Conversion of calcite from cockle shells to bioactive nanorod hydroxyapatite for biomedical applications

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Bioactive ceramics such as hydroxyapatite (HA) can mimic the organic structure of human bone. HA was successfully synthesized from animal bones, corals, and eggshells which have been studied for bone repairing treatment and as implant coatings. This study aims to synthesize nanorod HA from cockle shells via two routes: calcination and the hydrothermal method. The raw cockle shells were converted to calcite by calcination method at 450°C (CS450) and 800°C (CS800) for 2 hrs. The calcite calcium carbonate samples were reacted with diammonium hydrogen phosphate and hydrothermally treated at 110°C. The pH of the solution was kept at 10.5 throughout the synthesis step by adding drops of ammonia. Product obtained was labelled as HA450 and HA800 containing HA powder. Presence of calcite phase in the raw cockle shells was characterized by utilizing Thermogravimetric analysis (TGA), X-ray Diffraction (XRD) and Fourier transforms infrared spectrophotometer (FTIR) analyses and morphologically analyzed by Field emission scanning electron microscopy (FESEM). The best result was obtained from the HA800 sample where nanoparticle with rod-like shape was observed (aspect ratio = 7) while needle-like particle was seen in HA450 sample (aspect ratio = 20). High purity HA was developed in HA800 sample while HA450 showed small presence of calcite phase. In vitro bioactivity test of HA powder samples incubated simulated body fluid (SBF) for 1, 3, 8, 15 and 21 days showed high bioactivity in both samples by forming apatite agglomerate on the surfaces. Higher microhardness strength was observed in HA800 compared to HA450, CS450 and CS800 sintered pellet samples.

Key words: Cockle shells, Hydroxyapatite, Nanorod, Hydrothermal.

Introduction

Human bone is composed of inorganic particles and organic matrix materials. Inorganic material found in bone has a similar structure to hydroxyapatite (HA), with a chemical formula of Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ [1-3]. Synthetic HA has shown excellent biocompatibility and osteoconductivity in human body environment, such as in bone and teeth [4, 5]. In the case of bone fracture or bone loss, HA is the most appropriate biomaterial to be applied as bone repair or replacement [6]. Osteogenesis improves with the assistance of highly porous HA scaffold. Therefore, the time taken during the healing process of diseased or broken bone can be reduced by changing the morphology of synthetic HA. Other applications of HA include drug delivery, biosensors, adsorption of biomolecules, and metallic implant coatings [7-11].

Synthesis of HA includes the combination of calcium and phosphate precursors. Recently, numerous studies in developing HA bioceramics were reported and focused on producing nanoparticle structure [12, 13]. Previous studies reported that the smaller the particle size, the higher the porosity and surface area of bioceramics desired for tissue engineering scaffolds [14]. Various experimental methods have been used to synthesise nano size HA, including solid state reactions [12], sol-gel techniques [15-18], vibro-milling method [19], hydrothermal methods [20-24], mechano-chemical techniques [25, 26], precipitation methods [27], and wet chemical methods [28]. Since 1990s, a lot of research has recognized the potential to synthesise HA from natural resources such as natural bones, corals, clams, and eggshells [2, 19, 25, 29-31]. Cockle shells (Anadara granosa) are composed of ~98.70% Ca, along with other elements such as Mg (~ 0.05%), Na (~ 0.90%), K (~ 0.04%) and P (0.02%), similar to that in clams and corals [32, 33]. Therefore, cockle shells are suited for use as calcium source for HA synthesis. Cockle shells are also abundant and cultured on a massive scale, unlike coral, which is under serious conservation.

Cockle shells were used in the development of bioceramics, as reported by Mohammad et al [34]. They conducted a comparative study of pure cockle shells and synthetic HA-cockle shells. A mix of synthetic HA-cockle shell with 3 : 1 ratio was found to have a similar compressive strength to human bone. In a separate study by Zuki et al [35], a cockle shell based biocomposite scaffold was confirmed to have osteoconductive and osteogenetic effects. In vitro studies of synthetic HA in
simulated body fluid (SBF) gave a clear picture of its interaction with body fluid ions [12, 36].

