

Preparation and synthesis of hydroxyapatite bio-ceramic from bovine bone by thermal heat treatment

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Abstract

Calcium phosphate, particularly hydroxyapatite (HAp) is an important material in biomedical engineering applications. The development of HAp is continued rising due to the similarity and biomimetic requirements to the hard tissue of human body such as bone and dental. The purpose of our work was to produce and describe HAp bioceramic powder from environmental and cheap source (Bovine bone) by thermal process at various calcination temperatures. The analysis of Fourier transform infrared spectroscopy (FTIR) verified the formation of HAp because of the existence peaks related to phosphate and hydroxyl groups. The analysis of Raman confirmed findings of the FTIR the formation of HAp due to the appearance of peaks at 960 and 920 cm^{-1} related to a phosphate group. A result of Energy-dispersive X-ray spectrometry (EDS) also referred to Ca/P atomic ratio at 1000 °C was 1.6 that has been near stoichiometric hydroxyapatite (1.67) in human body.

Keywords: Calcium phosphate, Calcination process, Hydroxyapatite, Tricalcium phosphate
 Kulcsszavak: kálcium foszfát, meszesítési folyamat, hidroxiapatit, trikálcium foszfát

1. Introduction

Calcium phosphate (CaP) ceramics are a group of ceramic materials including calcium ions (Ca^{2+}), different phosphate ions [PO_4^{3-} , PO_4 , $\text{P}_2\text{O}_7^{4-}$], and sometimes hydroxide (OH^-) or carbonate (CO_3^{2-}) ions [1]. CaP can be divided into 3 kinds: Hydroxyapatite (HAp), Tricalcium phosphate (TCP), and Tetracalcium phosphate (TTCP) based on atomic ratio of Ca/P [2]. It is common knowledge that atomic ratio of Ca/P is the main factor in identifying the bioactivity and dissolution property of CaP. A decrease in Ca/P atomic ratio will increase the disintegration rate of CaP [3]. Atomic ratio of Ca/P in bone and dental of human body exceeds (1.67) represents HAp [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] in comparison with Ca/P atomic ratio of TCP (1.5) and TTCP (2) [4].

Due to the continued rising similarity and biomimetic requirements to the hard tissue of human body such as bone and dental, the use of HAp in biomedical and dental applications is rapidly increasing. In addition, the need for biocompatibility, osteoconductivity, and bioactivity in the biomedical application, tooth implants, and maxillofacial surgery, make this ceramic valuable for applications in the medical engineering.

HAp is the one of the major constituent of hard tissue (bone and teeth) of human body. Table 1 shows biological, mechanical, and physiochemical, properties of HAp [5]. HAp can be produced in two methods including chemical methods [6][7] and natural resources[8][9]. HAp typically synthesized from the chemical methods presents some drawbacks: they are having cytotoxicity [10], hazardous chemicals [11] and

relatively high cost [4]. On the other hand, HAp synthesized from the natural resources that can be used without any drawbacks in terms of similar chemical composition and physical properties [12].

Properties	Data	Properties	Data
Chemical composition	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ or $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$	Hardness (HV)	600
Ca/P molar ratio	1.67	Decomposition Temp. (°C)	More than 1000
Crystal system	Hexagonal	Melting point (°C)	1614
a=b c	0.942 nm 0.688 nm	Thermal conductivity (W/cm. K)	0.013
Young's modulus (GPa)	80-110	Biocompatibility	High
Elastic modulus (GPa)	114	Bioactivity	High
Density (g/cm³)	3.16	Biodegradation	Low

Table 1 Physiochemical, mechanical and biological properties of HAp [5][13][14]
 1. táblázat A HAp. Fizikokémiai, mechanikai és biológiai tulajdonságai

The purpose of our work was to produce and describe HAp bioceramic from environmental and cheap source (Bovine bone) by thermal process at various calcination temperatures. This study is divided into four steps.

- Calcine of the Bovine bone at different temperatures (600-1000) °C for 2 hours.
- Calculate the Ca/P atomic ratio of calcined bones at different calcination temperatures by using energy-dispersive X-ray spectrometry (EDS).

