ORIGINAL ARTICLE

Densification of Calcium Phosphate from Biogenic Waste for Biomedical Application

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ABSTRACT

Introduction: Calcium phosphate-based biomaterial is commonly employed in biomedical applications such as dental applications, bone substitution and filler tissue engineering. Its excellent biocompatibility and potential bio-implant material have attracted many researchers to broaden their hydroxyapatite (HA) studies. **Methods:** The present work used waste eggshells as the source of calcium precursor to synthesize HA via a solid-state reaction. The eggshells were calcined at 700°C and mixed with the phosphate precursor, dicalcium phosphate dihydrate (DCPD). Following this, the mixture was ball-milled at 400 rpm for 2h and then heat treated at 800°C to produce pure eggshell-derived HA powder. The synthesized HA powder was then consolidated by uniaxial pressing (~6.5 – 7.1 tons) and sintered at 4 different temperatures of 1100°C, 1150°C, 1200°C and 1250°C. **Results:** From the XRD analysis of the sintered HA samples, it was found that an increase in sintering temperature up to 1250°C did not affect the phase stability of the HA phase. Besides that, grain size, relative density and hardness of the sintered HA samples were also increased with sintering temperature. It was observed that HA dense sample prepared by compacting at 7.1 tons followed by sintering at 1250°C showed the best combination of mechanical properties among all samples with a relative density of 94.6% and a hardness of 3.7 GPa. **Conclusion:** The current result is significant in supporting the potential of the synthesized eggshell-derived calcium phosphate powder as an ideal alternative for the creation of cost-effective, biocompatible biomaterials for biomedical applications.
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INTRODUCTION

Calcium Phosphate (CaP) was first discovered by Abraham Gottlob, back in 1786. Due to their abundance in nature and existence in living organisms, calcium phosphates have been an interesting substance in many branches of study including biology, chemistry, geology, and medicine (1). The majority of calcium in the human body is stored in bone tissue as nanometric crystals of a calcium phosphate compound with properties resembling those of hydroxyapatite (HA).

In recent years, hydroxyapatite (HA) has been extensively studied due to its excellent biocompatibility and potential for its applications in biomedicine (2). Besides beta-tricalcium phosphate (β-TCP), HA is one of the most popular crystalline phases of CaP with a chemical composition of $Ca_{10}(PO_4)_6(OH)_2$ with a molar ratio of 1.67 for calcium to phosphorus (3) and crystal structure which are near to natural bone (4). Nevertheless, the CaP present in the mineral component of bones is better characterized as a non-stoichiometric, carbonated apatite, and calcium-deficient, which is different from the above formula.

In addition, HA is reported to not cause inflammatory reactions in clinical applications. Hence, studies on bone regenerative applications have been carried out

by mixing HA with soft materials such as polymers to complement the drawbacks (5). Despite that, HA as bioceramics is still lacking in its mechanical properties such as hardness and fracture toughness which is a major drawback for load-bearing applications.

The poor mechanical properties of calcium phosphates which are relatively low tensile stress, brittle, and low impact resistance have restricted the use of CaPs in wider clinical applications such as for load bearing applications (6-7). Despite that, CaPs have good compressive strength, being greater than that of regular bone. As a result, CaP is either employed as a coating on dental and orthopaedic metallic implants or as non-load-bearing implants for procedures including middle ear surgery and filling bone deformities in the oral cavity and skeleton (6).

Generally, HA can be extracted from natural resources and bio-wastes such as eggshells, seashells, coral, and animal bones (8) are non-stoichiometric due to the trace amounts of ions incorporated in its crystal structure, such as Fe^{2+}, Mg^{2+}, Si^{2+} and F^{-}. Therefore, the development of HA from natural resources is of great importance in its usage as bone-like implants. Around 80,000 tons of food waste are reported to be generated every day in Malaysia where eggshell waste contributed to the production of around 70,686 tons due to the large demand for egg consumption, especially in the food industry (4). The disposal of eggshell waste may indirectly cause pollution in landfills and cost a lot of money (9). Therefore, it is a wiser option to make use of the abundant amounts of eggshell waste by recycling. In addition, the high content of calcium carbonate ($CaCO_3$), as well as the presence of trace amounts of ions such as Na^{+}, Sr^{2+}, and Mg^{2+}, in eggshells makes it an attractive waste material to be used as a calcium precursor in the synthesis of HA (10).

