Hydroxyapatite induced microstructure by cooling rate modification of cancellous bone thermal treatment

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Hydroxyapatite was studied because of its wide set of applications in biocompatible materials. Natural hydroxyapatite obtained by heat treatment technique preserves the structure and the chemical composition of the raw material. The present work investigates the effects of the thermal treatment and the influence of the cooling conditions on crystallinity, morphology and porosity of the cancellous bovine bone using the scanning electron microscopy (SEM) and the Fourier transform infrared (FT-IR) spectroscopy techniques. After deproteinisation, the bovine bone samples were subsequently subjected to different calcination temperatures (ranging from 1000°C to 1300°C), being quenched in two different environments (air and frozen water). The SEM analysis showed that the trabecular bone matrix and its basic microstructure were preserved after calcination. The size of the apatite crystals has increased leading to an increased crystallinity with temperature. Additionally, an apparently increased apatite crystal size was observed in air-quenched samples, resulting a higher degree of compaction for the air-quenched samples than for frozen water-quenched ones. The FT-IR analysis identified bands of hydroxyapatite (500 – 700 cm^{-1}) and some bands (around 870cm^{-1} and 1400 – 1450 cm^{-1}) that are assimilated with the carbonate substitutions in the hydroxyapatite crystal lattice and no collagen or protein traces.

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1. Introduction

The bone represents a dynamic component of the living tissue due to its continuous change in time. It offers a mechanical support and plays the role of several important metabolic functions. The characterization of bone tissue has a significant importance as it is the most transplanted tissue after blood [1].

Autographs remain the most used materials for bone replacement, but the necessity of other surgery and the limited supply are significant disadvantages for today medicine. Allographs and xenographs are considered the most common alternatives [2], but they have a high risk of disease transfers and post-surgery rejection.

The bones are unique composites with approximately 30% organic component (collagen and other proteins and lipids) and respectively 70% inorganic component represented by hydroxyapatite (HA) [3]. Calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) was highly researched as a result of its wide set of applications in biomaterial and pharmaceutics industries, being considered a very good alternative due to their low production cost and unlimited supply.

Each application is strongly dependent on the hydroxyapatite structure, morphology and particle size resulting in a grown interest in this research area in the last years. Even though, there were some debates regarding the possible chemical and structural changes that might occur after the heat treatments, the late researche demonstrated that the mineral bone phase changes are not significant until complete degradation and remove of the organic matter (500°C) [2, 4-7]. In order to completely remove the bone organic phase it is necessary to continue the heating in the range of 500-650°C. Moreover, it might require bone heating to 800°C to ensure the elimination of protein and pathogen agents traces [2-7].

By continuing heating at higher temperature, bone suffers structural changes; the porous bone structure obtained as a result of complete organic phase removal condenses, leading to a highly interconnected structure [2, 4, 6-10]. Some research showed that the highly anisotropically strained state changed into one with significantly larger equidimensional crystals [11].

Besides the structural changes induced by the calcination temperature, it is necessary to take into consideration the quenching environment as it influences the properties of the heat treated bones. Even though in the last years multiple studies showed the influence of the temperature and heat treatment techniques [2], there is no quantification of the effects that heating and quenching have especially on the porosity levels and the hydroxyapatite crystallite size.

2. Materials and Methods

The bone samples used in this study were collected from local slaughter houses (Bucharest-Romania) being immediately frozen in order to adequately preserve them. The bone samples cut from the cancellous part of the bovine femur with a jigsaw. Accurate results require complete removal of the organic components to avoid immunologic contaminations with pathogenic agents or the transmission of diseases.

The first phase was to boil the bone samples in water (t=100°C and p = 1 atm) for 2 hours in order to remove any trace of macroscopic contamination. This process was repeated with the same parameters in clean water. Moreover, the samples were heated in air atmosphere, at 500°C with a heating rate of 10 °C/min and maintained for 2 hours to remove the proteins and collagen traces. The final heat treatments required the samples calcination in electrical furnaces in air atmosphere, at 1000°C, 1100°C, 1200°C and 1300°C and maintained for 2 hours. The heating rate was 10 °C/min. The cancellous bovine bone samples were quenched in icy-water or air atmosphere. The heat treatment temperatures were selected after a thermo-gravimetric analysis previously performed [3, 12].

