Acta Biomaterialia 9 (2013) 8403–8412

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Relevance of microstructure for the early antibiotic release of fresh and pre-set calcium phosphate cements

Cristina Canal [a,b,c,1], David Pastorino [a,b,c,1], Gemma Mestres [a,b,c], Philipp Schuler [a], Maria-Pau Ginebra [a,b,c,*]

[a] Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgy, Technical University of Catalonia (UPC), Av. Diagonal 647, 08028 Barcelona, Spain
[b] Centre for Research in Nanoengineering, Technical University of Catalonia (UPC), C/Pascual i Vila 15, 08028 Barcelona, Spain
[c] Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Maria de Luna 11, Ed. CEEI, 50118 Zaragoza, Spain

ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form 14 May 2013
Accepted 15 May 2013
Available online 23 May 2013

Keywords:
Calcium phosphate cement
Drug release
Hydroxyapatite
Antibiotic
Doxycycline

ABSTRACT

Calcium phosphate cements (CPCs) have great potential as carriers for controlled release and vectoring of drugs in the skeletal system. However, a lot of work still has to be done in order to obtain reproducible and predictable release kinetics. A particular aspect that adds complexity to these materials is that they cannot be considered as stable matrices, since their microstructure evolves during the setting reaction. The aims of the present work were to analyze the effect of the microstructural evolution of the CPC during the setting reaction on the release kinetics of the antibiotic doxycycline hyclate and to assess the effect of the antibiotic on the microstructural development of the CPC. The incorporation of the drug in the CPC modified the textural and microstructural properties of the cements by acting as a nucleating agent for the heterogeneous precipitation of hydroxyapatite crystals, but did not affect its antibacterial activity. In vitro release experiments were carried out on readily prepared cements (fresh CPCs), and compared to those of pre-set CPCs. No burst release was found in any formulation. A marked difference in release kinetics was found at the initial stages; the evolving microstructure of fresh CPCs led to a two-step release. Initially, when the carrier was merely a suspension of α-TCP particles in water, a faster release was recorded, which rapidly evolved to a zero-order release. In contrast, pre-set CPCs released doxycycline following non-Fickian diffusion. The final release percentage was related to the total porosity and entrance pore size of each biomaterial.

1. Introduction

Bone infections, like osteomyelitis or periodontitis, are associated with bone loss. Often, a bone grafting procedure is needed after infection elimination. The local adminstration of antibiotics in combination with a delivery matrix with osteoconductive or osteoinductive properties may allow combating the infection while simultaneously promoting bone regeneration. In this approach, it is essential not only to ensure drug access to the specific bone site, but also to maintain the drug concentration released within the therapeutic range for long periods of time. Therefore, the drug elution kinetics must be carefully controlled.

Calcium phosphate cements (CPCs) have great potential as carriers for controlled release and vectoring of drugs in the skeletal system due to their composition close to bone mineral, excellent bioactivity and possible use as injectable and degradable grafting materials [1,2]. Their cold self-hardening process mimics the processes taking place in the biomineralization phenomena. Moreover, their microstructure can be tuned by modifying different processing parameters, such as the chemical composition of reactants, particle size or presence of nucleating agents [3]. This has allowed the fabrication of pre-set solid scaffolds or granules with controlled textural properties, with porosities ranging from the nano- to the macroscale, using new CPC-based fabrication routes [4].

Despite the abundant literature generated in the last decades on CPCs as drug delivery matrices [1], there is still a need for more reproducible and predictable CPC delivery systems, and this requires a better understanding of the parameters governing the release process. In their role as drug-eluting systems, CPCs can be classified as non-swellable monolithic systems. Although some CPCs are resorbable, their degradation rate can be considered to be much lower than the rate of drug liberation, and it can be assumed that the drug release is mainly controlled by diffusion through the cement matrix, the microstructural features of the CPCs playing a

* Corresponding author at: Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgy, Technical University of Catalonia (UPC), Av. Diagonal 647, 08028 Barcelona, Spain. Tel.: +34 934017706; fax: +34 934016706.
E-mail address: maria.pau.ginebra@upc.edu (M.-P. Ginebra).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.actbio.2013.05.016

crucial role in the drug eluting process. However, there are two facts that add complexity to the CPCs as drug delivery systems, and which are often overlooked when designing drug-eluting CPCs [5–7]: (1) the setting reaction of the CPC will be influenced by the presence of the drug, not only in terms of the setting kinetics and the rheological properties, but also in terms of the microstructural development and therefore the final textural features of the material, which must therefore be carefully characterized; and (2) the microstructure of the CPC, which is the drug eluting matrix, will be evolving during the setting process, in a lapse of hours or even days, from a suspension of ceramic particles in a liquid phase to a network of entangled crystals, which will affect the drug eluting kinetics. This is critical when the material is intended to be readily injected in the host tissue [8]. In this case the drug eluting process starts immediately after mixing the two phases, and takes place simultaneously to the setting process of the cement paste. In contrast, the situation is completely different when drug-loaded pre-set CPC matrices are fabricated in the form of scaffolds, implants or microspheres [9–13]. Recently different techniques have been reported that utilize the calcium phosphate setting reaction to fabricate bioceramic implants at low temperature, compatible with the introduction of bioactive molecules of drugs, including cement casting [9], emulsion [10] and low temperature three-dimensional (3-D) printing [11–13]. This last technique is especially promising since it allows the production of drug-releasing scaffolds with a precise control of the geometry and localized deposition of biologically active molecules [12,13]. In these approaches the material is not intended to be implanted in a paste form, but as a pre-set construct, and therefore the drug release process is independent of the setting reaction of the cement.

