Original Article

Evaluating synthesis of Novel Hydroxyapatite from Egg Shell Compared with Chemically Synthesized HAP for Orthopedic application

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Abstract

Aim: The aim of the study is to synthesize Novel Hydroxyapatite (HAP) rich in Calcium phosphate that resembles the bone. These Novel HAP are synthesized from bio-waste that is from chicken egg shells. HAP can be synthesized from chicken egg shells which is considered as a biowaste. This technique helps to protect the environment from pollution caused by biowaste.The synthesized materials shows good biocompatibility as it possesses the hydrophilicity characteristics.

Materials and Methods: To synthesize the Novel Hydroxyapatite from bio-waste generated from chicken egg shells. This synthesized Novel hydroxyapatite powder will compare with chemically synthesized. Novel Hydroxyapatite powder used in conventional practice for orthopedic application.

Results: The synthesized Novel Hydroxyapatite from egg shells compared with Chemically synthesized one by qualitative analysis. In which the presence of phosphate ions and calcium ions was confirmed.

Conclusion: This research finds the replacement for HAP used in current bone repair. This replacement found the better choice as it is cost effect and readily synthesized from biowaste that pollutes the environment. This method indirectly helps in bioremediation.

Keywords: Novel Hydroxyapatite(bone substitute) from waste, Diammonium Hydrogen Phosphate, Orthopedic, Calcium Phosphate, Hydrophilicity, Bioremediation, Biocompatibility.

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INTRODUCTION

HAP can be synthesized from chicken egg shells which is considered as a Biowaste. Synthesis and production of Novel Hydroxyapatite(bone substitute) from waste, chemically is a time consuming process and cannot satisfy the need of the demand and is cost effective (Baláž et al. 2021). The main chemical component of human bone tissue is 70% hydroxyapatite (HAP) (Khandelwal and Prakash 2016). HAP is widely utilized in medical fields particularly as it was known to be a constituent of bone and teeth utilized for its substitute (Mucalo 2015). HAP is usually stoichiometric material (the quantitative relationship between constituents in a chemical substance) where as the

bone mineral components were non – stoichiometric consists of trace amount of ions like Zinc, Sodium, Magnesium, Potassium, Diammonium Hydrogen Phosphate, Calcium Phosphate, Carbonate etc(Gshalaev and Demirchan 2012). So to obtain the non – stoichiometric HAP with the beneficial ion presence can be extracted from the resources such as wood, algae, bones from animals and algae. The synthesized materials show good biocompatibility as it possesses the hydrophilicity characteristics.Being an egg shell is the wastage released as such into the environment that causes pollution (Goloshchapov et al. 2013). Hence, an attempt made to use this biowaste to a minimum extent will result in environmental protection (Abdulrahman et al. 2014). Many researchers use so many methods to synthesize the HAP and that research studies have proven that HAP is closely related to bone apatite and has strong biocompatibility, So HAP is an effective bioimplant material and this method helps in bioremediation (Salimi and Shahhosseini 2018).

The number of research articles published since 2018 on Evaluating the synthesis of novel hydroxyapatite from egg shells compared with chemically synthesized HAP for Orthopedic application are nearly 489 in Google scholar. Many publications that mainly focus on synthesis and production of novel hydroxyapatite from waste for bone substitutes. Few studies involved in the synthesis of Novel Hydroxyapatite by means and calculation of every day, a massive volume of eggshells is discarded, which are useless and trash. These eggshells encourage microbial activity and damage the environment (Abdulrahman et al. 2014). The food processing business produces roughly 250,000 tonnes of eggshell each year ("K Prabakaran, A Balamurugan, S Rajeswari, 2005, 'Development of Calcium Phosphate Based Apatite from Hen's Eggshell', Bull. Mater. Sci., Vol. 28, No. 2, Pp. 115–119. - References - Scientific Research Publishing" n.d.)).The eggshell makes upto 11% of the overall weight of the egg. These eggshells are predominantly composed of calcium carbonate (91-94%), calcium phosphate (1%), and other organic matter, making them ideal for the production of CaO("Rivera, E.M., Araiza, M., Brostow, W., Castaño, V.M., Diaz-Estrada, J., Hernández, R., et Al. (1999) Synthesis of Hydroxyapatite from Eggshells. Materials Letters, 41, 128-134. - References - Scientific Research Publishing" n.d.) et al). The techniques like XRD, SEM, SBF were used for the production of very pure and nanocrystalline hydroxyapatite powder using Eggshells as calcium source and used XRD and SEM to characterize the produced Nano Hydroxyapatite powder. Hence,the synthesized Nano Hydroxyapatite from Biowaste can be substituted as an implant material in orthopedic applications. The egg shells are a possible recycling material for producing HA powder, which can also help in waste management and keeping the environment clean (Tri Wahyudi et al. 2014).Our team has extensive knowledge and research experience that has translate into high quality publications(Bhansali et al. 2021; Jayanth et al. 2021; Sudhakar, Ravel, and Perumal 2021; Sathiyamoorthi et al. 2021; Deepanraj et al. 2021; Raju et al. 2021; Arun Prakash et al. 2020; Kamath et al. 2020; Shanmugam et al. 2021; Rajasekaran et al. 2020; Adhinarayanan et al. 2020; Rajesh et al. 2020; Aurtherson et al. 2021)

