Synthesis and Characterization of Hydroxyapatite Nanoparticles and β-TCP Particles

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Abstract - Hydroxyapatite (HA) was prepared by co-precipitation of calcium chloride and phosphoric acid while β-TCP was prepared using ammonium hydrogen phosphate and calcium chloride. The wet powders from both preparations were centrifuged, sonicated, autoclaved and calcinated to produce nanoparticles. They were characterized by SEM, XRD; FTIR; Vickers Microhardness Tester. Green and sintered disc of both samples were also prepared. The hydroxyapatite nanoparticles were of rod shape structure with dimension of 65±1.0 nm for length and 25±1.0 nm for width while β-TCP was larger with dimension of 200±10 nm for length and 100±10 nm for width. The hardness of sintered hydroxyapatite disc was found to be stronger than the sintered β-TCP.

Keyword: Hydroxyapatite; β-TCP; nanoparticles; SEM; XRD; FTIR; Vickers Microhardness Tester

I. INTRODUCTION

Synthetic calcium phosphates, such as calcium hydroxyapatite, HA; Ca_{10}(PO_4)_{6}(OH)_2, and β-tricalcium phosphate, β-TCP; Ca_(3)(PO_4)_2, and biphasic mixtures of these two have found use as bone substitutes [1-3]. HA or β-TCP implants exhibit relatively good tissue compatibility, and new bone is formed directly on the implants with no fibrous encapsulation [4]. However, sintered and well-crystallized HA ceramics usually demonstrated minimal in vivo resorption, with resorption times lagging the new bone formation rates [5-9]. Kilian et al. [10] showed that nonsintered HA could even be phagocytized and dissolved by macrophages and osteoclasts, while sintered ceramics were not degraded and remained at the site of implantation for years following the surgery. The β-TCP, on the other hand, has a significantly high solubility [11, 12] and typically fades away from the defect site even before the completion of new bone formation. An ideal skeletal repair implant should readily take part in the bone remodeling processes, and also allow for the direct anchorage by the bony tissues surrounding it (osteocoinduction) [13].

Nanosized size HA can provide large interfaces, giving high catalic activity and great adsorption capability in the catalysis and separation fields. Hydroxyapatite is non-inflamatory, causes no immunological and irritating response [14]. These scaffolds provide the necessary support as artificial extracellular matrices and permit cells to proliferate and differentiated functions. A new tissue will generate on the scaffold as the function of the scaffold as a template to guide the new tissue formation. For biodegradable scaffold in bone tissue engineering, the scaffold will be a temporary template that introduced at the defective area or lost bone. It initiated bone tissue regeneration and new bone tissue will be form. The biodegradable scaffold is gradually degrade and replaced by newly formed bone tissue. An ideal scaffold should be biocompatible, cytocompatible, controllable biodegradable, porous and have good mechanical strength [15, 16]. The objectives of this work were to synthesize hydroxyapatite and β-TCP nanoparticles and to characterize these particles by various instrumental methods and their properties were compared.

II. MATERIALS AND METHODS

A. Materials

Calcium chloride was purchased from Merck. Phosphoric acid was purchased from Systems. Tween 80 and ammonium hydrogen phosphate were purchased from Sigma-Aldrich. Liquid ammonia was purchased from R & M Chemicals. Ammonium hydrogen phosphate was purchased from Reidel-de Haën.

B. Synthesis of Sample HAP-A

The method conducted was according to the method reported [17] but with some modifications. A five neck flask was used. The flask was kept in a water bath at a temperature of 45°C. Different solutions were added through different necks of the flask while one neck of the flask was inserted with a thermometer and the other was for pH electrode. Aqueous solution of 1.0 M calcium chloride was vigorously stirred at room temperature. A solution of 0.6 M phosphoric acid was added slowly in a dropwise manner to the vigorously stirred calcium chloride solution. An aqueous solution of 5% Tween 80 was added into the vigorously stirred solution mixture of calcium and phosphate. A certain volume of 25% (v/v) ammonia solution was then added dropwise into the solution to maintain the pH of the solution at pH10. After 4 h reaction at 40 °C, the reaction mixture was allowed to age for another 16 h at room temperature to complete the reaction. The suspension was centrifuged at 10,000 rpm using a table-top centrifuge for 5 min. Wet powder of sample HAP-A was obtained.

C. Synthesis of Sample HAP-B
Aqueous solution of calcium chloride was vigorously stirred at room temperature. A solution of ammonium hydrogen phosphate was slowly added dropwise to the calcium chloride solution. Ammonia solution was added dropwise into solution to adjust the pH 10 [18]. After reaction at 40 °C for 4 h and the reaction maintain was stirred for another 16 h at room temperature. The white precipitate of HAP-B was formed.

