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Novel doped calcium phosphate-PMMA bone cement composites as levofloxacin delivery systems

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ABSTRACT

Antibiotic-loaded acrylic bone cements (ALABCs) are well-established and cost-effective materials to control the occurrence of bone and joint infections. However, the inexistence of alternative antibiotics other than those already commercially available and the poor ability to bind to bone tissue hampering its biological function are still major drawbacks of ALABCs clinical application. The concept of this research work is to develop a novel bone cement (BC) drug delivery system composed by Mg- and Sr-doped calcium phosphate (CaP) particles as drug carriers loaded into a lactose-modified acrylic BC, which, to the best of our knowledge, has never been reported. CaP particles are known to promote bone ingrowth and current research is focused on using these carriers as antibiotic delivery systems for the treatment of bone infections, like osteomyelitis. Levofloxacin is a fluoroquinolone with anti-staphylococcal activity and adequate penetration into osteoarticular tissues and increasingly being recommended to manage bone-related infections. Also, the lactose-modified BC matrix, with a more porous structure, has already proved to enhance antibiotic release from the BC inner matrix. This novel BC composite biomaterial has shown improved mechanical integrity, biocompatibility maintenance, and sustained release of levofloxacin, with concentrations over the minimum inhibitory concentration values after a 48 h while maintaining antibacterial activity over an 8-week period against *Staphyloccocus aureus* and *Staphyloccocus epidermidis*, common pathogens associated with bone infections.

1. Introduction

The use of antibiotic-loaded acrylic bone cement (ALABC) is still considered to be the standard of care for patients with chronic bone and joint infection, providing local delivery of high levels of antibiotics for an extended period of time without exceeding systemic toxicity, while being a more cost-effective procedure when compared to cementless implants (Jameson et al., 2015; Zilberman and Elsner, 2008). However, these ALABCs present major drawbacks, such as the incomplete and inadequate kinetic elution of the drug, the limited number of antibiotics available in commercially premixed formulas (Jiranek et al., 2006; Lewis 2009; Shi et al., 2010) and the poor bone tissue integration. Regarding the latter, one of the most described strategies to improve the biological performance of ALABCs, which are polymethylmethacrylate (PMMA) based cements, is the inclusion of osteoconductive materials aiming to enhance fixation at bony sites. PMMA-based bone cements incorporating calcium phosphate (CaP) ceramics to improve biological fixation between bone and cement have been one of the most recently reported materials in the field (Sa et al., 2015). Additionally, the properties of CaPs are not modified when incorporated in the PMMA matrix, maintaining its ability to promote bone ingrowth while the cement stays mechanically stable (Lopez-Heredia et al., 2012; Canul-Chuil et al., 2003). Therefore, loading CaPs into PMMA-based bone cement allows establishing a compromise between the desired mechanical and biological properties, combining the vast clinical experience of using PMMA, and the biological potential of CaP materials (Lopez-Heredia et al., 2012). Particularly, hydroxyapatite (HA) is an example of those CaPs due to the chemical and structural similarities with the inorganic phase of human bone,

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along with its biocompatibility, osteoconductive and osteophilic nature (Kim et al., 2004). More recently, doping of CaPs through the inclusion of foreign ions into the CaPs crystal lattice is described as an effective approach for improving CaPs osteointegration and mechanical properties (Boanini et al., 2010; Lima et al., 2011). Among others, magnesium (Mg^{2+}) is a particularly desired substitution ion for HA because it is associated with the mineralization of calcified tissues and indirectly influences mineral metabolism (LeGeros, 1991; Ren et al., 2010). Another interesting substitution ion is strontium (Sr^{2+}), which increases osteoclast apoptosis and enhances preosteoblastic cell proliferation and collagen synthesis, with consequent depression in bone resorption and increase bone formation (Guo et al., 2005; Pina et al., 2010).

The current study is intended at exploring important key-properties for the future development of a suitable system for the controlled release of antibiotics, circumventing the above-referred ALABCs handicaps. Hence, a lactose-modified acrylic bone cement matrix was chosen to favour drug release due to its more porous structure, when compared to plain bone cement, and proved mechanical and biocompatibility compliance (Frutos et al., 2009; Matos et al., 2014, 2015). The selected antibiotic was levofloxacin, a fluoroquinolone with high anti-staphylococcal activity and low toxicity, besides an adequate penetration into osteoarticular tissues above the minimum inhibitory concentration (MIC) for susceptible pathogens generally present in bone and joint infections (Zimmerli, 2015). Lastly, CaP particles, plain and doped with two different cations, Mg^{2+} and Sr^{2+}, were used as the carriers of levofloxacin to be loaded into the lactose-modified acrylic bone cement matrix.

In short, a novel bone cement (BC) composite was developed and tested, aiming at an *in vitro* release of the antibiotic, with concentrations above the MIC for the *Staphylococcal* spp., along with mechanical and biocompatibility compliance, for the potential application in bone and joint associated infections.

