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Drug delivery from injectable calcium phosphate foams by tailoring the macroporosity–drug interaction

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ABSTRACT

In this work, novel injectable calcium phosphate foams (CPFs) were combined with an antibiotic (doxycycline) to design an innovative dosage form for bone regeneration. The material structure, its drug release profile and antibiotic activity were investigated, while its clinical applicability was assessed through cohesion and injectability tests. Doxycycline had a clear effect on both the micro and macro structure of the CPFs, owing to its role as a nucleating agent of hydroxyapatite and to a drying effect on the paste. Doxycycline-loaded CPFs presented interconnected macroporosity, which increased drug availability compared with calcium phosphate cements, and was a critical parameter controlling the release kinetics which followed a non-Fickian diffusion model. Up to 55% (1 mg) of the drug was released progressively in 5 days, the percentage released being proportional to the macroporosity of the CPFs. All doxycycline-containing foams had immediate cohesion and were injectable. Moreover, antibacterial activity was observed against *Staphylococcus aureus* and *Escherichia coli*. Thus, in addition to enhancing osteoconduction and material resorption, macroporosity enables tuning of the local delivery of drugs from injectable calcium phosphates.

1. Introduction

Calcium phosphate cements (CPCs) show numerous attractive features when used as synthetic bone grafts. Their ability to set in vivo into calcium-deficient hydroxyapatite (CDHA) grants an excellent compatibility with the damaged bone and the possibility to be progressively replaced by new bone over time [1]. In addition, their use as local drug delivery systems for many drugs, mainly antibiotics, anti-inflammatories, anti-cancer or anti-osteoporosis drugs, has been widely investigated [2–6].

However, CPCs lack interconnected macroporosity. This hinders cell colonization, limits the possibility of circulation of nutrients and cell waste in the material, and impairs material resorption, thus preventing quick bone ingrowth [7]. When used as a drug delivery system, physiological fluids have reduced access to the centre of the matrix, potentially leading to an incomplete release of the active principle [8], particularly in slowly degradable CPCs, such as apatite cements [1]. On top of limiting the efficacy of the treatment, this can increase the risk of generating antibiotic resistance [9]. Different methods can be used to overcome these limitations. Specifically, drug-loaded polymeric porogens have been combined with CPCs with the purpose of generating macroporosity and releasing drugs in a controlled manner, as reviewed by Habraken et al. [10]. However, this method also has some drawbacks: (1) the macroporosity created by the porogen leaching is not available early enough to allow blood clotting in the graft; (2) leaching of the porogen might be detrimental to the regenerative process; (3) the proportion of porogen must be very high to generate an interconnected network of macropores; (4) the size of the porogen particles must be sufficient to generate macropores, and might thus be detrimental to injectability. The foaming approach used in this work presents an attractive, simple alternative to these drawbacks. Macroporous self-setting calcium phosphate foams (CPFs) can be obtained by foaming a surfactant-containing liquid and subsequently mixing with a reactive calcium phosphate powder [11–13]. This process generates an additional interconnected macroporosity to the already existing microporosity of CPCs, without losing injectability or requiring a subsequent step of porogen elimination. Thus, macroporosity is available instantaneously to

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the release media/corporal fluids. Different additives have been studied to foam the liquid phase of CPCs: low molecular weight surfactants (Sorbitol, Tween80) [11] or proteins, such as albumen [13] or gelatin [12]. The resulting foam, its stability and structure depend strongly on the chosen foaming agent, owing to the different mechanisms of action, such as the repulsive interactions between the adsorbed layers or the confinement of aggregates within the thin films [14].

A number of antibiotics, such as aminoglycosides (gentamicin), cephalosporins (cephalexin) and glycopeptides (vancomycin) have been proposed as active principles in combination with CPCs, in applications requiring both bone regeneration and local treatment of an infection. Although the local presence of the active principle enhances its efficacy, antibiotics can have side effects. For instance: gentamicin is thought to affect cell viability, proliferation and metabolism; cephalosporins inhibit osteoblast cells function, whereas vancomycin is less aggressive at low concentrations [15]. Interestingly, Kallala et al. [16] claimed that tetracyclines present some beneficial effects when targeting bone regeneration, i.e. enhancement of bone mineralization and induction of apoptosis of osteoclasts *in vitro*, thus limiting bone resorption [17]. The tetracycline employed in this work is doxycycline hyclate (Doxy). It has been used to treat a wide variety of infections including periodontitis [18], osteomyelitis [19–21] and methicillin resistant *Staphylococcus aureus* (*S. aureus*; MRSA) [22]. Beneficial effects on bone metabolism have been reported for Doxy even at low concentrations, i.e. 2–5 μg ml^{-1} [15].

In this work, for the first time, a new dosage form, intended for local treatment of infected bone defects, is proposed, based on self-setting injectable CPFs in combination with an antibiotic. Different aspects are investigated, such as: (i) the influence of the incorporation of Doxy on the porosity, macroporosity and pore interconnectivity of CPFs; (ii) the effect of the structural properties of the foam on the *in vitro* drug release kinetics; (iii) the relevance of interconnected macroporosity on the release and antimicrobial activity of the antibiotic.

