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Synthesis and Characterization of Hydroxyapatite from Bovine Bone

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Abstract: ***Hydroxyapatite (HA) was successfully synthesized from bovine bone by decomposition reactions at high temperature. Suitable condition of HA synthesis was selected by heating raw bone powder at 750 °C for 6 hours. XRD and FTIR highlighted the high purity and crystallinity of obtained material. FE-SEM observation showed the morphology of synthetic HA consisting of nearly spherical particles. EDX measurement indicated the Ca/P ratio of 1.64, nearly the theoretical value (1.67). In summary, this study presents a simple, quick processing for preparing HA material from bovine bone.***

Keywords: Hydroxyapatite, thermal treatment, biomaterial, natural, bovine bone, and crystallinity.

1. Introduction

Hydroxyapatite (HA) is a mineral with the formula $Ca_{10}(PO_4)_6(OH)$ and the crystalline structure in the hexagonal system. In the human bone, HA is the main inorganic component, accounting for up to 70% of the weight [1]. Hydroxyapatite has proved to be biocompatible, osteoconductive, non-toxic, non-inflammatory, and non-immunogenic. Furthermore, it is bioactive and can form chemical bonds with bone-defect positions after in vitro or in vivo assays [2].

Because of its excellent properties, HA material has been synthesized from different ways to apply in bone engineering and orthodontic surgery. Precipitation method is widely applied for HA synthesis, in which aqueous solutions of the calcium hydroxide $Ca(OH)_2$ and ortho-phosphoric acid H_3PO_4 was used to precipitate HA material following the controlled conditions. The synthetic powders with uniform and micro-size spherical particles can be obtained at low reaction temperature [3]. Hydroxyapatite material with one-dimensional structure (1D HA) has been successfully prepared by the hydrothermal technique. The obtained material showed micro-and nanostructures depending on several factors such as types of starting reagents, pH, time and stirring speed of reaction mixture [4]. HA nano-powder has been synthesized by using the sol-gel technique. The synthetic processing passed two main steps consisting of sol formation and gelation. The resulting gel was dried and then heated at several temperatures. The HA material with high crystallinity can be obtained for the sample synthesized at a pH of 7.5 and treated at 400 °C [5]. Other chemical syntheses have been reported such as the synthesis of HA material by microwave-assisted precipitation technique which permits to obtain the rapid formation of hydroxyapatite with nano-structure, template - assisted electro-deposition to prepare hydroxyapatite with nano-wires and nano-tubes, and thermal plasma processing to prepare hydroxyapatite with nano-sizes [6].

Besides chemical methods, HA material can be also efficiently separated from animal bones. HA material has been prepared from bovine, caprine and galline bones by using heating treatment from 500°C to 1100 °C. The obtained results highlighted that the natural HA from bovine bone was stable in the evaluated temperatures while those extracted from caprine and galline expressed an instability by the appearance of tri-calcium phosphate (TCP) after thermal treatment at the temperatures higher than 700°C [7]. Hydroxyapatite in nano-rod shape with an average length of 300 nm was synthesized by the thermal treatment of bovine bone at the temperature of 750°C for 6 hours [8]. Hydroxyapatite powder was extracted from Lates calcarifer fish bone by heating treatment in the range of temperatures from 200 °C to 1200 °C. The pure HA was achieved at a temperature of 800 °C [9]. The effects of the temperature and sintering time on the structural property of hydroxyapatite extracted from porcine bone were investigated. The experiments were effectuated by treating raw bone at 600 °C and 1000 °C for 1, 7, 20, and 50 hours. The obtained results showed an increase in crystallinity with sintering time [10].

In our previous study, hydroxyapatite (HA) has been successfully prepared from pig bone by thermal treatment without any chemical treatment. The suitable condition for HA synthesis was selected as a heating raw bone at 750 °C for 6 hours. Furthermore, the phase transformation of synthetic HA did not mention up to 1200°C [11]. In this study, we present the results related to the synthesis of HA materials from bovine bone. The synthetic processing consists of different steps of heating treatment without any chemical treatment. The obtained material was investigated by using several physic-chemical techniques such as XRD, FTIR, and FE-SEM combined with EDX. The in vitro experiment was also effectuated for bioactivity assessment of synthetic HA.

