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Role of ZnO additions on the β/α phase relation in TCP based materials: Phase stability, properties, dissolution and biological response

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Abstract

Guided by the need to manufacture smart biomaterials, capable to replace and regenerate the mineral component of the bone, with optimized mechanical performance and bioresoption rate under physiological conditions, modified synthetic Zinc doped monophasic and/or biphasic β/α-tricalcium phosphate, Znc-$Ca_{3-x}(PO_4)_2$ (Zn-TCP), dense bioceramics with different phase proportions and microstructures were synthesized by solid state sintering process.

The compositions studied, ranging from 0.125 to 1.000 wt% ZnO were formulated in the $Ca_3(PO_4)_2$-$Zn_3(PO_4)_2$ subsystem of the ternary ZnO-CaO-P_2O_5 system. The design and control of compositions and processing conditions, enabled to fulfill the requirement in which Zn^{2+} was incorporated in solid solution in the TCP structure. Consequently, biomaterials with controlled porosity, density, phase proportion and microstructural distribution of α/β-TCP polymorphs were developed.

The effect of ZnO content on the final properties was discussed and an improvement in relation to pure TCP samples was obtained. The influence of the surface physico-chemical characteristics on the mechanical performance and "in vitro" solubility in SBF was also studied. In addition "in vitro" biocompatibility was evaluated using MG-63 human osteoblast cells, obtaining synthetic Zn-TCP based biomaterials with improved cell-material interaction.

Keywords: Tricalcium phosphate; ZnO; Mechanical properties; Bioactivity; Raman spectroscopy; Biological response

1. Introduction

The complexity of the development of mimetic human bone tissue structures still remains a challenge these days. Achieving bone mimicking material structures is an important goal, which would lead to remarkable improvements in the treatment of bone diseases and it would have high impact on the lifestyle of patients after joint replacement surgery. Close linked efforts of medicine, cell biology and materials science and engineering are mandatory for this goal to be accomplished.

Due to the similarity with the inorganic component of bone, synthetic calcium phosphate based biomaterials in various forms have been used for the last decades in medical applications for damaged bone tissue treatment, reconstruction and replacement.[1–5] One of the most widely used calcium phosphate bioceramics is tricalcium phosphate (β/α-$Ca_3(PO_4)_2$-TCP) due to its excellent biocompatibility, osteoconductivity and resorbability. However, its high dissolution in the human biological environment, compared to new bone development during its progressive degradation, decreases the mechanical strength of TCP based bioceramics.[6,7]

Generally, the increase in the densification of TCP ceramics is beneficial for load bearing applications, in order to achieve a higher mechanical performance. However it is difficult to sinter high dense β-TCP polymorph based materials due to the low temperature α-TCP phase transition.[8,9] In addition the higher solubility of α-TCP ($K_{sp} = 10^{-25.5}$) and β-TCP ($K_{sp} = 10^{-29.5}$) compared to Hydroxyapatite-HAp ($K_{sp} = 10^{-58.6}$) at 37 °C[10] is a determining factor which must be taken into serious consideration when developing a desired bone-substitute material capable to withstand in the human body for a certain period of time. These are the main reasons why the high complexity

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inherent to calcium phosphate based biomaterials still requires further optimization nowadays.

The actual tendency to incorporate monovalent and/or divalent ions in TCP structure derives from the necessity to develop "intelligent" materials, in which the achievement of a tailored Ca and P ion release during the bioresorption process is fundamental, in order to optimize the time dependant mechanical strength of the implanted material.[11–13] Chemical modifications of TCP based biomaterials, satisfying the requirement of biocompatibility, are restricted to the use of anions and cations contained in natural human bone, namely CO_3^{2-}, SiO_4^{2-}, Mg^{2+}, Zn^{2+}, F^-, Cl^-, Na^+, K^+, etc. The choice of Zn^{2+} as a possible dopant, suggested in several studies,[14–16] is beneficial because it has been demonstrated to stimulate bone regeneration, osteoblast cell proliferation and synthesis of DNA, inhibiting at the same time osteoclast bone resorption "in vitro".[17–19] However, it must be taken into consideration that at elevated concentrations ZnO can be cytotoxic,[20] indeed the complete biological effect of ZnO has not yet been clearly established.

In an earlier work, the presence of Zn^{2+} in TCP structure induced the retention of the β-TCP phase up to higher temperatures modulating β/α polymorph phase proportions and distribution with temperature on the final microstructure.[21] As the solubility of β and/or α-TCP and Zn modified TCP based biomaterials depends not only on the chemical composition, phase proportion, microstructure assemblage, crystallinity, porosity and surface topography,[22] but also on the specific nature of the buffer chosen for "in vitro" characterization,[23] additional investigation needs to be carried out in order to understand the different parameters affecting in this sense.