2. Materials and methods

2.1. Liquid and solid phase preparation

α-TCP was used as a solid phase of CPFs and was obtained by heating in a furnace (CNR-58, Hobersal, Spain) in air a 2:1 molar mixture of calcium hydrogen phosphate ($CaHPO_4$; Sigma–Aldrich, USA) and calcium carbonate ($CaCO_3$; Sigma–Aldrich, USA) at 1400 °C for 15 h, followed by quenching in air. The α-TCP obtained was milled in an agate ball mill (Pulverisette 6, Fritsch GmbH, Germany) using 10 agate balls ($d = 30$ mm) for 15 min at 450 rpm; 2 wt.% of precipitated hydroxyapatite (HA; BP-E341, Merck, Germany) was added as a seed in the powder. The liquid phase was a solution of 1 wt.% of Polysorbate 80, herein Tween80 (Polysorbate 80, Sigma Aldrich, USA) in distilled water.

Doxycycline hyclate (Doxy; doxycycline hydrochloride hemiethanolate hemihydrate, Sigma–Aldrich, USA) in powder form was used as received. The schematic representation of the Doxy formula, i.e. $C_{22}H_{24}N_2O_8 * HCl * 0.5H_2O * 0.5C_2H_6O$, is show in Fig. 1.

2.2. Preparation of CPFs

Self-setting CPFs were prepared by foaming the liquid phase at 6000 rpm for 30 s using a domestic hand mixer followed by hand mixing with the solid phase. The liquid to calcium phosphate powder ratio was maintained constant and equal to 0.55 ml g^{-1}, as in previous works [11]. The amount of Doxy blended with the powder phase was a multiple of the lowest dose (D) corresponding to 0.88 wt.%. The different materials prepared, including the nomenclature used, amounts of liquid phase, calcium phosphate powder and antibiotic, and weight percentage of antibiotic in the material (C_{doxy}) are reported in Table 1.

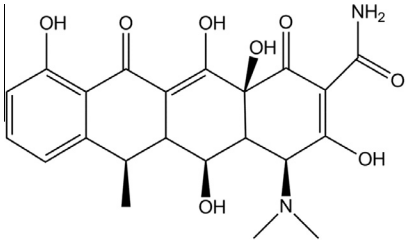

Fig. 1. Structural formula of doxycycline hyclate.

CPFs were then cast manually into Teflon cylindrical moulds of 6 mm in diameter and 12 mm high, and allowed to consolidate at 37 °C and 100% relative humidity for 1 h before immersion in water for 7 days for further reaction before subsequent characterization.

2.3. Material characterization

The surface tension of different dissolutions of Doxy in 1% of Tween 80 aqueous solution was measured. The concentrations chosen correspond to the doses of Doxy of each CPF formulation assuming total dissolution in the liquid phase, namely 0, 25, 50, 75 and 100 mg ml^{-1}. A tensiometer (K100, Krüss, Germany) with a Pt plaque was used to evaluate the surface tension.

The plastic limit of non-foamed CPC pastes containing different amounts of Doxy was evaluated via a simple technique, as described elsewhere [23]. Briefly, 1 g of powder phase was weighted and mixed with the adequate quantity of Doxy. Initially, 200 μl of distilled water was added to the powder phase and mixing was performed with a spatula until homogenization. A drop of water was then added and mixed again until homogenization. The process was repeated until the system had the consistence of a paste, and thus the plastic limit was reached. The paste was then weighted, and the liquid to powder ratio at the plastic limit was calculated.

A cohesion test was performed according to the protocol described in Montufar et al. [12]. Briefly, CPFs (0D-CPF, 1D-CPF, 2D-CPF, 3D-CPF, 4D-CPF) were freshly prepared. After 2.5 min, a small amount of the foamed material was injected into a cylindrical cavity 4 mm high and 8 mm in diameter in a commercial polyurethane sponge immersed in water at 37 °C. The integrity of the paste was evaluated visually using an arbitrary scale from 1 to 4, with 1 meaning no cohesion, i.e. paste disruption immediately after injection, and 4 meaning excellent cohesion, i.e. intact paste after injection and consolidated structure after 24 h. Three replicates were used for each composition.

To assess the effect of the addition of Doxy on injectability, CPFs were prepared and placed in a commercial syringe with a 2 mm aperture at the tip, a 13 mm cartridge and a nominal capacity of

Table 1
Nomenclature of the different CPFs, amounts of water, calcium phosphate (CaP) powder and antibiotic, and corresponding weight percentage of antibiotic with respect to the total weight of the CPFs (C_{doxy}).

Nomenclature	Liquid phase (ml)	CaP powder (g)	Doxy (mg)	C_{doxy} (wt.%)
0D-CPF	1	1.820	0	0
1D-CPF	1	1.820	25	0.88
2D-CPF	1	1.820	50	1.76
3D-CPF	1	1.820	75	2.64
4D-CPF	1	1.820	100	3.52

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