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Changes in the drug release pattern of fresh and set simvastatin-loaded brushite cement

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ABSTRACT

Calcium phosphate cements are synthetic bone graft substitutes able to set at physiological conditions. They can be applied by minimally invasive surgery and can also be used as drug delivery systems. Consequently, the drug release pattern from the cement paste (fresh cement) is of high clinical interest. However, previous studies have commonly evaluated the drug release using pre-set cements only. Therefore, the aim of this work was to determine if the time elapsed from cement preparation until immersion in the solution (3 min for fresh cements, and 1 h and 15 h for pre-set cements) had an influence on its physical properties, and correlating these to the drug release profile. Simvastatin was selected as a model drug, while brushite cement was used as drug carrier. This study quantified how the setting of a material reduces the accessibility of the release media to the material, thus preventing drug release. A shift in the drug release pattern was observed, from a burst-release for fresh cements to a sustained release for pre-set cements.

1. Introduction

Calcium phosphate cements (CPCs) were first described in the 1980s [1,2] and have been clinically accepted in many areas of orthopaedics and dentistry [3]. These cements set at physiological temperature through a dissolution–precipitation reaction when mixing a calcium phosphate-based powder with an aqueous solution. The resulting materials have chemical and physical similarities to the inorganic phase of bone tissue [4], which is one of the reasons why CPCs are bioactive, biocompatible and osteoconductive [5]. The use of cements is advantageous in comparison with sintered calcium phosphate materials, as they can be injected into the body using minimally invasive surgery techniques. However, the cement formulations must be chosen carefully to ensure a good cohesion of the paste and appropriate injectability, which will procure an adequate confinement at the target site [5,6] and clinical applicability.

The isothermal setting of CPCs together with their intrinsic porosity is advantageous when being considered as drug delivery systems since drugs generally maintain their integrity when loaded in the cement, and porosity facilitates their elution [7]. Drugs and growth factors that may either enhance bone regeneration or target specific skeletal pathologies have hence been evaluated in CPCs [7]. Enhancing bone growth is a key aspect to restore the mechanical function of the tissue, increase the osseointegration of the material and decrease the risk of posterior mechanical failure.

The drug release from CPCs has commonly been monitored using pre-set specimens, which were allowed to set for different periods, varying from 10 to 20 min [8], overnight [9], or 24 h [10–17] up to 7 days [10]. Moreover, in several studies [18–22] the conditions used were not indicated. Hence, most studies in the literature disregard the fact that the release pattern may change depending on the time the cement was set for; only Canal et al. compared the drug release patterns of cements set for different times (1 h vs 7 days), but this was done on apatitic cements only [10]. Although the importance of monitoring the drug release from fresh cement has been highlighted [7], to the best of the authors' knowledge, no previous studies have evaluated the drug release of a fresh, recently prepared cement paste. A previous study in our research group evaluated the release of simvastatin from premixed brushite cement during setting [23]. However, that study was performed on premixed cement, where the cement powder is mixed with glycerol and the setting reaction does not start until the mixture is put in contact with an aqueous solution, hence giving rise to a very different setting situation and drug release conditions than the aqueous mixtures commonly used.

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The aim of the current work was to evaluate the drug release pattern of brushitic, aqueous cement-mixtures directly after preparation, in comparison to the commonly used pre-set cement evaluations. Simvastatin was used as the model drug. This drug, besides being commonly prescribed to decrease hepatic cholesterol biosynthesis [24], has also been reported to potentially improve bone healing [25]. It stimulates the expression of bone morphogenetic protein 2 (BMP-2) in osteoblasts [26] and enhances alkaline phosphatase (ALP) activity and mineralization [27]. The drug release was monitored and modelled through Korsmeyer–Peppas (KP) equation. The handling properties of the cement paste were evaluated by cohesion, setting time and injectability tests. The structural properties of the fresh and set cements, as characterized by the crystalline phases, porosity, tortuosity, morphology and mechanical properties, were used to correlate the setting process of the paste to the release pattern of the drug. This study intended to highlight the importance of the time the cements are set for prior to implantation, since this may determine whether a therapeutic concentration is reached or not.

2. Materials and methods

2.1. Cement preparation

The cement powder was prepared by mixing 54 wt.% β-tricalcium phosphate (β-$Ca_3(PO_4)_2$, β-TCP, ref. no. 21218, Sigma Aldrich, USA), 44 wt.% ($Ca(H_2PO_4)_2 \cdot H_2O$, MCPM, ref. no. CA0211005P Scharlau, Germany) and 2 wt.% sodium pyrophosphate as a retardant [28] ($Na_4P_2O_7$, SPP, ref. no. 71499, Fluka, Switzerland) for 20 min in a powder mixer (Turbula®, Willy A. Bachofen AG — Maschinenfabrik, Germany). The MCPM was previously sieved (Retsch, Germany) down to particles smaller than 75 μm, to enhance the mechanical properties of brushite cement [29]. The cement was prepared by mixing the powder with an aqueous solution at a liquid to powder ratio (L/P) of 0.32 ml/g (the aforementioned L/P value was selected to ensure a good cohesion of the cements and an adequate setting time). The powder was manually mixed with respective liquid phase for 45 s before further characterisation or moulding.

