Structural and morphological induced modifications in hydroxyapatite obtained by bone thermal treatments

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As yet, production of hydroxyapatite through thermal treatment of animal hard tissues is one efficient and economical alternative to classical chemical synthesis and provides a suitable similarity with bone tissue. The production methods' optimization requires the proper assessment of tissue transformations induced by the variation of heat treatment parameters. The present study aimed to emphasize the chemical, structural and morphological modifications defined by heat treating the bovine bone at different temperatures (1000, 1100, 1200 and 1300°C) by subsequently characterising it using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). SEM results confirmed that increasing the heat treatment's temperature will lead to a growth of the hydroxyapatite grain size and a gradual decrease of porosity. The FT-IR analysis showed some modifications in the phosphate ions vibrations for the samples heat-treated at 1100 and 1200°C. After that, the structural evaluation performed by XRD identified some minor structural transformations after heat treating the samples at 1300°C, but the general analysis proved that increasing the heat treatment's temperature has no significant influence upon the crystallinity. The complementary analysis showed that the thermal treatment's temperature is one of the factors that influence the characteristics of bovine bone derived products.

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1. Introduction

The hydroxyapatite derived from various synthetic sources is the best match for the mineral bone architecture. One efficient alternative to chemical synthesis both economical and environment-friendly suggests the use of natural sources like bovine bone as raw materials for hydroxyapatite production [1, 2]. Different xenografts derived from bovine, ovine and swinish bone are already available for merchandising, and their major asset, towards the synthetic hydroxyapatite, is based on the ion traces assimilated in material's crystalline structure, with significant implications in bone metabolism.

Animal bone-derived products are generally prepared by thermal treatment, mostly for the removal of tissue's organic component. A suitable heat treatment allows for production of carbonate-free hydroxyapatite, with an improved crystallinity. Additionally, thermal treatment performed at approximately 850°C guarantees against the biological contamination of the material, thus eliminate one of the major risks of bone-derived products [3-9].

Bovine-bone derived hydroxyapatite stimulates bone regeneration, due to the presence of the characteristic ions, and has the ability to form a direct bond with the bone, without the existence of the intermediate tissue [4]. Its osteoconductivity may be ensured through a proper porosity control. The porosity may be categorized, based on the pores' dimensions', in: microporosity (pore dimensions smaller than 5 μm) and macroporosity (pore dimensions larger than 100 μm) from which the last one plays an extremely important role in tissue vascularization [10]. Pore dimensions and implicitly the biological properties of hydroxyapatite are strongly influenced by the heat treatment's parameters and especially by the heat treatment's temperature [11].

Besides porosity, some other characteristics are influenced by the heat treatment's temperature. Comparative assessment of the animal bone-derived products showed that temperature's variation may lead to a change of crystallite dimensions [11, 12] while heat treatments performed at elevated temperatures may contribute to new phase formation [3] or to an adjustment of the final product's mechanical properties [5].

Until now, the characteristics of bovine bone-derived products obtained at temperatures higher than 1000°C were not amply explored. In the present study, the

behaviour of bovine bone after heat treatment at various temperatures between 1000 and 1300°C was investigated. This paper discusses the influence of heat treatment temperature upon the morphology, composition and structure of bovine bone derived products. The characterization of temperature-induced variations and the assessment of other parameters' influence upon the osseous tissue represent critical actions for the optimization of bovine bone products' preparation.

2. Experimental

This study was performed on cancellous bone samples, obtained after the removal of cortical parts from bovine femur. The experiments began with the mechanical cleaning of the bovine bone samples in order to eliminate macroscopic impurities (ligaments and other tissue fragments), followed by a thermal treatment at 500° C for an hour, with a 10° C/min rate for the removal of organic components.

Further, the samples were heat treated at 1000, 1100, 1200 and 1300° C, with 10°C/min rate, for two hours. After air-cooling, the samples were ball-milled in an agate enclosure. The particles were then characterized in order to establish the influence of heat treatment's temperature.

The morphological changes were examined through scanning electron microscopy (SEM), using a Phillips XL 30 ESEM TMP equipment with a secondary electron detector in low vacuum and a solid state detector with two BSE diodes. The samples were mounted on aluminium stubs and were analysed without any preliminary coating of the material.

