



# Phase Stability and Morphological Evolution of Eggshell-Derived Hydroxyapatite Sintered at Different Sintering Temperatures

Mardziah Che Murad<sup>1\*</sup>, Ramesh Singh<sup>2,3</sup>, Nik Rozlin Nik Masdek<sup>1</sup>, Natasha Ahmad Nawawi<sup>1</sup>

<sup>1</sup>School of Mechanical Engineering, College of Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, MALAYSIA.

<sup>2</sup>Centre of Advanced Manufacturing and Materials Processing, Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, MALAYSIA.

<sup>3</sup>Mechanical Engineering Program Area, Faculty of Engineering, Universiti Teknologi Brunei, BRUNEI.

\*Corresponding Author (Tel: +60192771403, Email: mardziah31@uitm.edu.my).

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## Abstract

This study aims to investigate the morphological and grain size evolution of eggshell-derived hydroxyapatite (HA-Es) dense bodies after sintering at elevated temperatures. Prior to the fabrication process, the starting HA-Es powders were synthesized through a simple chemical precipitation technique using eggshell calcium and ammonium hydrogen phosphate as their precursors. The obtained powder was uniaxially pressed into cylindrical shape compacts, before being subjected to sintering at 1100, 1200 and 1250°. The XRD patterns confirmed that HA exists as a single phase even after sintering at 1250°C, indicating that it is thermally stable at high temperatures. In addition, a microstructural study via Field Emission Scanning Electron Microscopy (FESEM) showed that the densification of the HA-Es dense body progresses as the sintering temperature increases, which in turn increases its relative density and mechanical property. Sintering at 1250°C apparently resulted in a significant improvement of the compressive strength of the sintered HA by approximately 10%. These findings suggest that eggshell-derived HA has promising physical and mechanical properties for the development of hard tissue implants using biogenic waste material as one of its starting materials.

**Discipline:** Material Science

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# 1 Introduction

For many years, calcium phosphate (CaP) based biomaterials have been used in the biomedicine field, especially as bone repair material and replacement as well as dental implants due to their close similarity to the mineral component of bone (Sinusaite et al., 2021). CaP ceramics are mostly bioactive (such as hydroxyapatite and A-W glass-ceramic) or bioresorbable (such as tricalcium phosphate and calcium sulphate), which is why they are used extensively in the bone-tissue engineering field. One of the benefits of CaP biomaterials is that their biodegradability can be tailored accordingly to match the bone growth rate inside the human body. They are non-toxic, biocompatible, and have good osteoconductive properties, which allow them to integrate into the host bone without much difficulty (Ebrahimi & Botelho, 2017). Based on composition, synthetic calcium phosphates presently used as biomaterials are classified as calcium hydroxyapatite (HA), alpha- or beta-tricalcium phosphate, biphasic calcium phosphates, and unsintered apatites or calcium-deficient apatites.

The development of HA started in the 1950s and it is considered one of the most widely used types of calcium phosphate ceramic in the biomedical field. Numerous research works related to this bioceramic material are still being carried out progressively to this day. HA can be produced via various types of dry, wet, and high-temperature methods, using either high-grade chemical reagents or natural source precursors (Mardziah et al., 2021). The characteristics of the resulting HA particulates prepared from different synthesis methods and calcium sources would typically result in different mechanical and biological properties of HA. Even though the use of natural precursors to produce HA is considered new, current evidence has shown that the overall properties (mechanical and biological) of naturally derived HA are comparable to those produced using commercially available HA.

This present study aims to fabricate HA-dense bodies by using HA powders that are developed using waste chicken eggshells (HA-Es) as their calcium source. These powders are primarily synthesized through a simple precipitation method at controlled experimental conditions. Subsequent to oven drying, grinding and sifting, the HA-Es powders are uniaxially pressed using a hydraulic pressing machine, producing a cylinder shape compact. These green compacts are then sintered through conventional pressureless heat treatment in a thermolyne muffle furnace at different sintering temperatures under an air atmosphere. The sintering behaviour of HA-Es bioceramic is studied in order to evaluate its phase stability, and physical and mechanical properties after being subjected to a consolidation process at high processing temperature.

