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Dual-setting brushite–silica gel cements

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ABSTRACT

The current study describes a dual-mechanism-setting cement that combines a brushite-forming cement paste with a second inorganic silica-based precursor. Materials were obtained by pre-hydrolyzing tetraethyl orthosilicate (TEOS) under acidic conditions following the addition of a calcium phosphate cement (CPC) powder mixed of β-tricalcium phosphate and monocalcium phosphate. Cement setting occurred by a dissolution–precipitation process, while changes in pH during setting simultaneously initiated the condensation reaction of the hydrolyzed TEOS. This resulted in an interpenetrating phase composite material in which the macropores of the CPC were infiltrated by the microporous silica gel, leading to a higher density and a compressive strength ~5–10 times higher than the CPC reference. This also altered the release of vancomycin as a model drug, whereby in contrast to the quantitative release from the CPC reference, 25% of the immobilized drug remained in the composite matrix. By varying the TEOS content in the composite, the cement phase composition could be controlled to form either brushite, anhydrous monetite or a biphasic mixture of both. The composites with the highest silicate content showed a cell proliferation similar to a hydroxyapatite reference with a significantly higher activity per cell. Surprisingly, the biological response did not seem to be attributed to the released silicate ions, but to the release of phosphate and the adsorption of magnesium ions from the cell culture medium.

1. Introduction

Mineral biocements based on calcium phosphate chemistry set by a continuous dissolution–precipitation reaction, in which the cement reactants dissolve after contact with an aqueous phase, leading to a supersaturated liquid with regard to the setting product. The latter simultaneously precipitates from the liquid cement phase, leading to a hardening of the cement pastes after sufficient crystal entanglement [1]. The type of setting product formed by the reaction is affected by the pH conditions in the cement paste. While at neutral or basic pH nanocrystalline hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) is precipitated, a strongly acidic pH < 4.2 has been shown to lead to a matrix predominantly composed of secondary, protonated calcium phosphates such as brushite ($CaHPO_4{\cdot}2H_2O$) [2] or monetite ($CaHPO_4$) [3,4].

The final mechanical strength of set calcium phosphate cement (CPC) matrices was found to depend on several factors, such as the degree of cement conversion [5], the porosity of the cement [6], the kind of setting product [7], the crystal size [8] or the use of filler particles [9]. Due to their brittle behaviour and their low mechanical performance compared to polymeric cements, CPCs are indicated only for non- or low-load-bearing applications such as defects in craniomaxillofacial surgery [10]. Most approaches for the reinforcement of CPCs are based on an optimization of the above-mentioned parameters as well as on the addition of fibers [11] to increase the bending strength and work of fracture due to fibre pull-out and energy-dissipating mechanisms. However, fibre reinforcement has a detrimental effect on the rheological and injection properties of CPCs and the fibres used in many studies are of a non-degradable nature [11,12]. A relatively new approach involves the use of dual-setting CPCs, in which the cement liquid is modified by a water-soluble monomer, which is polymerized during cement setting such that an interconnecting hydrogel matrix within the porous cement structure is formed [13–15].

In the current study, we extended this dual-setting concept by combining brushite-forming cement paste with a second inorganic silica-based precursor. The latter is formed via the sol–gel process by the hydrolysis and condensation of tetraethyl orthosilicate (TEOS), forming a nanoporous silica network under acidic conditions. The reaction conditions of the cement pastes were altered in such a way that, following combination of the cement powder with the liquid silica precursor, both the cement setting reaction

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and silica gelation occurred simultaneously such that interpenetrating silica–brushite networks with a bimodal pore size distribution are formed in the set matrix.

2. Materials and methods

β-Tricalcium phosphate (β-TCP) was prepared by sintering $CaHPO_4$ (Baker, Renningen, Germany) and $CaCO_3$ (Merck, Darmstadt, Germany) in a molar ratio of 2:1 for 5 h at 1050 °C. The sintered cakes were crushed and sieved with 100 μm pore size mesh followed by ball milling in a planetary ball mill (Retsch, Haan, Germany) for 60 min at 200 rpm. Brushite cements were produced by mixing the β-TCP powder in an equimolar ratio with dicalcium phosphate anhydrous ($Ca(H_2PO_4)_2$, MCPA, Aldrich, Steinheim, Germany) in a coffee grinder for 30 s. Silica gel was produced by the sol–gel process under acidic conditions. Briefly, the precursor tetraethyl orthosilicate (TEOS, Sigma–Aldrich GmbH, Steinheim), 0.1 M HCl and deionized water were mixed and stirred to form an acid-catalyzed sol. Different H_2O:TEOS ratios (X) with $3 \leqslant X \leqslant 15$ were used to analyze the influence of this parameter on the composite properties. Finally, cement pastes were formed by manually mixing CPC powder and the liquid sol. The pastes were then transferred into silicon moulds and sealed. After 3 days of setting at 37 °C the cement blocks were transferred into a water bath with 100% relative humidity and 37 °C for a further 24 h. As reference, the CPC powder was mixed with either water or a 0.5 M citric acid solution and treated identically to the composite material. Modification with vancomycin hydrochloride as a model drug was performed by adding 5 wt.% vancomycin to the cement liquid phase. After setting, these cement samples (10 mm × 5 mm × 5 mm) were immersed in 1 ml PBS buffer at 37 °C, which was changed every second day. The vancomycin content of the eluate was determined by UV–vis spectroscopy at 280 nm.