In this context, pure TCP and Zn-TCP bioceramics with controlled β and/or α-TCP phase proportions and microstructural assemblages have been synthesized by conventional solid state sintering reaction.[21] The compositions selected for the present study were chosen taking into account previous research,[24] in which the solid solution extension region of Zn^{2+} in TCP structure in the rich tricalcium phosphate region of the ZnO-CaO-P_2O_5 system was delimitated. The aim of this work was to analyze the physico-chemical characteristics of the designed materials and to study their influence on the mechanical performance and dissolution rate "in vitro" in simulated body fluid (SBF). In addition the "in vitro" biocompatibility was also studied in cell culture tests using MG-63 human osteoblast-like cells.

2. Materials and methods

2.1. Material preparation

To obtain the different pure TCP (TCP) and zinc doped TCP (Zn-TCP) ceramics, the raw materials used in this study were $NH_4H_2PO_4$ (≥99.0% – Fluka), $CaCO_3$ (99.0% – Panreac) and ZnO (99.9% – Agalsa). The use of these ultra pure reactants was mandatory in order to minimize the presence of Zn^{2+}, Mg^{2+}, Sr^{2+}, Na^+, K^+ and SiO_4^{4-} ions among others in the powders. These impurities highly influence the polymorphic β → α-TCP transformation temperature and will consequently affect the final properties and the performance of the designed materials. Compositions summarized in Table 1 with 0, 0.125, 0.250, 0.500 and 1.000 wt% ZnO doped TCP were synthesized by conventional solid state sintering process with a previous calcination step.[21] Briefly, stoichiometric amounts of the previous optimized reagent powders were milled in an attrition-mill using ZrO_2 balls in isopropyl media for 2 h. After the milling step, the powders were oven dried at 60 °C for 24 h, passed through a 63 μm sieve and calcined at 900 °C for 2 h in order to remove H_2O, NH_3 and CO_2. This first heat treatment guaranteed the formation of β-$Zn_xCa_{3-x}(PO_4)_2$ phase and the absence of secondary phases in the powders.[21] Subsequently the stoichiometric powders obtained were isostatically pressed at 200 MPa into cylinders of 1 cm in diameter and 4 cm long and then cut into 10 mm diameter 2 mm thick disks. The pellets were sintered at a heating rate of 3 °C/min up to 1000, 1075, 1150 and 1250 °C in the case of pure TCP powders and 1150, 1200, and 1250 °C in the case of Zn-TCP powders for 12 h. Subsequently the samples were cooled down to room temperature at 3 °C/min.

Table 1
Chemical composition of the different samples prepared.

Compositions	wt%			Ca/P ratio
	ZnO	CaO	P_2O_5	
TCP	–	54.2350	45.7650	1.50 ± 0.01
0.125 Z	0.1250	54.1278	45.7472	1.50 ± 0.01
0.25 Z	0.2500	54.0207	45.7293	1.50 ± 0.01
0.5 Z	0.5000	53.8063	45.6937	1.50 ± 0.01
1.0 Z	1.0000	53.3775	45.6225	1.50 ± 0.01

2.2. Chemical and physical characterization

For all thermal treated compositions the (Ca + Zn)/P molar ratio was 1.50 ± 0.01, determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Rietveld refinement technique was carried out in order to identify and quantify the crystalline phases present in the sintered samples using the FullProf 2k program[25] and its graphical interface WinPLOTR.[26] The X-ray diffraction (XRD) patterns were performed at an angular range: $2\theta = 10–90°$, step scan = 0.0197 with a Vantec D8 Advance (Bruker, Spain).

Densification and porosity of the sintered samples was measured using the Archimedes method in water and by means of image processing and analysis program (Leica Qwin, Leica Microsystems Ltd., Cambridge, England) respectively. Theoretical density was taken to be 3.08 g/cm^3 for β-TCP and 2.86 g/cm^3 for α-TCP, respectively. The surface roughness of the sintered polished disks (10 mm in diameter × 2 mm thick) was determined using a Perthometer M1 (Mahr GMbH, Germany). The obtained roughness parameters were; R_a: average roughness and R_z: mean roughness depth (ISO 4287).

2.3. Mechanical testing

Mechanical properties were characterized performing Vickers microhardness measurements on polished samples, using a Zwick/Roell, Zhu 2.5. A load of 98 N for 15 s was applied and