The current study reports the conversion of cockle shells into hydroxyapatite through the formation of calcium carbonate precipitated from cockle shells. However, some traces of aluminium were detected in the final product. An attempt to synthesize HA using a calcination process from cockle shells gives mixture of HA, TCP, CaCO₃, and Ca(OH)₂ [37, 38]. In the present study, calcite (calcium carbonate, CaCO₃) is extracted from raw cockle shells through a simple calcination process. HA is synthesized by using this cost effective calcite source as a calcium precursor following a time-effective hydrothermal method. The main aim of this study is to produce pure HA with a nanorod shape from naturally abundant cockle shell waste. The main advantage of these nanorods is to increase the electrical sensitivity by increasing the electron mobility through higher aspect ratio (length to diameter ratio) particles [39, 40]. Subsequently, bioactivity and microhardness tests of synthesized HA from cockle shells were conducted to evaluate its viability as bone substitute.

**Materials and Methods**

**Preparation and characterization of calcite from cockle shells**

Raw cockle shells were collected from the coast of Kuala Selangor, Malaysia. First, distilled water was used to remove sand and dirt. Then, shells were soaked in 30% hydrogen peroxide (H₂O₂, supplied by Merck, Germany) to completely remove the outer slippery mucus layer. The cleaned cockle shells were then dried for 3 days at room temperature. Non-calcined cockle shells (CS) were completely crushed using a pestle and mortar before being sent for thermogravimetric analysis (TGA). The dried CS powder was calcined at either 450 °C (hereafter, CS450) or 800 °C (hereafter, CS800), for 3 hrs. These temperatures were selected for their calcite phase, according to the TGA thermogram of the cleaned raw shell-powder.

**Synthesis of hydroxyapatite**

Hydroxyapatite nanoparticles were prepared from calcined cockle shells using a hydrothermal method. In this method, the calcined calcite (CaCO₃) powder was prepared from cockle shells, and di-ammonium hydrogen phosphate ((NH₄)₂HPO₄) crystals (99% Sigma Aldrich, UK) were used as raw materials for both calcium and phosphorus sources. The reagent grade (NH₄)₂HPO₄ was used without further purification. Distilled water (H₂O) was added to the mixture of CaCO₃ and (NH₄)₂HPO₄ in order to dissolve the (NH₄)₂HPO₄ crystals and to facilitate the reaction. The 5 : 3 : 1 molar concentration of CaCO₃ : (NH₄)₂HPO₄ : H₂O was used to produce one mole HA according to a feasible chemical reaction (see reaction 1). Here, ammonia solution (32% Merck, Germany) was added drop-wise until the pH of the solution reached 10.5. The pH-adjusted solution was heated at 100 °C for 1 h in a closed vessel under continuous stirring in an active fume hood. An opaque white dilute solution appeared and gradually became thicker over a period of 1 h. Several washing steps using distilled water removed all traces of ammonia in the sample. All the procedures were performed in the fume hood since pungent ammonia gas was released to the air during the reaction process. Finally, the sample was dried in a drying oven (Memmert, USA) at 50 °C for 3 days to obtain dry white HA powder. The same process was used for both calcined (450 and 800 °C) powders separately to obtain differing morphologies of HA. The HA synthesized from CS450 and CS800 is denoted HA450 and HA800, respectively. Synthesized samples of HA450 and HA800 were a very fine white powder. The chemical reaction during the HA synthesis process can be expressed as reaction (1), below:

\[ 10 \text{CaCO}_3 + 6 \text{(NH}_4\text{)}_2\text{HPO}_4 + 2 \text{H}_2\text{O} \rightarrow \text{Ca}_{10}((\text{PO}_4)_6(\text{OH})_2) + 6 \text{(NH}_4\text{)}_2\text{CO}_3 + 4 \text{H}_2\text{CO}_3 \]  