The information regarding components and functional groups within the samples [3] were acquired after performing Fourier transform infrared spectroscopy (FT-IR) analyses in the region characteristic for hydroxyapatite (850 - 1150 cm^{-1}), using a Bruker Tensor 27 equipment with diamond ATR annex.

Finally, the phase evaluation for the heat treated materials and the structural properties interpretation [3, 13] were performed through X-Ray diffraction (XRD) using a Panalytical diffractometer for 2θ between 20 - 60°.

3. Results and discussion

The bone is the main calcified tissue in the human body. Its primary role is to ensure mechanical support while providing the appropriate amounts of calcium and phosphorus ions required by various metabolic processes.

Chemically, the bone tissue is a composite material constituted from a collagen-based phase (the organic component) and a calcium-phosphate phase (the mineral component). The mineral phase of the bone tissue is a non-stoichiometric form of hydroxyapatite, named "calcium deficient hydroxyapatite" (CDHA). According to its chemical formula, $Ca_{10-x}(HPO_4)_{6-x}(OH)_{2-x}$, many vacant calcium and hydroxide ions sites may be present in CDHA's structure. Also, the material may exhibit various ionic substitutions: calcium may be replaced by sodium, potassium, magnesium or strontium ions; phosphate is usually replaced by carbonate, and hydroxide may be substituted by fluoride, chloride and carbonate [14].

The thermal treatment of bone tissue will induce various modifications in the chemical, morphological and structural features of bovine bone tissue. All these modifications are strongly dependent of the thermal treatment's temperature.

The thermal decomposition of the bovine bone begins with adsorbed water removal. This phenomenon occurs until the temperature reach approximately 250°C and is characterized by mass loss and shrinkage. Visually, until reaching this temperature, the tissue will change its colour from ivory to different shades of brown.

At the same time with the water removal, the organic component of the bone begins its degradation, followed by combustion and complete removal at 500 - 600°C. However, the organic component's degradation is a complex process which is influenced by various factors, like the hydration degree, the proportion of organic substance, the bone type (cortical or cancellous). Mass loss is also characteristic for this stage, as well as carbon dioxide emission and water loss.

During the first stages of bone thermal degradation, its mineral component is not affected by the temperature rise, but the thermal treatments performed at more than 600° will lead to morphological and compositional changes of hydroxyapatite. It is assumed that those thermal treatments will start the hydroxyapatite recrystallization and will allow for the removal of carbonate groups from its crystalline structure. In this stage various modifications of crystalline lattice parameters may be observed along with an intense shrinkage.

The heat treatment performed at above 750° will ensure the complete biological decontamination and the hydroxyapatite will begin its decomposition at approximately 800°C. Its main decomposition products were found to be oxy-hydroxyapatite and oxy-apatite, which may further decompose in beta-tricalcium phosphate and calcium oxide. The decomposition is however strongly influenced by the initial chemical composition of the bone and is not documented in detail, due to the different chemical compositions of bovine bone from distinct animals. Also, the thermal treatment's environment (e.g. nitrogen or carbon dioxide) may decelerate the process so that the hydroxyapatite decomposition may begin at temperatures above 1000°C [7-9, 15-22]

3.1. Morphological evaluation through scanning electron microscopy (SEM)

Fig. 1a-1d presents the morphological aspects of the cancellous bovine bone samples, heat treated at temperatures between 1000-1300°C.