A cost effective method has not been optimized so far for synthesis of HAP. Since, this work focuses on the source as biowaste which is cost effective and controls the environmental pollution. The objective of our research is to synthesize the HAP from Biowaste that is from Eggshells as an implant material in orthopedic applications which will be advantageous in terms of cost.

MATERIALS AND METHODS

The research work is an in-house project that was done in the Genetics lab, Department of Bioinformatics at Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences. The number of groups are two and the sample size is two.

Preparation of sample Group 1

The Synthesis of Novel Hydroxyapatite from chemicals in the laboratory, The preparation of HAP was followed as per the protocol optimized by (Salimi and Shahhosseini 2018)). The chemicals required were- Nitric acid, Citric acid, Ammonium Hydroxide, Diammonium Hydrogen Phosphate, Calcium phosphate, Acetic acid, Ammonium Oxalate.

Preparation of sample group 2

The Synthesis of Novel Hydroxyapatite from Biowaste (chicken egg shell) in laboratory, The preparation of HAP was followed as per the protocol optimized by (Khandelwal and Prakash 2016). The chemicals required were- Nitric acid, Citric acid, Ammonium Hydroxide, Diammonium Hydrogen Phosphate, Calcium phosphate

Testing Procedure

The procedure for the Synthesis of HAP from egg shells is followed as firstly, the egg shell samples are washed and cleaned, then sun dried for two days. The dried and cleaned chicken egg shells were crushed and grinded with a blender into egg shell powder. Hydroxyapatite biomaterials are successfully synthesized from egg shells as a supply of calcium.The process is a wet chemical technique also known as chemical solution deposition by which the production of Novel Hydroxyapatite(bone substitute) from waste is done (Horowitz and Parr 1994). The calcined egg shells were subject to the precipitated carbonate (PCC). This precipitate was added with Dihydrogen Ammonium Phosphate to get the HAP with nonstoichiometric and the pH should be maintained as 9. HAP from Biowaste was checked for the presence of calcium phosphate ions Qualitatively and the results were compared with the testing results of chemically synthesized HAP. Synthesis of HAP from the chicken egg shell compared with chemically synthesized HAP for its chemical composition (Elliott 2013). From this we conclude the obtained HAP contains calcium carbonate.

RESULTS

The synthesized Novel Hydroxyapatite from egg shells compared with Chemically synthesized one by qualitative analysis. In which the presence of phosphate ions and calcium ions was confirmed. Once the presence of HAP was confirmed then the confirmation of hydrophilic nature is found to be essential. Since, the material synthesized was interacting with the body fluids which are hydrophilic in nature. This hydrophilicity helps to confirm that it should interact with body fluids and shows good biocompatibility. The chemically synthesized HAP is shown in Fig. 1. The biologically synthesized HAP is shown in Fig. 2. Fig. 3 shows the indication of calcium presence by the white precipitate obtained. Fig. 4 shows the indication of phosphate ion presence by the formation of yellow precipitate. Fig. 5 shows the indication of calcium ion presence by the white precipitate obtained in the HAP synthesized from the egg shell derived from chicken. Fig. 6 shows the indication of phosphate ion presence by the formation of yellow precipitate in the HAP synthesized from the egg shell derived from chicken. Fig. 7 shows the Synthesis of hydroxyapatite from chemicals. Fig. 8 shows the Synthesis of hydroxyapatite from Biowaste. Table 1 shows the Qualitative test to confirm the presence of HAP. Fig. 9 shows the Synthesis of HAP powder by Egg shell and Ammonium Hydroxide.

DISCUSSION

In this study, the Synthesis of Novel Hydroxyapatite from eggshells shows a similar chemical composition and suits for orthopedic application and the output of simple qualitative tests for both biological process and chemical process is the same. The results confirm that the Produced chemical by both the processes is HAP.

