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Hydroxyapatite nanopowders prepared in the presence of zirconium ions

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ABSTRACT

Hydroxyapatite nanopowders were prepared in the presence of different concentrations of zirconium ions. Such crystallization conditions yielded significantly reduced particle size and increased specific surface area. Cell viability and oxidative stress studies showed that biocompatibility was not impaired when compared to pure hydroxyapatite. Non-isothermal sintering implied the possibility for suppressing the reaction between hydroxyapatite and zirconia by limiting it to only calcium phosphates. Stress-induced transformation of tetragonal to monoclinic zirconia is facilitated by total hydroxyapatite to β-tricalcium phosphate phase transformation.

1. Introduction

The improvement of material properties without negatively affecting the biological response is a permanently targeted issue concerning bioceramics. Hydroxyapatite (HAp) is a bioactive material and one of the most suitable ceramics for bone and dental tissue reconstruction, but still lacks adequate mechanical properties. However, zirconia (ZrO_2) offers good mechanical response, but has a disadvantage of poor bonding to host tissue. Their composites are quite promising, though, offering many potential biological and structural advantages. HAp/ZrO_2 composites are usually prepared by physical mixing or colloidal dispersions of ZrO_2 with HAp [1]. The addition of other phases to HAp influences its bioactivity and phase stability during sintering [2]. Procedures that reduce the amount of reinforcing agents have already been investigated [3]. Also, pressureless densification of HAp/ZrO_2 is difficult because of the mismatch in thermal expansion coefficients and requires field-assisted sintering techniques and tetragonal ZrO_2 (t-ZrO_2) stabilization since there is a tendency to reactions between HAp and ZrO_2 [4,5].

In this study, the preparation of HAp nanopowders in the presence of different amounts of zirconium ions was performed under reflux conditions. Detailed structural and chemical characterizations were performed, while biocompatibility was determined in cytotoxicity and oxidative stress studies. Non-isothermal sintering was performed up to 1300 °C and the results were correlated with the phase composition.

2. Materials and methods

Chemical precipitation has been performed with following precursors: calcium nitrate tetrahydrate, $Ca(NO_3)_2 \cdot 4H_2O$, (Fluka Analytical, Germany), zyrconyl chloride octahydrate, $ZrOCl_2 \cdot 8H_2O$, (The British Drug Houses Ltd., United Kingdom) and ammonium dihydrogen phosphate, $NH_4H_2PO_4$, (Zorka Šabac, Serbia). Concentrations were adjusted to retain (Ca+Zr)/P molar ratio of 1.67 for different amounts of zirconium (0, 1, 5 and 10 at% with respect to Ca), denoted as HAp-*X* (*X*=0, 1, 5, 10), respectively. Reactants were dissolved in 80 ml of distilled water each and heated to 70 °C. The solutions of calcium and zirconium ions were mixed, followed by the addition of a phosphate solution and precipitation by the dropwise addition of 10 ml of NH_4OH to reach pH 11. The reaction batch (250 ml), enclosed in reflux conditions to provide constant pH in the presence of small amount of NH_4OH, was effectively stirred for 3 h at 70 °C, with afterward ageing, washing out to pH 7 and drying. The phase analysis was carried out using X-ray diffraction (Philips PW-1050). The FTIR measurement was performed in the spectral range of 400–4000 cm^{-1} (BOMEM, Hartmann& Braun). Particle size and morphology were investigated using transmission electron microscopy (TEM, JEOL 2100); elemental mapping was performed by EDXS analysis (JEOL ARM

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200 CF equipped with JEOL centurion 100). The specific surface area (SSA) was measured by Brunauer–Emmett–Teller (BET) technique with N_2 adsorption–desorption isotherms at −195.8 °C (Micromeritics Gemini 2370 V5).

For determination of the biological response we used HepG2 cells, which were obtained from the European Collection of Cell Cultures (UK) and were grown as described previously [6]. The cytotoxicity was determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to Mosmann [7], with minor modifications [6]. The HepG2 cells were seeded onto 96-well microplates (Nunc, Naperville IL, USA) at a density of 40,000 cells/ml and incubated for 20 h at 37 °C to attach. The medium was replaced by fresh medium containing 0, 0.00001, 0.0001, 0.001, 0.01 and 0.1% (w/v) of HAp-*X* (*X*=0, 1, 5, 10) samples and incubated for 24 h. In each experiment a negative control (non-treated cells) was included. The protocol was continued as described previously [6]. Cell survival was determined by comparing the optical density of the wells containing the treated cells with those of the negative control.

The formation of intracellular reactive oxygen species (ROS) was measured spectrophotometrically using the fluorescent probe DCFH-DA [8], with minor modifications [6]. The HepG2 cells were seeded at a density of 75,000 cells/ml into 96-well, black microplates (Nunc, Naperville IL, USA) in five replicates and incubated for 20 h at 37 °C to attach. After that, the cells were incubated with 20 mM DCFH-DA for 30 min, then DCFH-DA was removed, and cells were washed out with PBS and treated with concentrations 0, 0.00001, 0.0001, 0.001, 0.01 and 0.1% (v/v) of HAp-*X* (*X*=0, 1, 5, 10) samples. Negative control (non-treated cells) and positive control (0.5 mM tert-butyl hydroperoxide treated) were always included. The protocol was continued as described previously [6].

