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ScienceDirect
Acta Biomaterialia 5 (2009) 43–49
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

High-strength resorbable brushite bone cement with controlled drug-releasing capabilities

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Received 15 February 2008; received in revised form 2 July 2008; accepted 13 August 2008
Available online 26 August 2008

Abstract

Brushite cements differ from apatite-forming compositions by consuming a lot of water in their setting reaction whereas apatite-forming cements consume little or no water at all. Only such cement systems that consume water during setting can theoretically produce near-zero porosity ceramics. This study aimed to produce such a brushite ceramic and investigated whether near elimination of porosity would prevent a burst release profile of incorporated antibiotics that is common to prior calcium phosphate cement delivery matrices. Through adjustment of the powder technological properties of the powder reactants, that is particle size and particle size distribution, and by adjusting citric acid concentration of the liquid phase to 800 mM, a relative porosity of as low as 11% of the brushite cement matrix could be achieved (a 60% reduction compared to previous studies), resulting in a wet unprecompacted compressive strength of 52 MPa (representing a more than 100% increase to previously reported results) with a workable setting time of 4.5 min of the cement paste. Up to 2 wt.% of vancomycin and ciprofloxacin could be incorporated into the cement system without loss of wet compressive strength. It was found that drug release rates could be controlled by the adjustable relative porosity of the cement system and burst release could be minimized and an almost linear release achieved, but the solubility of the antibiotic (vancomycin > ciprofloxacin) appeared also to be a crucial factor.

Keywords: Calcium phosphate cement; Brushite; Mechanical properties; Controlled drug release; Bone cement

1. Introduction

Brushite-forming calcium phosphate bone cements have the advantage of being resorbable in comparison to hydroxyapatite (HA)-forming cements but suffer in application from their fast, water-consuming setting reaction and their low mechanical strength [1,2]. The maximum reported wet compressive strength value for non-compacted, i.e. hand-mixed and applied brushite-forming cements is 24 MPa [3]. Strength improvements for a brushite-forming cement up to 32 MPa could be attained by compaction during setting [4,5]. Apatite cement has been shown to still be workable whilst retaining high strength of up to 184 MPa for the compacted, and 67 MPa for the uncompacted system [6,7]. The inherent rapid setting of brushite cements, however, leaves insufficient time for both compacting and moulding during surgery, needed for strength improvements [8]. Comparing reported strength values for brushite-forming systems is complicated by variations in testing methods; often dry strength values are given, which are proportionally much higher than wet strength compared with HA cement systems [9], and sometimes the much lower tensile strength is measured which cannot be related to the load-bearing capabilities of the cement [10,11].

Unlike HA cements, which consume little (1 mol per 3 mol of powder reactant in β-TCP systems) or no water

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doi:10.1016/j.actbio.2008.08.005

(TTCP/DCPA systems) during setting, the brushite cement system consumes a lot water during setting reaction (up to 6 mol per 1 mol of powder reactant), theoretically allowing for the formation of cements with low or almost zero porosities. Eq. (1) shows the setting reaction for a brushite system made of β-tricalcium phosphate and monocalcium phosphate monohydrate [12]:

$$Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4CaHPO_4 \cdot 2H_2O \quad (1)$$

Calcium phosphate cements have been identified as potential drug delivery systems, although these studies mainly focused on non-resorbable hydroxyapatite-forming cements [13,14]. In a study by Bohner et al., in which a brushite-forming cement system was evaluated as a potential drug carrier, it was shown that the release of gentamicin could be controlled by adjusting the porosity of the cement, making encapsulation of the drug within polyacrylic acid unnecessary [15,16]. Recent studies showed the feasibility of drug release from brushite cements for periodontal application [17,18].

As the strength and especially the reaction kinetics (setting time) of brushite cements depend strongly on the particle size of the reactants [19], the aim of this study was to generate a high-strength, low-porosity hand-mixed brushite cement with controlled drug-releasing capabilities by adjusting particle size of the reactants. The drugs investigated were vancomycin, a highly water-soluble antibiotic used to treat severe staphylococcal infections causing osteomyelitis [20], and ciprofloxacin, a slightly water-soluble antibiotic widely used to treat bacterial bone infection [21].

