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Synthesis of bioactive calcium phosphate from cockle shell for biomedical applications

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ABSTRACT

The present work reports the synthesis of bioactive calcium phosphate from cockle shell via the combination of calcination and hydrothermal process. The raw cockle shells were pre-treated with 30 % of hydrogen peroxide for 4 days to eliminate the impurities. Afterward, the dried cockle shells were crushed and calcined at various temperatures ranging from 300 to 1100 °C. Subsequently, the calcined powders underwent hydrothermal process in di-ammonium hydrogen phosphate and distilled water at pH of 10.5 for 30 minutes. Lastly, the hydrothermal treated powders were dried in oven at 50 °C for 3 days. The results showed that the mixture of aragonite, calcite, hydroxyapatite, and calcium hydroxide was successfully synthesized at a calcination temperature of 900 °C and 1100 °C. In addition, the nanorods in the length of 80-300 nm were formed. The findings of this work indicate that the cockle shell could be transformed into valuable bioactive materials for biomedical applications.

Keywords: *cockle shell, aragonite, calcite, hydroxyapatite, calcium hydroxide, biomedical.*

1. INTRODUCTION

Biomaterials is defined as any materials that have been engineered to interact with biological substance for reconstructing the broken hard and soft tissues caused by injury, diseases, ageing or accidents [1]. Over the past decades, biomaterials have been extensively used for repairing ligament and tendons, ophthalmic applications, orthopaedic applications, cancer therapy, wound healing, implants for broken hard tissues, and manufacturing of surgical devices. Generally, biomaterials can be classified into four main groups, which are biologically inactive, bioactive, biomimetic, and biodegradable materials [2].Among these, bioactive materials have been attracted a great of attention owing to its ability to stimulate the biological responses such as hard tissues regeneration [3].

Bioactive materials can be derived from natural resources or synthesized in the laboratory via various techniques [4-6]. Natural resources such as mammalian bones, shell sources, aquatic, marine sources, plants, algae, and mineral sources have been intensively focused in order to enhance their biocompatibility properties[6-9]. Cockle (*anadaragranosa*) is an edible marine bivalve mollusc with a strong ribbed shell. Cockle shells are abundantly available in sandy or muddy areas on beaches with a high wave and current energy all over the world including South East Asian countries such as Malaysia, Indonesia, and Thailand coastal areas. Increasing numbers of cockle shell wastes lead to negative effects to the marine life where the unpleasant smell that comes from the untreated empty shells will cause the air pollution[10]. It is noteworthy to mention that cockle shell is a calcium-rich shell source, which mainly consists of calcium carbonate ($CaCO_3$). The Ca element of cockle shell could be further synthesized and transformed into valuable Ca-based compound such as hydroxyapatite (HAp), tri-calcium phosphate (TCP), calcite ($CaCO_3$), and calcium hydroxide $Ca(OH)_2$ for biomedical applications [11,12].

There has been numerous research studies conducted to transformed cockles shell into valuable bioactive materials. Buasri et al. [13] synthesized calcium oxide (CaO) from cockle shell via calcination methods. High purity of CaO powders were obtained at 1000 ^oC calcination temperature. The diameters of synthesized powders were ranging from 38-75 µm. On the other hand, Azis et al. [14] had successfully synthesized HAp using hydrothermal method at temperature of 140 ^oC for 16 hours. They reported that the specific surface area of synthesized HAp was 17.8 m^2/g. Rizkayanti and Yusuf [15] employed wet chemical precipitation to synthesize HAp. The results show that the optimum temperature to produce high purity HAp was at 40 ^oC. Furthermore, Laonapakul et al. [16] synthesized the bioactive calcium based compounds from blood cockle shell. It was found that the CaO powders were successfully synthesized at calcination temperatures of 600 - 800 ^oC. In short, various calcium based bioactive compounds can be synthesized via different techniques.

Calcite, a polymorphs of $CaCO_3$, is the most thermodynamically stable form compared to other polymorphs of $CaCO_3$ such as aragonite and vaterite. Natural calcite commonly existing in trigonal rhombohedral form. Owing to its thermodynamic stability under ambient conditions, it has been widely used in various biomedical applications [17]. Kumar et al. [18] have proven that calcite is highly biodegradable and bioactive

owing to the dissolve Ca ions from calcite enhance the supersaturation of body fluid.

Hap with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ has the most similar mineral composition with human bone and teeth [19-20]. HAp is a bioceramic which has been widely used as a bone substitute [21-22]. In addition, HAp has a dominant of inorganic phase component that have excellent biocompatibility, bioactivity, slow-degradation and also better osteoconductive, osteointegration, and osteoinduction properties [23]. Due to its excellent mechanical and biological activity responses, HAp gain researchers and manufacturers interest tofurther investigate and widen the application of HAp especially as biomaterial for orthopaedic and orthodontic applications [8].

