Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.13.042

Preparation Of Rod and Spherical Shaped Hydroxyapatite for Tissue Regeneration Application

George Franklin[1], Suganya Panneer Selvam[2], Satheesh Kumar Balu[3*], Ramya Ramadoss[4], Sandhya Sundar[5], Pratibha Ramani[6]

[1]Undergraduate Student, Department of Oral Biology, Saveetha Dental College and Hospital, Chennai - 600077

[2]Assistant Professor, Department of Oral Biology, Saveetha Dental College and Hospital, Chennai - 600077

[3]Researcher, Department of Oral Pathology & Microbiology, Saveetha Dental College and Hospital, Chennai - 600077

[4]Professor & Head, Department of Oral Biology, Saveetha Dental College and Hospital, Chennai - 600077

[5]Assistant Professor, Department of Oral Biology, Saveetha Dental College and Hospital, Chennai - 600077

[6]Professor & Head, Department of Oral Pathology & Microbiology, Saveetha Dental College and Hospital, Chennai - 600077

***Corresponding author:** Satheesh Kumar Balu, Researcher, Department of Oral Pathology & Microbiology, Saveetha Dental College and Hospital, Chennai - 600077

Submitted: 16 March 2023; Accepted: 13 April 2023; Published: 06 May 2023

ABSTRACT

The fundamental goal of current biomaterial research is to replace broken bones with affordable bioactive and biocompatible materials. In the current study, bone powder from the cuttlefish was employed as a precursor in the silicon oil bath-mediated precipitation method to create hydroxyapatite nanostructures (HAp NS). To generate HAp nanostructures with varied morphologies, the reaction was carried out across a range of time periods, such as 24 h at 80 C. The generated HAp NS was characterized using FTIR and FESEM. Additionally, it was demonstrated that the presence of BSA alters the shape of produced materials, which affects the reaction. FESEM revealed two distinct morphologies, including spherical and rod-like structures, both of which are very useful for a variety of biomedical applications.

Keywords: *Hydroxyapatite, Tissue engineering, Cuttlefish, Biocompatible, Bone, Osteoconductive*

INTRODUCTION

Hydroxyapatite, a naturally occurring form of calcium phosphate, is the main mineral component of bones and teeth. Natural hydroxyapatite and bone have similar physical and chemical characteristics that make them biocompatible. Its porous structure resembles native bone.

The biocompatibility, biodegradability and bioactivity make it extensively useful in interdisciplinary fields of sciences like chemistry, biology, and medicine. Cuttlefish bones are an inexpensive source of calcium carbonate, which are produced in large amounts by the marine food industry, leading to environmental contamination and waste.

The nontoxicity, worldwide availability and low production cost of cuttlefish bone products makes them an excellent calcium carbonate precursor for the fabrication of hydroxyapatite.

One of the best implant materials is hydroxyapatite (Ca10 (PO4)6 (OH)2 or HAp), which has great biocompatibility, especially in bone tissues(Traykova *et al.*, 2006; Lim *et al.*, 2021). The exceptional capacity of HAp to adsorb a wide range of chemical species on the surface is another important feature. For the purification and separation of proteins, the HAp chromatography has made use of this characteristic (Lim *et al.*, 2021). Sobczak-Kupiec et al reported that the physicochemical properties and morphology of HAP depended on the origin/preparation method. Synthetic hydroxyapatite exhibited low crystallinity, with high porosity and more surface area. On the other hand, HAP obtained from animal bone via calcination at 800˚C possesses the highest crystallinity(Sobczak-Kupiec *et al.*, 2018). Hydroxyapatite has the capability to form chemical bonds with surrounding hard tissues (Fujishiro, Hench and Oonishi, 1997) with the formation of a HAP interfacial layer (Wu *et al.*, 2022). The similar physical and chemical characteristics of natural hydroxyapatite with bone make it biocompatible.Calcium phosphate-based ceramics, such as HAP, are of great interest as synthetic bone graft substitutes due to their similarity in composition to bone mineral and bioactivity as well as osteoconductivity (Greish *et al.*, 2005).

Blocks of HAp have been studied for skeletal drug delivery systems because of their high affinity for macromolecules as drug scaffolds to delay drug release (Wong, no date). Due to claims that particle systems have improved bioavailability (Komlev, Barinov and Koplik, 2002; Tagaya *et al.*, 2011)) the preparation of HAp spherical shapes or granules has also been an intriguing topic. Recently, nanosized-HAp dispersion solutions for protein delivery systems were spray dried to create HAp microspheres . The microspheres were simple to inject into the desired area or subcutaneously. But in vitro experiments have frequently shown that the drug release patterns from HAp microspheres start off quite quickly. Although the first burst may be attributed to the desorption of macromolecules, which were not in close proximity to the HAp crystals (Liu *et al.*, 2005), the specifics of the interaction have not been fully clarified. These results also imply that additional compounds that delay the release of proteins may be required to adapt the system to long-term controlled-release dose forms, such as once-monthly injectable depot formulations for long-term therapy. A viable strategy for the regulated release of proteins would be to encase the HAp microspheres in biodegradable polymers like poly lactic acid (PLA) and poly lactic acid-co-glycolic acid (PLGA). Our team has extensive knowledge and research experience that has translate into high quality publications (Ramesh Kumar *et al.*, 2011; Anita *et al.*, 2020; Chellapa *et al.*, 2020; Kanniah *et al.*, 2020; Kumar *et al.*, 2020; Gowhari Shabgah *et al.*, 2021; Muthukrishnan, 2021; Samuel, Kuduruthullah, *et al.*, 2021; Samuel, Mathew, *et al.*, 2021; Ganapathy *et al.*, 2022)

