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Full Length Article

Influence of the modulated two-step synthesis of biogenic hydroxyapatite on biomimetic products' surface

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ABSTRACT

Processing calcium-rich natural resources, such as marble and mussel seashells, into biomimetic products could constitute an environmentally-friendly and economically sustainable alternative given their geographical widespread. Hitherto, their value for biomedicine was demonstrated only for seashells, with the technological exploitation approaches still facing challenges with respect to the identification of generic synthesis parameters capable to allow the reproducible and designed synthesis of calcium phosphate at an industrial-ready level.

In this study was targeted the optimization of Rathje synthesis method for the fabrication of biogenic calcium phosphates, by conveniently adjusting the chemical composition of employed reagents. It was shown that post-synthesis heat-treatment of compacted powders is the key step for inducing structural transformations suitable to attain biomimetic products for reconstructive orthopedic applications. The sintered materials have been multi-parametricallyevaluated from morpho-compositional, structural, wettability, mechanical and cytocompatibility points of view and the results have been cross-examined and discussed. Convenient and efficient preparation routes to produce biogenic hydroxyapatite have been identified. The functional performances of the as-prepared biogenic ceramics endorse their use as a solid and inexpensive alternative source material for the fabrication of various bone regenerative products and implant coatings.

1. Introduction

Exploitation of marine biogenic resources for preparing biologically relevant calcium phosphates (CaP) attracted considerable interest since the development, in 1974, of a synthesis method for coralline hydroxyapatite [1]. Although the method preserved the corals porous architecture, some disadvantages, such as uncontrolled resorption or inadequate mechanical properties, led to the

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development of alternative synthesis methods which allow the preparation of calcium phosphates powders with different compositions [2].

Transition to eco-friendly preparation methods, complying with the current exigent recommendations for environmental protection, impose adapting traditional synthesis procedures to exploit other various abundant waste-type raw materials. Currently, besides corals [1,3], waste materials such as marine animal shells (sea urchins [4] and sea shells [5–7]) are taken into account as CaP precursors in an attempt to satisfy the increasing demand of materials for biomedical applications by using the benefits of natural resources in a sustainable manner.

The frequently investigated synthesis methods use the calcium carbonate ($CaCO_3$) existing in various natural resources and a phosphate-based reagent (*e.g.* phosphoric acid (H_3PO_4)), which are chemically reacted to produce a CaP [7,8]. The reaction takes place at moderate temperature (150–220 °C) and requires up to 6 h [9,10]. More promising results have been obtained by applying an indirect synthesis route, which implies the thermal dissociation of $CaCO_3$ into calcium oxide (CaO), and its further treatment with H_3PO_4 [11]. Although it was initially was introduced for pure synthetic reagents, the method can be extended for the processing of natural raw materials by adapting the reaction parameters – the aim of this study.

The biomaterials prepared by such methods can be thermally processed and transformed into biphasic or multiphasic CaPs, whose biomedical performance depends on the ratio between the insoluble and the resorbable constituent phases. The phase composition can be conveniently adjusted, by designed variation of synthesis parameters, such as to induce desired properties (*i.e.* resorbability, bioactivity, osteoconductivity, or osteoinductivity), depending on the local requirements imposed by the implantation environment. Besides the biphasic or multiphasic CaPs the mixtures of thermally unstable CaPs (*e.g.* dicalcium phosphate dehydrate ($CaHPO_4{\cdot}2H_2O$, DCPD) or brushite, and anhydrous dicalcium phosphate ($CaHPO_4$, DPDA) or monetite) can be used as self-setting formulations. In this later case, the setting reactions and the final product characteristics are governed by each component fraction within the mixture. However, since there has been little discussion on the stability of such formulations and on the influence of compounds such as calcium oxide or calcium pyrophosphate, which sometimes occur during synthesis reactions [12], further clarifications are needed in order to develop competitive high quality products.

Most of the CaPs synthesis methods based on natural sources of calcium carbonate are difficult to reproduce because the synthesis parameters are not completely reported. Also, these studies primarily discuss the first stages of preparation, although further thermal processing of the obtained powders induces compositional and structural changes which influence considerably the final products performance [13–15]. Moreover, the variability of natural sources of calcium carbonate (due to their different origin and/or environment) influences their chemical composition, which in turn affects the thermal decomposition process into calcium oxide, and the phase composition of the prepared CaPs.