## 2 Literature Review

### 2.1 Development of HA Using Natural Origin Calcium Source

Natural HA can be produced from various biological sources such as mammalian bones, plants, and marine and biogenic wastes. Some of these natural resources can be converted to HA

directly, while some others are used as raw materials in the synthesis process of HA. Preparing HA using calcium from a natural source, especially from bio-waste, has now become a preference among many researchers due to its simplicity and cost-effectiveness. Fabrication of HA from bio-wastes is also more advantageous to the environment by giving out less carbon print (Oktar et al., 2017).

One study by Mondal et al. (2016) showed that Tilapia fish (*Oreochromis mossambicus*) scales derived from HA porous scaffold were successfully fabricated through a solvent casting technique, which has similar composition and structure to human bone tissue. The HA powders used to fabricate the scaffold were initially synthesized by thermal decomposition of the chemically treated fish scales at 1200°C. Mechanical and biological evaluations on the porous scaffold proved that it has mechanical properties comparable to human cancellous/cortical bone with excellent bioactivity. The Vickers hardness was recorded as 0.78 GPa at 35% porosity, compressive strength of 0.52 GPa and 190 MPa tensile stress after being sintered at 1200°C. On the other hand, in vitro and in vivo assessments indicate that the HA scaffolds are non-toxic and have good osteoconductive properties. From the histological analysis, the scaffolds are also shown to facilitate new cell growth on their surface, suggesting that they can be a promising candidate as a tissue augmentation substitute material with the load-bearing application.

Another group of researchers (Deb & Deoghare, 2019) also developed pure HA powders using fish scales but from a different type of fish known as *Puntius Conchoniis*. Prior to HA conversion at high temperatures, the fish scales were first pre-treated with acid, alkali and acid-alkaline for deproteinization. All of these pretreatment processes were carried out separately for comparison. The deproteinized fish scales were then dried at 50°C in a hot air oven for 10 hours after thorough washing with distilled water. To obtain HA powders, the fish scales were calcined at 900°C for 3 hours with a heating rate of 10°C/min, then left in the furnace to cool. XRD analysis confirmed the stability of the HA phase at 900°C and the formation of high crystalline powders at the same temperature for all three samples that underwent the pretreatment processes.

Nunez et al. (2018) studied the physicochemical properties of natural HA prepared by using different types of feedstock namely eggshell, clamshell and mussel shell. The HA powders were synthesized via the wet precipitation method using these feedstocks as the calcium precursor. The XRD and FTIR results showed that the resultant HA powders were composed of the HA phase only and were in a highly crystalline state. All powders have rod-like shape particles and the size is approximately 20 to 87 nm in diameter. This study proves that natural feedstock like eggshells, clam shells and mussel shells can produce high-quality HA powders just like those synthesized using synthetic chemical reagents. Not only are these bio-wastes available in large quantities, but the calcium content can also be extracted through a relatively uncomplicated and simple method. Therefore, it can be concluded that economically feasible HA nanopowders can in fact be developed at a large scale using these calcium-rich bio-wastes as feedstock.

Research conducted so far has shown a positive indicator that these natural resources can be easily converted into natural HA or calcium precursors for the synthesis of pure and thermally stable HA. In addition, HA synthesized from natural raw materials or bio-waste has more advantages than synthetic HA because it often contains beneficial ions, which are also found in the natural bone. These ions play an important role in bone growth and can be beneficial in the overall physiological functions of the body systems after implantation.

## 2.2 Sintering Behaviour of HA Bioceramics

With technological advancement, HA powders can now be translated into various physical forms such as particulates, granules, and dense and porous scaffolds for the application of bone grafting or tissue regeneration. Many improvements in synthetic HA have been made through the modification of its synthesis, fabrication method and thermal treatment with the intention of refining its mechanical and/or biological properties to enable a more diverse use for clinical applications. However, some of the approaches used to produce consolidated parts of HA sometimes require processing at high temperatures, which may deteriorate its chemical stability and subsequently affect its overall properties. According to Ruys et al. (1995), sintering of HA is pretty complicated because HA is a dehydrated phase that decomposes into other anhydrous calcium phosphate phases like TCP at around 1200°C to 1450°C. Thus, it is important to evaluate the phase decomposition and densification behaviour of HA bioceramics to identify any formation of undesirable phases after carrying out the sintering process at elevated temperatures.