The powders were uniaxially pressed under 100 MPa. Non-isothermal sintering was performed to 1300 °C in a heating microscope (New Heating Microscope EM201, Hesse Instruments) with the heating rate of 10 °C/min.

3. Results and discussion

XRD patterns of the prepared materials, Fig. 1a, showed the reflections characteristic for HAp. The only difference among these samples was the diffuse scattering in 20–40° 2θ range for HAp-10 sample, which could originate from ZrO_2 phase [9].

FTIR spectra, Fig. 1b, of the synthesized powders were almost identical, showing typical apatite phosphate (PO_4^{3-}) modes

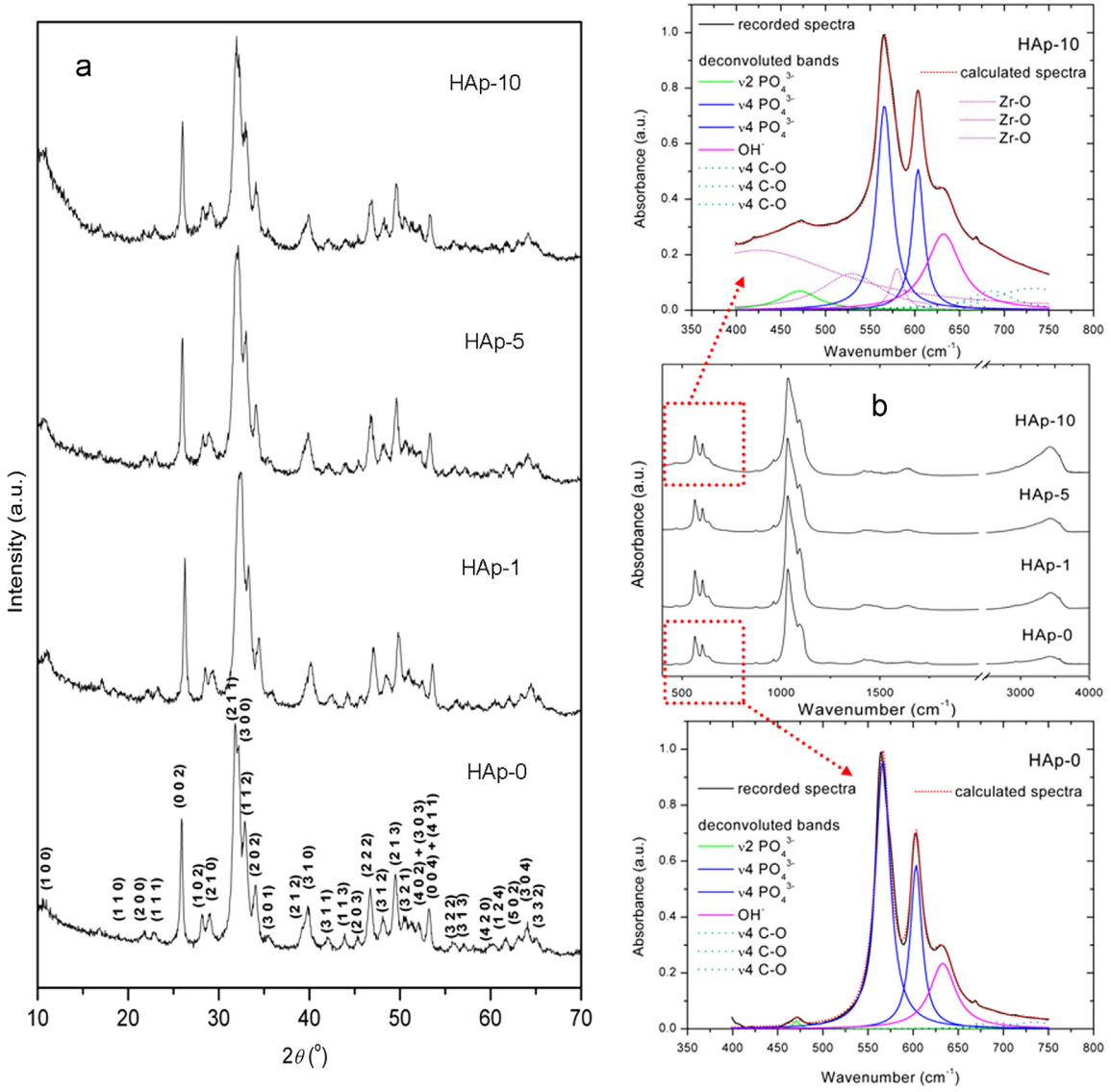

Fig. 1. (a) XRD patterns and (b) FTIR spectra of precipitated powders.