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Preparation of Natural Hydroxyapatite from Bovine Femur Bones Using Calcination at Various Temperatures

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Abstract

Calcium phosphate based biomaterials have received great interest since the main constituent of inorganic mineral component in human bone is hydroxyapatite (HAP). This study aims to extract the natural HAP from bovine femur bone at different calcination temperature with different particle size. The experiment starts with cleaning process of fresh bovine femur bones. Then bovine bones were ground into different particle size ($x \leq 45\mu m$, $45 < x \leq 63$ μm, and $63 < x \leq 125\mu m$). All bone samples were furnace heated at temperatures of 700°C, 900°C, and 1100°C for three hours. Obtained apatite was characterized using field emission scanning electron microscope (FESEM), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD). The results indicate that increasing calcination temperature leads to organic free and higher crystallite size of HAP. In terms of particle size, crystallite size is increasing as particle size increases but the increment crystallite size is not significant. The report here proposed that calcination temperature above 700˚C may utilize in bone tissue engineering application.

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Keywords: Hydroxyapatite; Bovine femur bones; Calcination; Temperature

1. Introduction

Over the past few decades, various engineering materials, including polymer, ceramics, metal and composites have been developed for bone clinical application such as bone repair, regeneration, and reconstruction [1]. The main constituent of inorganic mineral component of human bone is calcium phosphate. Synthesizing calcium phosphate based ceramic biomaterials such as hydroxyapatite (HAP), β-tricalcium phosphate (β-TCP), and biphasic

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calcium phosphate (BCP) has received great interest due to their excellent biocompatibility with hard tissue [2] and bioactive property.

Because of its physical and chemical similarities to inorganic mineral component of human bone and teeth enamel as well as its lower degradation rate than β-TCP [2, 3], synthetic HAP with chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ was the most frequently studied, clinically tested, and used for bone replacement and dental reconstruction. However, synthetic HAP cannot mimic apatite extracted from natural sources due to its absence of other trace elements (e.g., Na, Mg, and Al) in the bones [4, 5]. An alternative method to prepare HAP that can mimic apatites of human bone is extracting it from natural sources, such as fish bones [6] and bovine bones. Not only containing inherent inorganic minerals and other trace elements, but environmental friendliness and being economical are also the advantages of choosing natural materials as source for obtaining HAP.

In this study, bovine bones were used to obtain natural HAP via calcination. Through this synthesis technique, organic components in bones can be thermally decomposed eliminated and every genome sign of disease can be removes and thus provides high biological safety factors [7]. Extraction of natural HAP from bovine femur bones is done at different calcination temperatures and different particle sizes.

2. Experimental Methods

2.1 Bovine bone preparation

The raw material in this study was bovine femur bones obtained from local butcher. In the beginning, the bovine bones were boiled in water for 1 hour for defatting and easier removal of macroscopic adhering impurities. Afterwards, the bones were washed and cleaned well with water to evacuate all of the attached meat, tendons, bone marrows, and other soft tissues. The bones were then immersed in acetone for two hours and washed with water for several times afterwards. The process was followed by drying the bovine bones to evaporate the absorbed water. After that, the dried bovine bones were crushed into small pieces using mortar pestle and then milled into smaller particle sizes using rotary mill (Fritsch, Germany). Finally the bovine bones were sifted in the range 45-125μm using sieve shaker (Retsch, Germany) and were categorized in three groups according to the size of particles, i.e., less than 45μm ($x \leq 45$), between 45 and 63μm ($45 < x \leq 63$), and between 63 and 125μm ($63 < x \leq 125$).

2.2 Calcination

In the calcination process, the amount of 10 g of bone particles was placed in an open alumina crucible and then heated in a furnace (Carbolite, UK) to eliminate organic constituents contained in the bovine bones, leaving only HAP. All samples were heated at various temperatures, i.e., 700°C, 900°C, and 1100°C for 3 hours at heating rate 10°C/min and afterwards were cooled slowly to room temperature.

2.3 Characterization

The field emission scanning electron microscope (FESEM) was carried out to capture the surface morphology of the samples. To determine the functional groups present in raw and decomposed bones, Fourier transform infrared (FTIR) spectroscopy (PerkinElmer, Massachusetts) was performed over the range of 600 to 4000cm^{-1}. Information regarding phase composition and crystallinity of samples were obtained via X-ray diffractometer (XRD) using Bruker, D8 X-ray diffractometer with CuKα radiation. The XRD spectra were taken at 60 kVp and 80 mA over a range of 2θ angles from 10° to 80°. From XRD data, crystallite size is related to peak broadening and it can be calculated using Scherrer's formula.