Many methods have been employed to prepare HA with improved mechanical properties such as chemical precipitation, hydrothermal, and hydrolysis of other CaP [11-12]. These methods enable control over the morphology, crystallinity, and structure of HA. However, these methods are usually complicated, demand adept adjustment, and are time-consuming. For example, in the hydrothermal approach, the procedures can be carried out at either room temperature or a higher temperature and either normal pressure or high pressure [13]. The main drawback of wet methods is that they might occasionally introduce impurities into the structure of HA or other phosphate phases that are present with HA. Meanwhile, the dry method such as the solid-state reaction produced well-crystallized synthesized HA and was preferred for large-scale synthesis. This method can be conducted by first mixing the calcium and phosphate precursors powders via the mechanical activation milling method and followed by heat treatment.

There are different types of ball mills available to finely grind down powders such as attrition mill, horizontal mill, 1D vibratory mill, planetary mill, and 3D vibratory mill (14). In ball milling, the grinding jar spins in an orbit around the centre, just like in a planetary system. Meanwhile, the secondary arms are connected to the central shaft for attrition mill grinding, which rotates to propel the grinding balls. The grinding container itself is fixed (14). The process is then continued with heat treatment of the powder mixture at 800°C, followed by a sintering process at different temperatures (15). The sintering process can enhance the densification of HA composite and thus improve its mechanical properties (7).

Synthesizing HA by using the dry method such as the solid-state and mechanochemical process is straightforward and inexpensive. The chemical reactions that took place as a result of mechanical impact on solids became more specific and adaptable (16). If the starting powder has better powder qualities such as crystallinity, agglomeration, and morphology and is stoichiometric, dense HA ceramics with superior mechanical properties may be developed. Additionally, in improving the mechanical and biological capabilities of HA-based bioceramic materials, a reduction in grain size to the nanoscale in dense sintered materials is a desired parameter. It was discovered that 1250°C was the ideal sintering temperature for synthetic HA powder, producing the best overall combination of attributes, including a relative density of 97.7%, a Vickers hardness of 5.62 GPa, and outstanding fracture toughness (15).

Besides that, HA has also been prepared from organic calcium sources such as bovine, fish, and porcine bones as well as eggshells, coral, and seashells. The extraction technique used had a significant impact on the characteristics of natural HA. In contrast to synthesized HA, carbonate ions were frequently present in natural HA as an impurity. The naturally occurring carbonated ions in HA seem to be suitable components for bioresorbable bone replacements. The key benefit of obtaining HA from natural sources is that no toxic chemicals are used in the extraction procedure (17).

It is believed that with a proper processing regime, well-crystallized high-density sintered HA with enhanced mechanical properties can be produced. Hence, this study aims to prepare HA using eggshell waste in the form of calcium carbonate as Ca precursor via ball milling followed by heat treatment at a predetermined temperature. The sinterability of these compacted HA powders at 6.5 MPa will be investigated at different sintering temperatures ranging from 1100 °C to 1250 °C. In addition, 7.1 MPa compaction pressure was also used for the sample to be sintered at 1250 °C, for comparison purposes. Subsequently, the densification and mechanical behaviour of sintered eggshell-derived HA samples will be determined to investigate the effect

of various sintering temperatures on the phase stability and mechanical properties of eggshell-derived HA samples.

MATERIALS AND METHODS

In this study, the as-synthesised solid HA will be obtained through the solid-state route. The process is divided into two parts, the first part is the synthesis of eggshell-derived HA powder, followed by the preparation of HA green samples and its sintering regime.

Process 1: Synthesis of eggshell-derived HA powder
To synthesize HA from biogenic calcium carbonate, the food waste of eggshells was collected from local stalls. The eggshells were cleansed thoroughly with water to eliminate any remaining protein membrane layer and dried in a box oven overnight at 60 °C. Next, a mortar and pestle were used to crush the dried eggshells into a fine powder before being subjected to calcination at 700 °C for 2 hours to burn off any remaining volatile substances. The calcined eggshell powder will then be mixed with a phosphate precursor, dicalcium phosphate dihydrate (DCPD), at a molar ratio of 1.67 for calcium to phosphorus via a magnetic stirring process. Then the powder mixture was dried at 90 °C for 24 hours in the oven. Following that, the powder mixture was ball milled with a speed of 400 rpm and variable milling time of 2h with a one (1) time repetition. The resultant powder mixture was then sieved through a 212 µm mesh size to ensure a uniform size of powder mixture was obtained. Furthermore, the powder mixture was calcined at 800 °C to produce pure eggshell-derived HA powder.