(1)

Ammonium carbonate, (NH₄)₂CO₃, was further decomposed to ammonium bicarbonate and ammonia as per reaction (2):

\[ \text{(NH}_4\text{)}_2\text{CO}_3 \rightarrow \text{NH}_3\text{HCO}_3 + \text{NH}_3 \]  

(2)

Thermally-unstable ammonium bicarbonate, NH₄HCO₃, instantly degrades into carbon dioxide and ammonia gases. Therefore, repeated washing steps of the slurry are necessary to remove ammonia by products from the powder. The pH was maintained at 10.5 to control the particle shape and size distribution [28].

In another partial reaction (3) occurred due to the formation of Ca(OH)₂, which enhanced the extra HA in HA800 during the hydrothermal process. The calcium hydroxide (Ca(OH)₂) might have been produced during calcination at 800 °C (see reaction 4) followed by a reaction with adsorbed water (see reaction 5):

\[ 10 \text{Ca(OH)}_2 + 6 \text{(NH}_4\text{)}_2\text{HPO}_4 + 2 \text{H}_2\text{O} \rightarrow \text{Ca}_{10}((\text{PO}_4)_6(\text{OH})_2) + 12 \text{NH}_4\text{OH} + 8 \text{H}_2\text{O} \]  

(3)

\[ \text{CaCO}_3 + \text{heat (800 °C)} \rightarrow \text{CaO} + \text{CO}_2 \]  

(4)

\[ \text{CaO} + \text{H}_2\text{O} \rightarrow \text{Ca(OH)}_2 \]  

(5)

**Characterization of hydroxyapatite**

Thermogravimetric analysis (TGA) was employed for the cleaned raw powder, at temperatures ranging from room temperature to 1000 °C, at constant ramp of 10 °C/min in a nitrogen gas atmosphere, using thermogravimetric analyzer (TGA/SDTA851°Ultramicrobalance, Mettler Toledo, USA), in order to determine the proper sintering temperatures. Surface morphology and particle shape of the powder was studied using a field emission scanning electron
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The thermogravimetric curve of cockle shells powder illustrated in Fig. 1 shows multi-step decomposition of cockle shells. After losing water up to 200 °C, the organic molecules degrade between 190 and 455 °C. Then, carbon dioxide was evolved rapidly between 650 and 900 °C, indicating the maximum decomposition of calcium carbonate to calcium oxide. The derivative TG curve shows the two major peaks at 290 °C and 890 °C, corresponding to the TGA curve. The derivative TG curve shows a steady line from 400 to 500 °C, and the highest rate of decomposition at 890 °C.

XRD patterns of CS (non-calcined), CS450 (sintered at 450 °C), and CS800 (sintered at 800 °C) are depicted in Fig. 2 for structural phase comparison. Aragonite phase was identified from the CS sample as matched to the standard aragonite reference card (JCPDS 98-015-7997) and as resembles other studies [42]. The greatest peak of aragonite (111) was located at 2θ = 26.1°, with 100% intensity. A similar crystal phase was also detected by XRD, reported elsewhere [43] for cockle shells. An obvious shift of peaks occurred for CS450 and CS800 samples. A highly intense and sharp peak of calcite (104) can be seen at 2θ = 29.3° in both

In vitro bioactivity test

Simulated body fluid (SBF) was prepared by using the method developed by Kokubo et al. [36]. Each HA450 and HA800 powder were immersed in the SBF (Na+ 142.0, K+ 5.0, Mg2+ 1.5, Ca2+ 2.5, C 147.8, HCO3− 4.2, HPO4 2− 1.0, SO4 2− 0.5 m.mol/L) solution following the rule of 1 mg/ml buffered at pH 7.40. The samples were placed in a 37 °C incubator (Eppendorf, USA) and soaked for 1, 3, 8, 15, or 21 days. Both FESEM and whole frame EDAX were used to evaluate the surface morphology and apatite formations since they have been widely been used by other studies as both qualitative and quantitative characterization tools to identify in vitro bioactivity [41].