- Determine the best atomic ratio of Ca/P that is close to 1.67 and that represents HAp.
- Characterize the HAp by different techniques such as Fourier transform infrared spectroscopy (FTIR), Raman spectrum, Scanning electron microscopy (SEM), and atomic force microscopy (AFM).

2. Experimental procedure

2.1 Bovine bone preparation

Bovine bone femur was provided by a local meat shop and cut into small pieces. The bone pieces were boiled in water for 2 h and washed utilizing a strong water jet to take out the adhering meat. Drying process was taken at 50 °C for 1 h in a furnace and afterward dried at room temperature for 2 weeks. Finally, the bovine bones were submerged in acetone for 30 min and cleaned with water for several times. Fig. 1 shows the bovine bone after preparation with yellowish white color.



Figure 1 Bovine bone after preparation
1. ábra Szarvasmarha csont előkészítés

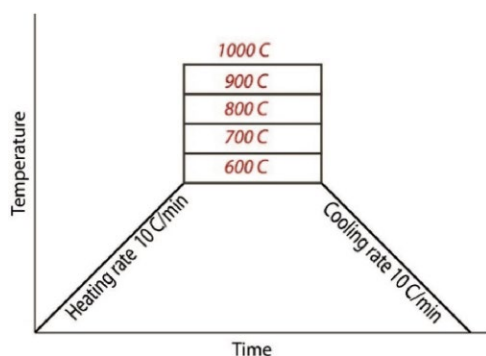


Fig. 2 Calcination process schedule
2. ábra Meszesítési folyamat leírás



Fig. 3 Bovine bone after calcination process
3. ábra Szarvasmarha csont meszesítési eljárás után

2.2 Calcination process

The bovine bones were calcined in a muffle furnace at various temperatures for 2 h. Calcination temperature was incrementally increased from 600 to 1000 °C with a step size of 100 °C. Fig. 2 shows the calcination process schedule. The heating and cooling rate were kept constant at 10 °C min⁻¹. Fig. 3 shows the color of bovine bone after calcination changes from yellowish white to white.

2.3 Characterization of calcined bone

Energy-dispersive X-ray spectrometry (EDS) was used to analyze localized chemical composition of calcined bone and determined the atomic ratio of Ca/P. Fourier transform infrared spectroscopy (FTIR) spectra were utilized so as to achieve the functional groups in calcined bone in the scope of 400 – 4000 cm⁻¹. The crystalline phases of the sample were determined by utilizing Raman spectroscopy. scanning electron microscopy (SEM) and Atomic force microscopy (AFM) were carried out to capture the surface morphology and topography of the calcined bone powder.

3. Results and discussion

3.1 Energy-dispersive X-ray spectrometry (EDS)

Table 2 shows EDS analysis of calcined bone at various temperatures. As can be seen, increasing calcination temperature from 600 to 1000 °C lead to the decreasing of Ca/P atomic ratio from 2.425 to 1.6. It was decided that the best calcination temperature for this study was 1000 °C due to Ca/P atomic ratio at this temperature was 1.6 that has been near stoichiometric hydroxyapatite (1.67) in human body. The result of EDS also referred to the presence of primary constituents such as Ca and P with some minor constituents such as Mg, Na, Cl, K, Si, and O. Piccirillo et al. [15] in their work on the characterization of HAp and TCP mentioned that these minor components such as Na and Cl improves on biocompatible and osteointegration.

temperature	600 °C	700 °C	800 °C	900 °C	1000 °C
Elements wt%					
C	2.878	2.373	2.628	2.567	3.050
O	29.474	35.277	34.945	37.080	42.348
Na	0.539	0.785	1.042	1.154	1.508
Mg	0.398	0.526	0.564	0.554	0.734
Si	1.644	1.959	2.146	1.360	1.012
P	15.424	16.042	16.114	16.557	17.095
Cl	0.248	0.101	0.103	0.087	0.095
K	0.997	0.864	0.952	0.671	0.442
Ca	48.398	42.073	41.507	39.972	33.716
Ca/P ratio	2.425	2.026	1.990	1.865	1.6

Table 2 Chemical composition (wt%) of calcined bovine bone at various temperatures
2. táblázat Különböző hőmérsékleten meszesített szarvasmarha csontok kémiai összetétele (tömeg %)