The sintering of ceramic material can be divided into initial, intermediate, and final stages. At the initial stage, bonding between particles is formed along with significant neck growth but the densification is only partial. Substantial densification of the powder compact takes place during the intermediate stage whereby the solid-porous phase is more connected. At the final stage, the grain growth is significant, in which the solid phase becomes fully connected but the pores are isolated. During this stage, the microstructure development is controlled by the interaction between pores and grain boundaries (Bordia et al., 2017). Other than the conventional pressureless method, numerous sintering techniques such as microwave sintering, hot isostatic pressing and spark plasma sintering have also been investigated by many researchers to produce bioceramics with fine grains, which subsequently improve the densification of HA.

## 3 Method

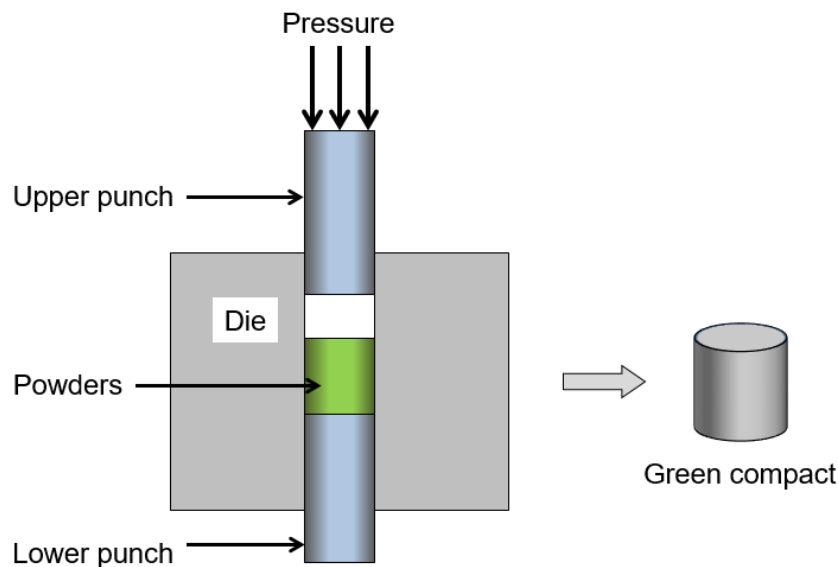
### 3.1 Materials

For the synthesis process of HA-Es powders, ammonium di-hydrogen phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ) (R&M Chemicals) and eggshell calcium were used as the starting precursors. Each precursor was measured accurately according to a Ca/P ratio of 1.67, as per stoichiometric HA. In order to increase the solution's pH to over 10, an ammonia solution ( $\text{NH}_4\text{OH}$ ) from R&M Chemicals was utilized. The solution mixture was continuously stirred for 3 hours while the temperature during synthesis was

kept at 90°C. After that, the suspension was allowed to age overnight for a complete reaction. The aged suspension was filtered and then dried in the oven at 100°C for 24 hours.

### 3.2 Preparation of HA-Es Green Compacts

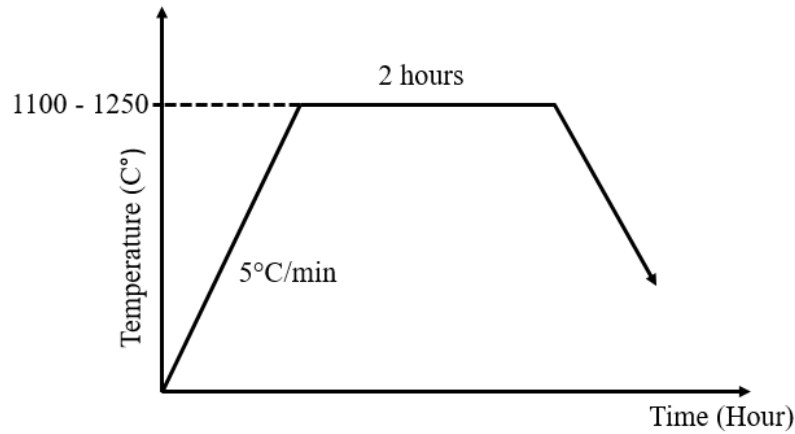
To prepare for the green compacts, the ground and sieved HA-Es powders were pressed into a cylindrical shape using a uniaxial pressing method with approximately 3-tonne force. The diameter-to-height ratio of the injected cylindrical samples was approximately 1:1.5. These green compacts were later sintered in a furnace at different sintering temperatures using a conventional sintering technique. An illustration of HA powder compaction using a uniaxial pressing machine is presented in Figure 1.