Results and Discussion

Characterization of aragonite and calcite from cockle shells

Fig. 1. TG and DTG curves of cockle shells powder demonstrate multi-step decomposition process. Stable line implies complete phase transformation of cockle shells along decomposition process.

Fig. 2. XRD patterns of aragonite in non-calcined cockle shells powder (CS) and calcite for cockle shells powder after calcination process at 450 °C (CS450) and 800 °C (CS800). Single Ca(OH)2 peak is detected for CS800 spectrum.
CS450 and CS800 samples along with other weak peaks, as seen in the standard calcite reference card (JCPDS 1-072-1937). No other extra peak for other materials, except for a small peak of calcium hydroxide \((\text{Ca(OH)}_2)\) formed at \(2\theta = 34.1^\circ\) in CS800 was found. The extra peak in CS800 for \(\text{Ca(OH)}_2\) indicates that the calcium oxide (CaO) formed from calcite at high temperature (800 °C) can easily be converted into \(\text{Ca(OH)}_2\) after reacting with adsorbed water (see reactions 4-5). Since the formation of CaO was not possible at lower calcination temperatures (450 °C) according to TGA, the CS450 sample didn’t show any peak for \(\text{Ca(OH)}_2\).

Fig. 3 illustrates FTIR spectra for the CS, CS450, and CS800 samples. The spectrum from the CS sample depicts aragonite characteristic bands which are attributed to stretching at 1085 cm\(^{-1}\) and bending modes at 857 cm\(^{-1}\) of \(\text{CO}_3^{2-}\). A sharp band of \(\text{CO}_3^{2-}\) can be observed at 1455 cm\(^{-1}\), assigned to the fundamental mode of vibration for \(\text{CO}_3^{2-}\). Weak double bands at 712 cm\(^{-1}\) and 700 cm\(^{-1}\) are attributed to bending modes of \(\text{CO}_3^{2-}\). The FTIR spectrum for CS powder confirms the aragonite structure, as reported elsewhere [44].

Meanwhile, the infrared spectrums of CS450 and CS800 samples are in agreement with the findings of calcite (CaCO\(_3\)) formation by XRD analysis. Common absorption bands assigned to calcite were observed in CS450 and CS800 infrared spectrums. Prominent absorption band of carbonate alky al of was seen at 1384 cm\(^{-1}\) instead of 1455 cm\(^{-1}\) in aragonite. The characteristic absorption bands of calcite can be observed at 875 cm\(^{-1}\) and a single peak at 714 cm\(^{-1}\), owing to vibrations of \(\text{CO}_3^{2-}\) molecules. Other small stretching bands of functional groups belong to amide at 1792 cm\(^{-1}\) and carboxylic acids at 2507 cm\(^{-1}\). A weak OH\(^{-}\) peak observed on CS800 spectrum located at 3642 cm\(^{-1}\) depicts the presence of \(\text{Ca(OH)}_2\) at this stage. Both calcined powder samples, mostly containing calcite, were used as a starting material for synthesis of HA and compared. Unlike other reported methods, formation of calcite in the present study does not involve mixing with other unwanted compounds [37, 38].

