Ceramics International (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Ceramics International

journal homepage: www.elsevier.com/locate/ceramint

Reactivity of $BaTiO_3$-$Ca_{10}(PO_4)_6(OH)_2$ phases in composite materials for biomedical applications

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ARTICLE INFO

Keywords:
Barium titanate
Hydroxyapatite
Biomedical composite
Solid state reaction

ABSTRACT

In this work the reactivity of barium titanate (BT) and hydroxyapatite (HA) composites from commercial and synthesized powders was studied. Raman spectroscopy, X-ray diffraction and scanning electron microscopy were used for characterization of sintered composites. Moreover, biotoxicity assays were performed to evaluate the effect of secondary phases in the sintered composites in relation to BT and HA phases. The formation of secondary phases and transformation of hydroxypatite into tricalcium phosphate depended on the reactivity of the starting materials. The piezoelectric voltage coefficient, calculed from permittivity and piezoelectric constant for composites containing 20 vol% HA, generated promising values for a good osteogenic response. The secondary phases formation during the sintering process favored the ions release to the incubation solution; consequently, the composite biocompatibility highly depended on samples composition. Composites made of synthesized HA and commercial BT produced valuable results for biomedical applications.

1. Introduction

The bone piezoelectric properties were reported by Fukada et al. in 1957 [1]. Bone tissue exhibits a very low piezoelectric coefficient of 0.7 pC/N [2] and responds to micromechanical stress such as body movement, leading to electrical dipole generation. These properties contributed to develop a new area of bone remodeling study. Consequently, new biomaterials with enhanced performance in bone regeneration can be designed considering these bone properties [3].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA), a calcium phosphate with similar composition to that of the inorganic bone, can be polarized at 300–400 °C applying a 2 kV/cm electric field [4]. Bodhak et al. showed that the osseointegration rate can be modulated with the samples polarization degree. The possibility of producing piezoelectric composites between HA and lead-free traditional piezoelectric materials which could be suitable to apply in the human body has been explored. Barium titanate ($BaTiO_3$, BT) is the most frequently piezoelectric material used in bone replacement and repair, due to its excellent biocompatibility properties. Moreover, piezoelectric materials stimulate the implant attachment to the bone tissue, promoting bone regeneration [5,6]. Therefore, there is a great challenge in the development of HA-BT bioactive composite materials to improve the *in vivo* performance.

Feng et al. [7] showed that polarized HA-BT materials promote bone growth in higher degree than only HA depending on the direction of polarization. Hwang et al. [8] studied the effect of polarizing BT substrate in the formation of a calcium phosphate layer (CP) on the surface. They found out that the layer was formed on the CP negatively polarized side, whereas it was not observed on the positive side. In that study, three different polarization conditions were compared; however, the relation between piezoelectric properties and the CP layer formation was not studied. Bowen et al. [9] studied the influence of composition on dielectric properties of HA-BT composites finding that the incorporation of HA abruptly decreases dielectric permittivity (ε_r) and piezoelectric coefficient (d_{33}) values. They observed that samples with BT addition lower than 80% of BT did not show significant values of piezoelectric coefficient.

Several studies have shown that when a BT-HA bioceramic is polarized, a permanent residual charge on the surface is created, which can influence the response and activity of cells deposited on it [9,10]. However, some published works reported contradictory results related to the type of surface charge that improves the biological response [4].

In the preparation of dense BT-HA composites, the most important issue focuses on the reactivity of the crystalline BT and HA phases during the sintering process. This reactivity is detrimental for the reached piezoelectric properties after polarization because a high

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http://dx.doi.org/10.1016/j.ceramint.2016.12.053
Received 5 October 2016; Received in revised form 7 December 2016; Accepted 9 December 2016

fraction of piezoelectric phases is missed after sintering. Dubey et al. [11] have reported the constituent HA and BT phases in HA-25 vol% BT and HA-35 vol% BT samples. However, they only showed X-ray diffraction patterns of HA-xBT samples before the spark plasma sintering. Baxter et al. [10] suggested the formation of secondary phases during the sintering process without clear experimental evidence.

This work aims at studying the reactivity of dense BT-20 vol% HA composites from commercial and synthesized powders of BT and HA, in order to evaluate the crystalline phases in the sintered materials. Raman spectroscopy, X-ray diffraction and scanning electron microscopy were used for characterization of sintered composites. Moreover, biotoxicity assays were performed to evaluate the effect of secondary phases in the sintered composites in relation to BT and HA phases. Additionally, the dielectric behavior on commercial BT and synthesized HA composites was analyzed considering the HA content.

2. Materials and methods

Commercial $BaTiO_3$ (cBT, Transelco Division, Ferro Corporation), commercial HA (cHA, Aldrich), synthesized $BaTiO_3$ (sBT) by mechanochemical method [12] and synthesized HA (sHA) by assisted precipitation process [13] were used as starting materials.

Approximately 6g of solid blend (80 vol% BT+20 vol% HA) were dispersed with isopropyl alcohol (10 mL) in a planetary mill (Fritsch Planetary Micro Mill Pulverisette 7) with zirconia balls (4 units) during 10 min at 890 rpm. Then, the blends were dried in an oven at 80 °C during 24 h. The obtained composite powders were used for sintering processing.

Three combinations were evaluated for reactivity: cBT/20 vol% cHA; sBT/20 vol% sHA and cBT/20 vol% sHA. Moreover, a series of cBT with 20, 40, 60 and 80 vol% sHA was prepared and dielectrically characterized.