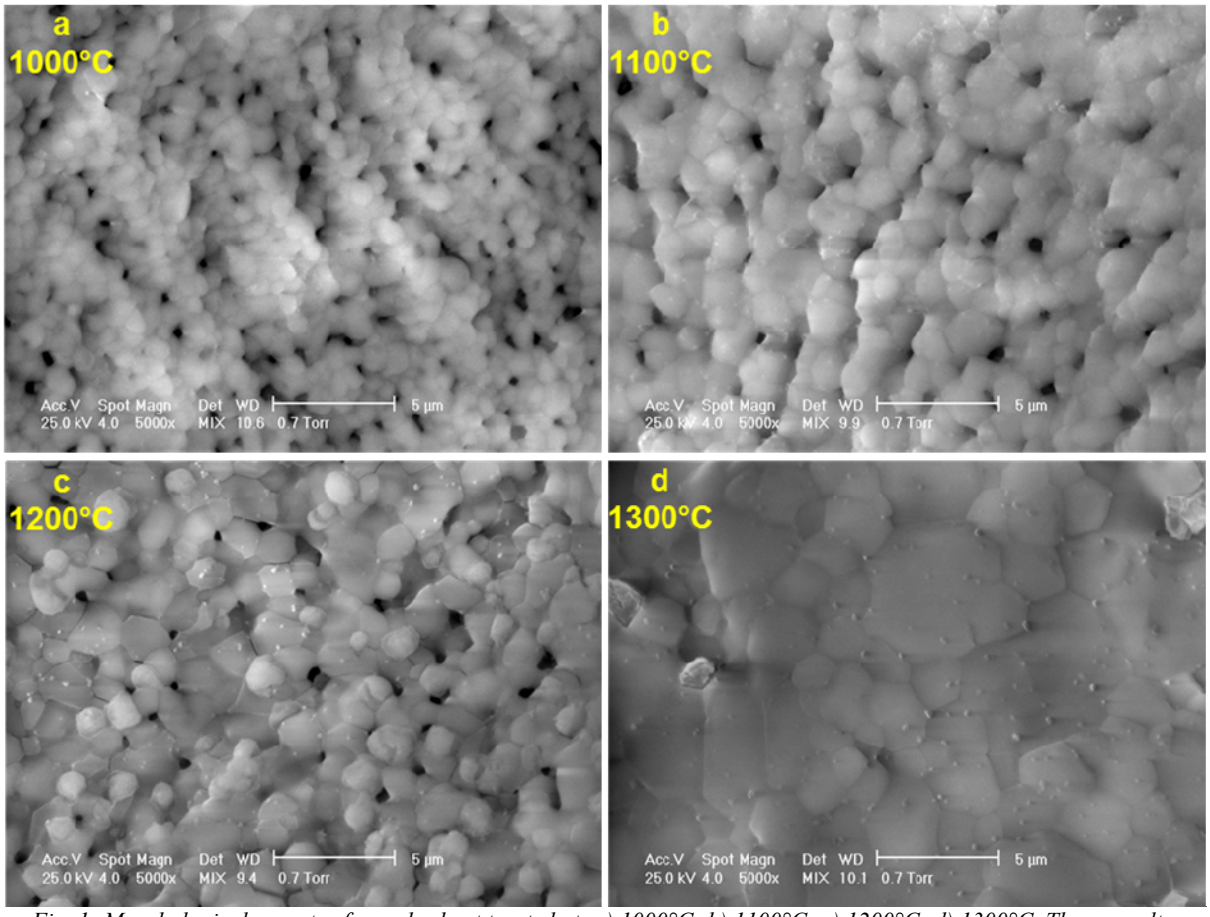

Fig. 1: Morphological aspects of samples heat treated at: a) 1000°C; b) 1100°C; c) 1200°C; d) 1300°C. These results evidence the influence of heat treatment's temperature on bovine bone's microstructure and porosity

These results clearly demonstrate the influence of thermal treatment's temperature upon the sample's microstructure and porosity. Sample heating performed at 1000°C led to the formation of hydroxyapatite crystals with granular morphology and porous architecture as a result of thermal degradation and removal of the bovine bone organic component.

Similar microstructural and porosity-related aspects for the bone mineral component were deserved in similar studies performed on bone tissue heat treated at 900-1050°C [1, 3, 5, 11, 21-24].

The increase of heat treatment temperature to 1100 and 1200°C caused a porosity decrease, followed by the complete extinction after heat treating the samples at 1300°C, and simultaneous with grain growth. This evolution is documented in other studies as well [1, 13] and is defined as "boundary diffusion" [1].

A similar grain growth coexistent with pore coalescence [5,6] was obtained after the heat treatment of bovine bone samples at the same temperatures, but in different conditions (4°C/min heating rate, for 4 hours, followed by cooling in the furnace) and was described more like a "grain fusion" than an increase in their dimensions.

These differences prove the major influences of all heat treatment parameters upon the bovine bone morphology and especially upon its porosity

3.2 Chemical evaluation through Fourier transform infrared spectroscopy (FT-IR)

The infrared spectrum may serve as a footprint for unknown samples' identification, by comparing it with a reference spectrum previously acquired. The characterization, and sometimes identification of a sample may be performed even without a reference spectrum, because some structural features of the molecule will generate characteristics and reproducible infrared signals. However, FT-IR results interpretation must be performed with respect to the sample preparation method and precedent processes.