2.1. Cement characterization

The initial setting time was measured by the Gilmore needle test in a humidity chamber at 37 °C and >90% relative humidity. Setting pH and temperature were measured at room temperature (22 ± 1 °C). For compressive strength measurements the CPC–silica gel composites were produced with different silica and CPC contents. The sol–gel component was produced with H_2O:TEOS ratios (X) between 3 and 15, and the powder to liquid ratio (PLR) was varied between 0.5 and 2.5 g ml^{-1}. For each parameter five samples (10 mm × 5 mm × 5 mm) were measured after setting for 24 h at 37 °C under wet conditions. Mechanical testing was performed using a static mechanical testing machine (440, Zwick, Ulm, Germany) and a 2.5 kN load cell, samples were loaded parallel to their long axis and tested at a constant crosshead displacement rate of 1 mm min^{-1}. Porosity characteristics such as pore size distribution, median pore size and pore volume were measured by nitrogen adsorption (BET-method, Autosorb-iQ-AG, Quantachrome, Odelzhausen, Germany) and mercury (Hg) porosimetry (PASCAL 140/440, Porotec GmbH, Hofheim, Germany). Set cement samples were dried at 37 °C for 24 h prior to measurement. For BET, the samples were additionally dried in vacuum for 48 h at 50 °C and outgassed for 72 h. Scanning electron microscopy (SEM; Zeiss DSM930) was used to analyze the gold-coated fracture surfaces of the ceramic monoliths. Samples were imaged using an accelerating voltage of 10 kV. X-ray diffraction (XRD) patterns of samples were recorded using monochromatic Cu K_α radiation (D5005, Siemens, Karlsruhe, Germany). Data were collected from $2\theta = 20–40°$ with a step size of 0.02° and a normalized count time of 1 s per step. The phase composition was checked by means of JCPDS reference patterns for brushite (PDF Ref. 09-0077), monetite (PDF Ref. 09-0080) and β-TCP (PDF Ref. 09-0169).

2.2. In vitro cytocompatibility testing

The osteoblastic cell line MG 63 (ATCC No. CRL-1427, Rockville, MD) was cultured at 37 °C and 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen Life Technologies, Karlsruhe, Germany). The culture medium was supplemented with 10% fetal calf serum, 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin (all from Invitrogen Life Technologies). The medium was changed in fixed intervals of 2 days. During the experiments cells were cultivated on polystyrene (PS), hydroxyapatite (PLR 3 g ml^{-1}), brushite (fabricated with H_2O or 0.5 M citrate acid; PLR: 1 or 2 g ml^{-1}) or CPC–silica gel composite (X: 15, 9 or 3; PLR: 1 or 2 g ml^{-1}). Previously every cement disk was washed for 24 h in 3 ml PBS at 37 °C. For biocompatibility tests samples were placed in quadruplicate into the wells of a 24-well plate and covered with cell suspension.

The cytocompatibility tests of the cement surfaces were performed by means of cell counting and determination of cell activity after 2, 6, 10 and 14 days in culture on all surfaces as described in detail elsewhere [16]. Briefly, cell proliferation was analyzed by electronic cell counting using a CASY 1 TTC cell analyzer (Schärfe System, Reutlingen, Germany), while cell viability was analyzed using the cell proliferation reagent WST 1 (Roche Diagnostics, Mannheim, Germany). After incubating the cells for 30 min with the WST reagent diluted 1:10 in DMEM at 37 °C, the absorption of the supernatant was quantified in a Tecan Spectrafluor Plus photometer (Tecan, Crailsheim, Germany). For each method and sample four readings were recorded and the mean values and standard deviations were calculated. Statistical analysis was performed using the ANOVA *t*-test in Microsoft Excel, in which CPC–silica gel composites were compared with the CPC reference (1 g ml^{-1} brushite). Ion concentrations (Ca, P, Si and Mg) in the used cell culture medium were determined using inductively coupled plasma mass spectrometry (ICP-MS, Varian, Darmstadt, Germany). The quantitative measurement was carried out against standard solutions (Merck, Darmstadt, Germany) containing defined concentrations of all ions of interest.

3. Results

With a decreasing X the setting time increased from few minutes (2.2 ± 0.3) of the pure CPC to 43.0 ± 2.6 min of the composite with X = 3 (Table 1). The composite shows a thixotropic behaviour during the setting reaction such that the paste could be refluidized by mechanical load until the cement was completely hardened. Both the CPC reference and the CPC–silica gel composites set at an acidic pH between 1.5 and 5 (Fig. 1a), whereas lower pH values were obtained with decreasing X due to the higher absolute HCl content in these cements. After 60 min the pH of the different composites increased by ~0.3 (X = 3) up to 1.5 (X = 15). The pure CPC shows a fast and exothermic reaction with a maximum temperature difference during setting ($\Delta_{max}T_{set}$) of 19.2 ± 0.8 °C (Table 1), which results in a maximum setting temperature of 41.2 ± 0.8 °C. For silica–cement composites with a high water content (X = 15) $\Delta_{max}T_{set}$ was found to be nearly the same (17.8 ± 1.0 °C), while a decrease of the water content in the liquid sol (X = 9) decreased $\Delta_{max}T_{set}$ to 8.5 ± 1.2 °C. The setting temperature of the CPC–silica gel composite with X = 3 showed no exothermic behaviour at all (Fig. 1b).

Brunauer–Emmett–Teller (BET) analyses of the pure CPC reference indicated only a marginal fraction of pores below 100 nm (Fig. 2a). As expected, the pure silica gel showed a clear pore size