Process 2: Preparation of HA green samples for sintering
The HA green samples were prepared by consolidating the eggshell-derived HA powder using uniaxial pressing with compaction pressure of 6.5 tons and 7.1 tons (only for one sample sintered at 1250 °C, for comparison purposes) followed by sintering at temperatures of 1100°C, 1150°C, 1200°C, and 1250 °C with a heating and cooling rate of 2°C/min conducted in an air atmosphere. These sintered samples are later described respectively to their sintering temperature namely ES-1100, ES-1150, ES-1200, ES-1250A (6.5 tons) and ES-1250B (7.1 tons).

Subsequently, thermal analysis was run for the powder mixture of calcined eggshells and DCPD using thermogravimetric analysis (TGA) to study its phase stability and reaction temperature. As for the phase stability, the eggshell-derived HA powder will be characterized using an X-ray diffractometer (XRD) (Rigaku Ultima IV). The determination of the specific surface area of the sintered powders was accomplished through BET analysis, utilizing a Micrometric 3Flex 3500 Adsorption Analyzer instrument. In this analysis, nitrogen gas adsorption facilitated the calculation of specific surface area, pore size distribution, and pore volume. Meanwhile, for the sinterability, the morphological characterization of the sintered HA samples will be evaluated using a Field Emission Scanning Electron Micrograph (FESEM) (Jeol JSM-7600F) instrument. Besides that, the hardness of all the compacted samples will be measured via the Vickers indentation method, whilst their bulk density will be measured using Archimedes Principle concerning the theoretical density of HA (3.156 gcm^{-3}) that will be used to calculate their relative density.

RESULT

The phase stability and reaction temperatures for the mixture of calcined eggshell and DCPD powder were determined via thermal analysis. Fig. 1 presents the TGA analysis of the untreated powder mixture. It can be observed that during the heating regime starting from room temperature to 40°C, there was a weight loss of 1.53%. It is believed that the weight loss was caused by the loss of moisture from the surface and the pores of the powder mixture. When the temperature was raised to 470°C, there was a further decrease of 2.98% in weight which was assumed to be linked with the loss of dehydrated water in the lattice. A partial reaction between $CaCO_3$ and $CaHPO_4 \cdot 2H_2O$ has occurred, as proven by the weight loss that was noticeable (approximately 10.50%) during the temperature range of 630°C to 720°C. The release of carbon dioxide from the decarboxylation of $CaCO_3$ is accountable for this weight loss. The minor weight loss above this temperature thus served as evidence that the reaction between $CaCO_3$ and DCPD had completed at around 750°C.

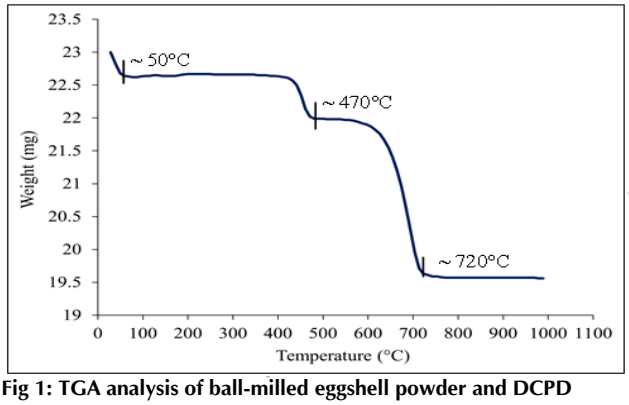

Fig 1: TGA analysis of ball-milled eggshell powder and DCPD mixture.

Meanwhile, the XRD pattern of the heat-treated synthesized HA powder and sintered HA disc samples are displayed in Fig. 2. In Fig. 2(a), sharper peaks are visible in the XRD pattern of the heat treated powder sample, which denotes high crystallinity of synthesized HA powder. The transformation reaction to phase pure HA was accomplished with no β-TCP peaks, which indicates that the HA phase was thermodynamically stable as the calcination temperature increased to 800°C (17). The peak positions of the samples are in good accord with the 09-0432 Joint Committee on Powder Diffraction Standards (JCPDS) card number proving that

