CHARACTERISATION OF POROUS HYDROXYAPATITE BEADS PREPARED FROM FISH SCALE FOR POTENTIAL BONE FILLER APPLICATIONS

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Abstract. The aim of this study is to prepare the different sizes of porous HA beads from fish scales hydroxyapatite (FsHA) using sieve technique. Different composition of porous agents (0 to 20 wt%) were used to prepare different porosity of FsHA beads. The FsHA beads was sintered at 1200 °C in open air for four hours at heating rate of 3 °C min^{-1}. The FsHA beads were characterized using Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD) and scanning electron microscope (SEM). The physical properties of FsHA beads such as density and porosity were determined using pycnometer method. The linear shrinkage was carried out by calculating the shrinkage percentage as sintered. The sintered FsHA beads was confirmed to be crystalline hydroxyapatite since the FTIR spectra shows the sharps absorption peaks at 550 and 600 cm^{-1} and 1100-1200 cm^{-1} which are corresponding to the phosphate groups. The XRD analysis has confirmed the pattern of hydroxyapatite with sharp peaks was observed at (211), (112) and (300), characteristics to the high crystallinity of hydroxyapatite. The results showed that as the amount of porous agent (tapioca starch) increase from 0 to 20 wt% , the porosity was increased from 33 to 61 %, meanwhile the density was decreased from 3.0 to 1.64 g cm^{-3}. SEM analysis shows the mesoporous structure of FsHA beads at different composition of porous agent. The percent of shrinkage of different composition of porous agent are slightly similar to each other which about 10 to 12 %. The high porosity with low density of hydroxyapatite bead has potential to be used as bone filler.

Keywords: Fish scale, hydroxyapatite, beads, bone filler

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Introduction

Bone fillers become a crucial material to fill the bone loss in the field of orthopedic, dentistry and bone substitute and the most frequently used materials in tissue transplantations. The demand of bone fillers is more than two million procedures every year in US with over $200 billion being spent annually [1]. Since the mammal bone especially human bone consists of 65% of calcium apatite, most of research had been focusing on the application of calcium phosphate (CaP) compound including HA [$(Ca_5(PO_4)_3OH$] as bone fillers. HA is suitable for bone filler due to its high biocompatibility that will stimulate osteoconduction and it can be replaced slowly by the human bone after implantation [2]. Synthetic HA is a bioactive ceramic widely used in bone grafting due to its very good biocompatibility and osteoconductive properties that has the ability to regenerate the osteoblasts in human bone [3]. Since the chemicals used in the synthesis of HA were expensive, thus the price of HA produced was also expensive. Another disadvantage of synthetic HA is they are low bioresorbable material which contribute to reduction of ingrowth of new bone formation [4].

Natural HA from the bio-waste materials recently have been actively research to replace synthetic HA due to their similarity in their biological properties [2]. Natural HA is cost effective due to high mass production of calcium phosphate content during HA extraction from the biological sources. Besides, the natural HA would be considered as an environmental-friendly because it is made up from the by-product (bio-waste) of natural sources in the food industry. Therefore, it gives benefit towards the solid waste management by reducing the environmental pollution. In comparison between natural and synthetic HA, the natural HA is proven to be more safety than that of the synthetic [5].

The natural HA has been extracted from mammal bones such as pig and bovine bone [6]. However, the HA extracted from bovine bones rise up an issue among the Hindu community as it is forbidden for them to consume cow-related products [7]. Similarly, pig bones are not Halal for Muslim users. Animal diseases such as bovine spongiform encephalopathy (BSE) or “mad cow” that transmitted among cows are reported to become serious safety issues [8]. Some other diseases that were transmitted by land-based animals are transmissible spongiform encephalopathy (TSE). As an alternative, natural HA has been extracted from aquatic source such fish bone, fish scale and corals [5]. Natural HA derived from the bones of sword fish (Xiphias gladius) and tuna (Thunnus thynnus) have been reported to be bioactive material [5]. Another study has shown that natural HA derived from the fish scale of fresh water fish (Labeo rohita) was found to enhance the cellular attachment and proliferation [9].