The sample preparation and analysis were performed in the laboratories of the University POLITEHNICA of Bucharest, Materials and Science Engineering Faculty. The SEM analyses were performed with an electronic microscope (Philips XL 30 ESEM TMP) equipped with a secondary electron detector in low vacuum and a BSE detector with two diodes. None of the samples required any plating with conductive material due to the special performances of the microscope. The FT-IR analysis were performed using a Bruker Tensor 37 spectrometer.

3. Results and discussion

The images presented (Fig. 1÷4) are the most representative for the observed properties during the cancellous bovine bone samples examination, showing a good hydroxyapatite sintering process without any organic component trace.

The SEM analysis presented a regulate morphology with a relatively uniform surface compositional distribution. The particles observed were approximately spherical shaped. The SEM images were acquired through the MIX technique (secondary electrons and backscattered electrons coupled signals).

During the samples analysis noticeable variations were observed not only between the different calcination temperatures, but also between the samples quenched in the two environments. The highest agglomeration tendency was observed at the both samples subjected to heat treatment at 1300°C and it decreased with temperature.

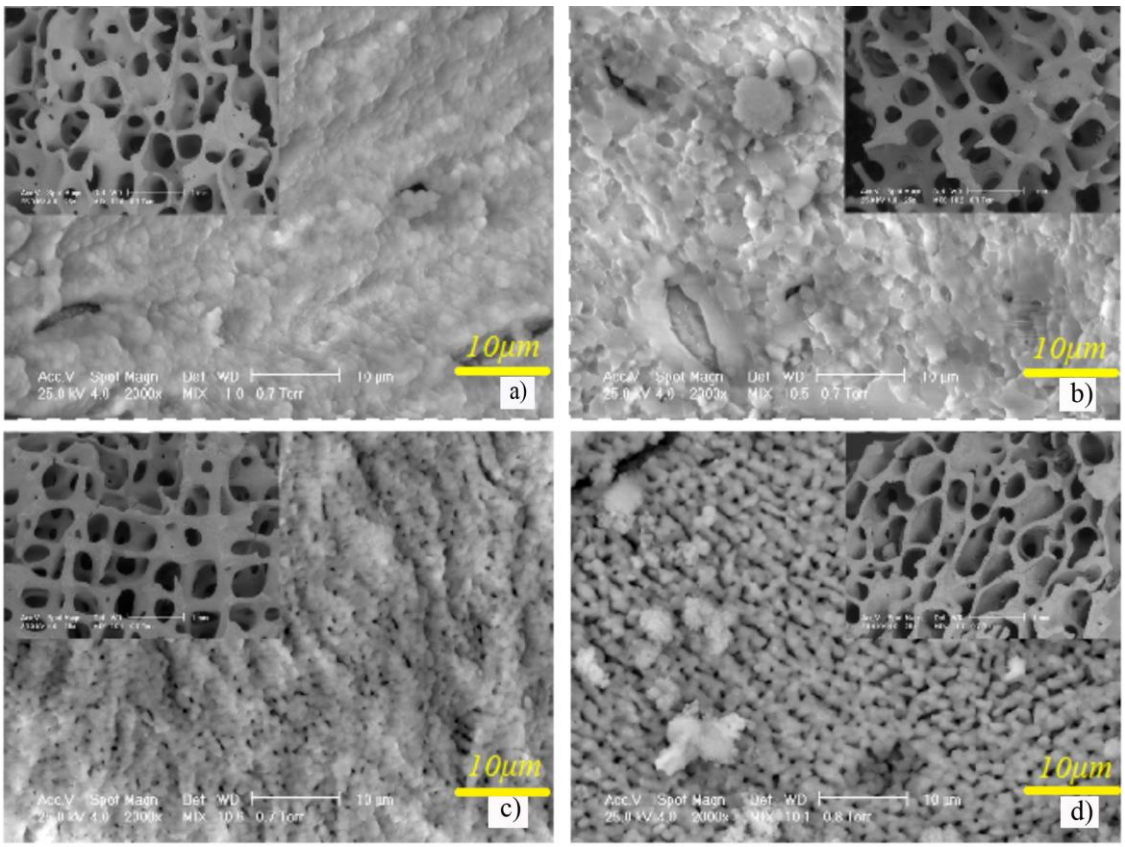

Fig.1. SEM images showing the heat treated at 1000°C and 1100°C bone samples morphology: a) and c) air-quenched; b) and d) frozen-water – quenched - the influence of the quenching environment on the bovine bone's microstructure and porosity