**Figure 1:** Diagram of HA-Es powder compaction via uniaxial pressing machine.

### 3.3 Sintering of HA-Es Bioceramic

Sintering is typically a final step in the ceramic fabrication process which converts loose powder compacts into consolidated parts. During sintering, individual powder particles start to bond together, therefore allowing the removal of the porosity in the green compacts. For this particular study, consolidation of the powders was carried out at 1100, 1200 and 1250°C for comparison. Other parameters such as heating rate and soaking time were set at a constant of 5°C/min and 2 hours, respectively. Figure 2 shows the graph of temperature versus time for the sintering process of the HA-Es green compacts.



**Figure 2:** Graph of temperature versus time for the sintering process of HA-Es bioceramic.

### 3.4 Characterization of Sintered HA-Es

Phase analysis was carried out using an X-Ray diffraction machine (Rigaku Ultima IV) with a scan speed of 5°/min and a step size of 0.05°. Peaks that correspond to the HA phase were assigned according to the International Centre for Diffraction Data (ICDD) PDF Card No. 01-079-5683. The morphology of the as-dried powders and sintered samples was evaluated using transmission electron microscopy (TEM) (FEI Tecnai G2 20S Twin) and Field Emission Scanning Electron Microscopy (FESEM) (FEI Quanta FEG450), respectively. Prior to the microstructural examination, both HA powders and sintered samples were first platinum coated using a sputter coater (Polaron, SC7620) to prevent the charging effect.

The average grain size of the sintered samples was determined by adapting the quantitative metallographic technique using FESEM images in accordance with the ASTM E112 standard. Using the linear intercept method, multiple measurements on the FESEM micrographs were recorded and the average values were calculated. The bulk density of the sintered samples was measured by using a density determination kit (A&D, EJ-303) based on Archimedes' method. The calculated values were then compared to 3.16 g/cm<sup>3</sup>, which is the theoretical density of stoichiometric HA. On the other hand, the compressive strength was measured on the dense samples using Universal Testing Machine (Instron) using a 5 kN load and at a crosshead speed of 0.5 mm/min.