3.2 FTIR and Raman of calcined bone at 1000 °C

Fig. 4 shows a FTIR spectra of calcined bone at 1000 °C. A typical HAp molecules structure is indicated by the presence of peaks matching to phosphate and hydroxyl groups. The

organization of HAp structure is dependent on this kind of groups [16]. Phosphate groups are a common part of FTIR spectra of HAp, which can be divided into three vibrational modes: symmetric stretch, asymmetric stretch and bending. The symmetric stretch mode is represented in FTIR spectra (Fig. 4) at the peak 960 cm^{-1} corresponding to PO_4^{3-} , asymmetric stretch mode is represented at the peaks $1090\text{-}600\text{-}568\text{-}460\text{ cm}^{-1}$ corresponding to PO_4 , and bending mode is represented at 1025 cm^{-1} corresponding to PO_4^{3-} . The peak at 630 cm^{-1} is represented due to the vibrational of the hydroxyl group. In addition, the presence of 850 cm^{-1} band in spectra corresponding to P-OH related to HPO_4 group. A remarkable feature of HAp is the presence of HPO_4 group. The phosphate and hydroxyl groups were corresponded in accordance with [16][13][17].

The other test carried out with Raman spectrum (Fig. 5) confirmed findings of the FTIR the formation of HAp due to the appearance of peaks at 960 and 920 cm^{-1} related to a phosphate group [18].

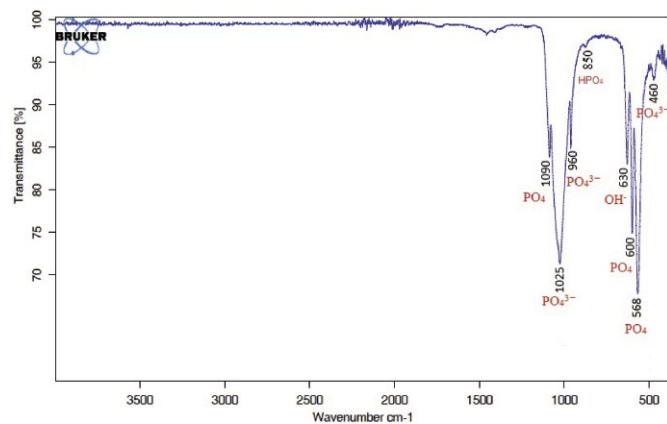


Fig. 4 FTIR spectra for calcined bone powder at 1000 °C calcination temperature
4. ábra 1000 °C -on meszesített portított csont FT-IR spektroszkópia

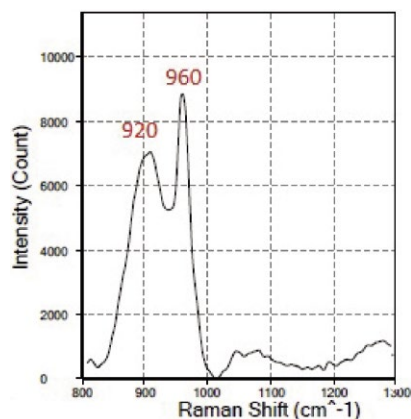


Fig. 5 Raman spectrum of Bovine bone heated at 1000 °C
5. ábra 1000 °C -on hevített szarvasmarha csont Raman spektroszkópia

3.3 SEM and AFM of calcined bone at 1000 °C

The result of SEM is presented in Fig. 6 with different magnifications. The shape of HAp particles was irregular like needle and polygonal and the size was between 10 nm and 150 nm as shown in Fig. 7. The results carried out with AFM confirmed findings of SEM as shown in Fig. 8.