**Characterization of hydroxyapatite from cockle shells powder**

XRD patterns of HA450 and HA800 samples were compared to the standard HA (JCPDS: 09-0432) with a hexagonal crystal system and a lattice dimension of \(a = 0.9418\) nm, \(b = 0.9418\) nm, and \(c = 0.6884\) nm, in order to determine the crystal structure. Some peaks of HA450 in Fig. 4 were matched to calcite in the XRD spectrum. An obvious large peak corresponding to the (104) plane of calcite was observed at \(2\theta = 29.3^\circ\). Other extra peaks for calcite are marked with red

**Table 1. Comparison of 2θ (°) and d-spacing (nm) values between standard HA (JCPDS 9-0432) and synthesized HA.**

<table>
<thead>
<tr>
<th>Miller lattice planes (hkl)</th>
<th>Standard HA</th>
<th>HA450</th>
<th>HA800</th>
<th>Standard HA</th>
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<th>HA800</th>
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<td>53.15</td>
<td>1.720</td>
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triangles, and the rest of the peaks which are in clear agreement with HA are marked with blue circle, as shown in HA450 of Fig. 4. Therefore, calcite is not totally converted into HA in the HA450 sample. In HA800, the maximum diffraction peak was observed at $2\theta = 31.8^\circ$, which corresponds to the (211) lattice plane. Other small peaks were also matched to the standard for HA. No other element appeared, including the peak of Ca(OH)$_2$ that was formed before the synthesis process. Here, the HA800 sample consists of single phase HA crystals which match the HA spectrum found elsewhere [15, 18, 45]. Comparing the diffraction angle ($2\theta$) and $d$-spacing values between both samples to the standard HA, it was found that the HA800 sample has the most similar profiles with the standard HA. In contrary, a slight deviation of the $2\theta$ and $d$-spacing values is depicted in the HA450 sample as illustrated in Table 1. This result proposed that particles in HA450 are composed of distorted hexagonal crystals. The Debye Scherrer Equation (1) is applied to estimate the crystallite size of the obtained HA powder[2].

$$D = \frac{k\lambda}{\beta\cos\theta}$$  

(6)

Where $D$ is the estimated crystallite size in nm, $k$ is a constant ($k \approx 0.9$), $\lambda$ is the X-ray diffraction wavelength i.e., 0.154056 nm, $\beta$ is the full width at half maximum (FWHM) in radian and $\theta$ is the Bragg's angle in degree. The approximate crystallite size was determined by applying FWHM and $\cos\theta$ values at (002) reflection into Equation (1). As a result, the crystallite size of the sample is estimated to be 39.9 nm (HA450) and 53.1 nm (HA800). These crystallite sizes of HA also resemble the HA developed by other studies [18]. The diameter of the HA nanorod will be measured from FESEM images to visualize the actual particle dimensions, since the Debye Scherrer equation can only be used to know the crystallite size.

The formation of functional groups for HA450 and HA800 samples evaluated using FTIR spectroscopy are shown in Fig. 5. The FTIR spectrum of HA800 as given in Fig. 5 depicts all important peaks for hydroxyl and phosphate groups corresponding to HA. The strongest sharp stretching band at 1019 cm$^{-1}$ is attributed to the vibration of PO$_4^{3-}$ group. Other major absorption bands that are assigned to phosphate group appeared at 1090, 962, 600 and 559 cm$^{-1}$. Then, a broad absorption band from 3200 to 3500 cm$^{-1}$ and a weak stretching mode at 633 cm$^{-1}$ of OH$^-$ are indicative of an adsorbed water peak. Two carbonate group stretching peaks at 1420 and 878 cm$^{-1}$ are attributed to the absorption of CO$_2$ that was evolved during the calcination process of the cockle shells. These FTIR results are consistent with HA synthesized elsewhere [15, 18, 27]. Similar bands assigned to hydroxyl, phosphate, and carbonate groups are detected in the HA450 infrared spectrum. However, the PO$_4^{3-}$ band at 1090 cm$^{-1}$ and the OH$^-$ band at 633 cm$^{-1}$ are almost flattened here. Thus, HA may not be well-crystallized in the HA450 sample, but is well formed in HA800 (since the small sharp molecular OH peak is observed at 3566 cm$^{-1}$ due to effect of sintering) [13]. Identification of standard HA characteristic bands in Fig. 5 suggested that main component of both samples is HA. Unlike the XRD results, the FTIR spectrum does not illustrate significant peaks for calcite formation in the HA450 sample.