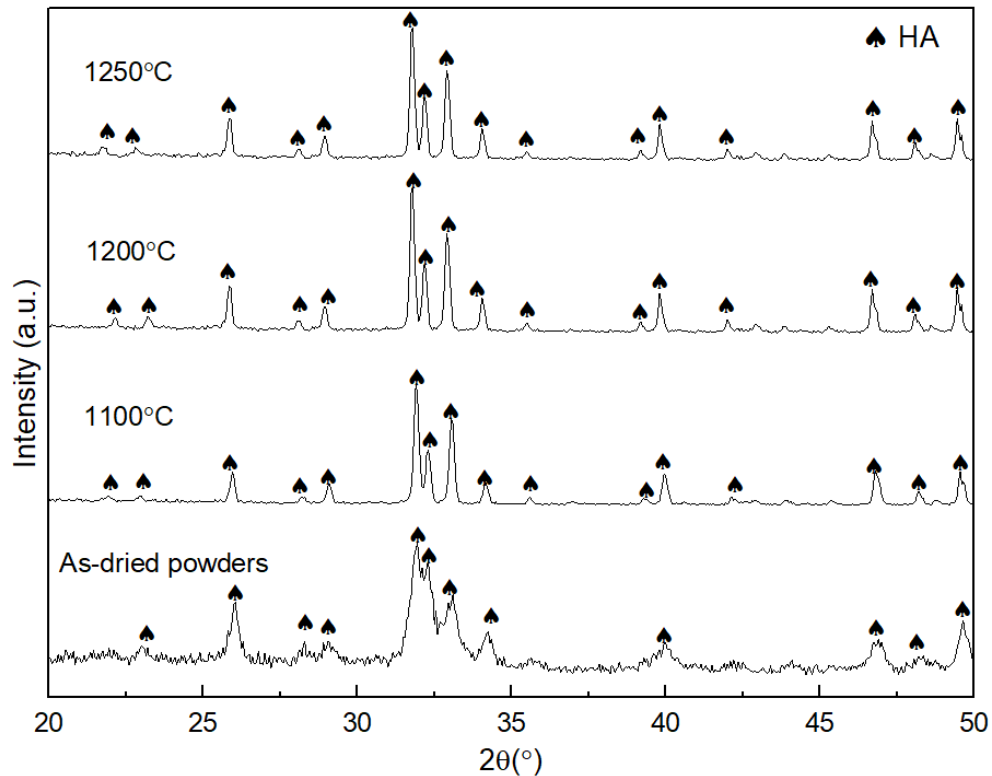
## 4 Result and Discussion

### 4.1 Phase Stability

The XRD patterns of dense HA-Es after sintering at different temperatures are shown in Figure 3. The intensity of the main peaks that correspond to the HA phase at around 32-35° is high for all samples, signifying that it remains as the sole phase with no secondary phase being detected up until sintering at 1250°C. Decomposition of HA typically occurs beyond the critical point of dehydroxylation at around ~1200-1450°C (Ruys et al., 1995). These temperatures are known as decomposition temperatures and they may differ depending on the initial HA powder characteristics. According to Ou et al. (2013), CaO is expected to form at high temperatures due to the HA decomposition of  $\beta$ -TCP and TTCP, accompanied by a non-uniform distribution of calcium and phosphorus throughout the material. They claimed that the formation of CaO could start as



early as 1100°C, but become more prominent when the temperature increases. However, in this present study, there was no indication of the CaO characteristic peak in the XRD patterns for all samples, even at the highest sintering temperature applied in this study (1250°C). CaO is known for its possible toxic effect on cells which may reduce the biocompatibility of HA (Ramesh et al., 2016). Therefore, the presence of its residual is typically undesirable in calcium phosphate bioceramics.



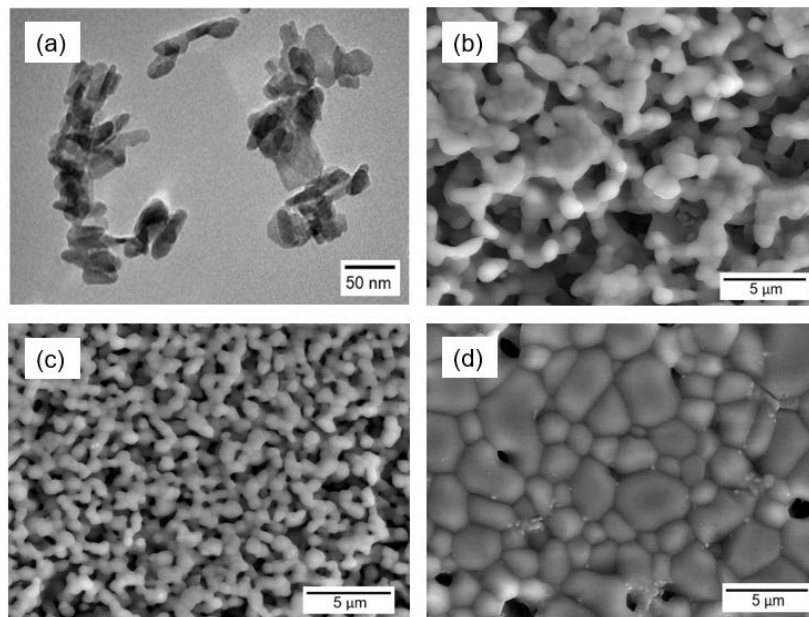
**Figure 3:** XRD patterns of HA-Es bioceramic. sintered at different temperatures.

