



Silicon-stabilized α -tricalcium phosphate and its use in a calcium phosphate cement: Characterization and cell response

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ABSTRACT

α -Tricalcium phosphate (α -TCP) is widely used as a reactant in calcium phosphate cements. This work aims at doping α -TCP with silicon with a twofold objective. On the one hand, to study the effect of Si addition on the stability and reactivity of this polymorph. On the other, to develop Si-doped cements and to evaluate the effect of Si on their in vitro cell response. For this purpose a calcium-deficient hydroxyapatite was sintered at 1250 °C with different amounts of silicon oxide. The high temperature polymorph α -TCP was stabilized by the presence of silicon, which inhibited reversion of the $\beta \rightarrow \alpha$ transformation, whereas in the Si-free sample α -TCP completely reverted to the β -polymorph. However, the $\beta \rightarrow \alpha$ transformation temperature was not affected by the presence of Si. Si- α -TCP and its Si-free counterpart were used as reactants for a calcium phosphate cement. While Si- α -TCP showed faster hydrolysis to calcium-deficient hydroxyapatite, upon complete reaction the crystalline phases, morphology and mechanical properties of both cements were similar. An in vitro cell culture study, in which osteoblast-like cells were exposed to the ions released by both materials, showed a delay in cell proliferation in both cases and stimulation of cell differentiation, more marked for the Si-containing cement. These results can be attributed to strong modification of the ionic concentrations in the culture medium by both materials. Ca-depletion from the medium was observed for both cements, whereas continuous Si release was detected for the Si-containing cement.

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1. Introduction

Calcium phosphate cements (CPC) have been successfully used as synthetic bone grafts for the last three decades due to their excellent biocompatibility, bioactivity and osteoconductivity [1]. However, nowadays there are still many efforts focused on improving their biological performance. Several strategies have been proposed, namely the development of porous cements to enhance material resorption, tissue colonization and angiogenesis [2], the addition of drugs and growth factors [3], and the incorporation of some specific ions which play relevant roles in bone metabolism [4,5], with the aim of using the cements as a kind of “ion eluting” material.

One of the most common reactants for CPC is α -tricalcium phosphate (α -Ca₃(PO₄)₂, α -TCP). Upon contact with water α -TCP hydrolyses to a calcium-deficient hydroxyapatite (HA) [6]. α -TCP

is one of the three polymorphs of TCP, which is stable above \sim 1125 °C [7]. Even though the low temperature β -polymorph is widely used as a ceramic for orthopedic applications for its well-known biocompatibility, α -TCP is a much more efficient reactant for CPC due to its lower density and higher free energy of formation, being more reactive and soluble than β -TCP. It is known that the relative stability of the α - and β -polymorphs is highly affected by the presence of some impurities [8]. Thus whereas Mg is known as an element that stabilizes the β -phase [9,10], Si is known to stabilize the α -form [11–13].

The interest of doping α -TCP with silicon is twofold. On the one hand, it can stabilize this phase at low temperature, facilitating the fabrication process, which requires high temperature thermal treatments and in many cases fast cooling or quenching to avoid reversion of the reconstructive $\beta \rightarrow \alpha$ transformation. On the other hand, silicon is expected to enhance the bioactivity [13,14] and the osteogenic potential of the material [13,15,16]. Indeed, silicon is a bone trace element with a specific metabolic role connected to bone growth, specifically during the initial formation stages [17]. There are several studies showing biological improvements associated with silicon, although the mechanism by which silicon improves the bioactivity and the cellular response of a material

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Table 1
Elemental compositional data for the HA used as the main reactant.

Ca (wt.%)	P (wt.%)	Mg (ppm)	Sr (ppm)	Al (ppm)	Fe (ppm)	Na (ppm)	K (ppm)	Si (ppm)	Mn (ppm)	Zn (ppm)	Ca/P	(Ca + Na)/P
51.7	27.9	9.8 ± 0.9	49.1 ± 0.7	5.7 ± 2.6	23.3 ± 7.8	1.67 × 10 ⁴ ± 1.01 × 10 ³	127 ± 41.5	3.1 ± 0.3	0.4 ± 0.1	180.2 ± 88.0	1.43 ± 0.72	1.51 ± 0.85

Ca, P, Na, K, Si and Fe were measured by ICP-OES and Mg, Al, Sr, Mn and Zn by ICP-MS. The molar ratios Ca/P and (Ca + Na)/P are also shown.

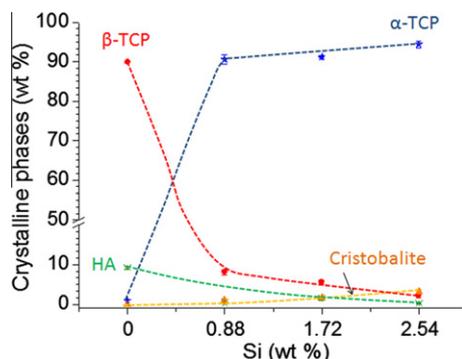


Fig. 1. Crystalline phase composition as function of the wt.% Si added to the HA. The samples were sintered at 1250 °C for 2 h and cooled down inside the furnace, quantified by the Rietveld method, including models for α-TCP (★), β-TCP (●), HA (✱) and β-cristobalite (▲). Error bars indicate the standard deviation ($n = 3$).

is still unknown [18]. Specifically, it is still not clear whether the behavior observed in Si-containing calcium phosphate ceramics can be attributed to silicon itself or to its indirect effects on the chemical and topographical properties of the material [19].

