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A comparative study of hydroxyapatite nanostructures produced under different milling conditions and thermal treatment of bovine bone

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ABSTRACT

In the present investigation the effects of milling parameters (time, atmosphere, and media) and chemical composition of raw materials on the mechanochemical synthesis of nanocrystalline hydroxyapatite (n-HAp) were studied. For a comparative study of the mechanically activated samples versus thermally treated specimen (natural origin), n-HAp was also produced via annealing of bovine bone at 800 °C for 2 h. The gained powders exhibited average sizes about 32 and 27 nm under air atmosphere, and about 32 and 34 nm under argon atmosphere. TEM images confirmed the formation of n-HAp with various morphologies under different experimental conditions.

1. Introduction

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$), as the major mineral constituents of vertebrate bone and tooth, is the most well-known bioceramic from the apatitic family. Due to its superior biocompatibility, osteoconductivity and bioactivity HAp is used in orthopedics and odontology in the coating of metallic implants, repairing of bone defects and bone augmentation [1]. In addition, HAp and its substituted structures have also been investigated for drug delivery [2], gene therapy [3], chromatography [4] and waste water remediation [5]. Depending on the application, there is often a need for the nanoparticles to be in a particular size range [6]. According to literature [7], nanocrystalline HAp (n-HAp) powders with higher surface area and lower particle size can provide higher biocompatibility, greater catalytic activity and good adsorption capability for use as biomaterial, catalyst and adsorbent. Therefore, many investigations have been carried out to synthesize n-HAp powders [8–11]. Nanostructures of HAp with desired properties can be achieved by control of the product characteristics such as particle size and shape, particle distribution and agglomeration [12].

Nowadays, there are several sources for the treatment of bone diseases, all of which are faced with restrictions [13]. As a result, research on production of calcium phosphates is likely to continue until suitable and cost-effective methods to be found. The obtained HAp from varied powder processing routes has great potential for bone substitute due to its excellent osteoconductive properties [14]. It has been found that the synthetic HAp can bond directly to tissues and promotes tissue growth, thus, has been considered in orthopedic and dental applications [15]. Generally, the fabrication methods of synthetic HAp nanoparticles can be classified into two groups: wet and dry [16]. The advantage of the wet process is that the by-product is almost water, as a result the probability of contamination during the process is very low.

Recently, HAp modified with different metal salts were prepared by wet-impregnation method and used as a catalyst for the synthesis of glycerol carbonate [17]. Also, a new group of adsorbents were prepared with different content of HAp and cement kiln to use in sorption of lead from aqueous solutions [18].

On the other hand, the dry process, as employed in this research, has benefit of high reproducibility and low processing cost [19,20]. Among different dry processes, mechanochemical treatment has recently been receiving particular attention as an alternative method to prepare nanocrystalline materials with appropriate structural characteristics [21–23]. The main features of the mechanochemical process are that melting is not essential and that the products have nanostructural characteristics [24]. Hence, when the mass production of n-HAp is required, mechanochemical method can be utilized. In this rout, milling media are often selected based on their high hardness, e.g. WC or SiC, or their chemical inertness, e.g. hardened stainless steel [25].

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The use of polymeric milling media has been proposed not only to annihilate contamination problem, but also to achieve the modified morphologies with high biomedical performance [26,27]. On the other hand, it has been reported that the size and number of balls had no significant effect on the synthesizing time and grain size of calcium phosphate ceramics, while decreasing the rotation speed or ball to powder weight ratio increased synthesizing time and the grain size of bioceramic [28]. These results suggest that the mechanochemical synthesis of calcium phosphates is affected by processing parameters. Thus, evaluation of milling parameters to synthesize a pure product with appropriate structural as well as morphological features is very essential.

Although, a variety of nanocrystalline calcium phosphates with various structural as well as morphological properties were known, but to author's knowledge, there are a few papers about the mechanical activation of calcium phosphates under inert gas atmosphere [29]. Mechanochemical synthesis of HAp nanoparticles under air atmosphere has been characterized by our group recently [26]. In this study, influence of milling parameters (time and atmosphere) and chemical composition of raw materials on mechanosynthesis of n-HAp were investigated. For this purpose, two distinct chemical reactions were activated for different milling times under air and a high purity argon (99.998 vol%) atmosphere. Furthermore, for a comparative study of the mechanosynthesized samples versus thermally treated specimen (natural origin), n-HAp was also prepared via annealing of bovine bone at 800 °C for 2 h. The phase purity of the HAp nanopowders, average crystallite size, lattice strain, volume fraction of grain boundary, fraction of crystalline phase (crystallinity) and morphological features of experimental outcomes were also determined.

