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Characterization of chlorhexidine-releasing, fast-setting, brushite bone cements

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Abstract

The effect of antibacterial chlorhexidine diacetate powder (CHX) on the setting kinetics of a brushite-forming β-tricalcium phosphate/monocalcium phosphate monohydrate (β-TCP/MCPM) cement was monitored using attenuated total reflection Fourier transform infrared spectroscopy. The final composition of the set cement with up to 12 wt.% CHX content before and after submersion in water for 24 h, the kinetics of chlorhexidine release and the total sample mass change in water over four weeks was monitored using Raman mapping, UV spectroscopy and gravimetry, respectively. Below 9 wt.%, CHX content had no significant effect on brushite formation rate at 37 °C, but at 12 wt.% the half-life of the reaction decreased by one-third. Raman mapping confirmed that brushite was the main inorganic component of the set cements irrespective of CHX content, both before and after submersion in water. The CHX could be detected largely as discrete solid particles but could also be observed partially dispersed throughout the pores of the set cement. The percentage of CHX release was found to follow Fick's law of diffusion, being independent of its initial concentration, proportional to the square root of time and, with 1 mm thick specimens, 60% was released at 24 h. Total set cement mass loss rate was not significantly affected by CHX content. On average, cements exhibited a loss of 7 wt.% assigned largely to surface phosphate particle loss within the initial 8 h followed by 0.36 wt.% per day.

Keywords: Brushite; Chlorhexidine; Drug release; Drug cement interaction; Setting kinetics

1. Introduction

Calcium phosphate cements, either forming hydroxyapatite (HA) or brushite (dicalcium phosphate dihydrate, DCPD) as final product, are used as artificial bone substitute materials [1,2]. A major problem with HA bone cements, comprising the majority of developed calcium phosphate cement systems, is their inability to degrade in order to allow full replacement by the surrounding tissues [1,2]. For this reason there has been in recent years increased interest in the development of degradable brushite-forming cement formulations [3–8].

Many calcium phosphate cements have been investigated as potential drug delivery systems. A large number of studies have focused on the release of antibiotics due to the common problem of bone infection after surgery and the many difficulties involved in treating this. Most studies have used the more robust non-resorbable HA-forming systems [9–11], although controlled release of the antibiotic gentamicin from brushite cements has also been demonstrated [12,13]. For the treatment of common

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infections in the oral cavity and jaw bone, extensive use of antibiotics, however, is of concern due to the potential for antibiotic resistance.

A highly effective non-antibiotic, antibacterial alternative is chlorhexidine (CHX, $[(CH_2)_3NH(C(=NH)NH)_2C_6H_4Cl]_2$). This is used extensively in dentistry, including within crosslinked gelatine (Periochip™) for the treatment of periodontal infections [14,15] and as a mouthwash (e.g. Corsodyl™). It has also been incorporated within various dental restorative materials to reduce recurrent caries [16,17] and is used in antiseptic creams and sprays (e.g. Savlon™). One clinical problem with solid, periodontal antibacterial devices such as Periochip™ has been difficulty in placement. Other concerns include movement from the site of application soon after placement due to lack of fixation and the relatively rapid release rate of the antibacterial agent [18].

An alternative, initially fluid, slower CHX-releasing brushite cement would enable easier filling of the periodontal pocket or infected bone around a perio-implant. Solidification of the cement would then provide fixation. After release of CHX, the cement itself could degrade, providing calcium and phosphate ions to aid surrounding bone repair.

In previous studies the period of release of gentamicin from brushite cement was limited due to the high porosity of these materials [12,13]. More recently, through improvements in setting retardant action, it has been possible to increase the powder-to-liquid ratio of brushite cements and thereby reduce the final porosity [19]. The presence of CHX, however, might interfere with the hydrolysis reaction of any brushite cement system, as their chemistry and setting rate can be very sensitive to the addition of additives [4,19,20]. The aim of this investigation was therefore to assess the effect of varying CHX concentration on the chemistry of brushite cements. The effects of drug concentration on the setting kinetics and final product chemistry/drug stability were assessed using attenuated total reflection Fourier transform infrared (ATR-FTIR) and Raman mapping, respectively. In addition, drug release and cement degradation rates were quantified using UV spectroscopy and gravimetry. It has been hypothesized that there may be a maximum drug level that can be added before any serious detrimental effects on cement setting chemistry occur. In addition, drug release is likely to be governed by a diffusion-controlled process but its rate may be reduced in a higher powder content formulation.

2. Materials and methods

2.1. Materials

The reactants for the cement powder mixture were phase pure sintered β-tricalcium phosphate (β-TCP) [21] and monocalcium phosphate monohydrate (MCPM) powder (Rhodia, Birmingham, UK) with median particle sizes of 11 and 62 μm, respectively (as determined by laser diffraction particle sizing) [22]. β-TCP was prepared by sintering a 2:1 molar mixture of dicalcium phosphate anhydrous (DCPA, Mallinckrodt-Baker, Griesheim, Germany) and calcium carbonate (Merck, Darmstadt, Germany) at 1050 °C for 24 h. The β-TCP sinter cake was crushed in a mortar until it passed through a 355 μm sieve and afterwards dry milled for 1 h in a planetary ball mill (PM400 Retsch, Haan, Germany) unidirectionally at 200 rpm in 500 ml agate jars with a load of 125 g β-TCP and four agate balls (30 mm).

Chlorhexidine diacetate salt hydrate (CHX) powder (Sigma, UK) was used as received (ACS grade, Sigma–Aldrich, UK). Chlorhexidine diacetate solubility in aqueous buffer solutions ranges from 3.3 to 10.1 mg ml^{-1} at pH 7 and 4, respectively [17]. Use of this salt in powder form, instead of, for example, the more water-soluble chlorhexidine digluconate (as in Periochip), should reduce interaction between drug and cement components. Spectra of pure potential products were obtained using DCPD, and dicalcium phosphate anhydrous (DCPA or monetite), both from Sigma–Aldrich.

2.2. Cement preparation

Equimolar amounts of β-TCP and MCPM were combined with increasing levels of CHX. These powders were then mixed with 800 mM citric acid solution at a powder-to-liquid ratio of 3.3 g ml^{-1} to form a cement paste and to start the setting reaction. A CHX content of more than 12 wt.% was found to make the mix too dry to form a homogeneous paste. Formulations with 0, 3, 6, 9 and 12 wt.% (relative to the total cement paste weight) CHX were therefore studied. In the presence of water, β-TCP and MCPM reacted to form a brushite cement structure, according to the following equation [3]:

$$\beta\text{-}Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4CaHPO_4 \cdot 2H_2O \quad (1)$$

Solid discs 8 mm in diameter and 1 mm deep were prepared using metal washer rings as moulds. Excess cement was removed with a razor blade before sealing top and bottom with acetate sheet. Samples were then left to set at 37 °C for 24 h.

2.3. FTIR setting reaction monitoring

In order to investigate the kinetics of setting, the unset cements were placed on the diamond of an ATR-FTIR Perkin Elmer Series 2000 spectrometer temperature controlled at 37 °C (Perkin–Elmer Beaconsfield, UK, with Timebase software) within 60 s of mixing the powder and liquid. This method is designed to mimic how the material might set in clinical use: at room temperature during mixing by the clinician but then raised to body temperature soon after placement. Spectra between 500 and 4000 cm^{-1} were then obtained with a resolution of 4 cm^{-1}, every 12 s from 60 s

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