3. Results and Discussion

3.1. Morphology

The FESEM micrographs in Figure 1(a) to (d), depict the morphology changes at the surface as calcination temperature increases. Unlike highly dense microsurface observed in raw bovine bone particles, calcinated bones contain multiple pores created by decomposition of organic substances [8]. Calcination temperature brings direct impact to changes in grain growth where higher calcination temperature yields increase in grain size. This grain growth may associate with the absorption of heat energy by the particles.

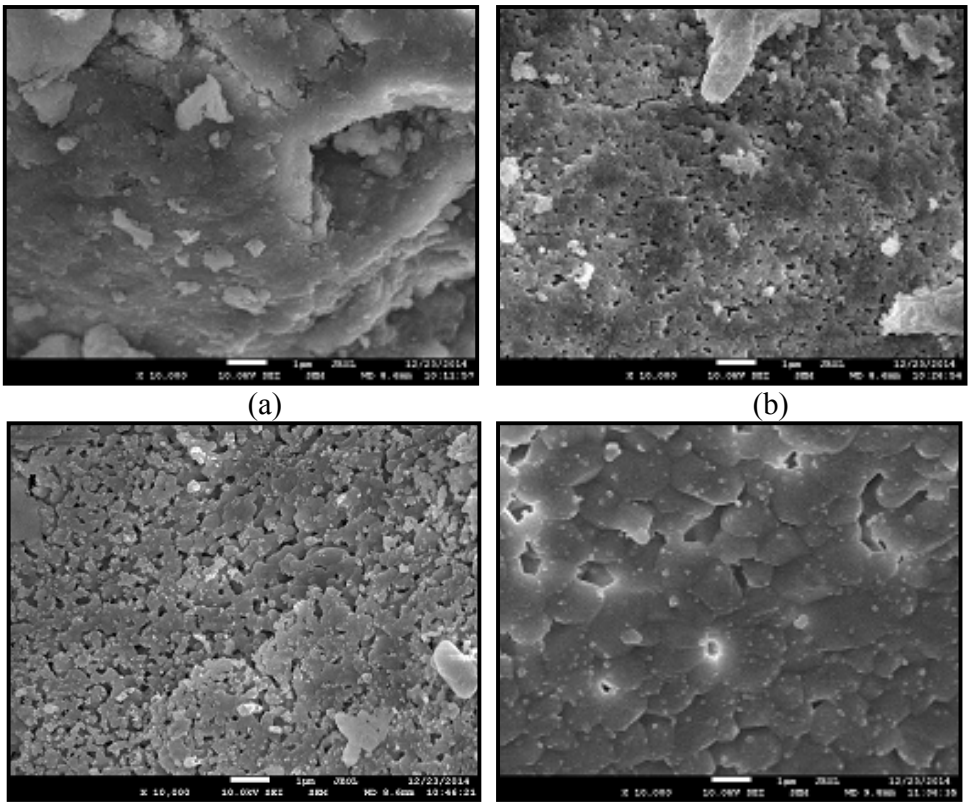

Fig. 1: Images of raw (a) and calcinated bovine bones at 700˚C (b), 900˚C (c) and 1100˚C (d).

3.2. FT-IR result

Figure 2 demonstrates the FTIR spectra of raw bovine bone and calcinated bone at different temperatures (700°C, 900°C, and 1100°C) with particle size of less than 45 μm. As shown in Figure 2, the spectra of raw bovine bone and heated ones are obviously different due to the changes in their chemical bonds during heat treatment. Through visual observation, the color of bone particles changes from yellowish white to white after calcination. This color changes implies the decomposition of organic substance (e.g., proteins and collagen) in heated bone powder [9, 10]. For affirmation, the FTIR band of 1640 cm^{-1} which corresponds to Amide I of collagen ([11]) that is present in the raw bovine bone is completely eliminated in calcinated samples.