## 4.2 Morphological Evolution

The TEM image in Figure 4 (a) displays the morphology of the HA-Es powder particles after drying at 100°C for 24 hours. It shows that the particles have a rod-like shape of approximately ~50 nm in length and ~10 nm in diameter. Previous research reported that nanoscale HA is more desirable in clinical applications due to its significant improvement properties over larger-size HA (Poinern et al., 2013; Sadat-Shojai et al., 2013). Moreover, the features of smaller HA particles closely mimic the features of natural bone during biomineralization (Cai et al., 2007). Not only nanocrystalline HA interacts better with the surrounding bone tissue, it also demonstrates better densification and sinterability, resulting in improved mechanical performances (Lin et al., 2014; Sadat-Shojai et al., 2013).

On the other hand, the microstructural evolution of HA-Es samples after undergoing a consolidation process is depicted in the SEM images of Figure 4 (b, c and d). The sintering temperatures were varied at 1100-1250°C because these temperatures are often characterized by a significant microstructural change in HA. Therefore, it is appropriate that the sintering study was carried out around these temperature ranges in order to evaluate the densification behaviour of HA-Es bioceramics. From the SEM images, it is clear that the HA-Es sample sintered at 1100°C and

1200°C still contains high porosity, most likely because sintering at this temperature merely induces necking between particles but could not provide sufficient driving force for effective consolidation. However, the densification of HA-Es at 1250°C remarkably improves, as demonstrated by the increasing contact between grains and decreasing size of pores. This indicates that the interaction between pores and grain boundaries at this temperature is quite strong (Bordia et al., 2017). Nonetheless, there are still residual porosities that can be observed in a form of closed pores, but it is postulated that this only has minimal effect on the overall strength of the sintered sample.

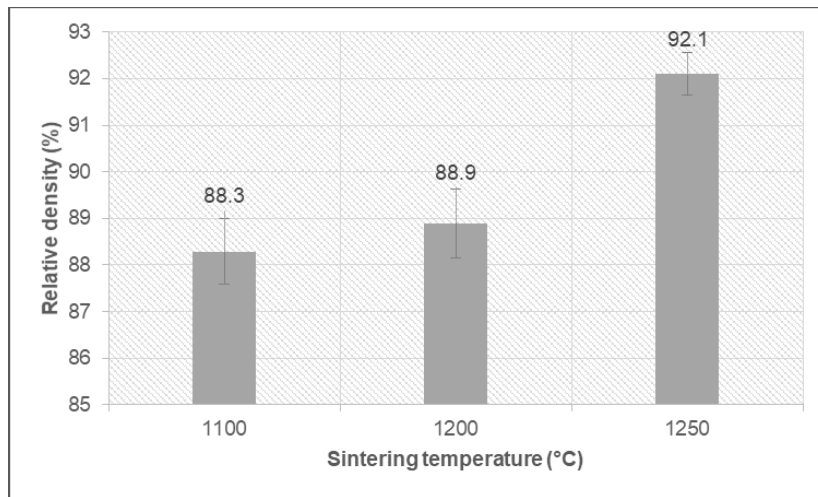


**Figure 4:** (a) TEM image of the HA-Es as-dried powders. SEM images of dense HA-Es sintered at (b) 1100, (c) 1200 and (d) 1250°C.

### 4.3 Relative Density

The difference in the relative density of all samples at various sintering temperatures is presented in Figure 5. It was found that the calculated relative density for the samples sintered at 1100°C and 1200°C was 88.3% and 88.9%, respectively, which is less than 90%. However, the relative density of the sample increases to over 90% when the sintering temperature reaches 1250°C. The trend in relative density values correlates with the SEM images which show that the microstructures of the samples sintered at 1100°C and 1200°C were composed of many voids and undiffused particles, suggesting that they only went through partial densification. On the contrary, the microstructure of specimens sintered at 1250°C was denser and more closely packed, therefore, it is to be expected that its bulk density is higher than the other two samples.