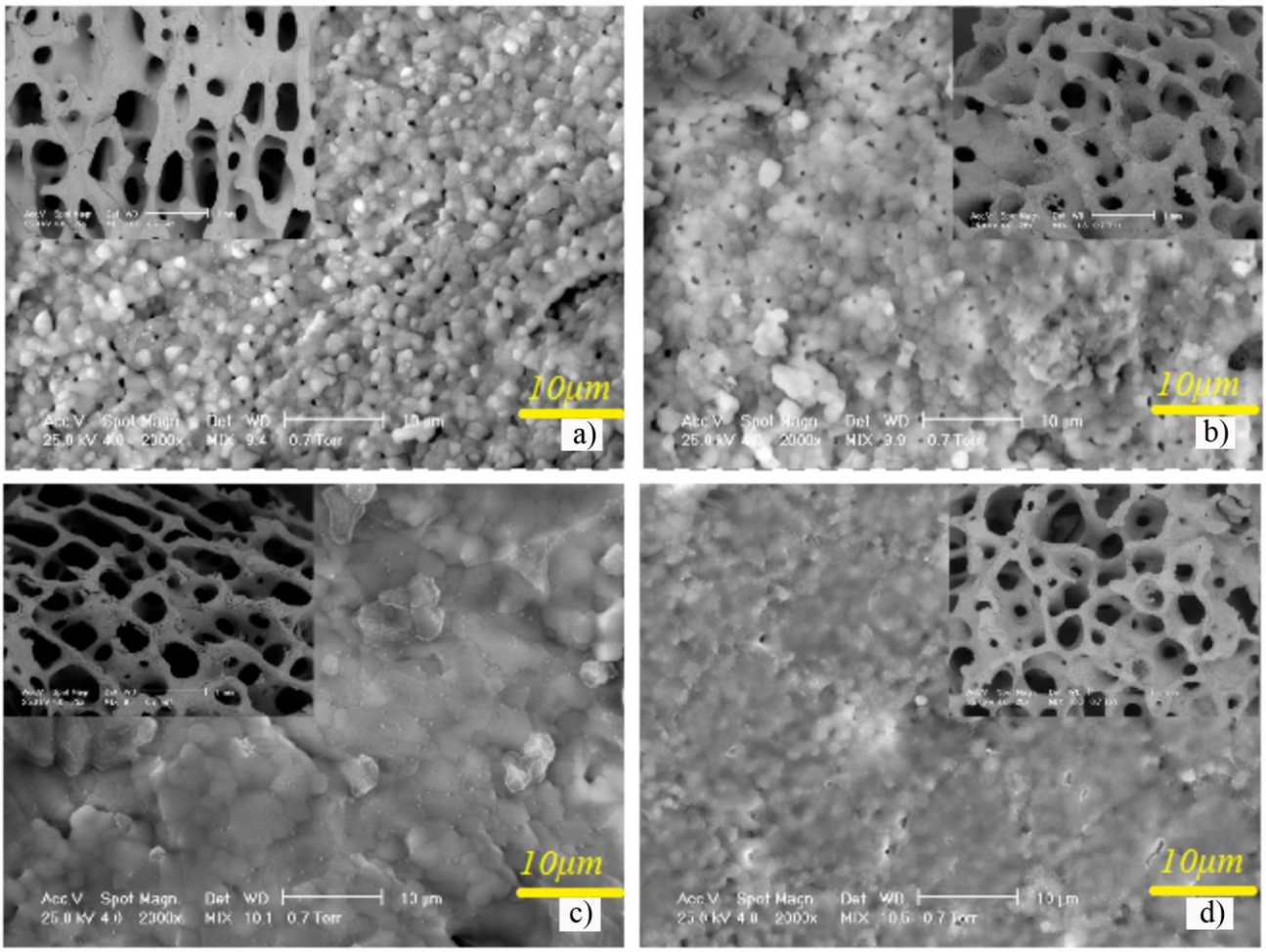

Fig.2. SEM images showing the heat treated at 1200°C and 1300°C bone samples morphology: a) and c) air-quenched; b) and d) frozen-water – quenched - the influence of the quenching environment on the bovine bone's microstructure and porosity

The HA crystal size increased with temperature, presenting a high crystallinity at 1300°C. Venkatesan and Kim show that the crystal size of HA and morphology increased from 0,3-1µm at 900°C to 0,5-2µm at 1200°C and assign the formation of microstructures to the tendency of particles to crystallize and agglomerate at high temperature [13].

