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Extraction of Biological Hydroxyapatite from Bovine Bone for Biomedical Applications

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Abstract. Current biomedical field demands intensive research on cost-effective and high availability materials to fulfil its various applications. Owing to its similar characteristic to human bone, biological hydroxyapatite (HAp) has been used as an alternative in bone replacement and implantation. In this study, biological HAp was extracted from bovine bones via calcination. Powders calcined at 700 °C and 900 °C showed the presence of HAp. The field emission scanning electron microscope (FESEM) analysis shows that the irregular morphology emerged and the size was increasing as the calcination temperature increased. By increasing the temperature of 1100 °C, β-TCP (beta-tricalcium phosphate) started to develop and influenced the ratio of Ca/P. At 900 °C, the Ca/P ratio obtained was 1.70, and closest to the theoretical ratio of Ca/P. The presence of trace elements like Ca, Mg, Sr Na, K and Zn in all samples are proved via energy-dispersive X-ray spectroscopy (EDS) analysis, and these elements help to enhance the bioactivity hence make it a good alternative in biomedical applications.

Introduction

Emerging biomedical world leads to a great reliance on more cost-effective and high availability materials to fulfil the wide range of applications in dentistry and orthopaedics [1]. Intense research efforts have been conducted to find out various bioceramics materials for this application [2]. Among bioceramics studied is hydroxyapatite (HAp). HAp with the stoichiometric formula of $Ca_{10}(PO_4)_6(OH_2)$ has similarity in structure and morphology to human bone [3] and its biocompatible nature making it is suitable for biomedical applications [4]. The extraction of HAp from natural resources allows recycling and reusing of waste materials which help lower the cost of HAp production [5]. Komur et al. stated that HAp can be extracted synthetically from reagent chemicals or can be synthesized from natural resources via hydrothermal transformation [2] and high-temperature calcination of different animal species including mammalian bones [6], fish scale and bone [7] and eggshell [3].

In this study, bovine was selected as the natural resource for bioceramic extraction. Owing to its beneficial nutrients and high content of iron, zinc, selenium, and protein [6], bovine has become a high demand product among the population. Bovine bones consist of 65–70% HAp and 30–35% organic compounds on a dry weight basis [8]. Besides, collagen also presents in the bovine bones as the main organic compound. Not knowing the advantages of bovine bones, people normally throw away the bones, and this can lead to huge waste production and thus, environmental pollution. Thus, in this study, HAp was extracted from the bone of bovine via the calcination method. This study aims to produce and characterise the HAp from bovine bone.

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Materials and Methods

Extraction of hydroxyapatite. Bovine bones were obtained from a local butcher stall. The bones were first cut into smaller pieces before they were boiled with distilled water at 100 °C for 3 hours to remove the organic substances and the unwanted meats. After the bones were soaked and rinsed, they were dried in a laboratory oven at 100 °C. The dried bovine bones then went through the calcination process in an electric furnace (Protherm) at the temperature of 700, 900, and 1100 °C with a heating rate at 10 °C/min for 3 hours. The final process before characterisation was the milling process using the Planetary Pulverisette machine (Fritsch). The calcined sample was milled for 2 minutes.

Characterisation. The mineralogy was analysed using X-ray diffraction (XRD, X'pert-PanAnalytical, Bruker Advance D8 XRD diffractometer, 40 kV, 40 mA, angle of incidence 1°2θ, scanning step 0.02°2θ). The morphology of powders obtained was examined using field emission scanning electron microscopy (FESEM, JFM- 7600F, JEOL, 15.0 kV). The elemental composition of the powder obtained was examined using an energy dispersive spectrometer (EDS, SU1510, Hitachi, 15.0kV).