2. Materials and methods

2.1. CaP particles preparation

Strontium and magnesium doped CaP particles were obtained by aqueous precipitation and then loaded with levofloxacin (Lev, on specimens designation) (Sigma-Aldrich). Briefly, the synthesis was accomplished by the slow addition of $(NH_4)_2HPO_4$ (Quality Chemicals, Spain) solution to a continuously stirred mixed solution of $Ca(NO_3)_2{\cdot}4H_2O$ (Quality Chemicals, Spain) and $Mg(NO_3)_2{\cdot}6H_2O$ or $Sr(NO_3)_2$ (Sigma–Aldrich). For all compositions the precursor's concentrations were designed to achieve a [Ca + (Sr or Mg)]/P molar ratio of 1.67. The concentration of each doping element was fixed at 5 mol% (*i.e.*, 0.95 × 1.67 mol of Ca + 0.05 × 1.67 mol of Sr, or similarly, 0.95 × 1.67 mol of Ca + 0.05 × 1.67 mol of Mg). Pure HA was also prepared and use as a standard control material. The synthesized particles were calcined at 800 °C (Pt30%Rh/Pt6%Rh thermocouple, Thermolab) and dry milled for 30 min in a high energetic ball mill, using a porcelain mill (weight ratio of alumina balls to powder of 3:1). The obtained mean particle size was ~300 nm.

A levofloxacin aqueous solution was prepared with a suitable concentration of ~3 wt.% ($w_{Lev}/w_{Particles}$) to impregnate an estimated amount of 2.5 wt.% of drug in CaP particles. The impregnation process was similar for the three CaP particles and consisted in immersing particles in the Lev solution for 24 h, in the absence of light, followed by freezing particles at –80 °C (Hettich, Germany) for 4 h, and freeze-drying at –51 °C under a pressure of 1.5 Pa (Labconco, USA) for 48 h. According to their compositions, the levofloxacin impregnated powders will be hereinafter referred to as Lev[HA], Lev[Sr-HA] and Lev[Mg-HA]. The non-loaded and levofloxacin loaded particles will be also generically referred to along the text as CaPs and Lev(CaPs), respectively.

2.2. Preparation of the BC composite specimens

Commercial acrylic BC CMW1® Radiopaque (DePuy Synthes Portugal), a high viscosity BC intended for digital application, was used to prepare the BC composite specimens. Lactose monohydrate (Merck Millipore, Portugal), L on specimens' designation, was added as the poragen additive. Parallelepiped and cylindrical specimens were obtained according to the ISO specifications (ISO 5833, 2002), at room temperature (23 ± 1 °C) and atmospheric pressure, maintaining the commercial supplier recommended proportion [CMW1® powder]:[Monomer liquid]. Lactose, CaPs and Lev(CaPs) were added over the CMW1® powder. After careful mixing of all components, the monomer was added up to obtain a dough with the desired consistency. The dough was then manually cast into aluminium moulds. Cure proceeded for 1 h at room temperature. All specimens were finished to careful polishing, measured with a digital micrometer (Mitutoyo Digimatic, Painesville, Ohio, USA) with an accuracy of 0.01 mm, and stored in a vacuum desiccator (at 23 ± 1 °C for 24 ± 2 h) before use. Cylindrical specimens were used for compressive strength determination and *in vitro* release studies and parallelepiped specimens were used for flexural strength and modulus determination. Table 1 presents the acronyms of the different samples.

2.3. Mechanical assessment of the BC composites

Tests were performed at room temperature in a servo-hydraulic universal machine (TIRAtest® 2705). Assay parameters used for the compressive strength, flexural modulus and flexural strength determination were in strictly accordance with ISO 5833 specifications (ISO 5833, 2002). At least five specimens of each BC composite were tested and results were expressed as mean±SD.

2.4. BC composites inner structure and outer surface analysis

2.4.1. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) analysis

The inner structure of the cylindrical BC specimens was analyzed and photographed through a thermal field emission scanning electron microscopy, FEG-SEM, model JSM7001F (JEOL, Japan) operated at 5 kV. Samples were mounted onto aluminium stubs and their surface was coated with a gold–palladium film (thickness of 30 nm) under vacuum in an argon atmosphere (Quorum Technologies, Polaron E5100). Images were made using a backscattered electron detector. Back-scattered emission (BSE) was applied to improve the surface contrast photographs and ease element analysis through the atomic weight. The elemental chemical composition of the samples was determined by energy dispersion spectroscopy (EDS) with an Oxford Inca Energy 250 spectrometer. Analyzed specimens were ~1 mm slices of a selected group of representative specimens, obtained using a cut-off machine (Struers Accutom-5®, Struers, Denmark), mounted onto aluminium stubs and their surface was coated with a gold–

Table 1
Sample codes and composition of the BC composite specimens, expressed as wt.% of CMW1® powder.

BC Composite	Lactose	CaP particles	Lev(CaP) particles
BC_L	10.0		
$[HA]BC_L$	10.0	2.5	
$[Sr\text{-}HA]BC_L$	10.0	2.5	
$[Mg\text{-}HA]BC_L$	10.0	2.5	
$[Lev(HA)]BC_L$	10.0		2.5
$[Lev(Sr\text{-}HA)]BC_L$	10.0		2.5
$[Lev(Mg\text{-}HA)]BC_L$	10.0		2.5