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Physical properties of continuous matrix of porous natural hydroxyapatite related to the pyrolysis temperature of animal bones precursors

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ABSTRACT

This study reports the physical properties of porous blocks of non-stoichiometric natural hydroxyapatite, received from shapes of porcine and bovine bones by heat-treatment at temperatures 400–950 °C, in nitrogen atmosphere. Raw and heat-treated samples were investigated using gas helium densitometry, DC electrical and ultrasonic measurements. Thermogravimetric analysis (TGA), chemical characterization by means of the CHN analysis (carbon, hydrogen and nitrogen content) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), as well as by infrared spectroscopy (FTIR) were also used to the study. Morphology of the samples was observed using scanning electron microscope (SEM). Special attention was paid on properties of matrix (continuous matter of porous block) of obtained materials. Monolithic shapes of pyrolysed bones were found to be porous (bulk porosity ~51%) of highly stiff matrix of dynamic elastic moduli up to 40 GPa. Density of matrix (true density) ranged from 2.078 g/cm^3 up to 3.094 g/cm^3–close to that of stoichiometric hydroxyapatite. Distinct ordering of matrix structure caused by heat treatment of bones at the highest temperatures was reflected by high anisotropy of stiffness and distinct increase in DC electrical conductivity. Beside of growth of 4–6 orders of the electrical conductivity, the natural hydroxyapatite still remained insulator with conductivity 10^{-6}–10^{-3} S/cm. Strong correlation between DC electrical conductivity and true density was detected. Big difference between properties of blocks of natural hydroxyapatite produced from different precursors (bovine or porcine bone) was not observed.

1. Introduction

As a result of the increasing importance of natural environmental protection and of lowering amount of non-renewable resources, development of new advanced materials from natural renewable feedstock becomes necessary. The renewable resources of a natural origin base generally on plants, rarely on animal waste. Cellular anatomy of plants and animal bones provides an attractive template for design of various materials [1–4]. Biomorphous materials, such as plant carbonization products/biomorphous composites were successfully applied as adsorbents [5,6], sensors or electrodes for glucose sensors [7], exceptionally as scaffolds for tissue engineering [8]. For bone substitutes, materials prepared from animal bones seem to be more promising.

Various reasons, such as tumors, trauma, disease, etc., create a demand for skeletal reconstruction. Although autograft and allograft are the standard treatments, they are sometimes accompanied by many medical problems. Therefore, many investigations have been conducted to find novel materials for bone substitutes. Among them, hydroxyapatite (HA) is perhaps one of the most promising materials. It is well-known that HA with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ is the major mineral component of bone. Due to its excellent biocompatibility and bioactivity, HA has been widely used as bone substitutes [9].

HA can be produced chemically [9–12] or from natural resources like corals [13], bovine bone [9,14–25], porcine bone [25–27] or cuttlefish bone [28]. Bone is composed of organic (about 30–40%) and inorganic components (about 35–45%), as well as of water (about 15–25%). The organic part contains mainly collagen and proteins, whereas the inorganic component is mainly HA with a small percentage of other elements such as carbon, magnesium, sodium, etc., being incorporated in the structure. The elements mentioned above are absent in the case of synthetic HA. It is well-known that the

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presence of trace elements is very important, because they play a vital role in the bone metabolism process [18,21].

High temperature heat-treatment of animal bones was found to be most often used technique to produce natural (or biological) HA. The natural HA obtainment usually takes few hours of thermal treatment, during which the organic components in bones are removed, leaving pure inorganic HA as the residue. The papers mentioned above presented investigations of powders or monolithic blocks of bones (bovine bones mostly), heated in an inert atmosphere or in the presence of oxygen, at final temperatures ranged from 400 °C to 1350 °C. Organic compounds were found to be completely removed from species of bovine bone upon heating at temperatures >600 °C. Bone pyrolysed between 800 °C and 1000 °C revealed the characteristics of a natural bone with the interconnecting pore network being retained in the structure. HA samples obtained from bovine bone were characterized by high porosity (>57%) and the interconnecting pore system. High volume porosity was found to be a result of high amount of macropores of diameter ~300 μm (97% of porosity). Smaller pores (~1.3 μm) account for only 3% of the total porosity. Minimum requirement for pore size in bone substitute is considered to be about 100 μm due to cell size, migration requirements and transport. However, pore sizes >300 μm and gradients in pore sizes are recommended, due to enhanced new bone formation [20]. The investigation of pyrolysed bone chemical composition revealed small amounts of other inorganic compounds such as $Ca_4O(PO_4)_2$, $NaCaPO_4$, $Ca_3(PO_4)_2$, CaO, and MgO. Besides trace amounts of aluminum, iron, magnesium, potassium, silicon, sodium, vanadium and zinc, pyrolysed bone contains probably carbonated apatite [20,22,23,29].