Results and Discussions

XRD analysis. The phase analysis of HAp powders calcined at 700 °C, 900 °C, and 1100 °C was performed using XRD (Fig. 1). The XRD patterns were validated by comparing it with the HAp standard (ICDD 00-003-0747). At 700 °C and 900 °C, all the peaks appear were correspond to the standard HAp peaks where the strongest intensity peaks can be found at Miller indices of (211), (300), (310), (222) and (213). Thus, it confirms that the XRD analysis of the sample at calcination temperature 700 °C and 900 °C were in pure HAp phases. As the temperature of calcination rising, the crystallinity of the sample also increased as the intensity of the XRD peak has been increased. At 1100 °C, the major peaks show a form of HAp. However, minor peaks belong to beta tri-calcium phosphate (β-TCP) starting to emerge at this temperature. This is owing to the HAp decomposition, which above a certain temperature, HAp started to decomposes, allowing β-TCP to produce. According to some research, HAp begins to decompose to β-TCP at a temperature $<$850°C [9]. However, there also many research proves that the pure HAp phases can be produced at a temperature of 900 °C and 1000 °C [10]. In addition, the presence of β-TCP was not given the disadvantage to HAp phases. Hence, the mixture of HAp and β-TCP in the powders improved mechanical properties and bioactivity of implants as it achieved controllable biodegradation rate and biological stability [11].

FESEM Analysis. The morphology of the extracted HAp from bovine bone was observed using FESEM. The FESEM micrograph in Fig. 2 shows that the HAp powder has an irregular shape for all calcination temperatures (700 °C, 900 °C, and 1100 °C). At the temperature of 700 °C, the presence of an uneven irregular-shaped HAp can be seen. The particle sizes of powder were ranging from 300 to 530 nm. As the temperature of calcination increase, the size of HAp structures begin to increase and it was further increased until temperature 1100 °C. The FESEM micrograph in Fig. 2 shows that the HAp particles tended to agglomerate as the temperature increased. In addition, Venkatesan et al. suggested that the complete removal of organic moieties by calcination method results in the particles growing [12]. Thus it supports the result shows in Fig. 2 that as the temperature increase, the size of the particle also increases.

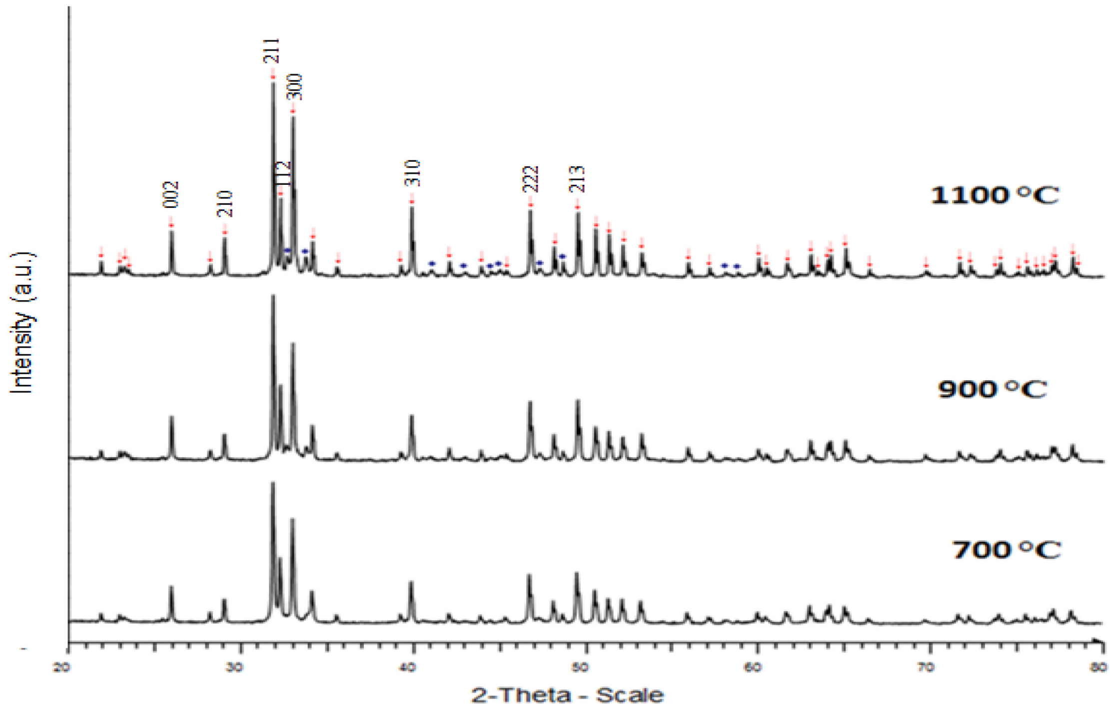

Fig. 1: XRD patterns of bovine bones calcined at 700 °C, 900 °C and 1100 °C (key (•) β-TCP and (!) HAp)

