

Sintering Properties of Hydroxyapatite Derived from Tuna Bone

Jin Sam Choi, Jeung-Soo Huh and Hyung Sun Kim*

Department of Metall. Eng., Kyungpook National University, Taegu, 702-701, Korea

**Department of Mat. Sci. and Metall. Eng., Suncheon National University, Suncheon, 540-742, Korea*

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Hydroxyapatite (HA) ceramic was prepared using tuna bone powders and was characterized on properties of sintered samples. The aim was to prepare dense and bulk HA sintered suitable for clinical applications. Thermal analysis, X-ray diffraction analysis, microscopic observation, and mechanical evaluation were applied to characterize sintered HA to find optimum processing condition. The major phase of sintered HA was observed up to 1300°C and β -TCP was observed as temperature increased. The density of sintered HA was increased up to 1350°C and decreased with further increasing temperature. The flexural strength of sintered HA at 1350°C showed 58 MPa as the highest value in this work. The results suggest that the crystal phase and transformation characteristics of HA prepared from tuna bone are the same as the behavior of HA powders made by chemical synthesis.

Key words: Hydroxyapatite (HA), Sintering, Tuna bone

I. Introduction

In HA powder preparation for bulk or coatings, there have been many methods used, including conventional wet synthesis, solid state transformation as a dry method, alkoxide method, sol-gel process, and hydrothermal reaction.¹⁾ Still, the most widely used method for HA powder preparation is the precipitation from an aqueous alkaline solution. In manufacturing HA, it is not formed initially but is the final product from a number of precursor phases using amorphous calcium phosphate, dicalcium phosphate and octacalcium phosphate. The nature of the precursor phase and the incubation time before complete HA transformation depend critically on total Ca and P ion concentration, pH, and temperature.²⁾ To overcome such problems dealing with many factors in the process, another method using natural living tissue has been suggested, which is a very simple method of converting the calcium carbonate skeleton into HA: calcination of animal skeletal bone, calcination of coral, and fish bone.³⁻⁶⁾ On the other hand, there have been objections to the use of bovine bone and corals as raw materials because of problems with reproducibility and the presence of impurities.²⁾ However, the HA powder derived from cod bone has high specific surface area, high porosity, and fine pores in its microstructure compared to some commercial HA powders and that has shown satisfactory results in the human body environment.⁵⁾ In addition, a strong advantage is that natural HA contains important minerals which are not found in synthetic HA. Thus, some of them have already been commercialized as "Interpore" and "HAP-B" for marine coral and cod bone, respectively.^{3,5)}

Using the tuna bone powders like cod bone as a fish bone, an in vivo study proved the good results to be compatible to synthetic powder.⁷⁾ However, the detailed information on the properties of HA derived from fish bone are not studied in detail; e.g., the effect of sintering temperature on the microstructure, crystal phases and mechanical properties of sintered samples.

In this study, the sintered products using the powder developed from tuna bone were characterized and compared to sintered HA ceramics produced by other chemical reagents.

II. Experimental Procedure

Tuna bone was treated by 1% NaOH solution at 80°C for 7 h in order to remove organic materials. By washing the bone with flowing water for 2 h, Na ion was cleaned from the tuna bone. After that, the bone was calcined at 850°C for 3 h in an electric furnace to burn out remaining protein.⁷⁾ In detail, the characteristics of powders is represented in Ref [7]. For sintering, the powder was prepared in the following procedures: After a wet milling for 24 h, and sieving with 1000 mesh sieve, the powders were pressed for bars (25×30×5 mm). The bars were then sintered in the temperature range of 1200-1450°C for 3 h in air.

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed using a thermal analyzer (TA, USA) with a heating rate of 10°C/m.

The phase analysis of sintered samples was carried out by X-ray diffraction (XRD, Cu target, Shimatsu XD-1) and fracture surface analysis was done by scanning electron micros-

copy (SEM, Hitachi S-4200). For the observation of the grain size of the sintered samples, a thermal etching was done at 1050°C for 30 m. The mechanical strength of sintered samples were evaluated by a 4-point bending test with a cross head speed of 0.5 mm/m.