It was reported that HA in the form of granules and beads are the most frequently used materials for bone fillers [1]. This was due to their excellent biological properties which allows the new bone to grow rapidly inside the inter-granular space of granule. The physical and chemical properties of porous HA granules were more likely similar to natural bone as compared to dense HA and it shown that the growth of new bone was successfully taken place between the gaps of the granules and also in the internal pores of HA [10]. Currently, none of researcher has developed the HA beads from fish scales for bone filler application using tapioca starch as porous agent Thus in this study, natural HA was extracted from fish scales (FsHA) and converted into porous FsHA beads with tapioca starch as porous agent using sieve technique for potential bone fillers applications

Materials and Methods

Preparation of Hydroxyapatite (HA) Powder from Fish Scales (FsHA)

FsHA was extracted from Tilapia (*Oreochromis niloticus*) fish scale. The scales were washed in order to remove impurities and then heated up to 800 °C at the rate of 3 ^{o}C min^{-1} in order to remove all the organic components that may be trapped in the scales. Then, the sample was wet-grinded by using ball mill to reduce the particle size below 10 μm. The sample solution was dried at 80 ^{o}C in the oven and grinded into powders.

Preparation of FsHA Beads

Dry FsHA powder was mixed with different percentage of porous agent (0 wt%, 5 wt%, 10 wt% and 20 wt%). The porous agent used was tapioca starch. The mixture of FsHA and starch was sprayed with water in the container and mechanically stirred. The mixture was then transferred into a sieve and were manually shook until the FsHA beads were formed. After that, the beads were sieved to obtain different sizes and dried in the oven. The FsHA beads produced were sintered at 1200 °C to produce porous beads.

Effect of Sintering on Shrinkage Percentage of Fsha Beads

The effect of sintering on beads size was determined by calculating the shrinkage percentage of FsHA beads. The diameter of each FsHA bead was measured by using a micrometer (SK Niigara Seiki, MC-25, 0-25 mm). Five measurements of FsHA beads before and after sintered had been taken in order to calculate the average and the shrinkage percentage was calculated by using the following formula;

$$\text{Shrinkage percentage} = \frac{\text{diameter before sinter-diameter after sinter}}{\text{diameter before sinter}} \text{ x } 100 \quad (1)$$

Density and Porosity Measurement

The density and porosity of FsHA beads were measured by using pycnometer method. This method is based on the following formula (2);

$$\text{Density } (\rho) = \frac{\text{mass (m)}}{\text{volume (V)}} \quad (2)$$

The pycnometer was cleaned, dried and weighed to obtain dry weight (W_1). After that, the pycnometer was filled with distilled water and reweight (W_2). The volume of the pycnometer (V_p) was calculated as the following equation (3);

$$\text{Volume of pycnometer, } V_P(cm^3) = \frac{(W_2 - W_1)}{\rho_w} \quad (3)$$

where, ρ_w is the density of water. Then, the pycnometer was dried again and weight (W_1) was measured. The pycnometer was filled with the FsHA beads and weighed (W_3). So the mass of sample was calculated as follows;

$$\text{Mass of sample} = (W_3 - W_1) \quad (4)$$

After that, the pycnometer was filled with HA and water was weighed (W_4).

$$\text{Mass of water} = (W_4 - W_3) \quad (5)$$

$$\text{Volume of water, } V_w(cm^3) = \frac{(W_4 - W_3)}{\rho_w} \quad (6)$$

$$\text{Volume of sample, } V_s(cm^3) = (V_p - V_w) \quad (7)$$

$$\text{Density of sample, } \rho_s(g\ cm^{-3}) = \frac{(W_3 - W_1)}{V_s} \quad (8)$$

$$= \rho_w \frac{(W_3 - W_1)}{(W_3 - W_1 + W_2 - W_4)} \quad (9)$$

where, the density of water (ρ_w) used was 0.997 $g\ cm^{-3}$. Five measurements of each sample were taken in order to calculate the average so that the measurements were more reliable.