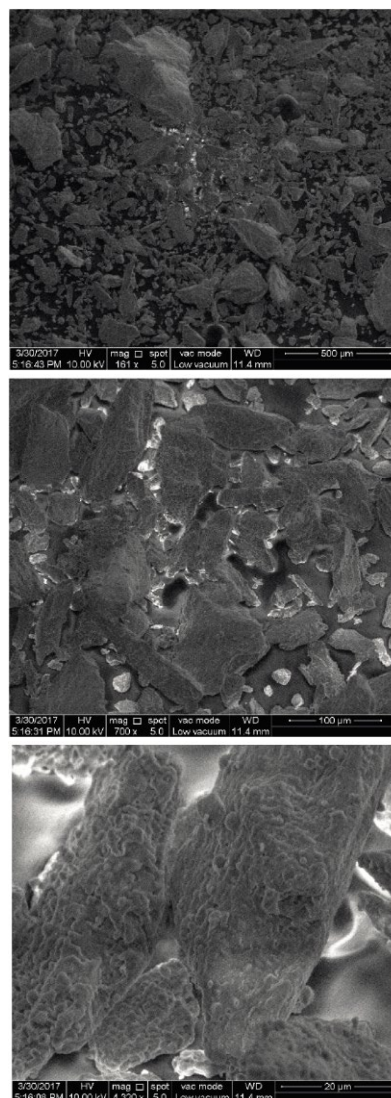


Fig. 6 SEM micrograph of bovine bone heated at 1000 °C showing the shape of HAp particles at three different magnifications
6. ábra SEM felvételek az 1000 °C -on hevített szarvasmarha csont szept Hap. szemecskékről három különböző nagyításban

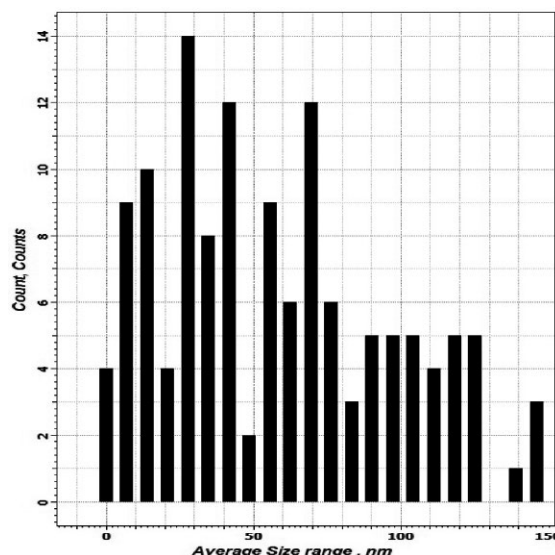


Fig. 7 Particle size of powder bone after heating at 1000 °C
7. ábra 1000 °C -os hevítés után szarvasmarha csont szemcsék mérete

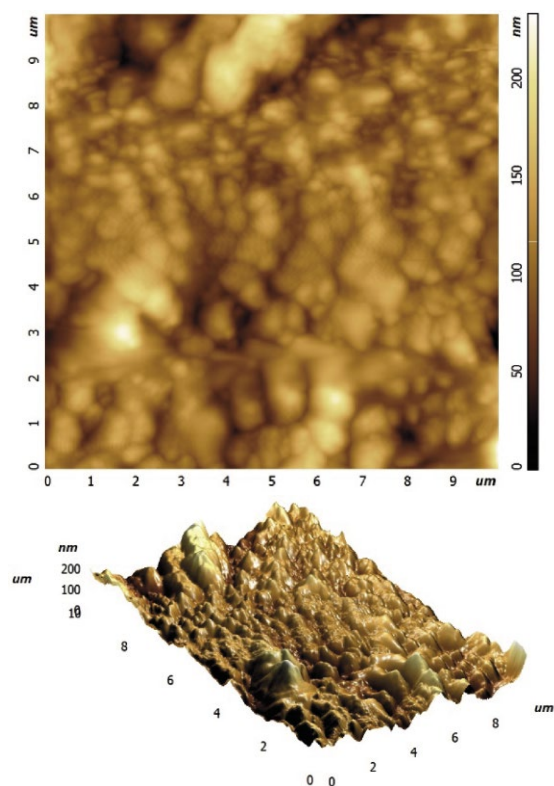


Fig. 8 Topography and surface properties of powder bone after heating at 1000 °C
8. ábra 1000 °C-on hevített szarvasmarha csont por topográfiai és felületi jellemzői

4. Conclusions

1- The result of EDS referred to the best calcination temperature for this study was 1000 °C due to Ca/P atomic ratio at this temperature was 1.6 that has been near stoichiometric hydroxyapatite (1.67) in human body.

2- FTIR and Raman analysis confirmed the formation of HAp by the presence of peaks matching to phosphate and hydroxyl groups which are of great significance in a scope of HAp structure.

3- The shape of HAp particles was irregular like needle and polygonal and the size was between 10 nm and 150 nm.

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