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Preparation and properties of macroporous brushite bone cements

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Abstract

In the present work a macroporous brushite bone cement for use either as an injected or mouldable paste, or in the shape of preformed grafts, has been investigated. Macropores have been introduced by adding to the powder single crystals of mannitol which worked as a porogen. The size of the crystals was in the range of 250–500 μm in diameter, suitable for cell infiltration, with a shape ratio between 3 and 6. From compression tests on cylindrical samples an elastic modulus in the range 2.5–4.2 GPa and a compressive strength in the range 17.5–32.6 MPa were obtained for a volume fraction of macropores varying between 15 and 0%. Thus the compressive strength exceeded in all tests the maximum value currently attributed to cancellous bone.

Keywords: Bone cement; Brushite; Cell adhesion; Porosity; Mechanical properties

1. Introduction

Calcium phosphate bone cements are used as mouldable or injectable pastes to fill bone cavities, defects or discontinuities [1,2]. These cements, which set and harden shortly after implantation, form highly biocompatible and osteoconductive scaffolds [3].

The chemical reaction which produces the biomaterial is activated by mechanically or manually mixing several calcium phosphate phases with a liquid phase. Depending on the pH of the reaction, two types of cements are obtained: for pH higher than ca. 4.2 the reaction product is hydroxyapatite (HA) [4], in the other case brushite (DCPD) [5] is obtained.

Brushite cements have raised considerable interest in the latest decade, because they are metastable under physiological conditions and can be resorbed more quickly than HA cements that are stable [6]. Cements of the first type may improve, therefore, the ingrowth of bone tissue from the surface to the inside of the implant, which means that they are more osteoconductive.

Unfortunately the setting time of brushite cements is generally too short, typically ranging between 30 and 60 s [7], to be efficiently used by surgeons.

To make such cements suitable for orthopaedic applications specific setting retardants, such as pyrophosphate ions or citrates [8,9] are usually added to slow down the setting process. The action of retardants essentially consists in inhibiting both nucleation and growth of calcium phosphate crystals [10,11]. In a recent work [8], a reduction of grain growth rate has been attributed to the action of pyrophosphate ions in concentration of about 4.3 wt.%. As a consequence smaller crystals with a better packing factor were formed, improving the tensile diametral strength of the cement as set.

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There are different philosophies concerning the choice of porogens. In some instances fast dissolving porogens (as e.g. mannitol) are used, while in other cases fibers of resorbable polymers are preferred to preserve mechanical strength in the initial stage of implantation. Inclusions of both types have been used by other authors to obtain an optimum compromise between the two requirements [12–14]. As to the pore size, which has to be large enough to admit bone cells as macrophages and/or osteoblasts, some authors [15] compared the amount of bone ingrowth in hydroxyapatite implants containing pores of different sizes from 100 to 600 μm and found best results when pores were interconnected and with a diameter in the range of 300–400 μm. For implants of resorbable materials the resorption time is the target parameter depending on the thickness of the pore walls. In this case the problem is quite complex, involving as inter-correlated variables the pore size and shape, as well as the resorption rate which, in turn, is related to the mechanisms of cell activation. According to the theoretical model by Bohner and Baumgart [16], considering macropores of spherical shape distributed in a CFC lattice, the optimum pore radius depends on the implant size and ranges between 200 and 350 μm.

Finally the in vivo ageing strongly depends on a number of factors having to do not only with the formulation of the reactants but also with characteristics of the implant, such as the animal model, the type of bone to treat, the size of the defect to heal [17] and the flow of the surrounding biological fluids [18].

This work is aimed at obtaining a macroporous resorbable cement that can be in situ injected or moulded. The first step was to prepare a microporous brushite cement with a nearly ideal composition, a setting time suitable for in vivo implantation and mechanical properties sufficiently high to allow for the introduction of macropores in a certain volume fraction, while preserving a compressive strength comparable to that of cancellous bone. Successively a macroporous cement was studied, obtained by adding to the calcium phosphate powder mannitol crystals as a biocompatible and water-soluble porogen, in different volume fractions. The experimental techniques used to characterize the brushite cement were IR spectroscopy and XRD, to check the degree of conversion of the reactants into the final product, and SEM analysis to investigate microstructure. Compression tests were carried out on samples with different volume fractions of macropores. Preliminary checks of biocompatibility and cellular adhesion on cements without and with macropores were performed with positive results.

2. Materials and methods

Macroporous cements were prepared with a solid phase made of equimolar quantities of β-tricalcium phosphate (β-TCP, assay $\geqslant$96%) (FLUKA, Germany) and monocalcium phosphate monohydrate (MCPM, assay min. 98%) (Sharlau, Spain) wherein mannitol single crystals in volume fractions between 0 and 15 vol.% were dispersed for use as a porogen. The liquid phase consisted of a sodium citrate ($C_6H_7O_7Na$) solution of molar concentration C equal to 0.5 M, saturated with mannitol to avoid an anticipated dissolution of the porogen. The whole was stirred manually for ca. 3 min. The ratio between the amounts of the solid and the liquid phase was fixed at a value of $R = 3\ g\ ml^{-1}$. The mannitol crystals were of acicular shape with shape factors between 3 and 6 and were sieved to keep the diameter in the range of 250–500 μm.

The pH of the sodium citrate solution was measured with a pH meter (WTW, pH 539 microprocessor pH meter).

The setting time of cements containing different fractions of mannitol was evaluated by a Vicat needle following the directions of ASTM C-191, on samples held at a temperature of 37 °C with 100% relative humidity.

Successively to mixing, the paste was poured into a Teflon mould of cylindrical shape to obtain samples 16 mm in length and 10 mm in diameter.

The samples, once hardened at a temperature of 37 °C with 100% relative humidity and kept in this condition for 24 h, were immersed in deionized water (which was refreshed every day) for 5 days, in order to let the porogen dissolve.

After this time the samples, in number of six for each macroporosity class, were tested in compression, as still wet, by a servo-hydraulic machine INSTRON 8501, with a crosshead speed of 1 mm min^{-1} starting from a preload of 20 N.

Identification of brushite was achieved by means of X-ray diffraction on powder, using a Philips PW3710 diffractometer. The sample was run in the interval of 2θ between 3° and 80°, with a generator potential of 30 kV, a generator current of 22 mA (using a CuKα radiation), a Ni filter, and a scan speed of 1 min^{-1}. The software used for XRD data reduction was Philips PC-APD Diffraction Software.

FT-IR spectra of samples in form of powder diluted in KBr (0.1%w/w) were recorded by a FT instrument Nicolet 6700, DTGS-KBr detector, 100 scans, and displayed in absorbance units.

Both XRD patterns and FT-IR spectra were obtained on samples without and with macropores after incubation in deionized water and revealed that no transformation of brushite could be observed. Moreover, micrometric measurements of sample dimensions were taken before and after incubation, to exclude any phenomenon of surface degradation.

The microstructure of a brushite cement without porogen and the disposition of macropores inside the cement matrix were observed by SEM.

To estimate the percolation threshold of the macropores contained in the investigated samples, some of them were charged with graphite rods similar in size and shape to the mannitol crystals and the electric conductivity between electrodes fixed to the bases was measured by an ohmmeter. The

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