Fourier-Transforms Infrared Spectroscopy (FTIR) Analysis

FsHA powder sample was analyzed using FTIR spectroscopy (Nicolet Thermo Nexus 6700) to determine the functional groups of the compound. The samples were grounded with KBr powders at the ratio of 1:9 and pressed into pellets before analyzed by the instrument.

X-Ray Diffraction (XRD) Analysis

The confirmation of crystal structure of FsHA beads was performed by XRD analysis (Rigaku MiniFlex). The analysis was carried out on the powder form of crushed FsHA beads with the usage of current and voltage was 15 mA and 40 kV respectively, scanning from 3° to 80° with the rate of 3.0° per minute.

Field Emission Scanning Electron Microscopy (FESEM) Analysis

Morphology of FsHA beads were analyzed using FESEM (Hitachi, SU8020, Japan). The micrograph obtained from the samples were used to determine the microstructure of the sample. In this study, energy dispersive x-ray analysis (EDX) was conducted to determine the element present in the samples. The results gave information about the calcium and phosphate composition in the FsHA bead samples.

Results and Discussion

Effect of Sintering on Particle Shrinkage

Figure 1 shows the FsHA beads photograph with average particle size around 2 mm produced in this study. The beads exhibit high porosity and white in colour. Most recently porous synthetic hydroxyapatite was fabricated using rice starch as porous agent by Masako et al. [11].

Figure 1: Photograph of hydroxyapatite beads

Table 1 shows the data obtained from percentage shrinkage of FsHA beads calculated from each formulation. The percentage shrinkage of beads was effected by composition of porous agent (0 to 20 wt%) used in the formulation. Based on the result in Table 1, the shrinkage percentage of FsHA beads slightly increased as the porous agent increases ranging from 10 to 13 %. During sintering process, the starch composition as porous agent will be burn-off when temperature reach about 400 ^{o}C and left behind holes or pores in FsHA matrix. The higher the composition of porous agent composition, the greater the holes or porous in the matrix. Further heating will make the pores inside the beads became smaller due to the shrinkage of FsHA matrix [12]. Thus, the higher the percentage of porous agent, the greater the shrinkage of FsHA beads.

Table 1: Shrinkage percentage of hydroxyapatite beads

Sample	Porous agent (wt%)	Shrinkage percentage (%)
A	0	10
B	5	12
C	10	12
D	20	13

Density and Porosity Measurement

In this section, the relationship between density and porosity of FsHA beads with different percentage of porous agent was elaborated in details. The density and porosity of FsHA were determined by using pycnometer method. Figures 2(a) and 2(b) shows the results of density and porosity of the FsHA as a function of starch composition respectively. Based on the result, it shows that as porous agent percentage increase, the porosity was increased

from 33 to 61 %, meanwhile the density of FsHA was decreased from 3.0 to 1.64 gcm^{-3}. Similar observation was reported by Sharmiwati et al. as the density of ceramic sponge decrease, the amount of porosity was increased [12]. Wu et al. [13] also reported that as percentage of starch increased, the porosity was also increased in diatomite-based ceramics. It was reported the theoretical density of HA is 3.156 g cm^{-3} [14] which is closed to the density of pure FsHA in this study i.e 3.0 g cm^{-3}.