Synthesis of Novel Hydroxyapatite chemically is a time consuming process and it is cost effective. For synthesis of HAP, the pH of the solution was maintained at 11 in order to get only monophasic nano HAP (Azis et al. 2021). A milky suspension was obtained which was then filtered and this filtrate washed with ethanol and finally with distilled water several times in order to remove contaminated ions and surfactant (Toibah et al. 2019). The synthesized materials show good biocompatibility as it possesses the hydrophilicity characteristics.This method was considered as most effective in controlling pollution and also gives HAP in pure form.Synthesis of HAP chemically is time consuming processes and cannot satisfy the need of the demand and cost effective (Mohan et al. 2018) and it is compared with chemically synthesized HAP for chemical composition and it suits for orthopedic applications (Khandelwal and Prakash 2016).The production of Novel Hydroxyapatite(bone substitute) from waste is considered as best way to replace the bone constituent and this method helps in bioremediation as the preparation procedure is easy and helps to satisfy the need of demand in the field of medical.

The limitation faced in our research study is the collection of chicken egg shells should be done in a protective way otherwise the results may be deviations. Biochemical studies such as cytotoxicity, bioprotectivity assays were helped to confirm its application in Orthopedics. Hence further study should be planned as evaluation of this synthesized HAP using Biochemical Assay.

CONCLUSION

The simple Qualitative test was performed in both the HAP derived from the chemicals and also from the egg shell biowaste obtained from chicken.This results support that the Novel Hydroxyapatite derived from egg shell can be the good replacement for the commercially available HAP for orthopedic applications. Synthesis of HAP from the chicken egg shell compared with chemically synthesized HAP for its chemical composition. This comparison confirms the HAP obtained from eggshell resembles the commercial one in composition.

DECLARATION

Conflict of Interest
The authors of this paper declare no conflict of interest.

Author Contribution
Author GP was involved in data collection, data analysis, manuscript writing. Author KK was involved in conceptualization, guidance and critical review of manuscript.