D. Autoclaved, Sonicated and Calcined

After aging of both samples, the solutions were transferred to the autoclave (HICLAVE HVE-50, HIRAYAMA), and autoclaved at 105°C for 4 h and 130 °C for another 4 h. The resultant wet powder was centrifuged using 10,000 rpm for 5 min. Ethanol was added to the pellet and the suspension was sonicated using ultrasonicator for 20 min and dried in an oven at 100°C for 3 hours. The dried powder was calcinated at 900 °C for 4 hours before sample analysis.

E. Preparation of Green and Sintered Disc

Powder samples were compressed into cylindrical tablets with a diameter of 12.80±0.02 mm and thickness of 2.00±0.03 mm under a uniaxial pressure of 5.0 MPa for 5 min to prepare green disc. The sintered disc were prepared by sintered the green disc at 1200ºC for 2 hours with same diameter and thickness.

F. X-ray Diffraction (XRD)

The structure of HAP-A and HAP-B were examined using XRD Rigaku Geiger-Flex Japan. The powder was examined with Ni filtered CuKα radiation generated at 40 kV and 30 mA. The powders were scanned from 3-100° 2θ with a scan speed of 2° per minute. The peaks obtained were compared with standard references in JCPDS file available in software for hydroxyapatite (09-0432) [19]

G. Fourier-transformed Infrared Spectroscopy (FTIR)

The functional groups of the hydroxyapatite were identified by FTIR Perkin Elmer using the KBr-disc method. The FTIR spectrum was scanned from 4000-400cm⁻¹ [20].

H. Vicker’s Microhardness Tester

Hardness measurements were performed on a Vickers microhardness tester (MITUTOYO MVK-HI). Loads of 2, 5 and 10 N was applied to the surface of each compressed disc for 15 s through a pyramidal diamond indenter. Diagonal length of the indentation was measured through a micrometric eyepiece with an objective lens of 40º. The tests were repeated 5 times for each sample. The Vickers hardness number was calculated using the equation VHN = (2Fsinθ2)/d², where F is the applied load in grams, θ is the angle between opposite faces of indenter (136°), and d is the diagonal length of indentation in micrometers [21].

III. RESULTS AND DISCUSSION

XRD pattern of the sample HAP-A showed the structure of the prepared sample was similar to the hydroxyapatite standard as shown in Fig. 1. There is a high consistency between the data of HAP-A and that from the standard data base, with lattice dimensions of a = b = 0.9418 nm, c = 0.6884 nm. No other impurity was observed in the XRD pattern, indicating that the chief inorganic phase of the sample is hydroxyapatite crystal. The result obtain was similar to [22] as reported.

Figure 1. XRD of (a) HAP-A nanoparticles and (b) library standard of hydroxyapatite.

XRD pattern of HAP-B as shown in Fig.2 showed that it is similar in structure to the standard β-TCP which has Ca:P ratio of 3:2. The result obtained for β-TCP was similar to that reported by [23]. No α-TCP phase and HA phase were detected in the XRD pattern of the powder β-TCP with lattice dimensions of a = b = 1.0429 nm, c = 3.7380 nm.

Figure 2. XRD of (a) HAP-B nanoparticles and (b) library standard of β-TCP.

FTIR spectrum as shown in Fig. 3 showed the characteristic absorption peaks of sample HAP-A.
nanoparticles. The broad bands at 3432 and 1642 cm\(^{-1}\) were attributable to adsorbed water, while sharp peak at 3571 cm\(^{-1}\) was attributable to the stretching vibration of the lattice OH- ions and the medium sharp peak at 633 cm\(^{-1}\) was assigned to the O-H deformation mode. The characteristic bands for PO\(_4^{3-}\) appear at 470, 568, 602, 964, 1041, and 1093 cm\(^{-1}\). The observation of the asymmetric P-O stretching vibration of the PO\(_4^{3-}\) bands at 964 cm\(^{-1}\) as a distinguishable peak, together with the sharp peaks at 633, 602, 568 cm\(^{-1}\) correspond to the triply degenerate bending vibrations of PO\(_4^{3-}\) in hydroxyapatite. Our FTIR result was similar to those reported [17, 18, 22].

![Figure 3: FTIR spectrum of HAP-A](image3.png)

The FTIR spectrum of HAP-B powders is shown in Fig. 4. The characteristic PO\(_4^{3-}\) absorption bands of \(\beta\)-TCP were observed with the broad bands at 3433 and 1631 cm\(^{-1}\) attributable to adsorbed water. The bands at 900–1200 cm\(^{-1}\) were the stretching mode of PO\(_4^{3-}\) group. The sharp peaks at 561 and 606 cm\(^{-1}\) represent the vibration peaks of PO\(_4^{3-}\) in \(\beta\)-TCP [23, 24]. The absence of a sharp peak at 3571 cm\(^{-1}\) indicating the absence of O-H functional group in the sample.