![Fig. 6. Surface morphology of synthesized HA (a) HA450 and (b) HA800 under FESEM. (Top insets are higher magnified images; Bottom Insets are EDAX spectrums demonstrate Ca/P ratios). Particle morphology of synthesized HA (c) HA450 and (d) HA800 under AFM.](image)

Surface morphological and particle shapes of the non-stoichiometric HA samples were investigated by using FESEM micrographs (see Fig. 6). Needle-like and rod-like particles were clearly identified in both HA450 (Fig. 6(a)) and HA800 (Fig. 6(b)) samples from the FESEM micrographs. Particles of HA450 had a mean length of 458 ± 1 nm and a mean diameter of
24 ± 4 nm, significantly longer than HA800 particles (mean length: 207 ± 5 nm; mean diameter: 27 ± 8 nm). Particles of HA450 and HA800 had average aspect ratios (i.e., length to diameter) of 20 and 7, respectively. The fact that smaller standard deviations were found during HA800 crystal measurement showed more the uniform shape and size of the HA crystals. The mean diameter of the nano-rods of the present HA is much lower than the width of the other studies [42], and the aspect ratio higher than reported elsewhere [45]. In this regard, the morphology of the HA particle can be changed with different processing routes and heat-treatments from the same source material like cockle shell [37, 46]. EDAX results in Figs. 6(a-b) (inset spectrums) reveal the presence of only Ca, P and O elements in both HA samples. There was no other element found in the EDAX study even after scanning on the whole frame EDAX. The Ca/P elemental molar ratio value is ~ 1.76 (HA450) and ~ 1.8 (HA800), which is higher than the stoichiometric ratio of HA (~ 1.667). This agrees with similar studies using cockle shells [37, 38]. The needle-like smaller-diameter particles of HA450 (Fig. 6(c)) and rod-like larger particles of HA800 ((Fig. 6(d)) have also been found in the AFM study. This study also supports the FESEM analysis results and implies a higher aspect ratio of cockle shell HA processed at 450 °C, compared to 800 °C.

**Microvicker’s hardness**

The microvicker’s hardness of typical pellets of CS45, CS800, HA450, and HA800 samples is depicted in Figs. 7(a-d), respectively. The hardness values are illustrated in Table 2. The surface hardesses of the CS and HA samples processed at 800 °C were found to be higher than those of corresponding CS and HA samples processed at 450 °C. Therefore, it is clearly an indication of the effect of heat-treatment on the ceramic sample. It seems probable that this mechanical property (surface hardness) became higher where processed at 800 °C because more crystalline or ordered phases were developed at 800 °C compared to at 450 °C, as indicated by sharp peaks in the XRD study (see Figs. 2 and 4). Hardness of both HA samples was higher than that of their corresponding CS samples. This may be the effect of more ordered and new crystalline phases present in the HA samples.

**Bioactivity evaluation**

Deposition of tiny bone-like apatite agglomerates occurred in both HA450 (Fig. 8) and HA800 (Fig. 9) samples after soaking in SBF (1, 3, 8, 15 and 21 days). The overall amount of apatite agglomeration increased following the soaking time. HA800 shows a higher rate of agglomerate formation, taking place after just 1 day. The apatite formation was also clearly visible after 3 days of immersion in SBF of the HA450 sample. The

![Fig. 8. FE-SEM micrographs of HA450 powder after soaking in SBF at 37 °C for (a) 1, (b) 3, (c) 8, (d) 15 and (e) 21 days. EDAX readings and Ca/P ratios of the respective sample (Top Inset).](image)