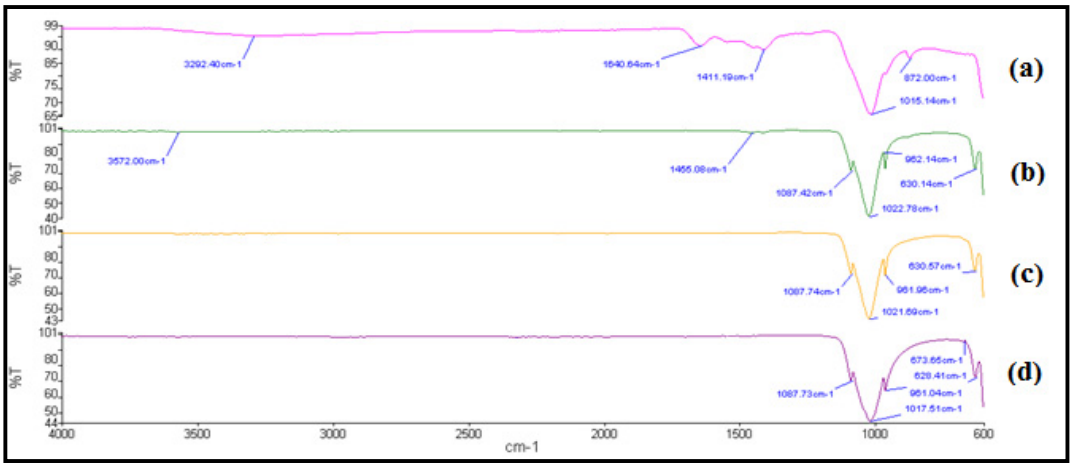

Fig. 2. FTIR for raw bovine bone (a) and calcinated bones at 700°C (b), 900°C (c) and 1100°C (d) for particle size less than 45µm.

Through FTIR spectra, it is also revealed the presence of phosphate (PO_4^{3-}), carbonate (CO_3^{2-}) and hydroxyl (OH^-) groups. These spectra are more clearly appeared in calcinated samples because the calcination process has destroyed the cross linked structure in the raw bovine bone. A strong and broad band which appears at 962 cm^{-1}, and other bands at 1022 and 1087 cm^{-1} in calcinated bone particles are associated with the phosphate group. With increasing temperature, two peaks at 872 and 1411 cm^{-1} in Figure 1(a), which is attributed to carbonate group, decrease gradually. The raw bovine bone particles exhibit a wide band near 3292 cm^{-1} which corresponds to the absorbed water molecule in the sample. The sharp narrow band at 630 and wide band at 3572 cm^{-1} are associated with hydroxyl group where these two peaks prove the presence of HAP phase. When a critical dehydration temperature is achieved, HAP starts to decompose onto TCP. Ooi et al [10] noted that there is a small amount of TCP when bones are annealed at temperature above 1100˚C. Meejoo et al [12] also found that after heat treatment at 900˚C or above, the hydroxyl absorption band disappeared and TCP shoulders begin to show up. However, in this experiment, the hydroxyl group of all samples remains even after calcination at higher temperature (1100˚C) and thus this finding implies that there is no secondary phase is detected except HAP.

3.3. XRD result

Figure 3 demonstrated the XRD patterns of raw bovine bone (a) and calcinated bovine bones at temperatures of 700˚C (b), 900˚C (c), and 1100˚C (d) with particle size of less than 45µm. The obtained XRD spectra were compared to the JCPDS 09-0432 standard HAP data. As shown in the Figure 3, all the crystalline peaks in XRD spectra closely matched with peaks in standard HAP which means that the thermal process has produced natural HAP. For the calcinated samples, there are three main peaks, namely, 211, 112, and 300 planes at 2θ near 31.8˚, 32.2˚, and 32.9˚, respectively. It is obvious that the intensity of diffraction peaks increases with the calcination temperature while the peaks became sharper and narrower. Heating at temperature 700˚C resulting in broad diffraction peaks which correspond to a poor crystalline apatite, probably due to the presence of minor concentration of carbonated groups in the samples which were detected in FTIR testing. Increasing the heating temperature to 900˚C resulting in more intense, sharper, and narrower diffraction peaks which denote the increasing of crystallinity and crystal size. Haberko et al [13] also found that the concentration of carbonate groups decreases when calcination temperature is above 700˚C. This implies that the amorphous raw bovine bone was transformed into crystalline phase with decreasing organic phase and carbonates as temperature increases from 700˚C to 1100˚C.

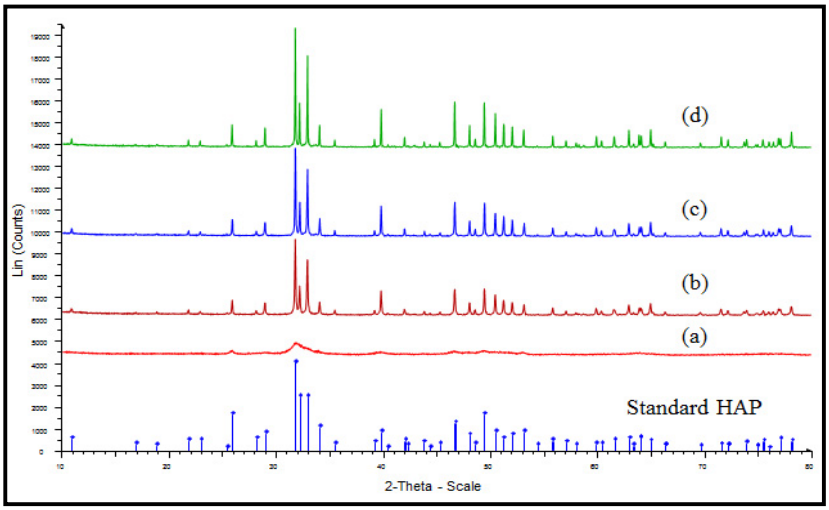

Fig. 3: XRD for the raw and heated bovine bone.

