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Comparison of physicochemical properties of bio and commercial hydroxyapatite

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ABSTRACT

This article reports a physicochemical comparison of synthetic and biological Hydroxyapatite (HAp). Eight samples were separated into two groups: bio and commercial hydroxyapatite (bio-HAp and commercial-HAp). The bio-HAp group containing *defat*, *alkaline*, and *calcined* samples taken from bovine bone were obtained by using three different treatments, in order to establish their effect on the final product quality. The commercial-HAp group, from different sources: *NIST*, *sigma*, *apafill G*, *coralina*, and *biograft*, were analyzed and compared with the bio-HAp results. Thermogravimetric analysis (TG) was used in order to establish thermal degradation of the samples; structural behavior was then analyzed by X-Ray Diffraction (XRD) to found the crystalline phases, as well as the crystalline quality. Fourier Transform Infrared Spectroscopy (FTIR) was performed in order to identify the corresponding HAp functional groups within the samples. The surface morphology was analyzed by Scanning Electron Microscopy (SEM) and the elemental composition was established by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). It was found that the calcination process obtains HAp with comparable quality to the commercial samples. A crystallinity greater than 62% after the *alkaline* process was found. Additionally, the surface of the *alkaline* sample presents a transition behavior between dense and porous morphology.

1. Introduction

Bone is a living tissue, which is basically composed of an organic phase (20–30 wt%), an inorganic phase (60–70 wt%) and around 5 wt% of water. The organic matrix is composed mainly of collagen, but there are other compounds in small concentrations, such as lipids and non-collagenous proteins. The organic phase provides elasticity, flexibility, and resistance to the bone. On the other hand, hydroxyapatite (HAp) – chemical formula $Ca_{10}(PO_4)_6(OH)_2$ – is the main component of the inorganic matrix. There are other ions, such as magnesium, fluoride, and sodium that form the minor components of the inorganic matrix; the whole matrix gives hardness and stiffness to the bone [1–6].

The understanding of both bone components is important for biomedical applications such as prosthesis and partial bone replacement among others [3,7–9]. The main component of the inorganic matrix (HAp) has gained significant attention due to its excellent biocompatibility, bioactivity, non-inflammatory behavior, non-immunogenicity, and high osteoconductive and/or osteoinductive nontoxicity properties, as well as its easy processing [3,4,10–12]. Thanks to these properties, HAp has been widely used in dental applications and hard tissue surgery, in part due to its ability to bond with bone tissue after the procedure [13–15].

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However, HAp does not have the required mechanical properties for such applications; typically, its use demands the capacity to bear a high load. In relation to this point it is important to note that there have been many attempts to reinforce and combine HAp with other ceramics, polymers and bioactive glasses in order to improve the mechanical and biological properties through a composite of materials [14,16].

HAp can be obtained from natural and synthetic sources, and these have been used for bone replacement and ingrowth. The synthetic HAp has a stoichiometric distribution of its components; for this reason it does not have the same mineral traces of natural bone. The mineral traces play an important role in the osteointegration process. Therefore, HAp from natural sources, e.g., coral and bovine bone is more similar to human bone tissue. Synthetic HAp, unlike HAp from natural sources (bio-HAp), does not have the same behaviors in different applications [3,7,9,10,17].

There are different production methods for both HAp types: hydrothermal, mechano-chemistry, microwave irradiation, precipitation, sol–gel, hydrolysis, and micro-emulsion methods, among others, that have been used to obtain synthetic HAp [19]. In the natural HAp case, calcination, chemical, and thermal treatments have been used to remove the organic material in order to prevent infections, disease transfer, and immunological defensive reactions [3,8,18,20]. In most cases, bovine bone has been used as the HAp source for medical applications [10].

N.A.M. Barakat et al. [3] proposed three methodologies to clean bovine bone in order to establish a good process to eliminate the organic materials present in a sample. This study showed that by means of the thermal decomposition treatment produced carbonate-free HAp with better crystallinity than both the subcritical and alkaline treatments. On the other hand, D. Tadic and M. Epple [12] reported a complete study of different types of commercial calcium phosphates used in bone substitution from different sources (synthetic, animal and human), and the manufacturing processes. They reported that in some cases there are different HAp phases depending on the sample, e.g. tricalcium phosphate (TCP), octacalcium phosphate (OCP), calcium oxide (CaO), and in some cases, organic material like collagen and bioactive.

