

Effect of annealing treatment on the characteristics of bovine bone

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The effect of annealing on the properties of bovine bone was investigated over the temperature range of 400 °C to 1300 °C at a ramp rate of 10 °C/minute. The XRD results indicated that annealing at between 600 °C and 1100 °C resulted in phase pure hydroxyapatite (HA). However, decomposition of HA to form tri-calcium phosphate was observed for samples annealed above 1000 °C. Thermo-gram analysis of bovine bone revealed the existence of organic combinations that was completely removed from the structure during heat treatment above 600 °C. Annealed bovine bone between 800 °C-1000 °C exhibited features of interconnecting pore structure, suitable for biomedical application. The study revealed that proper heat treatment through the control of annealing temperature represent a viable method to produce a HA structure from bovine bone.

Key words: Hydroxyapatite, Bovine bone, Annealing, Microstructure.

Introduction

Hydroxyapatite (HA) is a biocompatible material that is widely used in various medical applications for bone repairs and replacement of damaged hard tissues [1-3]. HA can be obtained from different synthetic methods and also natural sources like bovine bone and sea shells [4]. HA is amongst the best material for orthopaedic and dental applications due mainly to its chemical similarity and similar calcium to phosphate ratio with that of human bone [5]. Nevertheless, a major weakness of synthetic HA is concerned with its rather poor mechanical properties particularly when exposed in wet and moist environments [6-8]. Therefore, their clinical applications has been limited to non-load bearing applications including spinal merger, parts adjacent to implants, bone defect cavities, filling of periodontal pockets and tooth root substitutes [9, 10]. Recently natural HA from bovine bone has received great attention mainly because of its biocompatibility and lower production cost [5, 6, 11]. The bovine HA is morphologically and structurally similar to human bone which is consider as a bio-waste material, simple to manufacture and it is accessible in limitless amount with lower production cost compared to other available biomaterials. Bovine HA has also been proven to promote bone bonding to regenerate and fill up the bone missing part without intermediate connective tissue formation at a faster rate

than other materials [8, 9]. From material studies, the bovine bone is a complex material, combined of organic and with a mineral matrix consisting of carbonated calcium hydroxyapatite that has an ability to bind in the human bone. The majority of organics substances are collagen and proteins, whiles the main component of mineral part is HA with a low percentage of other minerals such as carbonate, magnesium (Mg) and sodium (Na) that is integrated in the bone structure [7, 10, 11]. Heat treatment processes have been extensively investigated to extract the HA from bovine bone [6, 12, 13]. The HA derived from bovine bone has been employed in bone transplant to fix and replace the damaged bone owing to its high biocompatibility, excellent osteoconductivity, non-inflammatory behavior, non-toxicity, and non-insusceptible properties [9, 10]. It is also capable of promoting bone regeneration and bonding directly to regenerated bone without intermediate connective tissue at a faster rate than other materials [14-16]. According to the literatures, the temperature at which bovine bone is annealed is the most important factor that can affect the morphology and properties of bovine HA [11, 13]. In addition, the phase stability of sintered HA is dependent on the sintering temperature and researchers have shown that the HA decomposed to other phases of calcium phosphate such tri-calcium phosphate (TCP) when it is sintered at temperatures more than 1200 °C [17, 18]. Thus the sintering temperature play a significant role in controlling the HA phase stability and ultimately the mechanical properties. This study aim at investigating the effect of heat treatment on bovine bone in terms of the HA phase stability, sintering properties and microstructural evolution at various temperatures ranging from 400 °C to 1300 °C.

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Experimental Procedures

The femur bone from an adult bovine (2-3 years old) was obtained from a local slaughterhouse for this research. The bone was defatted and cleaned thoroughly to remove the flesh from the bone surface. Samples were prepared by cutting the as-prepared bone into small cubic pieces of size 10 mm × 5 mm × 5 mm. The samples were subsequently heat treated in air atmosphere in an electric box furnace at several temperatures ranging from 400 °C to 1300 °C, using a heating rate of 10 °C/min and a soaking time of 2 hours.

The bovine bone thermal stability during calcination was studied using a TG/DTA analyzer (Mettler Toledo, Switzerland) under flowing nitrogen (50 cm³/min) from 30 °C to 1000 °C with a heating rate of 10 °C/min. The phase analysis of the bovine sample was done by X-ray diffraction (XRD) of calcined samples at room temperature using CuK α as the radiation source at a scan speed of 0.5 per minute and a step scan of 0.02. The crystalline hydroxyapatite phase present in the samples as well as other calcium phosphate phases were identified based on the standard JCPDS files available in the system software. The calcium to phosphate (Ca/P) ratio of calcined samples was subsequently obtained from compounds comprising both elements. The microstructure evolution of the bovine hydroxyapatite (bovine-HA) was investigated using a field emission scanning electron microscope (FESEM) whereas the energy dispersive X-ray spectroscopy (EDX) was employed to determine the element present in the structure.