Using Scherrer's equation, crystallite size of the samples was calculated. Table 1 shows crystallite size of HAP prepared at 900˚C and 1100˚C for particle size $x \leq 45\mu m$ (a), $45 < x \leq 63\mu m$ (b) and $63 < x \leq 125\mu m$ (c) at planes of [002] and [310]. The calcination process at higher temperature causes changes in crystallite size. Broader diffraction peaks obtained at 700˚C reflect smaller crystallite size compared to other peaks at 900˚C and 1100˚C. The increment of crystallite size is relatively small when different particle sizes of samples were subjected to the same calcination temperature.

Table 1: Crystallite size of HAP as a function of calcination temperature.

Calcination temperature (˚C)	Crystallite size at plane [002] (nm)			Crystallite size at plane [310] (nm)		
	(a)	(b)	(c)	(a)	(b)	(c)
900	91.94	101.89	102.31	94.95	108.31	108.31
1100	118.14	128.032	134.42	115.24	116.29	118.45

4. Conclusion

Through the calcination method at different temperatures of 700, 900, and 1100 ˚C, natural HAP was obtained from the bovine femur bone as the raw material. FTIR and XRD results revealed that the calcination temperature performed on bovine bone did not bring secondary phase transformation except the presence of the HAP. Calcination temperature and particle size of bovine bone affect the composition, crystallinity, and crystallites size of the extracted natural HAP. At 700˚C, organic phase was eliminated but carbonate groups still exist in the HAP. Increasing calcination temperature to 1100˚C leads to higher crystallite size of HAP. When different particle sizes of bovine bone were subjected to the same calcination temperature, there was no significant change in crystallite size. It can be said that calcination of bovine bone at 700˚C and above can produce organic free and crystalline natural HAP.

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References

[1] K. J. L. Burg, S. Porter, and J. F. Kellam, Biomaterial developments for bone tissue engineering, Biomaterials 21 (2000) 2347-2359.

[2] S. V. Dorozhkin, Calcium orthophosphate-based bioceramics, Materials. 6 (2013) 3840–3942.

[3] R. Z. LeGeros and J. P. LeGeros, Calcium Phosphate biomaterials: an update, Int. J. Oral-Med Sci 4 (2006) 117-123.

[4] A. F. A. Yazdi, A. Yazdani T. T. Khozani and M. Kalantar, Extraction and viability checking of various carbonated hydroxyapatite by Wharton's jelly mesenchymal stem cell, Sci. Int. (2013) 132–138.

[5] M. Akram, R. Ahmed, I. Shakir, W. A. W. Ibrahim and R. Hussain, Extracting hydroxyapatite and its precursors from natural resources, J. Mater. Sci. 49 (2014) 1461–1475.

[6] K. Prabakaran and S. Rajeswari, Development of hydroxyapatite from natural fish bone through heat treatment, Trends Biomater. Artif. Organs. 20 (2006) 20–23.

[7] E. Hosseinzadeh, M. Davarpanah, N. Hassanzadeh, and S. A. Tavakoli, Fabrication of a hard tissue replacement using natural hydroxyapatite derieved from bovine bones by thermal decomposition method, Int. J. Org. Transplant. Med. 5 (2014) 23-31.

[8] R. Murugan, K. Panduranga Rao and T. S. Sampath Kumar, Heat-deproteinated xenogeneic bone from slaughterhouse waste : Physico-chemical properties, Bull. Mater. Sci. 26 (2003) 523–528.

[9] E. Kusrini and M. Sontang, Characterization of x-ray diffraction and electron spin resonance: Effects of sintering time and temperature on bovine hydroxyapatite, Radiat. Phys. Chem. 81 (2012) 118-125.

[10] C. Y. Ooi, M. Hamdi, and S. Ramesh, Properties of hydroxyapatite produced by annealing of bovine bone, Ceramics Int. 33 (2007) 1171–1177.

[11] N. A. M. Barakat, M. S. Khil, A. M. Omran, F. A. Sheikh, and H. Y. Kim, Extraction of pure natural hydroxyapatite from the bovine bones bio waste by three different methods, J. Mater. Process. Technol., 209 (2009) 3408-3415.

[12] S. Meejoo, W. Maneeprakorn, and P. Winotai, Phase and thermal stability of nanocrystalline hydroxyapatite prepared via microwave heating, Thermochimica Act. 447 (2006) 115-119.

[13] K. Haberko, M. M. Bućko, J. Brzezińska-Miecznik, M. Haberko, W. Mozgawa, T. Panz, A. Pyda, and J. Zarębski, Natural hydroxyapatite—its behaviour during heat treatment, J. Eur. Ceram. Soc. 26 (2006) 537–542.