2. Experiments

2.1. Synthetic processing

The natural bovine bone was removed the residues of meat, fat, and outer membrane. After that, the bone was boiled for

6 hours to remove mechanical impurities and organic compounds. The clean bone was cut into pieces. The thermal synthesis consists of two main processes. The first one, raw bone was preheated at 200 °C for 3 hours to burn the organic compounds. The second one, the resulting bone was heated at several temperatures of 750, 800, and 900 °C for different times.

2.2. In vitro assay

The obtained HA was investigated in its bioactivity by soaking powder sample in the simulated body fluid (SBF) at several times. The SBF has an ionic composition similar to human blood. This solution was synthesized in the laboratory according to Kokubo's method [12].

2.3. Characterization

The phase and crystallinity of synthetic HA were identified by X-ray diffraction (XRD) on a Bruker D8 Advance diffractometer with monochromatic copper radiation (CuKα) of wavelength λ = 0.15406 nm. HA powder was mixed with cyclohexane and put on the surfaces of plastic tablets. The prepared samples were dried for solvent and then introduced into diffractometer. The XRD spectra were obtained with a scanning speed of 1°/min. The functional groups in the structure of synthetic HA were identified by the Fourier transformed infrared absorption spectroscopy (FTIR) on a Bruker Equinox 55 spectrometer. The samples were prepared by pressing a mixture of 1 mg of ceramic powder and 400 mg of dried KBr under reduced pressure. The resulting pellets were introduced into the spectroscopy. The FTIR spectra were achieved in the range of 4000-400 cm^{-1}. Scanning electron microscopy (SEM) was used to observe the surface morphology of synthetic HA.

3. Results and Discussion

3.1 XRD investigation

To assess the formation of HA materials according to the temperature and time, the XRD diagrams of raw bone, bone samples treated at 750 °C for 3 and 6 hours were regrouped together for comparison (Fig. 1).

The observation clearly showed the change in spectral shape from the untreated bone to bone sample heated at 750 °C for 6 hours such as new peak formation, peak sharpness, and increase in peak intensity and displacement of the peaks. The sample treated at 750 °C for 6 hours, all HA peaks were identified according to the XRD standard JCPDS no. 09 432. The obtained result allows the separation of HA material from the bovine bone at a temperature of 750 °C and for a heating period of 6 hours.