III. Results

3.1 Hydroxyapatite stabilization

TGA result (Fig. 1) shows that the weight loss started at around 660°C, which resulted from the dissociation of carbonates and the elimination of hydroxyl groups. The carbonates and elimination of hydroxyl groups are also found in bovine bone and Indian coral.^{4,5)} The decreasing trend was continued up to 1200°C, which is the maximum temperature used in this work. In addition, a synthetic HA powder decomposed to eliminate hydroxyl groups at temperatures higher than 800°C.⁸⁾ As shown in Table 1, the wide decomposition temperature seems to be dependent upon the constituents of bones. Since human bone (cortical bone) and tuna bone have 65 and 67.5 wt% of ash (total inorganic), respectively and human bone has 5.8 wt% of carbonate in the composition,³⁻⁵⁾ the natural bones in Table 1 show the weight loss at a temperature lower than the synthetic HA that does not contain carbonate. The three peaks observed in the DTA trace (Fig. 1) are related to burning organics remained in the powder, crystallisation of amorphous calcium phosphate, and transformation of HA at around 230, 780 and 1310°C, respectively. At 780°C, the lower exothermic peak suggests that the content of amorphous phosphate

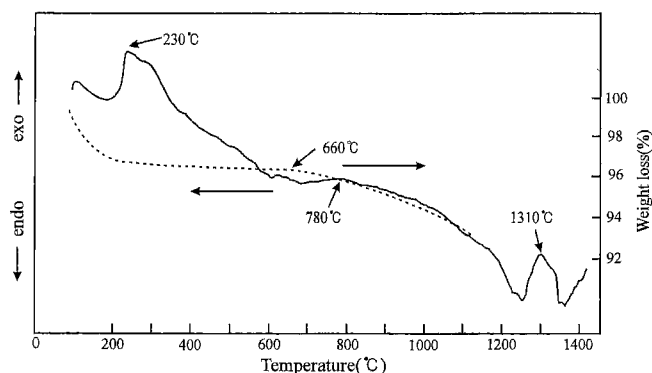


Fig. 1. DTA and TGA traces of tuna bone powder.

Table 1. The Decomposition Temperature of Bones.

Materials	Decomposition temperature (°C)	Weight loss (%)	Reference
Synthetic HA	>800	1.8	11
Synthetic Ca carbonate	898		3
Coral	680	45	3
Bovine	800	2-5	6
Tuna	660	2	this work

Table 2. XRD Phases Sintered at Different Firing Temperatures with Inorganic Materials Derived from Tuna Bone

Temperature (°C)	HAp*	β-TCP
1200	M	–
1250	M	–
1300	M	–
1350	M	m
1400	m	M
1450	–	M

*HAp=Hydroxyapatite, β-TCP=β-Tricalcium phosphate, M=Majority, m=minority

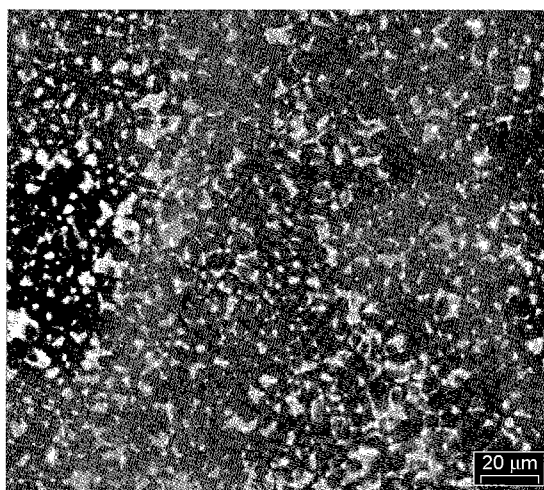


Fig. 2. Thermal etched microstructure of a sample sintered at 1350°C for 3 h.

is small in the tuna bone.