2. Materials and methods

2.1. Mechanosynthesis of n-HAp powders

Details of milling conditions and composition of initial powder mixtures are given in Table 1. Starting materials including calcium hydroxide ($Ca(OH)_2$, Fluka), anhydrous dicalcium phosphate ($CaHPO_4$, Merck) and calcium carbonate ($CaCO_3$, Merck) with given stoichiometric proportionality within the reagents, were milled using a high energy planetary ball mill under air and a purified argon (99.998 vol%) atmospheres. Mechanical activation was performed in polyamide-6 and tempered chrome steel vials (vol. 125 ml) using Zirconia balls (20 mm in diameter) for 15, 40 and 80 h. In all experiments, the weight ratio of ball-to-powder and rotational speed were 20:1 and 600 rpm, respectively. To control temperature and prevent excessive heat, the millings were completed in 45 min milling steps with 15 min interval pauses. In order to investigate the effect of chemical composition of raw materials on the mechanochemical process and purity of final products, two distinct chemical reactions were utilized as follows:

$$6CaHPO_4 + 4Ca(OH)_2 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O \quad (1)$$

$$4CaCO_3 + 6CaHPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 4CO_2 + 2H_2O \quad (2)$$

2.2. Thermal synthesis of n-HAp powders

To thermal synthesis of n-HAp powders, a femur of an adult bovine was prepared and cleaned by boiling to remove flesh and fat. The impurities that were sticking on the bone surface were shaved and removed, and then irrigated with a brush in running water, followed by boiling in distilled water for 2 h. This cleaning process was replicated three times till it yielded a white and clean bone. After that, the cleaned bone was heated at 60 °C for 24 h to remove moisture. This procedure to remove organic substances was done to avoid soot formation in the sample during the annealing process. The as-received bone was then cut into small pieces of approximate size 10 mm × 10 mm × 10 mm and heated at 400 °C for 3 h. This heating process was carried out to ensure that the organic compounds were completely removed and that the material is safe and to avoid any microbial contamination. Finally, the obtained bone ash was annealed in an electric furnace, under ambient condition, at 800 °C using a heating/cooling rate of 5 °C/min with 2 h holding time. The output of this process was white HAp nanopowder that was utilized for a comparative study.

2.3. Characterization of n-HAp powders

Phase analyses of products were carried out by X-ray diffraction (Philips X-ray diffractometer (XRD), Cu-K_α radiation, 40 kV and 30 mA). For the analysis of the diffraction patterns and determine the structural properties of the samples, "*PANalytical X'Pert HighScore*" software was used. The obtained patterns were compared to standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS), which involved card #24-0033 for HAp, #09-0080 for $CaHPO_4$, #01-0837 for $CaCO_3$ and #04-0733 for $Ca(OH)_2$. Crystallite size and lattice strain of the samples were determined using the XRD data according to the following equations [21,30]:

$$D = \frac{K\lambda}{(b_{obs} - b_{std})(\cos\theta)} \quad (I)$$

Table 1
Details of milling conditions and composition of initial powder mixtures.

Sample	Composition	Milling atmosphere	Milling time (h)	Milling media
HA1	100 wt% ($CaHPO_4 + Ca(OH)_2$)	Air	40	PA6[a]
HA2	100 wt% ($CaHPO_4 + Ca(OH)_2$)	Argon	40	PA6
HA3	100 wt% ($CaHPO_4 + Ca(OH)_2$)	Air	80	PA6
HA4	100 wt% ($CaHPO_4 + Ca(OH)_2$)	Argon	80	PA6
HA5	100 wt% ($CaCO_3 + CaHPO_4$)	Air	40	PA6
HA6	100 wt% ($CaCO_3 + CaHPO_4$)	Argon	40	PA6
HA7	100 wt% ($CaCO_3 + CaHPO_4$)	Air	80	PA6
HA8	100 wt% ($CaCO_3 + CaHPO_4$)	Argon	80	PA6
HA9	100 wt% ($CaHPO_4 + Ca(OH)_2$)	Argon	40	TCS[b]
HA10	100 wt% ($CaCO_3 + CaHPO_4$)	Argon	40	TCS
HA11	100 wt% ($CaHPO_4 + Ca(OH)_2$)	Argon	80	TCS
HA12	100 wt% ($CaCO_3 + CaHPO_4$)	Argon	80	TCS
HA13	Bovine bone	–	–	–

[a] PA6: polyamide-6.
[b] TCS: tempered chrome steel.