![Figure 4: FTIR spectrum of HAP-B](image4.png)

The results of XRD and FTIR indicated that the HAP-A prepared was hydroxyapatite while the HAP-B was identified as \(\beta\)-TCP.

Scanning electron microscope (SEM) was used to examine the morphologies of the samples HAP-A and HAP-B which have been autoclaved, sonicated and calcinated. Rod shape nanoparticles of sizes in the range of 50–70 nm was obtained for the sample HAP-A as illustrated in Fig. 5. The image showed clearly the clear contour of rod shape structures of nanoparticles with average dimension of 65±1.0 nm for length and 25±1.0 nm for width and no agglomeration was observed. From the other report [22], it shows needle-like morphology with particle width ranged from 30–60 nm and the length from 100–400 nm. Aili et al. [17] reported that the average diameters and the average lengths of the resultant HAP nanorods increased from 14.9×24.0 nm to 17.5×56.1 nm with increasing the autoclaving temperature from 100 to 200 °C.

![Figure 5: Scanning electron micrograph HAP-A nanoparticles](image5.png)

The size of the green pellet is 12.80±0.04 mm for diameter and thickness 2.00±0.03 mm. After sintering at 1200°C for 2 hours, the size of pellet was slightly reduced to 12.00±0.04 mm for diameter and 1.90±0.03 mm for thickness. The shrinkage of the green pellet and sintered pellet for sample HAP-A and HAP-B is not much different. The grain size of sintered disk as shown in Fig. 6 is 1.5±0.5 μm is larger than that before sintering.

![Figure 6: Scanning electron micrograph of sintered HAP-A disc](image6.png)
The hardness of disc pallet HAP-A were 1400, 1370 and 1050 MPa after indented with forces of 2 N, 5 N and 10 N respectively (Fig. 7). The hardness of hydroxyapatite disc becomes greater when sintered at 1200°C for 2 h, which were 4260, 3701, 2852 MPa after indented with forces of 2, 5 and 10 N, respectively as shown in Fig. 7. Kanana et al. [25] found that the hardness for the hydroxyapatite disc which was pressed at 80 MPa and sintered at 1200°C for 2 hours determined using vickers hardness tester under the load of 10 N was 297.45 ± 9.53 MPa. Timothy et al. [26], reported that the compression strength of zero-porosity value of HA structures which processed and sintered at 1100°C to 1300°C was 6.00±0.7 GPa.

![Figure 7](image7.png)

Figure 7. Comparison of hardness of for green and sintered HAP-A discs at different loading strength

![Figure 8](image8.png)

Figure 8. Scanning electron micrograph of HAP-B nanoparticles

Scanning electron micrograph of HAP-B is shown in Fig. 8. The image shows rod shape structures of particles with dimensions in the range of 200-500 nm with an average dimension of 200±10 nm for length and 100±10 nm for width similar to that reported [18]. The particles prepared were not fused together with other crystals. It can be inferred that majority of the particles were of single crystals, regular shape and cleaner contours with no agglomeration, which are highly beneficial for coating of nanoparticles onto biomedical implants.

The grain size of β-TCP as shown in Fig. 9 is 2.0 ± 0.5 μm. The grain size is larger before sintering same as that reported [27].

![Figure 9](image9.png)

Figure 9. Scanning electron micrograph of sintered TCP nanoparticles

The hardness of green disc HAP-B were 1050, 1106 and 954 MPa after indented with forces of 2 N, 5 N and 10 N respectively. The hardness of disc becomes greater when sintered at 1200°C for 2 h, which were 3751, 2942, 2200 MPa after indented with forces of 2, 5 and 10 N respectively as shown in Fig 10. Biqin et al.[23] reported the compressive strength, flexural strength, elasticity modulus and the fracture toughness of the sintered pieces β-TCP at 1125°C were 291±15 MPa, 93.0±8.7 MPa, 72.4±7.5 GPa and 0.92 ±0.04 MPa·m0.5 respectively at a load 2.0 kg.

![Figure 10](image10.png)

Figure 10. Comparison of hardness of green and sintered HAP-B discs at different loading strength

IV. CONCLUSION

Hydroxyapatite and β-TCP nanoparticles have been synthesized by using different chemicals followed by centrifugation, autoclaving, drying and calcinations. Hydroxyapatite nanoparticles and disc demonstrated better physical properties than β-TCP and has the potential for biomedical and biotechnological applications.

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