In spite of differences between the natural HA and native bone, the most important properties with respect to the use of such mineral as a biomaterial for filling bone defects are maintained. Thus, natural HA is potentially a better basis for bone grafting than synthetic material. Appropriate mechanical properties of 'artificial' bone and its desired architecture for new tissue formation are very important in tissue engineering. The major problem with the use of synthesized HA as bone regeneration grafts is its weak mechanical strength. Therefore, to solve this problem various composites containing HA were elaborated [4,11].

As it was mentioned previously, natural HA is considered generally to be basis for bone grafting. But it can be also promising precursor for production of heavy metal ions [24] or high toxic fluoride [30–32] adsorbents. HA was also used successfully to prepare electrodes for supercapacitors [33,34]. Changes of the bone composition and structure following heat treatment at different temperatures could affect the HA efficacy in these applications. Investigations described in the literature were made mainly for powdered bones, rarely for monolithic samples. Monolithic blocks of bone substitute of required shape would be more useful than those made from powders. What is more, monolithic porous blocks of bones pyrolysed at high temperatures can be modified by impregnation with other substances, e.g., with natural polymer—chitosan. Summarizing, they could be promising materials in various potential applications: for dividing organic compounds in purification processes of food products, as adsorbents of harmful substances in the environmental protection, as electrodes for electric double-layer capacitors (EDLC), etc.

The aim of this work was to study monolithic blocks of both bovine and porcine bones, heat-treated at different temperatures between 400 and 950 °C and to characterize anisotropic properties of the obtained materials using helium gas pycnometer, measurements of both ultrasonic wave velocity and DC electrical conductivity. Special attention was paid to these parameters, that were rarely or not reported at all in the literature for pyrolysed bones: both density and dynamic elastic modulus of continuous matter of porous bone (bone matrix), as well as the DC electrical conductivity.

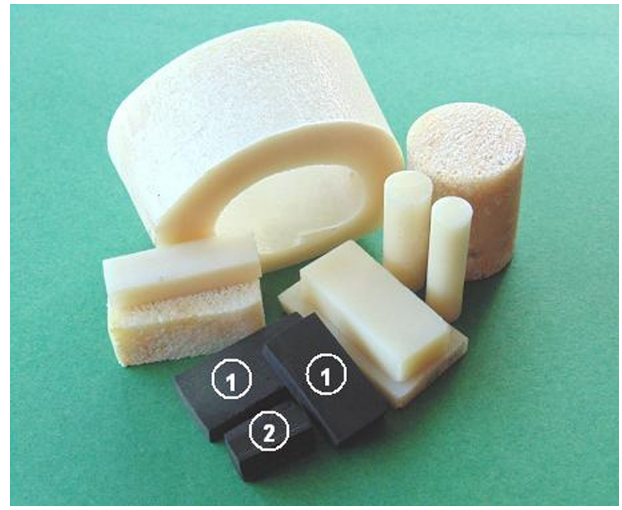

Fig. 1. Monolithic blocks cut from the bones: raw (light samples) and pyrolysed (black samples). Notation: ① —BB400, ② —PB550.

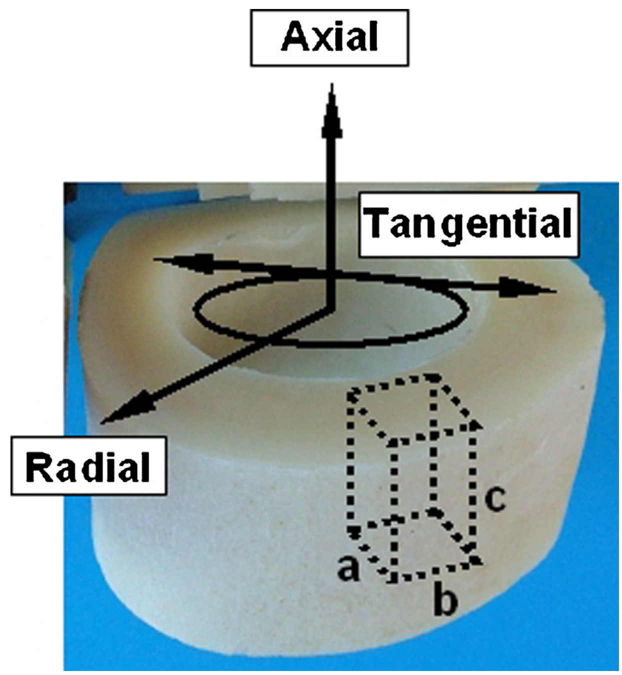

Fig. 2. Principle directions used for describing physical properties of bone sample, studied in the shape of cuboid. Notation: (a) radial direction, (b) tangential direction, (c) axial direction.

2. Experimental

2.1. Preparation of samples

Bones of swine (porker) and cow were used as the starting materials for pyrolysis. Femora of adult animals were procured from local slaughterhouse and next hydro-thermally treated to remove visible tissues and substances on the bone surface. Rectangular monoliths were cut from the ring-shaped bones (Fig. 1). The final form of the samples was a cuboid with sides of about 10 mm × 10 mm × 30 mm, which were parallel to three orthogonal axes of the femur (axial, radial, and tangential axes) (Fig. 2). Pyrol-