Results and Discussion

A general observation that was made during the calcination of the samples was the colour change of the bone with increasing temperature as presented in Table 1. The colour of raw bone changed from cream at room temperature to dark black at 400 °C to light grey at 600 °C. However, the samples became fully white when

Table 1. Colour change due to annealing of bovine bone.

Sample No.	Temperature (°C)	Colour
1		
<i>as-received bovine bone</i>	Room temperature	Light yellow
2	400	Black
3	500	Dark grey
4	600	Light grey
5	700	White
6	800	White
7	900	White
8	1000	White
9	1100	White
10	1200	White
11	1300	White

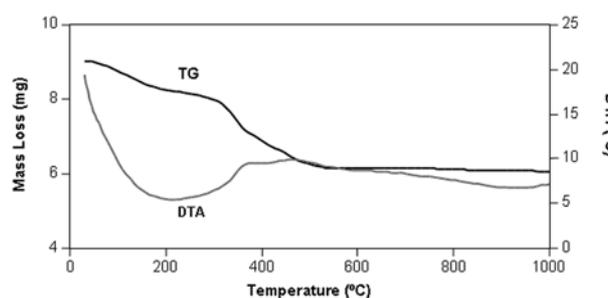


Fig. 1. The TG and DTA curve of as-received bovine bone as measured from room temperature to 1000 °C.

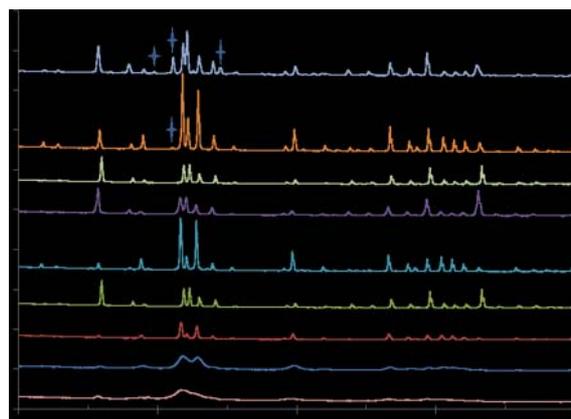


Fig. 2. XRD trace of raw bovine bone and bone annealed from 600 °C and 1300 °C. All the unmarked peaks belong to that of HA phase. The marked peaks shown for the 1200 °C and 1300 °C belongs to that of β -TCP.

the annealing increased above 700 °C, indicating complete removal of organic substance such as protein and collagen.

The DTA analysis of the bovine bone presented in Fig 1 confirmed the removal of the incorporated hydroxide group from within the bone structure as heating proceeded from room temperature to about 200 °C. This is indicated by the small endothermic transmission at about 200 °C as shown in Fig. 1. The continuous weight loss observed in the TG curve between 250 °C and 550 °C is attributed to the burning of the organic substance from the sample. Further heating above 600 °C did not reveal much fluctuation in both the TG and DTA curves, thus indicating the formation of a stable hydroxyapatite phase.

The phase analysis of the bovine bone calcined at varying temperatures is shown in Fig. 2. The intensity of the hydroxyapatite (HA) peaks increased progressively with increasing temperature and became more prominent as the temperature increased beyond 600 °C. This substantial increase in the peak height and the reduction in the peak width are a reflection of the improved crystallinity and an increased in the crystallite size of the HA phase, respectively. However, calcination above 1200 °C resulted in partial HA phase decomposition to form beta tricalcium phosphate (β -TCP). This phase decomposition for samples observed at 1200 °C and 1300 °C (Fig. 2) is attributed to partial dehydration of