Table 2. Microvicker’s hardness of the CS450, CS800, HA450, and HA800 sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average diagonal length (d) ±SD (mm)</th>
<th>Microvicker’s hardness number (HV) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS450</td>
<td>100.97 ± 0.56</td>
<td>41.74 ± 0.51</td>
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<tr>
<td>CS800</td>
<td>56.72 ± 1.34</td>
<td>60.87 ± 2.45</td>
</tr>
<tr>
<td>HA450</td>
<td>92.00 ± 1.18</td>
<td>43.86 ± 1.14</td>
</tr>
<tr>
<td>HA800</td>
<td>75.21 ± 2.83</td>
<td>65.84 ± 5.19</td>
</tr>
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</table>

![Fig. 7. Microvicker’s hardness of the typical pellets of (a) CS45, (b) CS800, (c) HA450, and (d) HA800 samples.](image)
agglomerated apatite layer had completely covered the surface of HA800 on 21st day. The corresponding Ca/P ratios of both HA450 and HA800 the samples are illustrated in Figs. 8 and 9. The Ca/P ratio of HA450 increased to 2.03 on the 3rd day then reduced to 1.76 on the 8th day and then increased again to 2.04 after the 21st day of immersion. For HA800, the Ca/P ratio kept increasing to a maximum of 2.30 on the 8th day before reducing to a Ca/P ratio of 1.74 on the 21st day. The different Ca/P ratio values on the surface of HA substrate indicate the influence of the HA substrate on the newly formed apatite layers. The total Ca/P ratio also strongly depends on the surface charge mobility of the substrate [47]. Since dissolution of Ca²⁺ or PO₄³⁻ ions from the HA substrate also takes place simultaneously with apatite formation from the SBF solution, the Ca/P ratio captured from the whole frame EDAX result may not follow a linear trend or stoichiometric ratio. As a result the desolation of PO₄³⁻ ions from the HA substrate compensates more than the Ca/P ratio at Ca-poor layer. Thus, the overall Ca/P ratio fluctuates. Moreover, it was related to the fact that the surfaces of wet apatite is covered with hydrogenophosphates in equilibrium with water at pH = 7.4, resulting in calcium phosphates with a Ca/P ratio of nearly 1 along with left-over apatite and thus a higher level of Ca²⁺ ions and Ca/P ratio. Therefore, this result proved that HA synthesized from cockle shells has a high bioactivity due its ability to form an apatite layer in SBF. FESEM micrographs in Figs. 8 and 9 clearly indicate bone-like apatite formation which increases with immersion time. In addition, bone-like apatite formation was more prominently revealed in HA800 with immersion time. This result indicates that the mixture of HA and calcium carbonate in the HA450 sample is also bioactive but with less apatite formation rate compared to HA800. Bioactivity is in addition to the proven property of non-toxicity of this material as reported previously [38].

**Conclusions**

Nanorod particle HA was successfully developed by using Malaysian cockle shells (*Anadara Granosa*) through calcination and a hydrothermal method. It was found that high purity nanoparticle HA can be produced via this simple and cost-effective hydrothermal method. Cockle shells calcined at two different temperatures (450 °C and 800 °C) were selected and prepared as starting material prior the synthesis procedure. The best results in terms of fully crystallized pure HA with a nanorod shape and high hardness value were produced from the HA800 sample. Through this method, formation of other calcium phosphate phases could be prevented. Other impure elements in raw cockle shells were also eliminated. Needle-like and rod-shaped HA nanoparticles with aspect ratios of 20 and 7 were observed for HA450 and HA800 samples, respectively.

The sintering effect has a significant role on the morphological changes which take place to HA due to different crystallization phenomena [13, 18]. However, the processing conditions can have a significant effect on growth morphology of the HA [42]. Processing conditions had a significant effect on the formation of HA and can control the morphology. HA powder synthesized showed high in vitro bioactivity and thus can be an excellent bone substitute and implant coating. As an advantage, cockle shells can be found in bulk to scale up the production of hydroxyapatite. In addition, no further heat treatment, milling or grinding process required to obtain nanorod particles. This nanorod HA can potentially also be used in biosensing applications. Further study in development of porous HA for potential scaffolds and improving of mechanical properties is important.
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References