$Ca(OH)_2$, or known as slaked lime, is obtained by mixing the water with calcium oxide. It has been widely used in endodontic applications such as pulp-capping agents, intracanal medicament, and root canal sealers. On top of that, it has been embedded with antimicrobial formulation for the treatment of root perforations, root resorption and root fractures. The nano formulations of $Ca(OH)_2$ are able to neutralize the bacterial acid metabolites, induce hard tissue formation, antimicrobial effects and inhibit tissue dissolution [24,25].

The aim of this study is to synthesise bioactive compounds from cockle shell using combination of calcination and hydrothermal methods as a single method is not productive to convert the $CaCO_3$ of cockle shell to calcium phosphate. In the present study, the effect of calcination temperatures on the formation of bioactive compounds was investigated for medical applications; particularly in orthopaedics and other surgical subspecialties. Besides, this study can also reduce the environmental impacts caused by the improper disposal of cockle shells.

2. MATERIALS AND METHODS

2.1. Synthesis of calcium phosphate from cockle shell.

The raw cockle shells were obtained from Sungai Lurus, BatuPahat, Johor, Malaysia. The cockle shells were soaked with 30% of hydrogen peroxide (H_2O_2) for 4 days to remove the outer slippery mucus layer (residues). Subsequently, the cockle shells were rinsed with distilled water and all the residual trays that attached to shells were peeled off using brush. Then, the cockle shells were dried by using oven at temperature of 100 °C for 1 days mainly to make sure the cockle shells were dried completely. Next, the cockle shell was crushed using the pestle and mortal and sieved to get the particle size less than 5 µm. After that, the cockle shell powder was calcined in furnace at temperature ranging from 300 to 1100 °C for 3 hours.Next, the calcined powders were mixed with di-ammonium hydrogen phosphate and distilled water at 5:3:1 molar ratio of $CaCO_3$:$(NH_4)_2HPO_4$:H_2O and 32 % of ammonia solution were added until the solutions reach up pH10.5. The mixture was undergoing hydrothermal reaction at 100 °C for 30 minutes. Lastly, the mixture was filtered and washed several times using distilled water prior dried in oven at 50°C for 3 days.

2.2. Characterization.

The phase structures of the powder were determined using X-ray diffraction (XRD) (Bruker diffractometer, Model D8 Advance) with CuKα radiation at 40 kV and 40 mA, and angle of incidence of 1° and a scanning step of 0.02°.Fourier transform infrared spectroscopy, FTIR (Spectrum 100, PerkinElmer) was used to examine the functional groups of synthesized powders.The spectra were recorded for the range of 4000–600 cm^{-1}.The morphologies of powders were examined using field emission scanning electron microscopy (FESEM, JFM- 7600F, JEOL, 15.0 kV).

3. RESULTS AND DISCUSSION

3.1. Phase Structures of Powder.

Figure 1 shows the patterns of the synthesized raw cockle shell and bioactive compounds of cockle shell at calcination temperature of 300-1100 °C and hydrothermal reaction at 100 °C. As can be seen, the peaks of raw cockle shell powder corresponded to a poorly crystallised aragonite phase of $CaCO_3$.Aragonite, polymorphs of $CaCO_3$, is a carbonate material thatcan only be found in precipitation of $CaCO_3$ or on shell. It can be formed through biological (natural) or physical processes. It has been intensively studied by many researchers owing to its excellent biocompatibility and larger surface area. The result obtained is in agreement with the results reported by other researchers [26,27].

For the sample calcined at 300 °C, the intensity and amount of aragonite significantly decreased, and moreover, HAp and calcite were observed to comprise the powder. By increasing the calcination temperature to 500 °C, the greater intensity and amount of HAp and calcite were observed.The same trend was observed in the sample calcined at a temperature of 700 °C, However, the peak of HApwas significantly decreased. Further, at calcination temperature of 900 °C, it can be observed that the intensity and amount of HAp and calcite significantly decrease.Great intensity and amount of $Ca(OH)_2$ were observed in the sample. The same trend also can be observed for the sample calcined at temperature of 1100 °C. Therefore, the following conclusions may be made from the results:

- At calcination temperature ≤ 700 °C, phase transformation of poorly crystallised aragonite $CaCO_3$ into highly crystallised calcite $CaCO_3$. Laonapakul et al. [16] reported that this occured due to the instability of the aragonite $CaCO_3$ at low temperature.
- The formation of HAp is due to the hydrothermal process after calcination. As suggested by Mohamad Razali et al. [28] and Azis et al. [14] the reaction between calcite anddi-ammonium hydrogen phosphate resulted in precipitation of HAp owing to the addition of phosphate ions in the compound. The chemical reaction could be explained as follows:

$$10CaCO_3 + 6(NH_4)2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)CO_3 + 4H_2CO_3$$