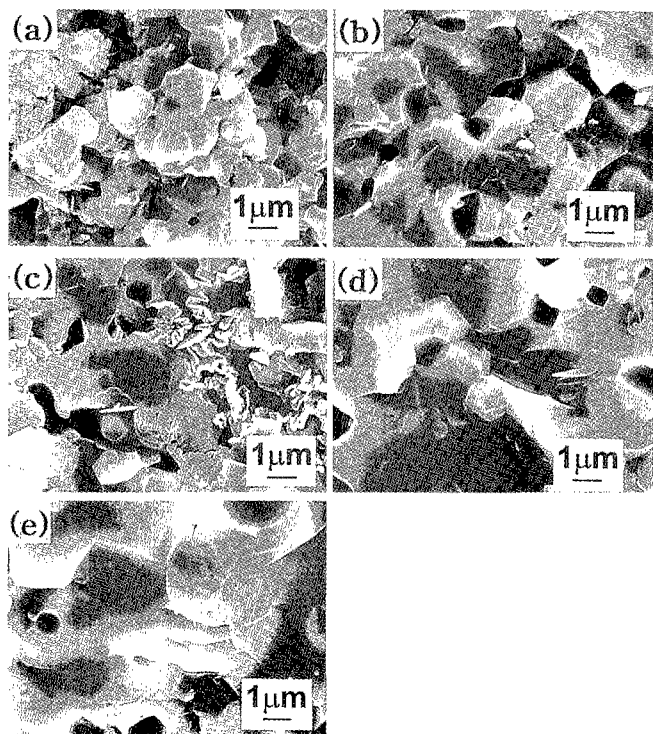
3.2. Properties of sintered samples

The phase of sintered ceramics was HA up to 1300°C and showed mixed phases of HA and β-TCP ($\text{Ca}_3(\text{PO}_4)_2$, tricalcium phosphate) which is a major one above 1350°C (Table 2). As the sintering temperature increased, β-TCP formation increased relatively as shown in Table 2. In this work, the CaO phase was not detected, although the phase was appeared along with HA from living bone above 950°C.⁹⁾ This is thought to be a different extraction method for HA in the preparation of powders. It was used with 1% NaOH solution, while LeGeros used an enzyme for the extraction of HA. HA is found to be stable at high temperatures in bovine, fish bone and coral.³⁻⁶⁾ The β-TCP was also observed in the sintering of commercial powders, and the phase transformation was observed in the range of 1200-1458°C.¹⁰⁾ In this work, the transformation to β-TCP occurred at 1310°C as in Fig. 1.

Table 3 shows the density and the porosity of the sintered samples. The porosity decreased drastically down at 1300°C

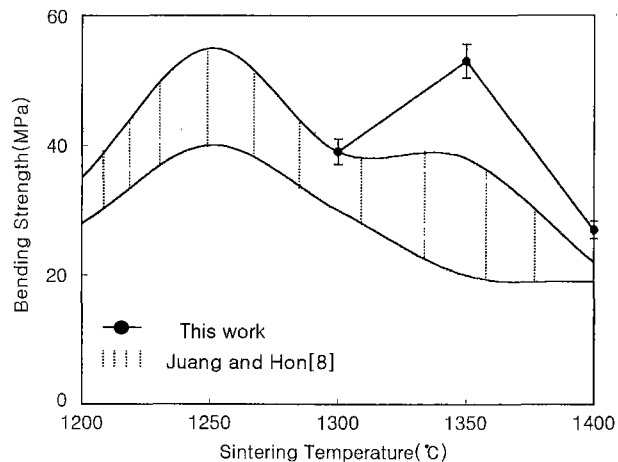
Table 3. Open Porosity and Bulk Density of Sintered Specimens with Various Temperature

Sintering temperature (°C)	1200	1250	1300	1350	1400	1450
Open porosity (%)	19.5	12.3	1.5	0.3	0.7	2.1
Bulk density (g/cm ³)	2.453	2.7245	2.9454	2.9583	2.8546	2.8236

**Fig. 3.** Morphology of fracture surface samples sintered at 1200 (a), 1250 (b), 1300 (c), 1350 (d) and 1400°C (e).

and then slightly increased with increasing temperature. The typical microstructure of sintered samples is represented in Fig. 2, in which the average grain size is found to be about 5-6 μm for the sample sintered at 1350°C. Considering that the powders average size of about 1 μm is prepared from the raw material, the grain size after sintering was changed slightly to a larger size as shown in the fracture morphology (Fig. 3). The fracture surface morphology of the sintered samples shows the effect of sintering temperature on the porosity, grain size and β -TCP formation. At low temperature of 1200-1250°C, necking appeared as the initial stage of sintering. Grain growth was observed as the temperature increased 1250°C; the size increased from 1 μm after the 1200°C sintering (Fig. 3(a)) to 5-6 μm after 1350°C sintering (Fig. 2 and Fig. 3(d)). Figs 3 (c) and (e) showed spherical and large pores in the microstructure, which indicate the amorphous phase formed at high temperature. This is attributed to the elimination of CO_2 caused by substitution of PO_4 for CO_3 at high temperatures.^{11,12} The highest density and lowest porosity at 1350°C as shown in Fig. 2 and Table 3 were obtained by sintering.