**Figure 5:** Relative density of sintered HA-Es at different sintering temperatures.

#### 4.4 Compressive Strength

Table 1 presents the relationship between sintering temperatures and compressive strength for all samples. Sample sintered at 1100°C has the lowest compressive strength with  $60.3 \pm 0.48$  MPa. In contrast, raising the sintering temperature to 1250°C resulted in the highest average compressive strength of  $89.5 \pm 0.11$  MPa, about a 10% increase compared to the samples sintered at 1200°C ( $81.4 \pm 0.25$  MPa). The measured compressive strength in this present study is considered low compared to the strength of the human cortical bone which is around 130-180 MPa (Prakasam et al., 2015). However, it should be noted that bone ingrowth after 3-6 months of implantation typically would increase the mechanical strength, resulting in improved *in vivo* mechanical performance. Thus, it would be appropriate to report that the HA-Es dense sample produced in this research is quite encouraging. Nonetheless, it is hypothesized that the strength of HA-Es can be further improved by the incorporation of metal ion dopants such as strontium, magnesium, zinc, manganese and iron. Therefore, future works on metal ion-doped HA-Es should be done to investigate the effect of the dopants on their overall properties.

**Table 1:** Compressive strength values of HA-Es samples after sintering at different temperatures.

Sintering temperature (°C)	Compressive strength (MPa)
1100	$60.3 \pm 0.48$
1200	$81.4 \pm 0.25$
1250	$89.5 \pm 0.11$

## 5 Conclusion

In this work, HA-Es nanopowders were successfully prepared through the precipitation method using calcium precursors derived from waste eggshells. These powders were oven dried at 100°C, uniaxially pressed then subjected to pressureless sintering at different temperatures (1100, 1200 and 1250°C) to study their thermal stability and physical and mechanical properties. Based on the findings in the present work, it was determined that HA-Es is thermally stable at every sintering temperature. Up until 1250°C, HA remained as the only phase since no other secondary phases such

as  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) or tetracalcium phosphate (TTCP) were detected. It was also revealed that HA-Es sintered at this temperature exhibited the highest compressive strength with  $89.5 \pm 0.11$  MPa. The improvement in the compressive strength was most likely attributed to its low percentage of porosity as well as close-packed microstructure due to more enhanced densification, which ultimately resulted in better mechanical performance.

This research provides insight into the feasibility of using natural calcium sources obtained from waste eggshells as a starting precursor in the development of HA bioceramics as a bone substitute or hard tissue implant in the biomedical field.

## 6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

## 7 Acknowledgement

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**Mardziah Che Murad** is a Senior Lecturer at School of Mechanical Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam, Malaysia. She holds a Master of Science in Materials Engineering at International Islamic University of Malaysia and received her PhD degree in Mechanical Engineering from University of Malaya, Malaysia. Her research focuses mainly on the Development of Calcium Phosphate ceramics for Biomedical Applications.



**Ramesh Singh** is a Senior Professor at Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, Malaysia. He received his Bachelor, Master Degree and PhD in Mechanical Engineering from University of Sunderland, United Kingdom. Some of his significant research works include the development of an aging resistant zirconia as well as fabrication of a novel nanostructured hydroxyapatite bioceramic derived from eggshells and bovine bones for orthopedic applications.



**Nik Rozlin Nik Masdek** is currently serving as a Senior Lecturer at the School of Mechanical Engineering, Universiti Teknologi MARA. She received her Bachelor Degree in Biomedical Engineering and Master of Science in Mechanical and Materials Engineering from University of Malaya, Malaysia. She obtained her Ph.D from the University of British Columbia, Canada. Her research interests include Corrosion, Nanocrystalline Coating and Electrochemical Processes.



**Natasha Ahmad Nawawi** is a Senior Lecturer at School of Mechanical Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam, Malaysia. She holds a Master of Science in Materials Engineering at International Islamic University of Malaysia and received her PhD degree in Mechanical Engineering from University of Malaya, Malaysia. Her research focuses mainly on the Development of Hydroxyapatite and Metal-Doped Calcium Phosphate for Biomedical Applications.

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