Although FT-IR is a method generally associated with organic samples analysis, it provides a useful characterization tool for inorganic samples as well, because all ionic or coordinative compounds will generate a characteristic signal, influenced by compounds' structure or orientation [25]. Hence, FT-IR analysis is an appropriate method for the characterization of bovine bone

mineral component and provides information regarding the types of molecular bonds existing in the samples and sometimes, regarding material's structure. The bone tissue spectrum includes the characteristic bonds of collagen and hydroxyapatite, along with some new bonds produced by the carbonate substitutions from the crystalline lattice of hydroxyapatite [12].

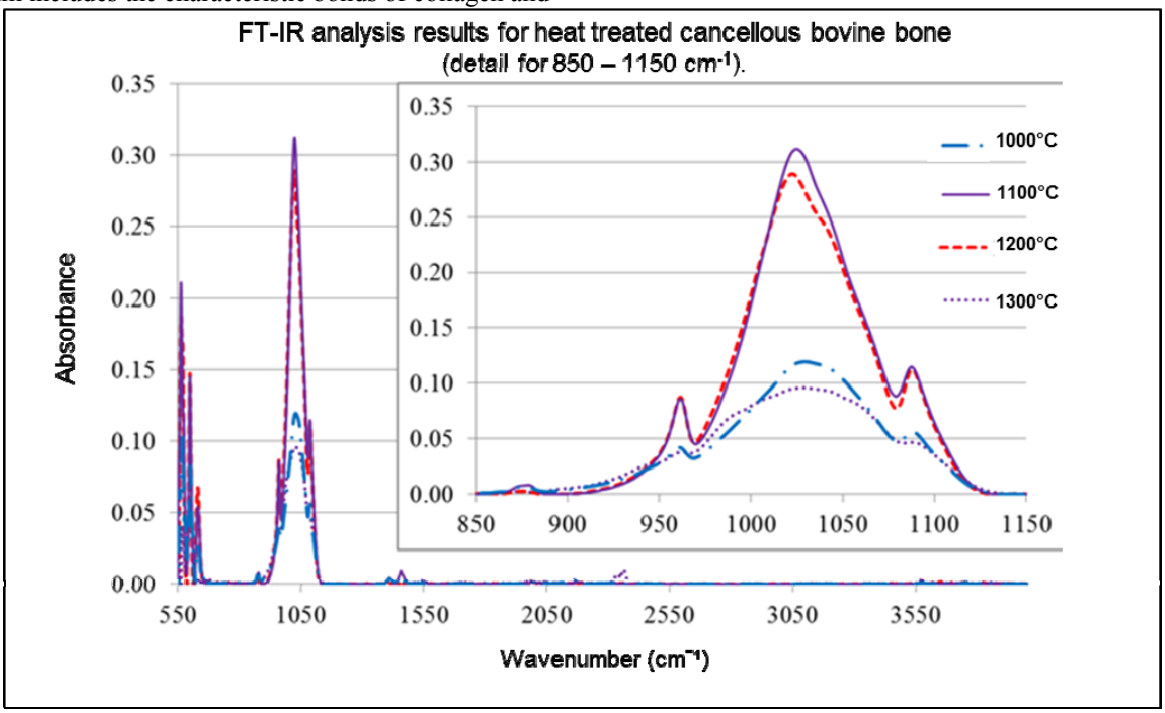

Fig. 2. FT-IR analyses results, between 850 and 1150 cm^{-1}, for the samples heat treated at 1000°C - 1300°C. Major variations of vibration modes can be observed between 950 – 1100 cm^{-1}, after the heat treatments performed at 1100 and 1200°C

Fig. 2 reveals the heat treated samples' features captured by the FT-IR analysis performed between 850 and 1150 cm^{-1}, which is a range characteristic for the phosphate groups $(PO_4)^{3-}$ from hydroxyapatite's structure. The peaks identified at approximately 960 cm^{-1} are associated with the symmetric stretching of $(PO_4)^{3-}$ groups while the peaks identified between 1000 and 1090 cm^{-1} are normally assigned to the asymmetric stretching of the same groups. Furthermore, the peaks recognized near 870 cm^{-1} are typically attributed to the carbonate groups $(CO_3)^{2-}$ [3, 7, 16, 21, 26-29].