- At calcination temperature ≥ 900 °C, the $CaCO_3$ underwent thermal decomposition and transform into calcium oxide (CaO). The results obtained agreed with previous work reported elsewhere [13,28]. Further, the formation of $Ca(OH)_2$ is mainly due to the reaction of CaO with water during the hydrothermal process. Therefore, this can explain why $Ca(OH)_2$ only presented on samples

prepared at calcination temperature ≥ 900 °C. To explain the reactions, Buasri et al. [13]suggested the followings:

$$CaCO_3 + \text{heat (900 °C)} \rightarrow CaO + CO_2$$
$$CaO + H_2O \rightarrow Ca(OH)_2$$

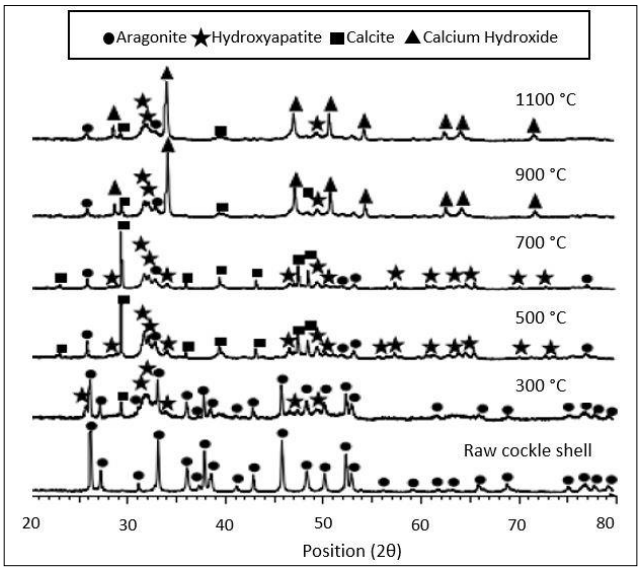

Figure 1. XRD patterns of the raw cockle shell andthe synthesized bioactivecompounds from cockle shell after calcination at temperature 300-1100 °C and hydrothermal reaction at 100 °C.

3.2. Structural Characteristic of Powder.

The FTIR spectrum of synthesized bioactive compounds from cockle shell at calcination temperature of 300-1100 °C is shown in Figure 2. The sample calcined at temperature of 300 °C shows the presence of phosphate (PO_4^{3-}), carbonate (CO_3^{2-}), and broad hydroxide (OH^-) absorption bands, indicating that the carbonated HAp was formed. However, the OH^- absorption band was not observed from the samples calcined at a temperature of 500 °C and 700 °C. With continued calcination temperature up to 900 °C and 1100 °C, a sharp peak of OH^-stretch vibration band appeared at 3643 cm^{-1}, suggesting that the presence of $Ca(OH)_2$, which is in consistent with XRD results [29].

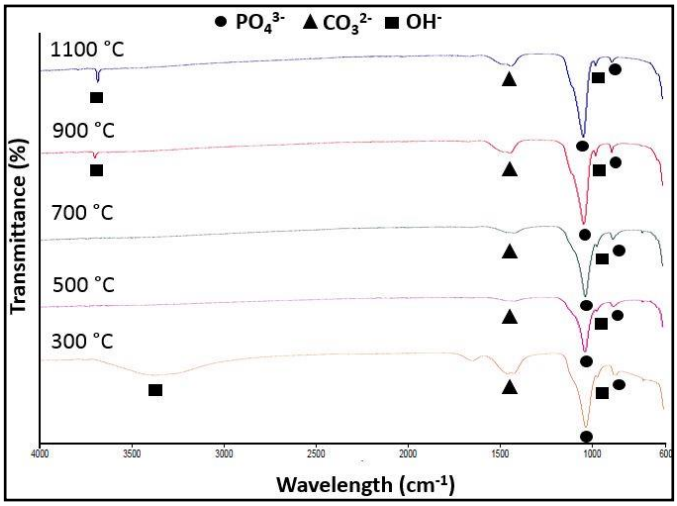

Figure 2. FTIR spectra of synthesized bioactivecompounds from cockle shell after calcination at temperature 300-1100 °C and hydrothermal reaction at 100 °C.