Studies reveal that the formation of the hydroxyapatite crystals characterized by porous architecture is representative for heat treated bone samples at 1000°C [3, 12, 16].

Moreover, the SEM images show the beginning of the sintering process for both air-quenched and frozen-water - quenched samples. However, a higher agglomeration tendency is noticed for the frozen water quenched sample.

The samples heated at 1100°C show a higher tendency to crystalize and agglomerate and a decreased porosity. Similar studies performed on heat treated bovine bones show that at this temperature it was observed an early stage of sintering [14-17]. Moreover, it is noticeable that the particles start to connect to each other confirming the sintering process.

The SEM images from Fig. 3 show that the porosity highly decreased with temperature for the both quenching environments. Studies performed at the same heat treatment temperature confirm the obtained results [12, 19]. Furthermore, researchers [17] discovered that at 1200°C the sintering process was at the end of the first stage, being characterised by the beginning of particle coalesce and the growth of the contact area between them.

The samples heat treated at 1300°C show the lowest degree of porosity of the investigated samples. Due to decreasing porosity tendency it is acknowledged that the degree of compaction is close to that of the raw bone [2]. Moreover, it has been reported that 1300°C is the critical sintering temperature for obtaining optimal hydroxyapatite properties [19]. Additionally, it is noticeable a high sintering process for both quenching environments. According to other studies [17, 20-22], heating the bone samples above 1200°C leads to achieving the second stage of sintering characterised by the densification and the removal of most of the specimen porosity. During this stage the development of grains boundaries can be clearly observed.

Furthermore, the apatite crystallite size increased, resulting an increased crystallinity with temperature.

The mechanical properties of the bones are highly influenced by the organic matrix degradation during the heat treatments [12]. The SEM images showed significant differences of the cancellous bovine bone structural architecture depending on heat treatment temperature and quenching environment. Moreover, the heat treatment increasing temperature from 1000°C to 1300°C led to a porosity major decrease coupled with grain growth boundary fusion determined by the completed sintering process.

SEM microscopy offers only bi-dimensional images of the bone structure, the mezostructure being characterised only at qualitative level. Consequently, the electronic microscopy analysis were coupled with Fourier transformed infrared spectroscopy (FT-IR.)

The FT-IR principles are based on the infrared radiation absorption during the vibrational transitions from the covalent bonded atoms. The frequencies and intensity of the bands offers important information regarding the nature of the molecular bonds, environment and the relative quantity of the analysed samples [1].