Sintering processes were carried out on disc-shaped samples (12 mm diameter×1 mm thickness) in triplicate, prepared by the compactation of powder with PVA binder at 10 MPa using a hydraulic press. The compacted samples were then sintered in a Carbolite RHF 17/6 S in air at 1250 and 1300 °C for 1 h at a heating rate of 10 °C min^{-1}. Sintered samples were then ground using an agate mortar prior to characterization. The starting powders and the sintered samples were examined by X-ray diffraction (XRD) using a PANalytical, X'pert Pro diffractometer equipped with a Ni filtered CuKα radiation (λ=1.5406 Å).

Density values of the composites (ρ_S) were determined by the Archimedes' method using a Sartorius Balance (YDK01) with a density kit. Theoretical density (ρ_T) was calculated using Eq. (1).

$$\rho_T = (1 - V)\,\rho_{HA} + V\,\rho_{BT} \quad (1)$$

where ρ_{BT} is the BT theoretical density (5.61 g/cm^3), ρ_{HA} is the HA theoretical density (3.16 g/cm^3) and V is the initial filler volume fraction. The porosity (*P*) represents the "void space" of the sample and was calculated from % P=100 ($1-\rho_S/\rho_T$). Determinations were made in triplicate.

Thermogravimetric analysis and differential thermal analysis (TGA and DTA) of starting mechanical mixtures were performed employing a Shimadzu TGA-50 and DTA-50 under air atmosphere and using a 10 °C/min heating rate. In these assays, 20 mg of samples were employed.

Raman microspectrometric analyses of composites were performed on a multichannel Renishaw In Via Reflex microspectrometer. Excitation was provided by the 514 nm line of an Ar laser. To enhance the signal-to-noise ratio, 30–50 scans were accumulated, each one having a 15 s exposure to laser power ranging between 30 and 300 mW.

Morphologies of the samples were investigated by scanning electron microscopy (SEM), employing a Jeol 6460LV microscope after coating the samples with a thin gold layer. The obtained images were analyzed by means of the free access imaging software tool, ImageJ, to describe morphology and pore diameter of the studied materials. The composition of the ceramics was analyzed by x-ray Energy Dispersive Spectroscopy (EDS) characterizations, using an EDAX Genesis XM4 - Sys 60, equipped with Multichannel Analyzer EDAX mod EDAM IV.

Finally, samples were painted using silver paste and dielectric measurements were performed with a Hioki 3522-50 and a Hioki 3535 Impedance Analyzers from 1 Hz to 10 MHz. In order to test the piezoelectric coefficient, samples were polarized under a direct current (dc) electric field of 2160 V/mm in a silicone oil bath at 140 °C for 20 min. The piezoelectric constant d_{33} was measured using a piezo d_{33} meter (YE2730A d_{33} METER, APC International, Ltd., USA).

Biotoxicity assays were performed on dissolution products of milled sintered samples. For this test, embryonic zebrafish (*Danio rerio*) were used since they prove to be superior to conventional *in vivo* models. This test provides universal availability, relative low cost, rapid development and growth, and compliance with ethical rules for the use of experimental animals [14,15].

Powder of the samples was incubated in embryonic medium with a 1 wt% concentration (Hanks solution 10 wt%, pH=7) during 7 days in an orbital shaker at 37 °C to obtain the dissolution products. Then, the extracts were centrifuged and filtered with a 0.22 μm filter. In some extracts, it was necessary to adjust the pH to 7. Subsequently, dechorionated zebrafish embryos (48 h post fecundation) were incubated at 28.5 °C in plates during 72 h in an embryonic medium (control) or embryonic medium containing the dissolution products of each sample. The mortality rate was determined each 24 h between 0–72 h. To end the experiment, embryos were anesthetized with Tricaine and fixed in 4 wt% paraformaldehyde in Phosphate Buffered Saline (PBS). Assays were performed in duplicate with n=20 embryos for each sample. This assay determined the biocompatibility of the studied composite materials. The dissolution of Ba^{2+} from the incubated samples was quantified by X-ray fluorescence analysis (XRF, PW4025/24 Minipal2 X ray spectrometer Panalytical with a Cr tube anode).

3. Results and discussion

3.1. Characterization of synthesized and commercial powders

Fig. 1 shows the XRD patterns and the morphological feature of the starting materials. The diffraction patterns of commercial and synthetic barium titanate powders correspond to a perovskite structure (PDF#75-1169). Furthermore, synthesized powders present traces of $BaCO_3$ (PDF#1-0506) as a minority phase in the XRD pattern of Fig. 1a). The XRD pattern of cBT shows a doublet in the resolution of the signal at 2θ=45°, which is associated with the stabilization of the tetragonal BT phase.

The diffraction patterns of commercial and synthesized HA (Fig. 1b) show peaks corresponding to crystalline hydroxyapatite (PDF# 73-0294). Differences in the peaks' width and intensity can be attributed to different values of the crystallinity degree, crystal size and composition (different Ca/P molar ratio) of the samples.

Through scanning electron microscopy, the medium particle size and particle morphology were analyzed (Fig. 1). Particle size of synthesized BT (mean particle size lower than 10 nm) was smaller than that of the commercial BT (mean particle size of 0.9 μm).

Morphological features of used HA particles are completely different as it is shown in Fig. 1b. Commercial HA presents plate-like particles with 2.1 ± 0.4 μm thickness and a 40 μm side. On the other hand, synthesized HA particles present a rounded form because of the agglomeration of small particles. Therefore, agglomerates of 10–15 μm were formed due to the high superficial energy of nanoparticles.