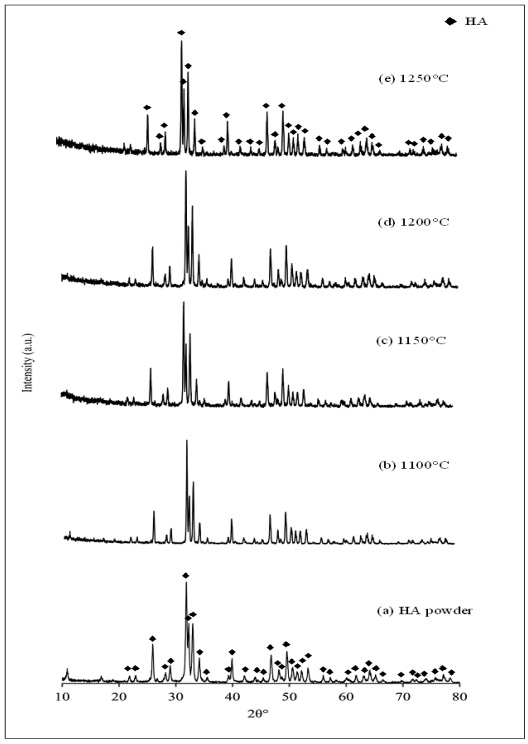

Fig 2: XRD patterns of (a) eggshell-derived HA powder and HA sampes sintered at (b) 1100°C , (c) 1150°C ,(d) 1200°C, and (e) 1250

the synthesized HA powder contained a phase of pure HA.

Meanwhile, all XRD peaks of sintered HA samples in Fig. 2b to Fig. 2d matched those in the JCPDS Card Number 09-0432 for HA, thus indicating that a phase of pure HA was obtained. This result demonstrated that the stability of the HA phase was not compromised when sintered between 1100°C and 1250°C. Nonetheless, HA phase decomposition may happen due to further sintering at 1300°C and 1350°C, forming minute amounts of α-tricalcium phosphate (α-TCP) and tetracalcium phosphate (TTCP) (15).

In addition, Fig. 3 shows the FESEM images of sintered HA samples. It was observed that at 1100°C, sintered HA was less dense with the presence of pores and had the smallest average grain size of 0.86 ± 0.02 µm as depicted in Fig. 3a and Fig. 3c. However, when the sintering temperature increased from 1150 °C to 1250 °C, the samples exhibited more densely packed microstructure. However, even though both ES-1250A and ES-1250B samples (Fig.5d & 5e) were sintered at the same temperature, sample ES-1250B has a denser packed microstructure as it was consolidated with higher pressure (7.1 tons) which giving it average grain size of 2.03 ± 0.02 µm than ES-1250A which was compacted with a pressure of 6.5 tons with 1.74 ± 0.02 µm of average grain size.

This finding is in agreement with the surface area of the sintered HA powder samples. From the BET results, it becomes evident that the surface area of the ES-1250B

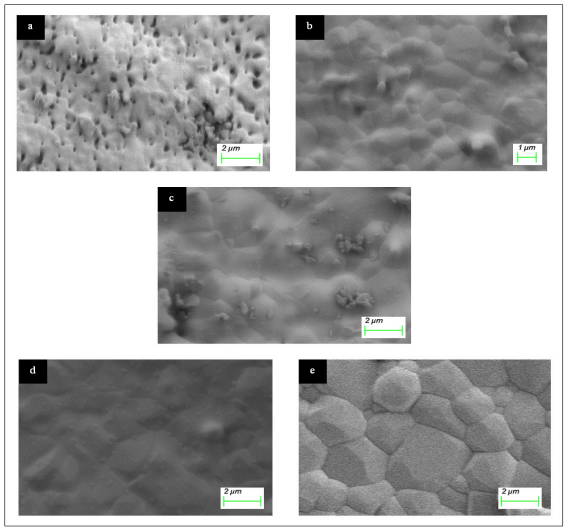

Fig 3: FESEM images of eggshell-derived HA samples sintered at different sintering temperatures: (a)1100°C, (b)1150°C, (c)1200°C , (d)1250°C (6.5 tons) and (e) 1250°C (7.1 tons)

sample outdoes that of ES-1100, recording values of 45.020 m²/g and 28.733 m²/g, respectively. This difference implies that the powder particles in the ES-1100 sample were much smaller than the particles in ES-1250B. This observation aligns with the expected behaviour, as higher sintering temperatures tend to promote particle growth as confirmed in the FESEM images analysis. Similarly, the total pore volume within the ES-1100 sample significantly outpaces that of ES-1250B, with respective values of 0.105 cm³/g and 0.006 cm³/g. Meanwhile, Fig. 4 shows the important correlation of relative density, Vickers hardness and grain size of HA samples at various sintering temperatures.