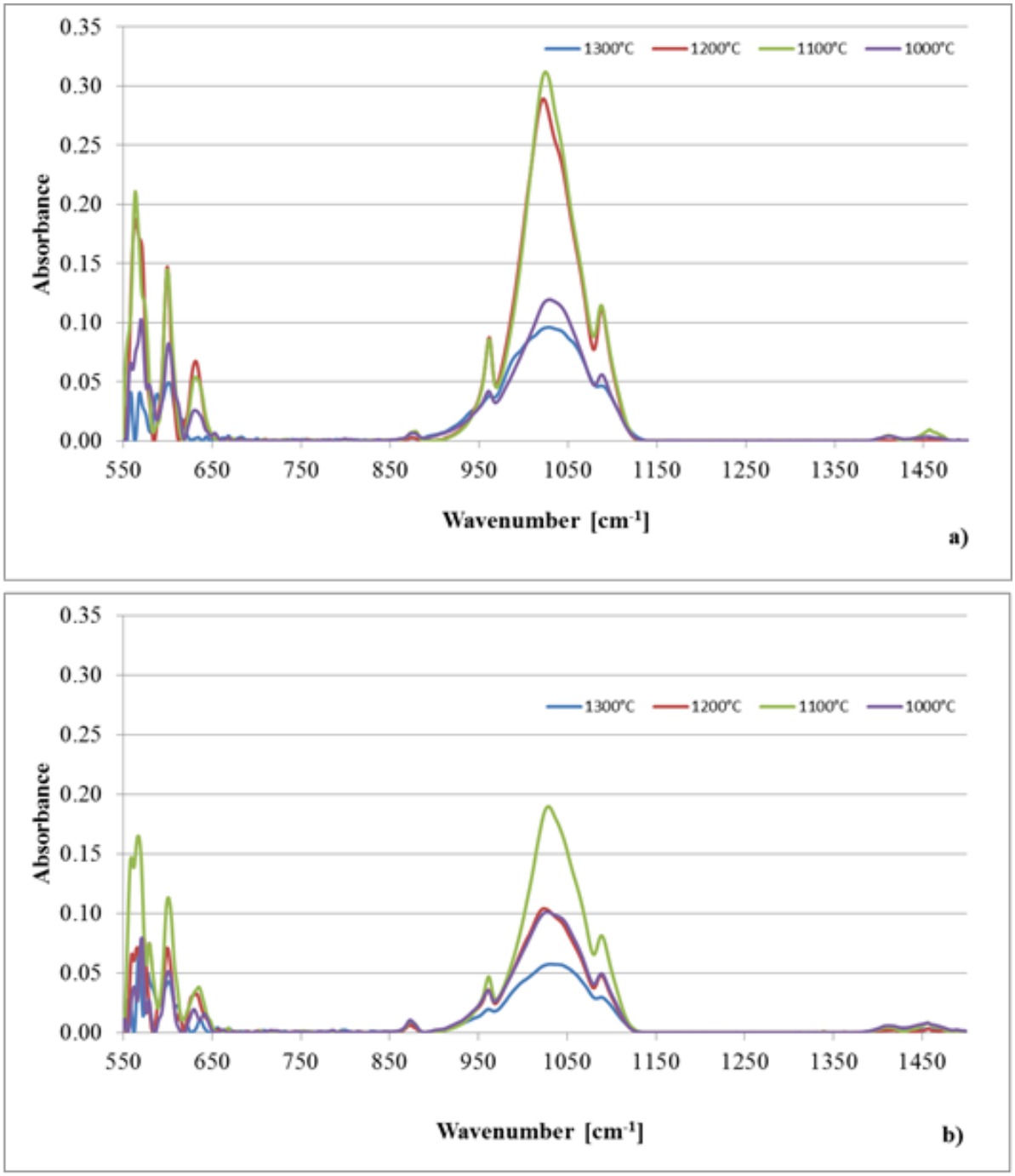

Fig.3. FT-IR Spectra of cancellous bovine bone heat treated at 1000°C, 1100°C, 1200°C and 1300°C – a) air-quenched; b) frozen-water – quenched

The bone composite material characteristics are mainly observed when comparing the bone spectrum with the hydroxyapatite and collagen ones. Fig. 5 shows the cancellous bovine bone spectrums ranged between 550 and 1500 cm^{-1} after being heated at 1000°C, 1100°C, 1200°C and 1300°C.

Studies reveal that hydroxyapatite bands are ranged between 500 - 700 cm^{-1} and 900 – 1200 cm^{-1}, while bands that are assimilated with carbonate groups (CO_3^{2-}) in the crystal lattice of the hydroxyapatite are visible around 870 cm^{-1} and 1400 – 1450 cm^{-1} [1, 23-26].These bands that are characteristic to CO_3^{2-} are visible in Fig. 5 spectra around 870-880 cm^{-1} (as single band) and around 1400 – 1450 cm^{-1} (as double band). Moreover, the CO_3^{2-}peaks are not visible above at 1200°C heat treated samples due to complete removal of the carbonate groups at temperature lower than 1200°C.

As a consequence of the heat treatment, typically for hydroxyapatite there are three bands in the range 500 - 700 cm^{-1} (around 560, 600 and 630 cm^{-1}). The bands are more intense for the air-quenched samples.

The phosphate (PO_4^{3-}) fundamental vibration stretching is observed at approximately 560 and 1035 cm^{-1}. Moreover, the FT-IR analysis show that the collagen associated peaks disappeared. The well distinguished absorption bands indicate a higher crystallinity degree.

3. Conclusions

This paper results show a significant influence of the calcination temperature on the cancellous bovine samples properties, morphology and structure. More pure forms of hydroxyapatite with a higher level of crystallinity and with crystallite increased size were acquired. The porous structure decreased with calcination temperature increase. Moreover, the samples heated at the same temperature, but quenched in different environments present morphology and sintering differences.

The FT-IR spectrums present a complete removal of the organic component as a result of thermal treatments temperature higher than 500°C. Moreover, the analysis demonstrated the obtaining of a carbonated hydroxyapatite, the carbonate groups being eliminated while increasing temperature.

The porosity level decreased with temperature. At 1300°C it was observed a grain size and a sintering tendency increase compared with the other calcination temperatures.

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