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Figures and Tables

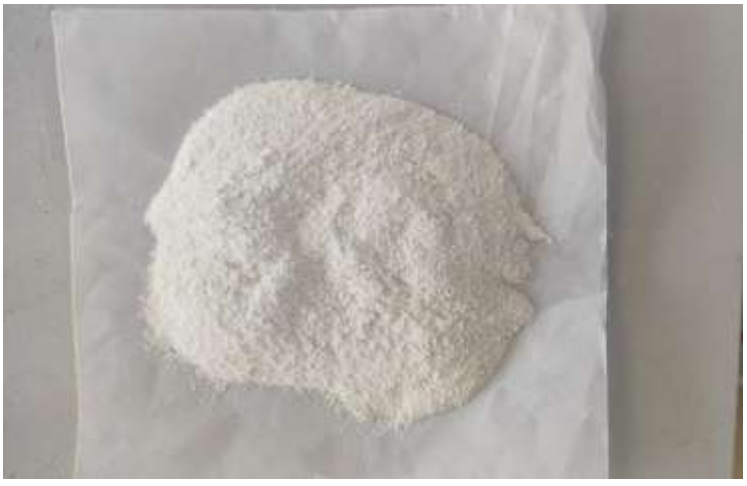

Fig. 1. Chemically synthesized HAP

Fig. 2. Biologically synthesized HAP

Fig. 3. Confirmation of Calcium ion in chemically synthesized HAP

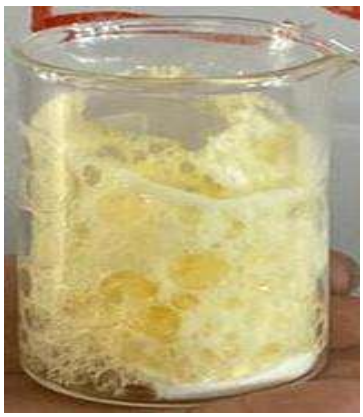

Fig. 4. Confirmation of Phosphate ion in chemically synthesized HAP

Fig. 5. Confirmation of Calcium ion in HAP synthesized from egg shell

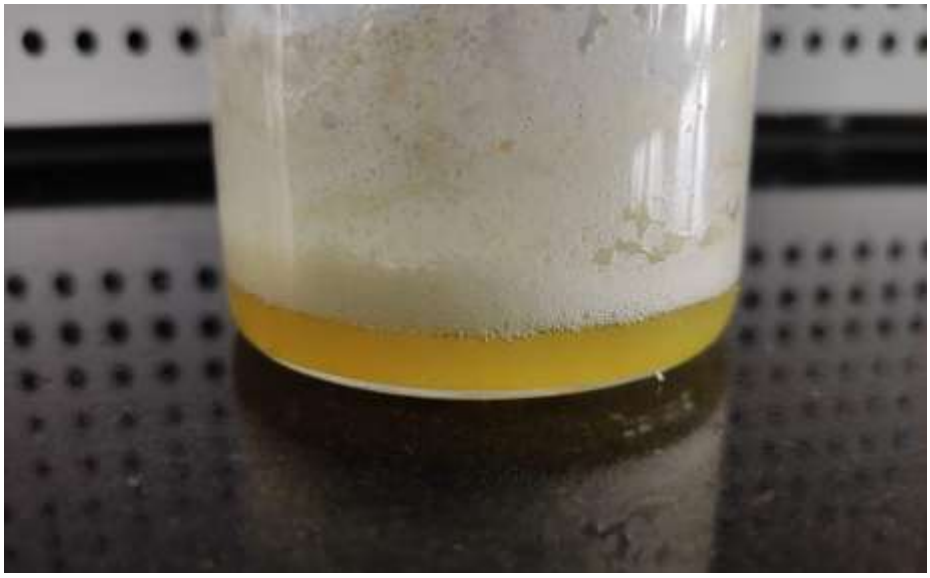

Fig. 6. Confirmation of Phosphate ion in HAP synthesized from egg shell

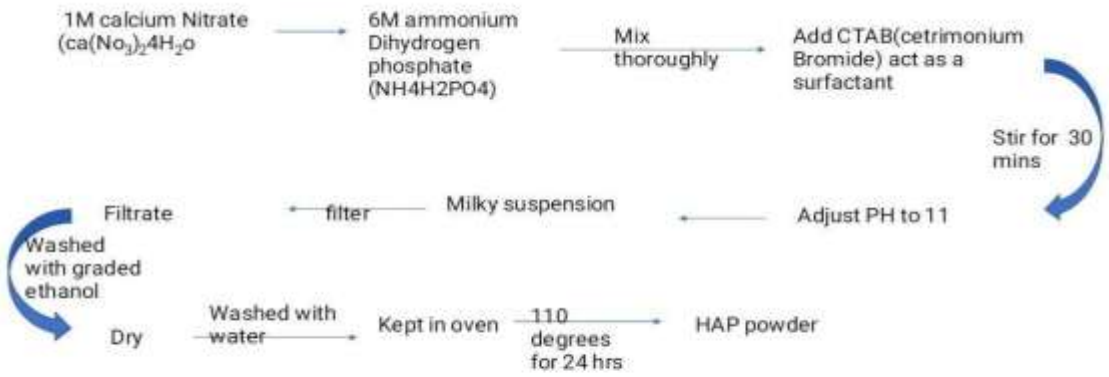

Fig. 7. Synthesis of hydroxyapatite from chemicals

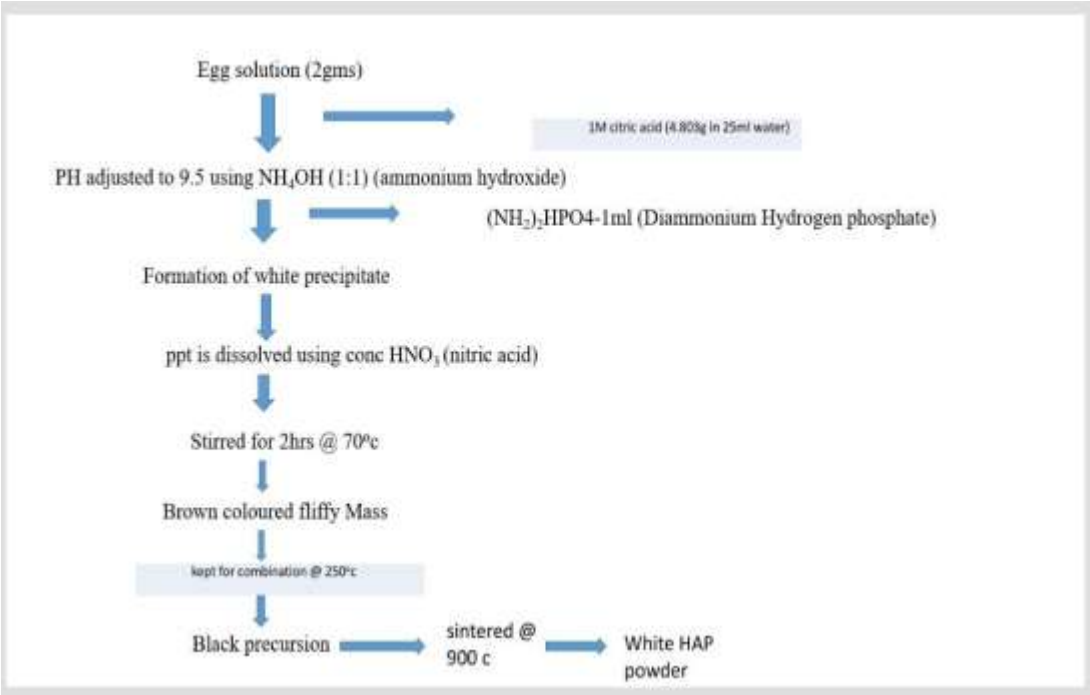

Fig. 8. Synthesis of hydroxyapatite from Biowaste (chicken egg shell)

Table 1. Qualitative test to confirm the presence of HAP

SAMPLE	PROCEDURE	OBSERVATION	INFERENCE
Chemically synthesized HAP	Dissolve the obtained precipitate in acetic acid and boil it, add a few drops of ammonium oxalate to the above solution and then add ammonium hydroxide.	White precipitate is obtained.	This confirms the presence of Calcium ions in the precipitate**.**