In this paper a comparison between the physicochemical properties of HAp obtained from bovine bone (bio-HAp) and commercial-HAp is reported. Three samples obtained from bovine bone by means of different treatments: *defat*, *alkaline*, and *calcined*, and five commercial samples obtained from companies and universities: *NIST*, *sigma*, *apafill G*, *coralina*, and *biograft*, were studied in order to establish the differences among the samples. By using TG analysis the mass loss as a function of the temperature was obtained in order to determine the presence of water and organic material in the samples. The structural characterization was performed by using XRD and FTIR techniques. The presence of HAp phase, the crystallite quality, as well as crystallinity percentage were determined by XRD. FTIR was used to identify the corresponding functional groups of HAp, and also to identify if carbonates and organic material are present. SEM analysis was performed in order to determine the morphology of the samples. Elemental analysis was carried out with ICP-OES in order to establish the mineral content in the samples.

2. Material and methods

8 different bio-HAp and commercial-HAp samples were studied. The bio-HAp group was comprised of 3 biological samples obtained from bovine bone using different treatments: 1) Treatment to remove fat: *defat*, 2) Alkali treatment: *alkaline*, and 3) Thermal treatment: *calcined*. All these samples were obtained from cortical bovine bones (2 years old) collected from the local slaughterhouse (folio number SDA-537295, 2011).

The commercial-HAp group comprised of 5 samples from different sources: 1) 1400 standard of bone ash certified by the National Institute of Standards & Technology: *NIST*, 2) Commercial-HAp from Sigma Aldrich (289396-synthetic): *sigma*, 3) Apafill G (Reg. No. 76LYC – synthetic, Centro de Biomateriales of La Habana University–Biomat): *apafill G*, 4) Coralina (HAP-200 – Reg. No. 47.174.92 – marine corals, Centro Nacional de Investigaciones Científicas – La Habana, Cuba): *coralina*, and 5) Biograft (Ref. 16140301, SN. 806330, LOT. DR-0005-08, human bone powder 1 cc): *biograft*, were analyzed.

2.1. Bone cleaning process

One of the most important procedures to obtain pure HAp from biological sources is the bone cleaning process; it aims to remove organic compounds such as fat and protein. In order to prepare bio-samples (bio-HAp), the first step in all cases was to cut the bone into smaller pieces and manually remove adhering soft tissue. Thereafter, the fluids in bone, marrow, and any remaining soft tissue were eliminated by boiling these small pieces of bone in deionized water during 30 min. The bone was then subjected to vacuum drying and a milling process using a stainless steel mill (Oster-USA) until powder was fine enough to pass through a mesh 100 (149 μm) sieve. After that, the three different processes mentioned above: *defat*, *alkaline*, and *calcined* were carried out respectively.

Process 1 – Defat: this consists in the removal of fat from the bone with solvents; firstly, the bone powder was treated with petroleum ether with constant agitation at 30 °C and then it was air-dried. After this step, the dried bone powder was immersed in acetone under ultrasound for 2 h, and after that, the powder was dried in a vacuum oven at 1.33 Pa and 70 °C for 5 h.

Process 2 – Alkaline: the bone powder was treated with sodium hydroxide solution at 8% p/v changing pH and temperature in order to eliminate proteins present in the bone. The powder was dried in a vacuum oven at 1.33 Pa and 70 °C for 5 h.

Process 3 – Calcined: the bone powder sample was heated from room temperature to 400 °C using a heating rate of 0.4 °C/min, and then from 400 to 900 °C using a heating rate of 1.4 °C/min. It was kept at this temperature for 3 h. The first ramp was to eliminate the organic materials without promoting the generation of carbonate phases; the second part of the ramp was to obtain the desirable HAp quality [8]. These temperatures ensure both the presence of the HAp phase, and that there will not be any organic material remaining [8].

All bio-samples were obtained after basic cleaning process. After the cleaning, samples were subject to different processes as follow: *defat* sample consist of bone with process 1, *alkaline* sample obtained after apply process 1 and process 2, and *calcined* sample obtained after apply process 1 and process 3.

2.2. Commercial sample preparation

The particle size of commercial samples was reduced until they passed through a mesh 100 (149 μm) sieve. In all cases, the samples were milled in an Agatha mortar.

2.3. Thermal behavior: TG analysis

The thermogravimetric curves and their derivative in relation to temperature were obtained by using TG Q500 equipment (TA Instruments). The sample mass was 12.0 ± 1.0 mg of each sample and these were placed in the platinum crucible of thermobalance (TA