The flexural strength of the samples was determined as a

**Fig. 4.** Flexural strength of samples sintered at around 1300°C and with others data, Juang and Hons result [8].

function of sintering temperature and the result is reported in Fig. 4. The highest strength of 58.8 MPa (the sample was calcined at 850°C for 3 h) was obtained by sintering at 1350°C.

IV. Discussion

In the case of the bovine bone, HA contains 40% of amorphous calcium phosphate⁵ and the crystallisation of amorphous calcium phosphate occurs at around 670-700°C. It is supposed that the different process for the preparation of powder causes a weak thermal reaction to indicate the low exothermic peak at 780°C (Fig. 1). Although the powder was washed with 1%NaOH solution to remove organics and amorphous phosphate, some of them still remained in the powder. Additionally, calcination of tuna bone was done at 800°C for 5 h before the DTA measurement, but thermal analysis showed that it was not completely calcined. This is likely related to the powder characteristics, that is: the tuna bone powders are mixed with cortical and cancellous bones, which have different elastic modulus, density and microstructure.¹ Thus, calcination processes would affect the powder characterization. It was revealed that increasing the calcination temperature results in a larger mean value of particle size and particle size distribution becomes narrower.³

The bulk density of sintered HA showed a transient range at 1300-1350°C, which is similar to the trend of the porosity of samples. It is thought that β -TCP producing temperatures are related to the maximum density and minimum porosity when they are compared with results of Table 2.

The density of sintered HA at around 1300°C was 2.94 g/cm³ and is closed to the theoretical value of 3.13 g/cm³. The density of the sintered sample is thought to be resulted from the appearance of mixed crystalline phases in the sintered sample, which is composed of different densities of HA (3.13 g/cm³) and β -TCP (3.07 g/cm³).¹¹ The HA→ β -TCP phase transformation begins at 1200-1450°C range and the extent of the range depends on raw powder property.¹⁰ Since β -TCP is known as a significant biodegradable phase in comparison with HA, an optimum sintering temperature is a temperature below the HA→ β -TCP transformation in order to avoid β -TCP formation.^{1,9,10} The phase transformation temperature (HA→ β -TCP) of synthetic HA is generally lower than 1300°C, while the HA prepared from the tuna bone showed that β -TCP is formed above 1300°C. Thus, the result reflects the relative thermal stability of sintered tuna bone.

As shown in Fig. 5, Juang and Hon's data shows the highest bending strength, 54.8 MPa, for the sample obtained by calcinations at 900°C and sintering at 1250°C⁸ of a commercial HA powder. They have reported that the change of strength of the sintered sample depends on the particle size distribution and packing density. This is consistent with the results of the specimen prepared from uniformly distributed commercial HA powders, which showed highest flexural strength of 130 MPa after sintering at 1250°C.^{11,12} Being compared to others with commercial HA powder, the mechanical property in Fig. 4 suggests encourageable results for the application to cortical or cancellous bone, which needs the flexural strength of 50-150 and 10-20 MPa, respectively.¹ The mechanical property is purely due to the microstructure relationship between porosity and grain size which appeared below 1300°C as shown in Fig. 3(c) and (d). Without revealing the fracture toughness and the elastic constants of the sintered samples in this work, the HA derived from the tuna bone is not to be applicable to human bones as a replacement. However, in future the sintered material would have a wide potential importance in ion exchanger and support for chromatograph as well as biomedical materials because of the bioactivity and the special composition.

V. Summary

It was demonstrated that the crystal phase and transformation of HA prepared from tuna bone are similar to those of HA powders made by chemical synthesis. The major phase of sintered specimen was HA up to 1300°C and then

HA mixed with β -TCP as temperatures increased. The density of sintered samples increased up to 1350°C and then slightly decreased with increasing temperature. An optimum microstructure was obtained at around 1350°C, where grain bonding was strong and porosity was minimal. The highest flexural strength of sintered samples, however, was 58 MPa after sintering at 1350°C rather than at 1300°C. The tuna bone HA showed higher thermal stability compared to the chemically driven HA as evidenced by the formation of β -TCP at elevated temperatures above 1300°C.

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