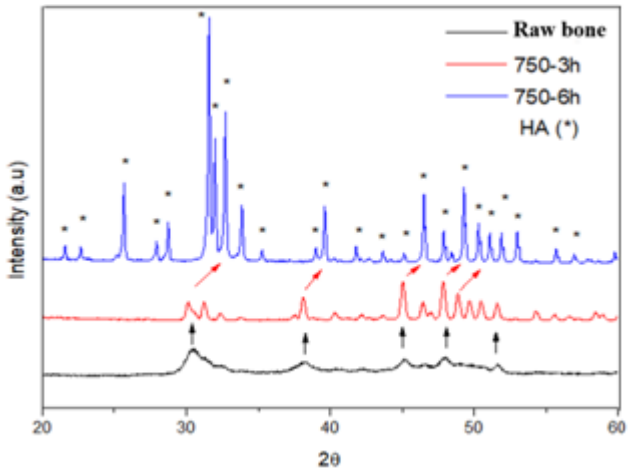


Figure 1: XRD diagrams of samples heated at 750 °C for 3 and 6 hours

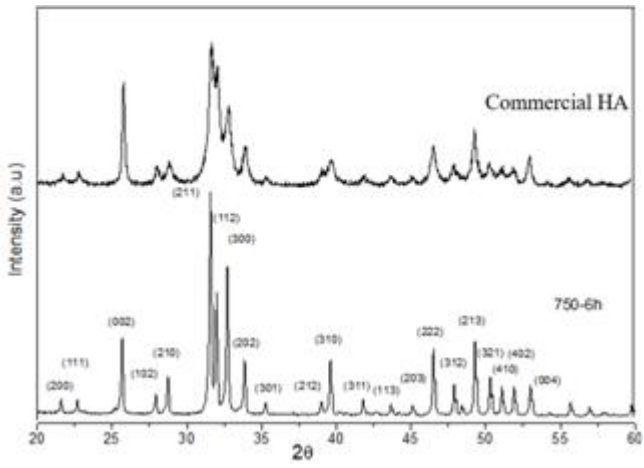


Figure 2: XRD diagrams of sample heated at 750 °C for 6 hours compared to commercial HA

The XRD diagram of sample heated at 750 °C for 6 hours was compared to the commercial HA as seen in Fig. 2. The XRD diagram of treated sample revealed all peaks of HA when compared to the commercial sample, including the main peaks in the 26°, 32° and 50° regions as well as other sub-peaks. Any strange peaks were determined. This result confirms that the bone sample treated at 750 °C for 6 hours was completely pure HA material.

Through XRD analysis, HA material can be separated from bovine bone at 750 °C and within 6 hours of calcination. To confirm this condition, raw bone samples were calcined at higher temperatures of 800 and 900 °C in the same heating period of 6 hours. The XRD data are presented in Fig. 3. The observation shows that when the temperature increased, the characteristic peaks displaced to the left, and the intensity of the peaks decreased. This result confirmed a phase change when HA material was calcined at higher temperatures. This result is completely consistent with previous study, in which the phase transition of HA was observed in the temperature range of 800-1350 °C. At temperatures above 800 °C, HA can be decomposed into α-TCP, β-TCP, and CaO components [11].