This study aims at elucidating the relevance of these two phenomena in a doxycycline-hyclate-containing CPC. Doxycycline hyclate (Doxy), of the family of tetracyclines, is an antibiotic commonly used in the treatment of bone infections, especially in periodontitis, due to its strong activity against periodontal pathogens and its broad-spectrum antibiotic effects [14]. It was the primary objective of this work to characterize the effect of Doxy on the microstructural and textural properties at the micro- and nanoscale of an apatitic CPC, and correlate them with the release properties. Also, the influence of the setting process on the release of the antibiotic and subsequent antimicrobial activity was assessed, by comparing the in vitro drug release from either fresh or pre-set CPCs, in order to identify the effect of the microstructural evolution during the first hours of setting on the release kinetics.

2. Material and methods

2.1. Liquid and solid phase preparation

α-TCP was used as the cement's solid phase, and was obtained by heating an equimolar mixture of calcium hydrogen phosphate ($CaHPO_4$, Sigma-Aldrich) and calcium carbonate ($CaCO_3$, Sigma-Aldrich) in a furnace (Hobersal CNR-58) in air at 1400 °C for 15 h followed by quenching in air. The α-TCP obtained was milled in a planetary mill (Pulverisette 6, Fritsch GmbH) using an agate bowl and ten agate balls (d = 30 mm) for 15 min at 450 rpm. 2 wt.% of precipitated hydroxyapatite (Merck, Germany) was added in the powder as a seed. The particle size distribution of the powders was analyzed by laser diffraction (LS 13 320 Beckman Coulter), after dispersing the samples in ethanol in an ultrasonic bath to minimize aggregation during the measurement. The specific surface area (SSA) of the powders was analyzed by N_2 adsorption following the Brunauer–Emmet–Teller (BET) method (ASAP 2020 Micromeritics). The properties of the powder used are summarized in Table 1.

The drug loading method of the CPC plays a significant role in different parameters [1,15], such as setting time or drug distribution within the CPC matrix. Preliminary studies conducted here reflected better homogeneity when Doxy, an antibiotic freely soluble in water [14], was incorporated into the CPC from the liquid phase than when it was added as a powder to the solid phase, where antibiotic aggregates were observed in the set cement (results not shown). Therefore, the liquid phase employed consisted of either MilliQ water for pristine CPCs or 50 mg ml^{-1} aqueous solutions of doxycycline hyclate (Sigma-Aldrich, doxycycline hydrochloride hemiethanolate hemihydrate, $C_{22}H_{24}N_2O_8\cdots HCl\cdots 0.5H_2O\cdots 0.5C_2H_6O$; MW: 1025.89).

2.2. Cement preparation and characterization

Cements were prepared with two different liquid-to-powder (L/P) ratios of 0.35 and 0.65 ml g^{-1}. The powder phase was mixed with the liquid phase (either MilliQ water or drug solution) in a mortar for ~1 min. The cements set due to the dissolution of α-TCP followed by the precipitation of calcium-deficient hydroxyapatite (CDHA) according to the following reaction [3,16]:

$$3Ca_3(PO_4)_{2(s)} + H_2O_{(l)} \rightarrow Ca_9(HPO_4)(PO_4)_5(OH)_{(s)} \quad (1)$$

Initial and final setting times were measured with the standard Gillmore needles method (ASTM C266–08). Cement samples were fabricated by introducing the paste with a spatula in 6 mm diameter × 12 mm height cylindrical Teflon molds. CPCs obtained were named C35 or C65 after the L/P ratio used, being 0.35 or 0.65 ml g^{-1}, respectively, and referred to as "water" or "Doxy" depending on the liquid phase employed. The samples were allowed to set in Ringer's solution (0.15 M sodium chloride solution) for 7 days at 37 °C.

The evolution of the pH during the setting reaction was measured in an α-TCP aqueous slurry at an L/P ratio of 200 with a Crison pHmeter connected to data acquisition software. The setting reaction was followed by X-ray diffraction analysis (XRD) in a PANalytical X'Pert powder X-ray diffractometer. The XRD measurements were obtained by scanning in Bragg–Brentano geometry using CuKα radiation. The experimental conditions were: 2 h scan step 0.016 between 4 and 100, counting time 50 s per point, voltage 45 kV and intensity 40 mA. The diffraction patterns were compared with the Joint Committee on Powder Diffraction Standards for α-TCP (JCPDS No. 9–348) and HA (JCPDS No. 9–432). The textural properties of the set cements were determined by N_2 adsorption–desorption by using a Micromeritics ASAP 2020. The samples were previously degassed under vacuum for 24 h, at 100 °C. The surface area was determined using the BET method. Total porosity and entrance pore size distribution were measured by mercury intrusion porosimetry (MIP, Autopore IV 9500, Micromeritics). BET and MIP measurements were done in duplicate for each formulation. The cross-section microstructure was imaged by Field emission scanning electron microscopy (SEM, JEOL JSM-7001F). The compressive strength was measured in wet samples (n = 6) in a universal testing machine (Bionix 858, MTS Systems) at a cross-head speed of 1 mm min^{-1}.

2.3. Drug release from the cements

Firstly, the stability of doxycycline in the release media was assessed by preparing dissolutions of different concentrations (from 1 to 100 μg ml^{-1}) in phosphate buffered saline (PBS), which were stored in the dark at 37 °C. Its stability was also studied for several hours at different pHs found in the setting reaction of cements by preparing NaOH solutions in PBS at pH 7.5, 8.5 and 9.5. UV-vis spectroscopy (Infinite M200 Pro Microplate reader TECAN,