This study proposes an adaptation of the conventional indirect synthesis method (known also as Rathje method) [11] for preparing CaPs using dolomitic marble and *Mytilus galloprovincialis* seashells raw source materials. Dolomitic marble represents a new proposal for extending the range of natural calcium carbonate sources used for preparing CaPs.

The as-prepared and thermally-treated products have been thoroughly characterized, aiming to correlate the composition, morphology, structure, and wettability of the biogenic materials with their mechanical and biological performance, in the search for relevant triggers capable to support a controlled bio-functionality.

2. Materials and methods

2.1. Samples preparation

The raw materials used in this study are dolomitic marble (Ruschiţa, Romania) and Mediterranean mussels *Mytilus galloprovincialis* (Black Sea coast, Romania). After a mechanical cleaning, the raw materials were calcined in air at 1300 °C for 6 h, in an electrical furnace. After calcination, the materials were cooled in air and stored in Petri dishes.

The reagents amounts for CaPs synthesis were initially calculated based on the stoichiometric reactions described in the Rahtje method [11,16–18]: for 10 g of $Ca(OH)_2$ powder (derived from heat-treated marble/seashells), 200 ml of distilled water, and 5.5 ml of H_3PO_4 (≥85%, Sigma-Aldrich) were used. Further, the experiments were conducted separately for the marble and seashells-derived powders, using 1.0, 1.1, 1.2, and 1.3 × stoichiometric acid quantity. The marble and seashell-derived powders were firstly hydrated in distilled water and then treated with H_3PO_4 by drop-wise addition at a rate of 1 ml/min. The solutions were magnetically stirred during the synthesis for 2 h, at 700 rpm. Further, the prepared solutions were washed, filtered and dried at room temperature (RT) for 168 h, and then in oven at 100 °C, for 24 h, until the powders were obtained. The prepared powders were mixed with normal saline solution and pelletized by cold isostatic pressing at 0.1 MPa. The pellets were dried at RT for 24 h, and sintered in an electrical furnace at 1200 °C, for 10 h.

The samples are further referred to by H_3PO_4 quantity (*e.g.* 0 = stoichiometric quantity; 3 = 1.3 × calculated H_3PO_4 quantity) and by the raw material (M = marble; S = seashell). The as-synthesized powders will be referred AS and the thermal-treated (sintered) samples TT (example: 1S-TT = Thermal Treated pellet prepared from **S**ea**s**hells-derived powder treated with 1.1 (+10%) × stoichiometric H_3PO_4 quantity). A bovine hydroxyapatite (HA) previously prepared and completely characterized [19] was used as reference material in the study. The reference material was analyzed as-synthesized (HA-AS) and after sintering (HA-TT) in the same conditions as the other samples.

2.2. Bulk and surface physical-chemical characterization techniques

a) The morphology and composition of the AS powders and TT pellets were analyzed by scanning electron microscopy (SEM) (Philips Xl 30 ESEM TMP) coupled with energy dispersive spectroscopy (EDS) (EDAX Sapphire spectrometer), at an acceleration voltage of 25 kV and working distance of 10 mm. The SEM and EDS investigations were performed in three randomly chosen areas.
b) The samples structure was evaluated by X-ray diffraction (XRD) (Bruker D8 Advance diffractometer equipped with a LynxEye detector), in Bragg-Brentano geometry, with Cu K_α (λ = 1.5418 Å) radiation. The scattered intensity was scanned in the 2θ range 10–60°, with a step size of 0.04° and a dwell time of 1 s.
c) The bonding architecture and functional groups presence was evaluated by Fourier Transform Infrared (FTIR) Spectroscopy (Perkin Elmer Spectrum BX II spectrometer) in attenuated total reflectance (ATR) mode (PikeMiracle head). The spectra were recorded in the range 500–4000 cm^{-1}, with a resolution of 4 cm^{-1} and a total of 32 scans/experiment.
d) The mechanical compression testing of the sintered materials was performed on cylindrical pellets with a Walter + Bai AG LFV

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