In this study, the desorption behavior of bovine serum albumin (BSA), a model protein, on industrial hydroxyapatite (HAp) microspheres and its control were examined. The ion exchange reaction on HAp was used to discuss the desorption of BSA from the HAp microspheres in different solutions. The solid-in-oil-in-water (S/O/W) emulsion solvent evaporation technique was used to encase the BSA-loaded HAp microspheres with PLGA for controlled release of protein, and the BSA release profile was investigated in physiological buffer solution.Cuttlefish have been investigated for their neurobiology and cognitive capacities in addition to its possible use in biomaterials. Researchers looking into the evolution of intelligence and behavior in animals have found their highly developed neural systems and complicated behaviors of interest.

Overall, cuttlefish are a fascinating subject of research in their own right and have potential applications in a variety of domains, including biomaterials and neurology, even if they may not immediately relate to the topic of the manufacture of hydroxyapatite for tissue regeneration.

MATERIALS AND METHODS

Chemicals and Reagents

Cuttlefish bones (CB) (CaCO3) from biowaste were gathered at the neighborhood fish market in Kasimedu, Chennai, Tamil Nadu, India. Chemicals of the analytical grade, such as

$C_4H_8O_2$ (Sigma-Aldrich, MW 88.11 g/mol, purity - 99.9%), NH_4OH (Merck, MW 35.05 g/mol, purity - 25%), $(NH_4)_2HPO_4$ (Merck, MW 132.06 g/mol, purity - 99%), CNa_2O_3 (Sigma-Aldrich, MW 105.99

Synthesis of Hap and Hap@BSA

HAp NS was produced using a temperature-controlled oil bath precipitation technique. Using a lancet, the cuttlebone's inner and outer sections were separated. The inner part, known as the lamellae matrix, was then cleaned by first using distilled water, then by acetone and ethanol to remove any surface pollutants. A hot air oven (Indfurr model OR- 3795) was used to dry the cleaned cuttlebone pieces for 24 hours while a high energy ball mill (VB Ceramic Consultants, Chennai) was used to grind 20 g of the CB pieces for 5 hours at a speed of 400 rpm. In order to conduct future research, the ground-up powders were kept at room temperature in a desiccator.

In a flask with a round bottom, 1 M (M) cuttlebone powder was added drop by drop together with 0.6 M $(NH_4)_2HPO_4$. The pH of the reaction process was measured and increased using NH_4OH from 8 to 12. The final precipitate was washed with distilled water and ethanol after the reactant was agitated for 12 hours at 80°C. The same procedure was used to make Hap@BSA, but instead of adding $(NH_4)_2HPO_4$ in drops, the bovine serum albumin (BSA) was incorporated first. The resulting powder was then annealed for 6 hours at 800 C.

Characterizations

The presence of functional groups in the HAp was verified using an FTIR spectrometer. Then, to evaluate the shape of the produced HAp nanostructures, field emission scanning electron microscopy (FESEM) was employed.

Antimicrobial activity

Using the agar well diffusion method, the antibacterial activity of HAp and Hap@BSA produced was examined. The National Center for Microbial Resource provided the pure cultures of the bacteria (E. coli and S. aureus) (NCMR, Pune). Nutrient Agar was used to culture the bacterial pathogens (SRL Chemicals, India). Fresh microbial cultures were then applied to the sterilized nutritional agar using sterile cotton swabs in a petri dish. By making holes in the nutritional agar using a sterile micropipette tip, wells were created. The wells received additions of the HAp nanocomposites at concentrations of 20, 40, 60, 80, and 100 g/mL. The zone of inhibition was determined after the plates had been incubated for 24 hours at 37°C. To appropriately dispose of the microorganisms and protect the environment, an autoclave was used in the end.

RESULTS AND DISCUSSION

The FTIR spectra of HAp nanostructures generated from cuttlefish at different times are shown in Fig. 2(b). The stretching vibration of the -OH group is shown by the peaks, which range from 3600 to 3000 cm1. There are peaks for carbonyl and carbonate at 1734 and 1465 cm-1, respectively. The produced hydroxyapatite included the B-type carbonate, which was recognised. Phosphate is responsible for the peaks at 1222, 1010, 615, 564, and 423 cm 1. As the reaction time was prolonged, the carbonyl peak decreased. The release of carbon dioxide might occur as a consequence of the extended holding duration over a longer length of time.