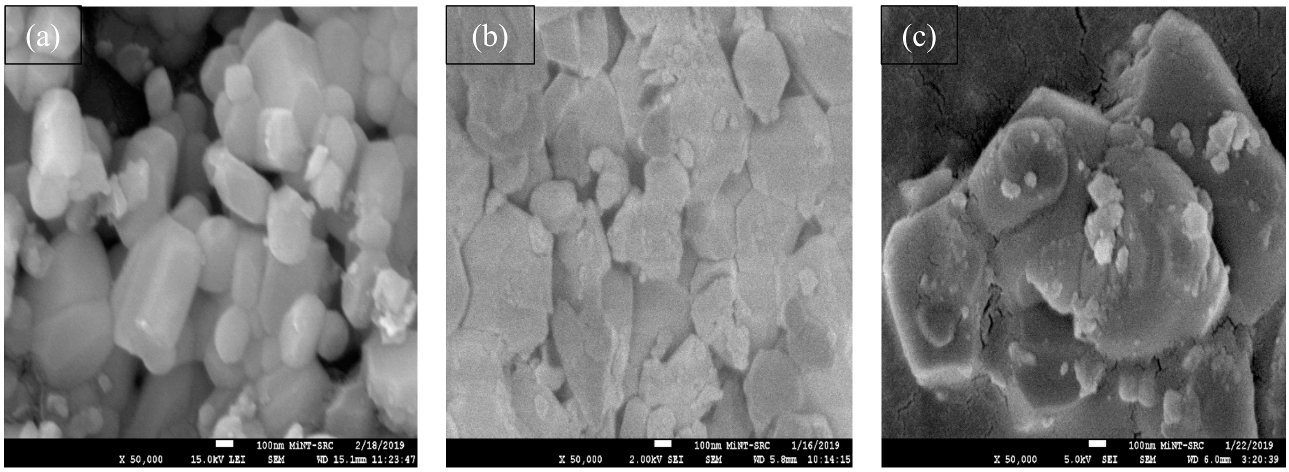

Fig. 2: FESEM micrographs of bovine bone powder calcined at (a) 700 °C, (b) 900 °C, and (c) 1100 °C.

EDS analysis. The element analysis was done using EDS. The results in Fig. 3 shows that there were various trace elements were found in the HAp samples. It can be seen that Na, Mg and Sr were found in the entire sample. While, trace element like Zn (700 °C), and K (900 °C) was present in certain sample. The presence of traces elements such as Mg, K, Zn and Sr in a natural source such as mammal bones are common [13]. The present of the trace element in natural source mimics the apatite from the natural human bone [10, 14]. According to Akram et al. the trace elements play important role in the regeneration of the bone, and it also accelerates the process of bone formation [3]. Some research shows that the presence of Sr ions enhance the differentiation and activity of osteoblast and also inhibit osteoclast proliferation [15].

The elemental analysis from EDS enables for the Ca/P ratio to be calculated. At 700 °C, the ratio of Ca/P obtained was 1.60, and owing to the presence of these trace elements. However, as the temperature of calcination increased to 900 °C, the ratio of Ca/P was increase to1.70 and the closest to the HAp theoretical ratio which is 1.67. At the calcination phase of 800 °C - 1000 °C, monophase of HAp started to maintain and this phase resulting in a close ratio of Ca/P [16]. After hitting a calcination temperature of 1100 °C, the ratio of Ca/P dropped to 1.52 owing to the presence of β-TCP. In the CaP phase diagram, it shows that the Ca/P ratio of β-TCP is 1.5[17, 18]. Thus the presence of β-TCP in HAp sample reduced the Ca/P ratio of the sample.