Chemically synthesized HAP	A small amount of sample is acidified with concentrated Nitric acid to which the little amount of ammonium molybdate was added	Bright Yellow precipitate Layer was obtained	This confirms the presence of Phosphate ions
HAP synthesized from biowaste egg shell	Dissolve the obtained precipitate in acetic acid and boiled it add a few drops of ammonium oxalate to the above solution and then add ammonium hydroxide.	White precipitate is obtained.	This confirms the presence of Calcium ions in the precipitate**.**
HAP synthesized from	A small amount of sample	Bright Yellow precipitate	This confirms the

biowaste egg shell	is acidified with concentrated Nitric acid to which the little amount of ammonium molybdate was added	Layer was obtained	presence of Phosphate ions

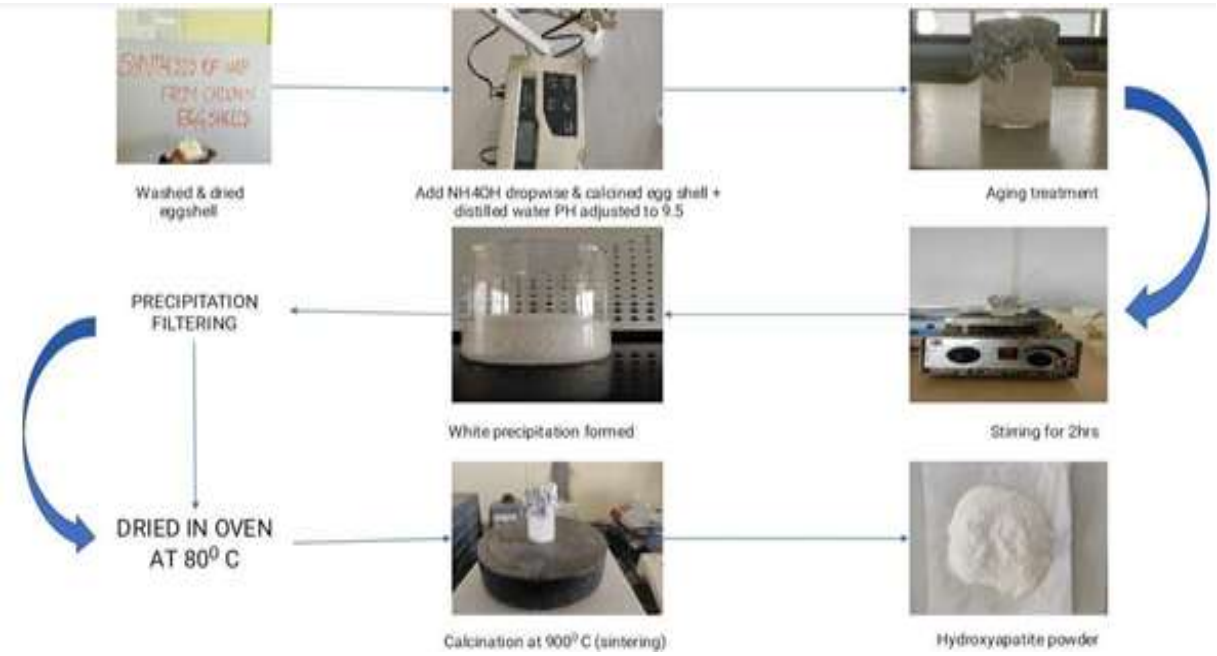

Fig. 9. Synthesis of HAP powder by Egg shell and NH_4OH