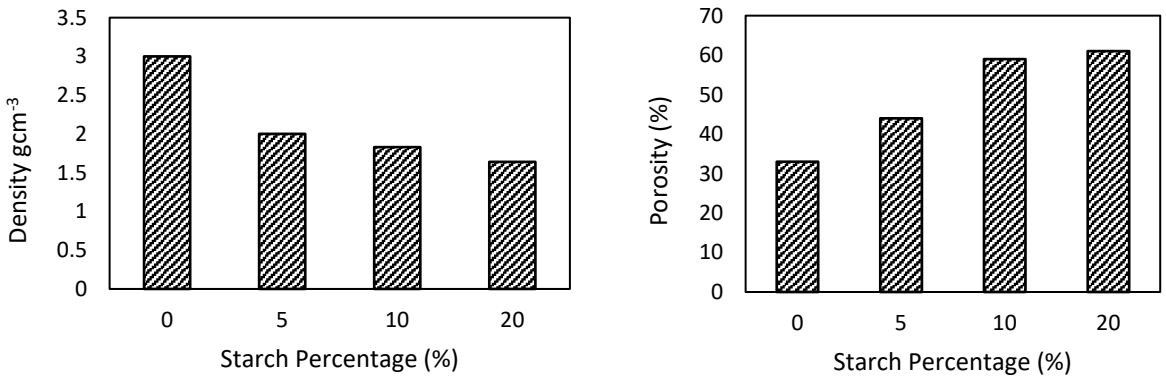

Figure 2: The (a) density and (b) porosity of hydroxyapatite beads

Fourier-Transforms Infrared Spectroscopy (FTIR) Analysis

In this study, FTIR analysis was performed to identify the functional groups of the FsHA beads. From the analysis, the absorption peaks in FTIR spectrum represent the functional groups present in the sample. The FTIR spectrum of sintered FsHA bead is shown in Figure 3. The spectrum shows several sharp peaks appeared in the range from 473 to 3570 cm^{-1}. According to Muhamad et al., the peak at 3570 cm^{-1} and 1989 cm^{-1} indicated the stretching and bending of hydroxyl group (OH) respectively which present in the hydroxyapatite [15]. Similar observation was also reported by Sharma et al., as they reported that a small sharp peak at around 3572 cm^{-1} was indicated the OH groups stretching [16].

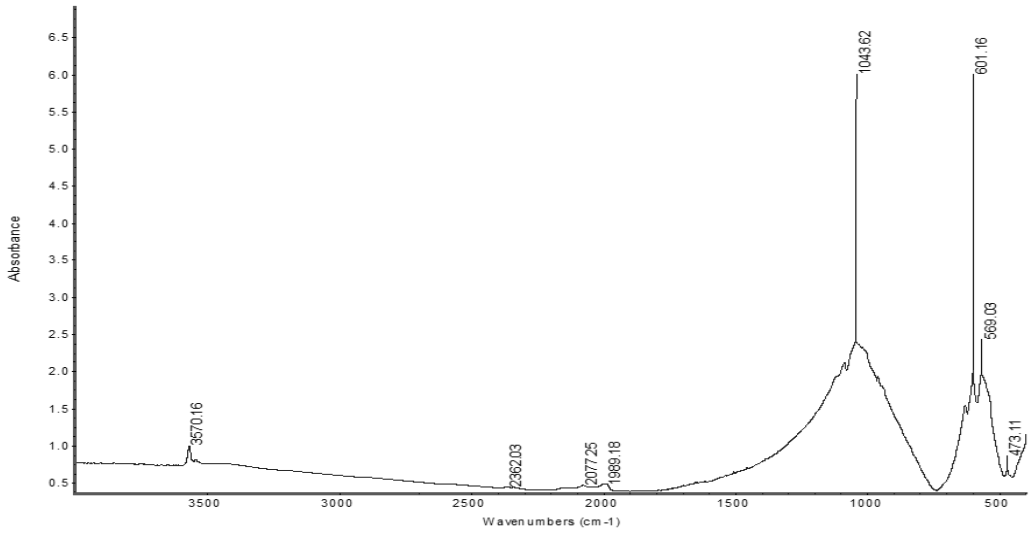

Figure 3: FTIR spectrum of sintered FsHA beads

The next functional group found in the sample was phosphate (PO_4^{3-}) groups. From the FTIR analysis, the broad peak at 1043 cm^{-1} represent the stretching of PO_4^{3-} groups while the sharp peaks at 601 cm^{-1} and 569 cm^{-1} represent the bending mode of PO_4^{3-} [15]. The FTIR analysis proved that the prominent peaks appeared in Figure 3 is characteristic to the functional groups of standard hydroxyapatite.

