97500 and 9012137) and α-TCP (COD: ICSD-923).