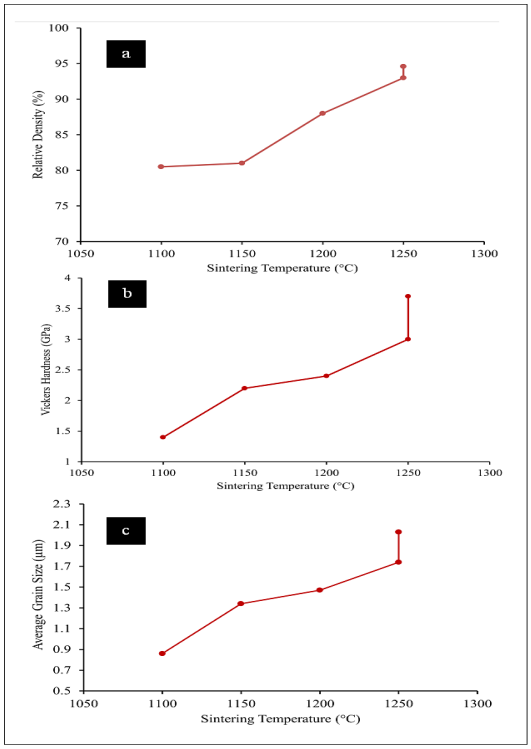

Fig 4: The effect of sintering temperature on (a) relative density, (b) Vickers hardness and (c) average garin size of sintered HA samples

DISCUSSION

It was observed that findings for the relative density of HA Fig. 4(a) at various temperatures agreed with the FESEM images (Fig. 3) as there was a constant increase of relative density with sintering temperature, which coincides well with the densely packed microstructure of the solid HA samples as the sintering temperature rises from 1100 °C up to 1250 °C (15). The trend increases from having a relative density of 80.5 % when it was sintered at 1100 °C to 94.6 % when the sample was compacted with a pressure of 7.1 tons and subsequently sintered at 1250 °C. Moreover, a previous study reported that a constant increase in density was seen as the sintering temperature was increased up to 1350 °C. The findings suggested that although the relative densities of the samples sintered at 1300 °C and 1350 °C were generally high, in the range of 98-99%, the number of secondary phases observed by XRD in these samples must be negligible (15).

It was noted that grain size imparts a significant impact on the sintered sample hardness. The hardness and the average grain size of the dense HA samples were found to be increasing with sintering temperature as depicted in Fig. 4b and Fig. 4c. This observation demonstrates the correlation between the densification of HA samples with its hardness. At 1250°C, the highest hardness value is attained for the ES-1250B sample with a value of 3.7 GPa. ES-1250A which was sintered at the same sintering temperature as ES-1250B had a 3.0 GPa value of hardness as it was compacted with a lower pressure value than ES-1250B. Meanwhile, the lowest value of hardness among the five dense HA samples was obtained for ES-1100 with a hardness of 1.4GPa. Hence, it can be generalized that pressing pressure applied during the preparation of green samples also plays a vital role in their densification.

Nevertheless, a previous study conducted by Ramesh et al. (2016) reported a similar trend for the result of eggshell-derived HA sintered from 1100°C to 1250°C although a different processing method (attrition milling) was used [15]. Notably, the ball milling process employed in this study has successfully produced pure eggshell-derived HA-sintered samples with comparable properties.

CONCLUSION

Pure HA samples were successfully prepared using discarded eggshells as a direct source of calcium precursor via a solid-state sintering technique. After preheating the powder mixture of calcined eggshell and DCPD at 800°C, a pure phase of highly crystalline synthesized HA powder was obtained. Besides that, the phase stability of the HA samples was not disrupted at all sintering temperatures. The sintered HA samples were also observed to have greater grain size than the sintering temperature. A similar trend was also observed for both the densification and hardness of the sintered HA samples. In addition, the result obtained showed that pressing pressure applied onto the samples also plays a vital role in their densification. Overall, the study showed that ES-1250B sintered at 1250°C showed the best mechanical properties among all samples with the highest relative density of 94.6% and a hardness of 3.7 GPa. This result is significant in expanding the potential of the synthesized eggshell-derived calcium phosphate powder as an ideal alternative for the creation of cost-effective, biocompatible biomaterials for biomedical applications. This approach not only offers economic advantages but also aligns with sustainable and environmentally friendly practices.

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