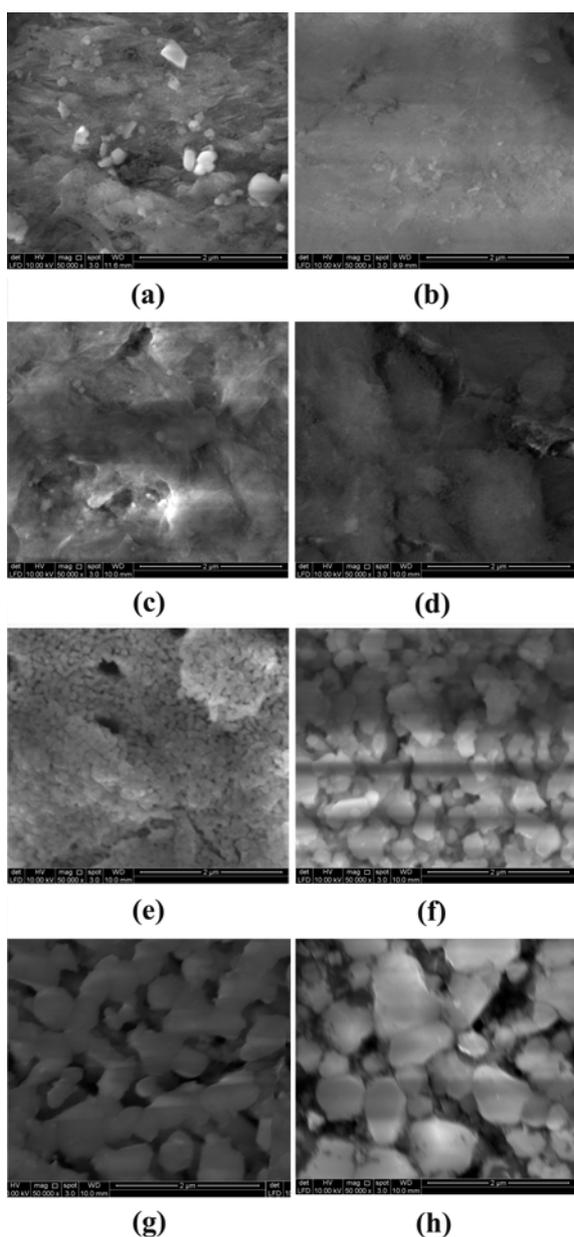


Fig. 3. FESEM images of (a) as-received bovine bone and bone annealed at (b) 400 °C, (c) 500 °C, (d) 600 °C, (e) 700 °C, (f) 800 °C, (g) 900 °C and (h) 1000 °C.

the hydroxyapatite during heating [19, 20].

The microstructure evolution of the as-received and heat treated (400–500 °C) bovine HA is shown in Fig. 3 (a, b, and c). As a result of the presence of collagen and protein parts in the bovine bone lattice, the lattice structures of these samples seemed to be dense. A porous structure of bone sintered at 600 °C–800 °C shown in Figs. 3 (d, e, and f) which resulted from the removal of organic substances from the bone. A typical interconnected porous microstructure of annealed samples were observed at 900 °C and 1000 °C as shown in Figs. 3 (g and h).

The EDX examination of the heat treated bone sample shown in Fig. 4 confirmed the presence of Ca, P, Na, C, Mg, and O. The Ca/P ratios of bone heat

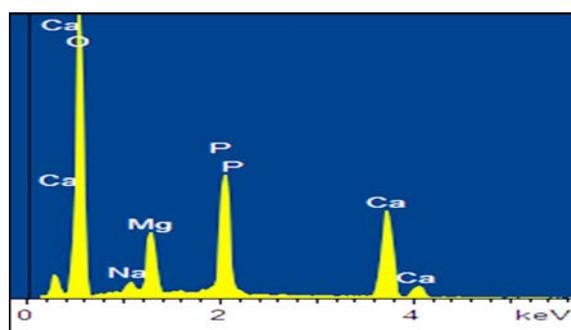


Fig. 4. EDX analysis of bovine HA annealed at 900 °C.

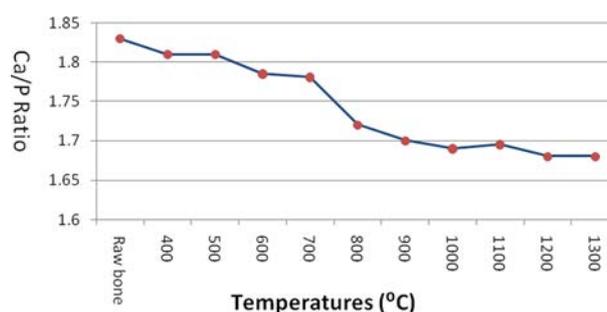


Fig. 5. Effect of annealing temperature on the Ca/P ratio of bovine bone.

treated at various heating point is indicated in Fig. 5. The Ca/P ratio of raw bovine bone was 1.83 at room temperature and it reached to 1.81 when treated at temperature of 400 °C. The Ca/P ratio was 1.68 when annealed at 1300 °C. In comparison to the theoretical Ca/P molar ratio based on the molecular formula of standard HA is 1.67 [21, 22]. The present results indicated that HA derived from bovine bone has great potential for medical usage as a bone and hard tissue substitute.

Conclusions

The production of HA extracted from bio-waste bovine bone from annealing above 600 °C is presented in this research work. XRD analysis of the calcined bovine bone showed that the HA phase stability was not disrupted in the bone lattice when annealed between 600 °C and 1100 °C. In addition, the crystallinity of the HA in the bone increases with increasing annealing temperature. SEM investigation revealed that the sintered bone between 700 °C and 1000 °C resulted in the formation of interconnecting pore structure of natural bone. Through proper control of annealing temperature, it is possible to control the Ca/P ratio of the bovine bone to attain as close as the stoichiometric value thus rendering this bio-waste material suitable for use in clinical application.

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