2. Materials and methods

The reactants for the brushite system were phase pure sintered β-TCP, made in house as described previously [22], and commercially available monocalcium phosphate monohydrate (MCPM) powder (Rhodia, UK).

The β-TCP sintercake was crushed in a mortar until it passed a 355 μm sieve and afterwards dry-milled for 1 h in a planetary ball mill (PM400 Retsch, Germany) unidirectionally at 200 rpm in 500 ml agate jars with a load of 50 g β-TCP and 4 agate balls (30 mm). For the experiments requiring a β-TCP powder with a much higher or lower particle size, commercial β-TCP (Plasma Biotal, Tideswell, UK) or further wet milled β-TCP (24 h in ethanol) was used, respectively.

The MCPM powder was fractioned into four different particle size distributions (<45, 45–63, <63, >63 μm) using a 63 μm and a 45 μm sieve.

Particle size distributions were determined by laser diffraction particle size analysis (Mastersizer S, Malvern, UK) of powder suspensed in pure ethanol. To avoid the generation of agglomerates the suspensions in the flow cell were exposed to ultrasound throughout the measurements. Median particle size d_{50} and the span (relative width) of the particle size distribution $(d_{90} - d_{10})/d_{50}$ were achieved from triplicate measurements.

The specific surface area of dry powder reactants was determined using nitrogen absorption (BET) at 77 K (ASAP 2000, Micromeritics, Norcross, GA).

The morphology of the powders was examined using scanning electron microscopy (JEOL JSM-5300 LV, UK). Specimens of powder were sprinkled on an adhesive stub and gold-sputter-coated. Images were recorded at 20 kV acceleration voltage.

To produce the cement, equimolar amounts of β-TCP and MCPM powders (1.23 g β-TCP per 1.00 g MCPM) were hand-mixed with a spatula in a weighing boat at powder to liquid ratios (PLR) varying from 2.5 to 4.0 g ml^{-1} using 500 and 800 mM citric acid solutions as a retardant. For the drug release measurements 2 wt.% vancomycin or ciprofloxacin (Sigma Aldrich, Steinheim, Germany) were added to the cement paste.

The cement slurries were cast without compaction into a PTFE mould to produce 6 mm diameter cylindrical samples with an aspect ratio of 2. After 1 h of drying at 37 °C the samples were immersed in double distilled water for another 23 h at 37 °C for the compressive strength measurements.

The wet compressive strength of the samples ($n \geqslant 6$) was measured with a Universal testing machine (Instron 5544, UK) with a 2 kN load cell and a crosshead speed of 1 mm min^{-1}.

The initial times of the cement slurries were determined by standard Gillmore needles test [23].

The strut densities of the set dried samples were determined by helium pycnometry (Accupyc, Micromeritics, UK). The average densities were calculated on the basis of 10 measurements. By measuring the apparent wet densities (by measuring the dimensions and weight of wet samples) and then calculating the dry densities (derived from weight loss measurements on wet samples until completely dried), the relative porosities (RP) were calculated with the formula RP = 1 – (dry density/strut density).

The release studies were carried out on wet cylindrical samples (6 × 12 mm) which were extracted directly from the moulds after their final setting time (less than 10 min for all compositions used [24]) and immersed in a shaking water bath set at 37 °C. The release medium was phosphate buffered saline (PBS, 0.1 M, pH 7.4, 50 ml) containing 0.02 wt.% sodium azide resulting in a PBS volume to sample surface ratio of around 0.18 ml mm^{-2}. One millilitre of the release media was taken out at each time interval and replenished with fresh buffer immediately. The antibiotic content of the samples (6 × 12 mm cylinders weighing between 0.632 and 0.763 g) was analysed by measuring absorbance with a Unicam spectrophotometer at 280 and 272 nm for vancomycin and ciprofloxacin, respectively. The amount of drug released was assayed by comparison with a calibration curve for the individual drugs made in PBS.

ID	Title	Pages
2343	High-strength resorbable brushite bone cement with controlled drug-releasing capabilities	7