3.3. Morphology of Powder.

Figure 3 shows the FESEM micrographs of synthesized bioactive calcium phosphate from cockle shell at calcination temperature of 300-1100 °C. It can be observed that different morphology forms and size of synthesized powder are formed when the calcination temperature isvaried. At calcination temperature ≤ 700 °C, agglomerated irregular flakes-like shapes were observed. A transition temperature of morphology from agglomerated irregular flakes-like shape to nanorodsshape occurred at calcination temperature of 900 °C. The nanorods with 150-300 nm in length were observed. The change from morphology was corresponding to the formation of calcium hydroxide.As the temperature of calcination increased to 1100 °C, the length of nanorods was significantly decreased to 80-100 nm. According to Raut et al. [30] nano structure of hydroxyapatite (calcium phosphate) improve the hardness and toughness of hydroxyapatite, thus it will be exelent features for the calcium phosphate itself.

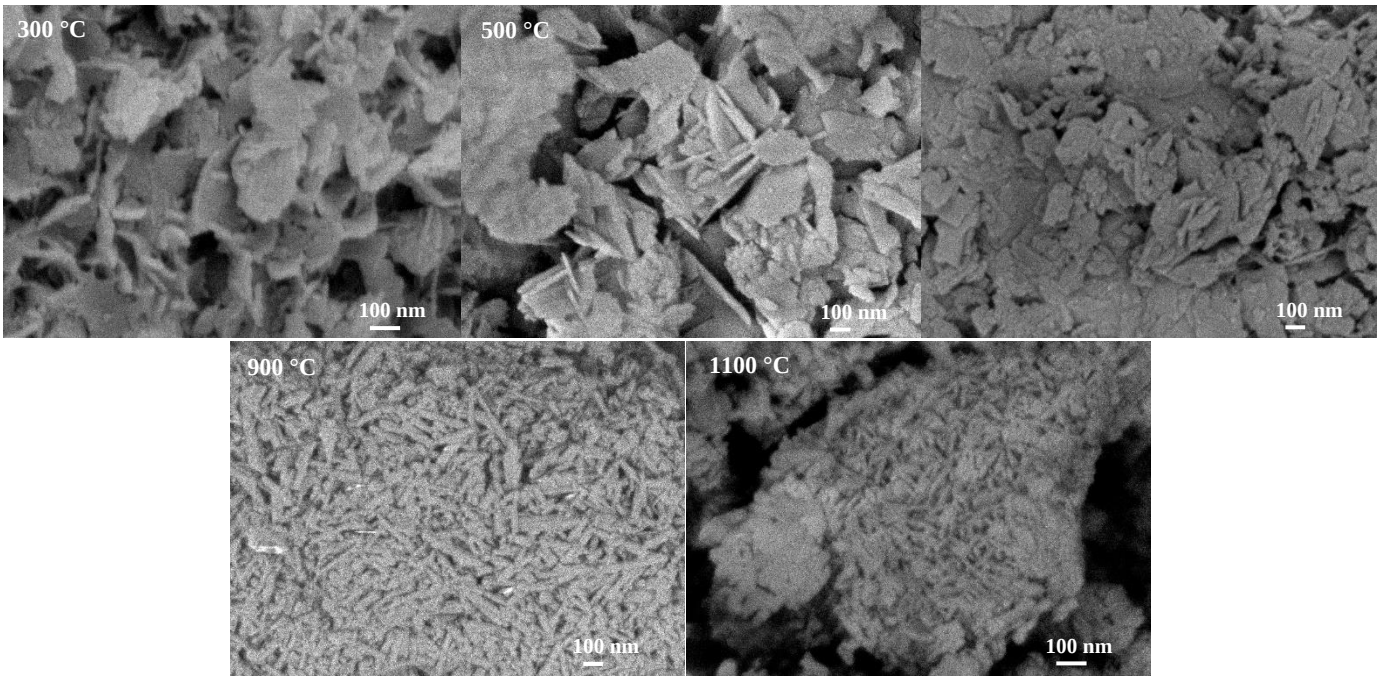

Figure 3.FESEM micrographs of synthesized bioactive compounds from cockle shell at calcination temperature of 300-1100 °C.

4. CONCLUSIONS

In summary, the calcination temperature has significant influence on the phase structure, structural characteristics, and morphology of synthesized bioactive compounds from the cockle shell. At calcination temperature ≤ 700 °C, agglomerated flake-like of aragonite, HAp, and calcite mixture weresynthesized. On the other hand, $Ca(OH)_2$ presence in the mixture when the calcination temperature ≥ 900 °C owing to the reaction of CaO with water during hydrothermal process. Additionally, nanorods with length ranging from 80-200 nm were successfully synthesized at calcination temperature ≥ 900 °C. Further testing such as in vitro and in vivo should be performed to verify the biocompatibility of bioactive compounds synthesized from cockle shell.

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