X-ray Diffraction (XRD) Analysis

The XRD pattern of FsHA beads presented in Figure 4 shows very sharp peaks indicated that the sample has a very high degree of crystallinity. The XRD pattern of FsHA was identified at the three peaks of intensity that are located at angular zone of 2 theta in between 30° to 40°. These peak positions correspond to the Miller indices of (211), (112) and (300) are characteristic to the hydroxyapatite materials. The diffraction peaks of HA were obtained with the d-spacing values of 2.80 Å, 2.78 Å and 2.75 Å. This results are almost similar with the one obtained by the previous study which the values of d-spacing were of 2.82 Å, 2.79 Å and 2.72 Å [16]. The XRD results further confirm the FsHA obtained in this study is highly crystalline hydroxyapatite.

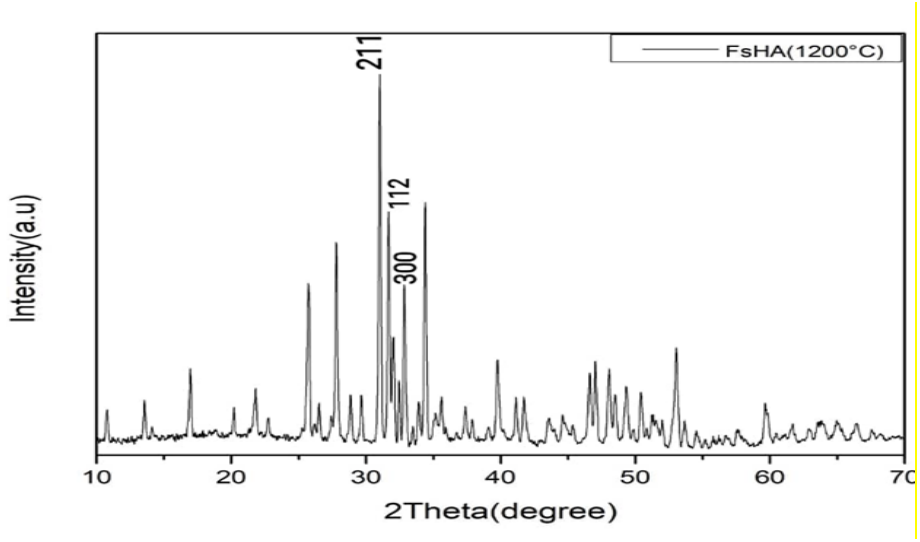

Figure 4: XRD diffractogram of FsHA beads

Surface Morphology Analysis

The FESEM was carried out to study the fracture surface morphological structure of FsHA beads. In Figure 5, the SEM micrograph shows difference pore size and porosity of FsHA beads with different composition of porous agent (0 wt%, 5 wt%, 10 wt% and 20 wt%). The results show the FsHA particles are fused together to form mesoporous structure and the pores are interconnected to each other. From the results, it can be seen that the higher the composition of porous agent in the sample, the higher the number of pores. The highest number of porosity can be seen in sample (d) (20 wt% of porous agent) while the lowest number of porosity can be seen in sample (a) (0 wt% of porous agent). This results support the results obtained from porosity study as shown in Figure 2(b). Similar observation was reported Masako et al., while they are using rice starch as porous agent. The pore sizes for all samples are similar to each other which ranging from 218 nm to 985 nm. The size of pores

and their interconnectivity full fill the requirement of the materials for bone fillers and bone tissue engineering [3].