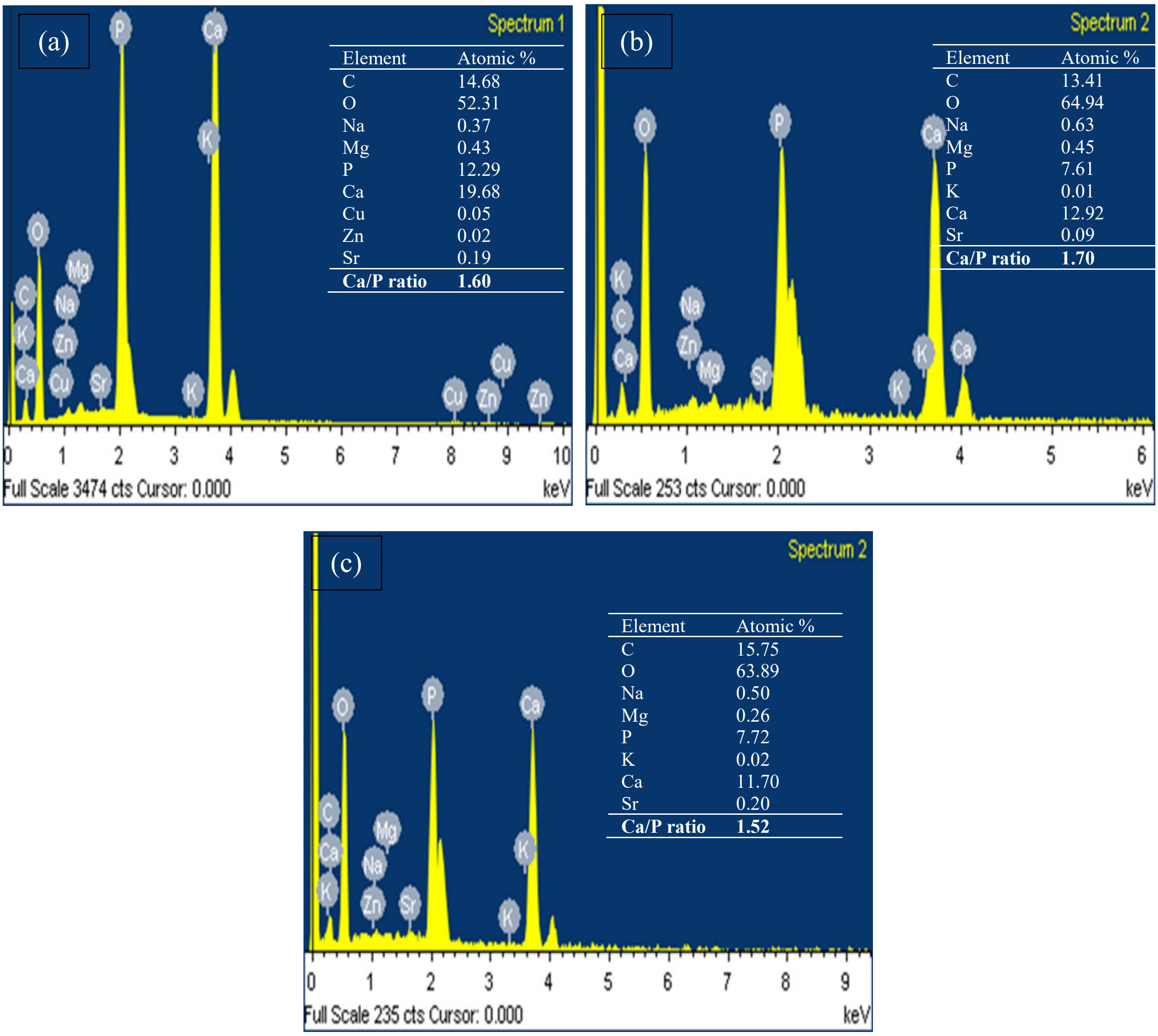

Fig. 3: The EDS spectrum of bovine bone powder calcined at (a) 700 °C, (b) 900 °C, and (c) 1100 °C.

Summary

HAp has been successfully extracted from bovine bones. The powders were calcined at the temperature of 700 °C, 900 °C and 1100 °C. XRD analysis shows that at 700 °C and 900 °C, HAp peaks were obtained. As temperature rises, β-TCP started to appear. FESEM analysis found that the sample has irregular shape morphology. In EDS analysis, powders calcined at 900 °C has the Ca/P ratio of 1.70 and the closest to the theoretical ratio of Ca/P that is 1.67. The presence of trace elements such as Na, K, Mg, Zn and Sr was also detected which give good properties to the sample. Thus, this biological HAp extracted from bovine bone may present a promising future for biomedical applications.

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