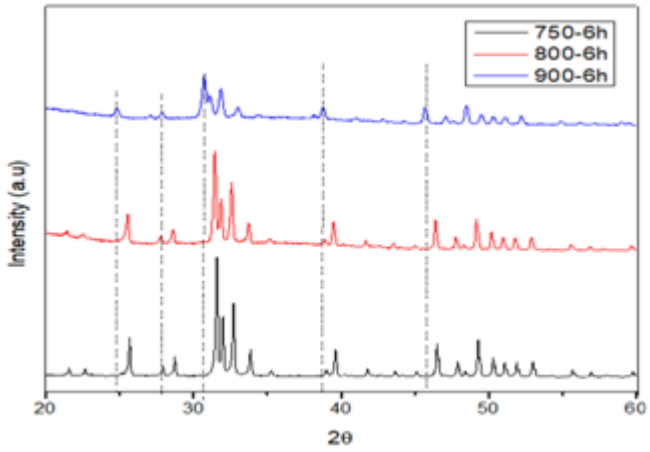


Figure 3: XRD diagrams of sample heated at different temperatures for 6 hours

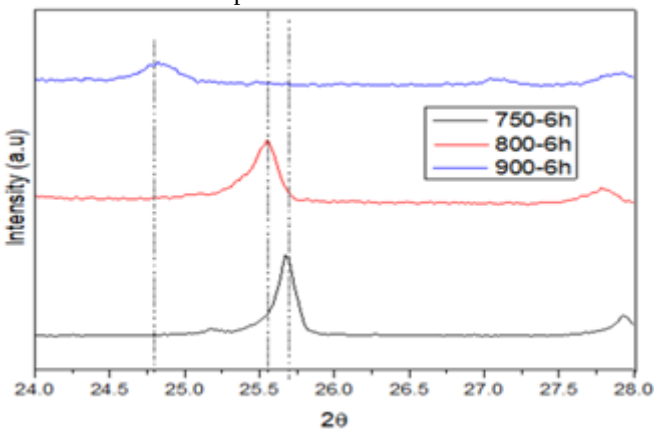


Figure 4: XRD diagrams of material samples enlarged at 26^{o} region

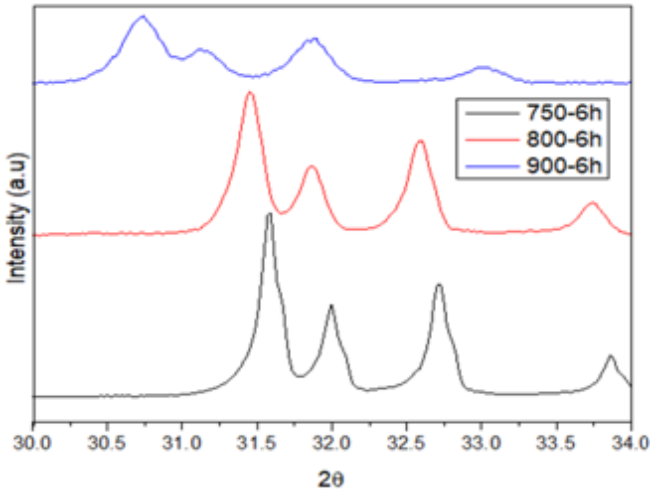


Figure 5: XRD diagrams of material samples enlarged at 32^{o} region

In order to observe the change in spectral feature when the heating temperature is increased, the XRD diagrams of samples are combined and enlarged in the main peak regions at about 26 and 32^{o} (2θ). Fig. 4 and Fig. 5 represent the XRD diagrams of samples which were enlarged. The results show a clear change in peak position and peak intensity when the heating temperature of samples is increased.

Therefore, the HA separated procedure was found to be calcining dried bone powder at temperature of 750 °C for 6 hours. The effects of heating temperature and processing time were assessed through XRD analysis. The HA material with high quality was achieved by heating sample at 750 ^{o}C for 6 hours. This sample was selected for further evaluation.

3.2 FTIR analysis

Fig. 6 shows the IR spectrum of HA separated from bovine bone compared to commercial HA. It is obvious that the synthetic HA has the full characteristic bands when compared to the commercial standard product [11]. Specifically, the spectral bands characterize to the oscillations of the PO_4^{3-} groups in the HA crystal structure; spectral bands of CO_3^{2-} and OH-, explained by the uptake of CO_2 and water vapor during sample storage as well as the transfer of samples for infrared spectrometry.

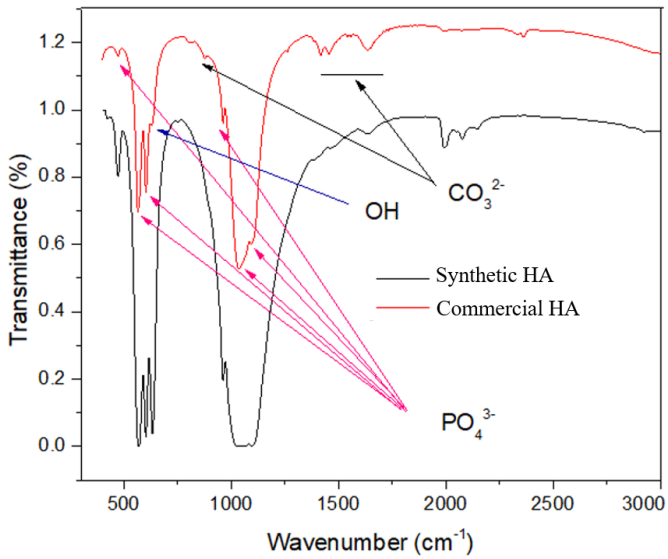
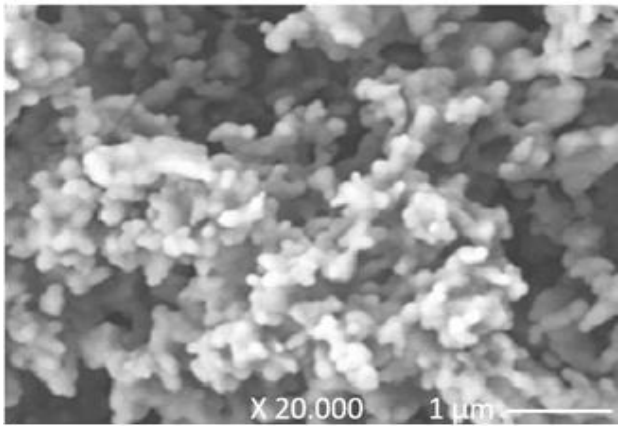