Several variations of stretching modes were observed in the 1000 – 1100 cm^{-1} interval after the increase of heat treatment's temperature at 1100 and 1200°C.

These infrared spectra transformations may be associated with the development and disintegration of various phases and phosphate compounds from the bone mineral component [1], the bands' increasing intensity being considered a usual consequence of the thermal treatment of apatites [13].

Furthermore, the presence of well distinguished absorption bands is associated with a higher crystallinity degree [3]. The carbonate peaks were not detected after heat treatment at 1200°C [3], which suggests that the removal of carbonate groups related to the bone thermal treatment finishes between 1100 and 1200°C.

3.3 Structural evaluation by X Ray Diffraction (XRD)

The structural features of cancellous bovine bone, identified by XRD analyses, are presented in fig.3.

The results are typical for the hydroxyapatite structure [1, 13, 28, 30-32] and the present study confirms some results documented in similar studies, which deduced that increasing the heat treatment temperature above 1000°C does not influence the samples' crystallinity [13].

The development of additional compounds was studied by other authors, but in the case of calcium oxide (CaO) [13] for example, the concentration was insignificant hence the phase could not be detected by XRD.

Other chemical compounds developed through similar heat treatment studies were β-tricalcium phosphate (β-TCP) [16], tetracalcium phosphate (TTCP) [28] and fluorapatite (FA) [5, 6], but a rigorous comparison is difficult to perform due to the major influences induced in the bone tissue by the additional heat treatment parameters (heating rate, soaking time and cooling method).

4. Conclusions

This study aimed for the evaluation of transformations induced by heat treatment's parameters applied to cancellous bovine bone. The major parameter targeted by the present comparative analysis was the heat treatment temperature. This is one of the key-factors involved in the preparation of bovine-bone derived products, whose accurate control allows for both the exclusion of biological risks associated with xenografts, and the maximization of mandatory properties. The main objective of the study was to identify the variations induced by the heat treatment temperature of cancellous bovine bone regarding its morphology, composition and structure, using specific analysis methods.

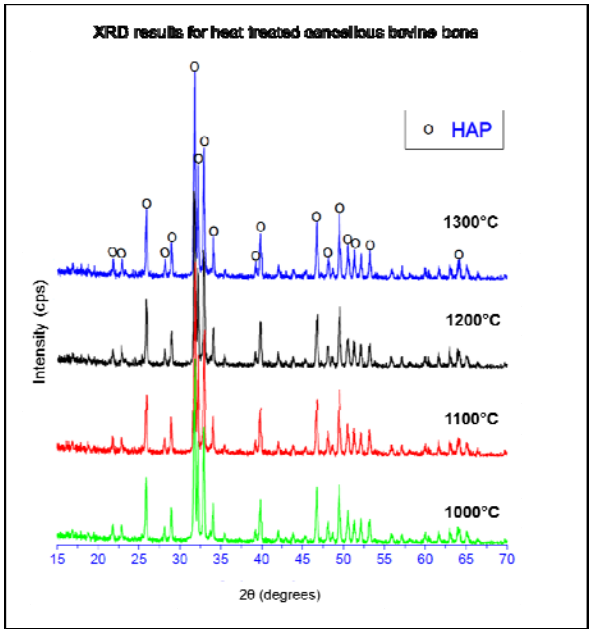

Fig. 3. Comparative overview of the structural features of cancellous bovine bone heat treated at 1000–1300 °C. An increase of the thermal treatment's temperature above 1000 °C does not influence the samples' crystallinity

The thermal treatment performed at 1000°C led to the development of hydroxyapatite with coarse morphology and homogenous porosity. A temperature rise above this level coincided with porosity and microstructure alterations, and with some variations of the stretching modes of some of the functional groups incorporated in the material's structure. Furthermore, no significant changes regarding samples crystallinity were identified.

The general conclusion of this study validates the significant influence of the heat treatment temperature upon the samples' morphology and upon the behaviour of functional groups normally identified in the cancellous bone tissue.

A proper results comparison with the ones submitted by similar studies is however problematic due to the diversity exhibited by the other heat treatment parameters (heat treatment environment, heating rate, soaking time and cooling method). Hence, a thorough study of all the aspects involved in bovine bone-derived products is still recommended in order to guarantee an optimum control upon the features of these materials.

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