The preparation of silicon-containing CPC is a field that is attracting increasing interest. The most common approach is based on the combination of calcium phosphate powder with either calcium silicate powders [20,21] or a silicate-containing liquid [22]. A different strategy consists of doping the calcium phosphate precursor with Si [16].

The aim of this paper was to assess the effect of silicon on the stabilization of the α-TCP phase, and on reversion of the β → α transformation. A calcium-deficient HA that did not retain the α-TCP polymorph upon slow cooling was selected as a precursor and it was modified with different amounts of silicon. As a second objective the properties of the cement obtained by hydrolysis of the Si-stabilized TCP were studied. Special attention was paid to ion release from the Si-containing CPC and its Si-free counterpart, and the effect on cell proliferation and differentiation.

2. Experimental

2.1. Silicon-stabilized α-TCP

2.1.1. Physico-chemical characterization of the reactants

As a first part of the study the effect of silica addition on the stability of α-TCP was investigated. A commercial HA (CalbioChem, reference No. 391948) with a low level of Mg contamination was selected as starting reactant, since Mg is known to be a β-stabilizer [9,10]. An aqueous dispersion of colloidal silica (SiO₂, Cab-O-Sperse® 1030 K), composed of amorphous spherical silica nanoparticles between 20 and 200 nm in size, was used as the silicon source.

The chemical composition of the HA was measured by inductively coupled plasma (ICP) spectroscopy. Ca, P, Na, K, Si and Fe were analyzed by ICP optical emission spectroscopy (ICP-OES) (Perkin Elmer Optima 3200 RL); Mg, Al, Sr, Mn and Zn by ICP mass spectrometry (ICP-MS, Perkin Elmer Elan 6000). The HA was dissolved in an acidic aqueous solution (2% HCl) at a concentration of 750 ppm. The elemental composition data for the HA, analyzed in triplicate, is

shown in Table 1. It was a calcium-deficient HA (Ca/P atomic ratio 1.43 ± 0.72), with a high Na content (>15,000 ppm). It is known that sodium ions can substitute for calcium ones in the apatite crystal structure [23]. The concentration of Mg, an element known to be a β-phase stabilizer, was less than 10 ppm.

The specific surface area (SSA) of the reactants was analyzed by N₂ adsorption (Micromeritics ASAP 2020) following the Brunauer–Emmett–Teller method (BET). In the case of SiO₂ the dispersion was previously dehydrated and manually crushed. The SSA values, analyzed in duplicate, were 71.1 ± 2.0 for HA and 74.5 ± 6.4 m² g⁻¹ for SiO₂.

2.1.2. Si-α-TCP and α-TCP preparation

HA and SiO₂ were mixed at different SiO₂/(HA + SiO₂) weight ratios, namely 0, 1.88, 3.69 and 5.43, which correspond to 0, 0.88, 1.72 and 2.54 wt.% Si, respectively. The procedure was as follows. Twenty grams of HA were mixed with the corresponding amount of SiO₂, which was previously sonicated with 30 ml of water for 5 min. An extra 20 ml of water was added to the reactants in order to improve the homogeneity of the mixture. The slurry was milled in a planetary ball mill (Fritsch Pulverisette 6) using three agate balls (diameter 30 mm) at 350 rpm for 30 min. The mixture was dried for 24 h in a furnace at 110 °C and the resultant powder was sintered at 1250 °C for 2 h, cooling the sample inside the furnace. The heating rate was 5 °C min⁻¹. Si-free α-TCP was prepared by heating the HA to 1450 °C for 2 h followed by quenching in air, to be used as a control.

2.1.3. Thermal analysis

Differential thermal analysis (DTA) (Netzsch STA-409) was performed at a heating rate of 5 °C min⁻¹ in steps from room temperature to 1300 °C, with an air flow of 80 ml min⁻¹. The samples (~60 mg) were placed in Al₂O₃ crucibles, and Al₂O₃ powder was used as the standard. Four series were analyzed, namely the HA used as the main reactant, the SiO₂/HA mixture with 2.54 wt.% Si, and the sintered doped (2.54 wt.% Si) and undoped α-TCP powders.

2.1.4. Phase quantification by X-ray diffraction

The phase compositions of the reactants and the sintered doped and undoped α-TCP were assessed by high resolution X-ray diffraction (XRD) (PANalytical, X'Pert PRO Alpha-1). The X-ray powder diffraction measurements were obtained by scanning in Bragg–Brentano geometry using CuK_α radiation. The experimental conditions were: 2θ scan step 0.020° between 4° and 100°, counting time 150 s per point, voltage 45 kV and intensity 40 mA. The diffraction patterns were compared with the Joint Committee on Powder Diffraction Standards for α-TCP (JCPDS No. 9–348), β-TCP (JCPDS No. 9–169), HA (JCPDS No. 9–432), tridymite (JCPDS No. 42–1401) and β-cristobalite (JCPDS No. 89–3607) [24]. Rietveld refinements were carried out in triplicate in order to quantify the phases present. For this purpose the Inorganic Crystal Structure Database (ICSD) was used, including structural models for α-TCP (ICSD No. 923), β-TCP (ICSD No. 6191), HA (ICSD No. 151414) and β-cristobalite (ICSD No. 75483) [25]. Quality fits for phase quantification were obtained by refining the instrument displacement, scale factors and lattice parameters using a Thompson–Cox–Hastings pseudo Voigt * Axial divergence, full width half maximum (FWHM)/shape parameters, preferred orientation and background.

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