Figure 6: FTIR spectrum of synthetic HA

3.3 SEM observation

Fig. 7 show the SEM images of hydroxyapatite (HA) synthesized from bovine bone, at magnifications of 20.000 and 30.000 times. The obtained HA showed the rod shapes interconnected to form the porous structure. The synthetic HA has structure similar to that of human bone in term of porosity and shape.



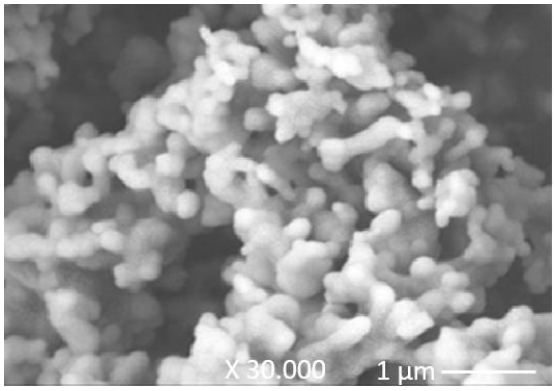


Figure 7: SEM images of synthetic HA at different magnifications

3.4 In vitro bioactivity

Fig. 8 shows the SEM image of HA before and after immersion in SBF solution. After soaking in SBF solution, it was noticed that the surface of material was completely covered with crystalline particles.

After 1 day of soaking in SBF, small particles began to appear due to the new crystal layer formed on the old HA substrate.

After 5 days of soaking in SBF, this crystal layer was strongly developed to form the spherical crystals. This proves that the new crystal layer was formed after 5 days of immersion in SBF.

After 10 days of soaking in SBF solution, new crystals developed with a larger size of spherical particles. The formation of new crystalline layer on the surface of HA confirmed its bioactivity.

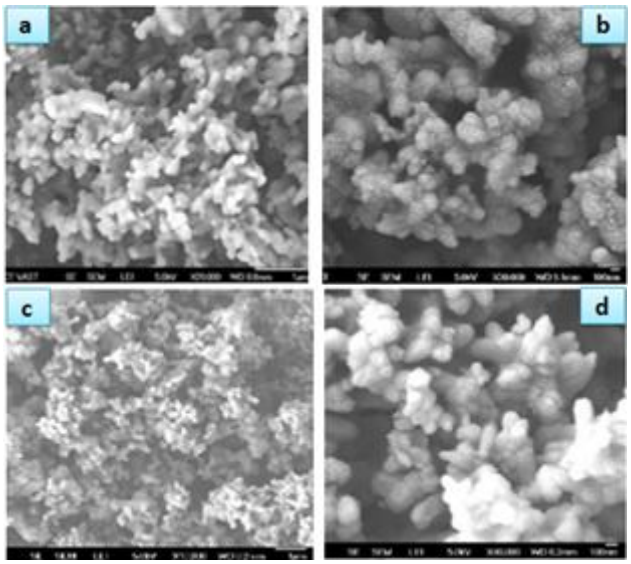


Figure 8: SEM images of HA a) before and after b) 1 day, c) 5 days and d) 10 days of immersion

4. Conclusion

Hydroxyapatite (HA) has been successfully extracted from bovine bone. The thermal processing was effectuated by burning raw bone to remove organic components and then heating samples to synthesize hydroxyapatite powder. The XRD investigation indicated that the suitable condition of HA preparation is the thermal treatment of raw bone at 750 °C for 6 hours. The obtained HA is pure and highly crystallinity. SEM observation showed the morphology of synthetic HA, including rod shapes. The in vitro experiment confirmed the bioactivity of synthetic HA by the formation of a new mineral layer on its surface after immersion in SBF.

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