Simvastatin (Sigma Aldrich, ref. no. 79902-63-9, USA) was used as the model drug. Whereas lipophilic molecules of simvastatin (lactone) are metabolized in the liver for the reduction of cholesterol, a more hydrophilic form (hydroxyacid) is needed in hard tissues applications to potentiate its pharmacological effect [30,31]. The simvastatin was therefore hydrolysed to convert its isolated β-hydroxyacid form into an open ring by adding the simvastatin in an alkaline solution of ethanol/NaOH 0.1 M and heating it at 50 °C for 2 h [32]. The simvastatin solution was then neutralized to pH 7.4 and stored at −20 °C. The liquid phase of the cement used was either distilled water for pristine cements; named C; or hydrolysed simvastatin at a concentration of 2 mM for simvastatin-loaded cements; named s-C (0.27 mg simvastatin/g brushite). The concentration of the simvastatin solution was selected with three considerations in mind: 1) a simvastatin concentration between 0.01 and 1 μM has been shown to promote osteoblastic differentiation in vitro [27,33–35], 2) there is a dynamic exchange of interstitial fluids in vivo, flow rate in pig bones was estimated to be 7.5–21.3 ml/min/100 g [36] and, 3) 1 h-set cement released almost half of the dosage in 1 day (preliminary study using a simvastatin solution of 2 mM).

2.2. Characterisation of the handling and structural properties

Pristine cement (C-XX) and simvastatin-loaded cement (s-C-XX) were characterized fresh (3 min after mixing the phases, C-3 m and s-C-3 m), set for 1 h (C-1 h and s-C-1 h) and set for 15 h (C-15 h and s-C-15 h). Hereafter XX indicates the time between mixing and measurement. s-C-3 m aimed to simulate a clinical scenario in which a fresh cement was applied. Cements set at two additional time points showed the release occurring from pre-set cements, where the two times were chosen in an attempt to correlate material microstructure to release profile, based on preliminary testing.

a) *Handling of fresh cement*

The cement paste, prepared without and with simvastatin in the liquid phase was evaluated in terms of cohesion, setting time and injectability. The cohesion of the cement paste was evaluated by injecting the paste 3 min after mixing the phases using a 2 mm aperture syringe (BD, ref. no. 309658, USA) into a PBS solution. After 1 h at 37 °C, the integrity of the paste in PBS was evaluated by visual inspection [37]. The initial and final setting times were determined by means of Gillmore needles after setting the cements in air (T = 22 ± 2 °C) [38]. Briefly, the assay consists in determining the time needed after the start of mixing in order not to create an indentation on the cement surface. The initial and final setting time refer to the use of low and high pressure tips, respectively. Duplicates were used to determine the cohesion of the paste and triplicates to evaluate the setting times.

Injectability of the cement pastes was evaluated using an experimental setup reproducing a minimally-invasive procedure (Fig. 1) by means of a universal testing machine (Shimadzu, AGS-X, Kyoto, Japan) with a 5 kN load-cell (SSM-DAK-5000 N, Shimadzu, Japan). The cement paste (prepared using 4 g of powder) was transferred into a 2 mm aperture syringe (BD, ref. no. 309658, USA) connected to a stainless steel G13 needle (OptiMed, ref. no. 1384-1013, diameter of 2.4 mm, length of 100 mm, Germany). Extrusion started at half of the initial setting time (i.e. 3 min) [39]. The paste was injected into a foam [40] mimicking the cancellous bone structure at a cross-head speed of 15 mm/min until the applied force reached 150 N, corresponding to the established force that can be applied to a syringe by hand [39,41]. The foam used was a cellular rigid polyurethane foam (Sawbones®, density of 0.12 g/cm^3, 2.5 × 2.5 × 2.5 cm^3; ref. no. 1522-09, USA), filled previously with a bone-marrow like substitute, i.e. 2.5 wt.% carboxymethyl cellulose sodium salt [42] (CCS, ref. no. 419273, Sigma Aldrich, USA). The foam was placed into a 5-faces Teflon cube and was immersed into a beaker filled with phosphate buffered saline (PBS, ref. no. P4417, Sigma Aldrich, USA) used to mimic a physiological solution. The force applied to the syringe piston during injection (extrusion force) was monitored, and the average of this force between 10 and 30 mm of the piston's displacement was calculated. The percentage of paste injected was calculated using Eq. (1).

$$\text{Injectability}(\%) = (w_i - w_a)/(w_i - w_e) * 100 \quad (1)$$

where w_i is the weight of the cement filled syringe, w_a is the weight of the syringe after injecting the paste, and w_e is the weight of the empty syringe. The injectability tests were performed using triplicates.

The spreading pattern of the injected cement paste was evaluated by means of micro-computed tomography (μCT, Skyscan 1072, Bruker, Kontich, Belgium) using the triplicates produced in the injectability test. Specimens were acquired using a source voltage of 80 kV, a current of 124 μA, a 0.5 mm aluminium filter, and an isotropic pixel size of 27 μm^2. Reconstruction of cross sections and 3D reconstructions were performed using software package NRecon and CTvox (SkyScan, Bruker, Kontich, Belgium). The reconstructed images were binarized to separate the cement from the foam and the background, using a global threshold. The diameter and sphericity of the injected cement was calculated using CTAn (SkyScan, Bruker, Kontich, Belgium). Sphericity (Ψ) of an