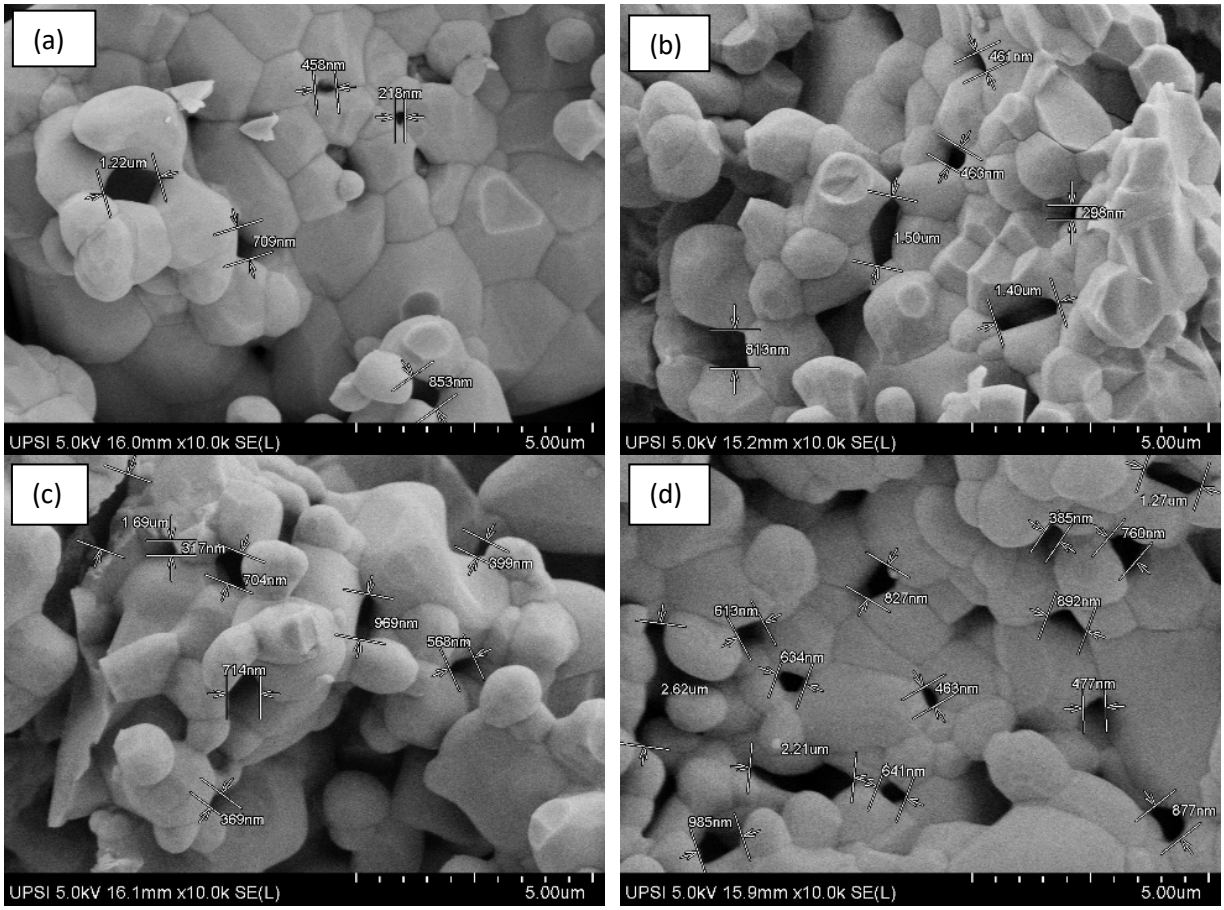

Figure 5: FESEM micrographs of (a) sample A, (b) sample B, (c) sample C and (d) sample D at 10, 000x magnification

Conclusions

Porous natural HA beads from fish scales have been successfully developed using sieve technique with tapioca starch as porous agent. The analysis have proved that the natural HA extracted from fish scales (FsHA) has similar structure to synthetic HA. The incorporation of porous agent (tapioca starch) significantly increase the porosity of the beads that has potential to be used as bone filler. This is because the physical and chemical properties of porous FsHA beads are more likely similar to natural bone compared to dense HA. Overall, results from the analysis show the porous natural HA beads have potential to be used as bone filler.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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