FESEM micrographs of the HAp and HAp@BSA powders are shown in Fig. 2. Nanosized rod-like and spherical formations with various reaction agents were seen in the morphology of nanoparticles. It was noted that the HAp nanoparticles in particular had rod-like shape of 80-120 nm in length, with uneven outlines. Due to its biocompatibility, bioactivity, and osteoconductive qualities, hydroxyapatite (HA), a bioceramic substance, has been widely used in tissue engineering and regenerative medicine applications. The main inorganic component of bone tissue is HA, which has a long history of application in bone tissue engineering. In order to meet the needs of particular tissue regeneration applications, HA can be synthesized in a variety of forms and sizes, including rods and spherical particles.Numerous techniques, including precipitation, hydrothermal, sol-gel, and spray-drying methods, can be used to create hydroxyapatite crystals with rod and spherical shapes. For instance, in the precipitation process, calcium and phosphate ions are mixed in a controlled atmosphere to produce a solid HA precipitate that may be formed into rods or spherical particles. In the hydrothermal process, HA crystals can be formed into rods or spherical particles by heating a solution of calcium and phosphate salts under intense pressure in water. By hydrolyzing calcium and phosphate precursors, the sol-gel technique creates a gel that is then calcined to create HA, which can also be formed into rods or spherical particles.final HA's qualities, including as its crystal structure, shape, and surface area, might affect the material's biocompatibility and osteoconductive properties. These properties can be influenced by the method of preparation used. For instance, compared to HA prepared using other methods, HA prepared using the sol-gel approach has been shown to have a greater surface area and smaller particle size, which can improve the material's bioactivity and osteoconductivity(Guo *et al.*, 2015) Different tissue regeneration applications, including bone repair, dental implant coatings, and drug delivery systems, have used rod- and spherical-shaped HA. For instance, rod-shaped HA has been employed as a scaffold for bone tissue creation, where it offers mechanical support and encourages mesenchymal stem cells (MSCs) to differentiate into osteogenic tissue(Wei *et al.*, 2016). Dental implants have been coated with spherical HA, which improve osseointegration and lowers implant failure rates(Zhang *et al.*, 2017) . In drug delivery systems, HA's spherical shape has also been exploited as a carrier since it may release medications under controlled conditions, increasing their effectiveness ((Yu *et al.*, 2014)

Therefore, as mentioned in earlier research, synthesis techniques and BSA have a crucial role in altering the dimension of HAp, which significantly influences their biological characteristics. However, FESEM analysis of the HAp nanostructures in this work showed a non-porous, rod-like shape that would affect the materials' biocompatibility, particularly when employed as scaffolds. This study thus confirms that calcination temperature adjustment is essential to achieve porosity in HAp for enhanced biocompatibility. The findings also demonstrate how difficult it is to manage the particle size and degree of crystallinity of HAp, given the variety of chemical synthesis procedures used to create micro and nano-HAP. Additionally, secondary metabolites created by the chemical activation of unreacted species degrade the terminal action of biomaterials. Similar issues can arise when using physical techniques like selective laser sintering.

Gram-negative bacteria are the major cause of nosocomial infections due to their inherent resistance for antibiotics. The obtained results are displayed in Fig. 3. The antibacterial activity of nanostructures against gram-negative bacteria was more than gram-positive bacteria due to the variations in peptidoglycan layer thickness. Among the various bacterial strains, the NPs showed enhanced antimicrobial activity against K.pneumoniae due to the variations in cell structure, diffusion rate, metabolism and interaction of the nanoparticles with the microorganisms (Fig. 3).

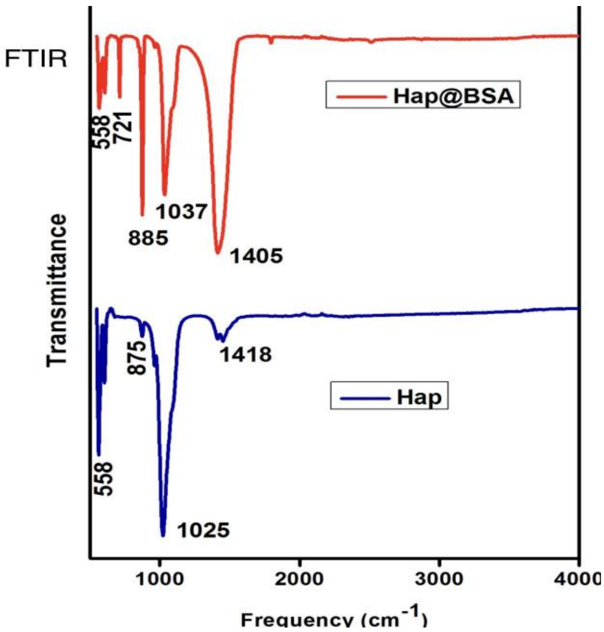

FIGURE 1: FTIR spectra of prepared Hap and Hap@BSA.

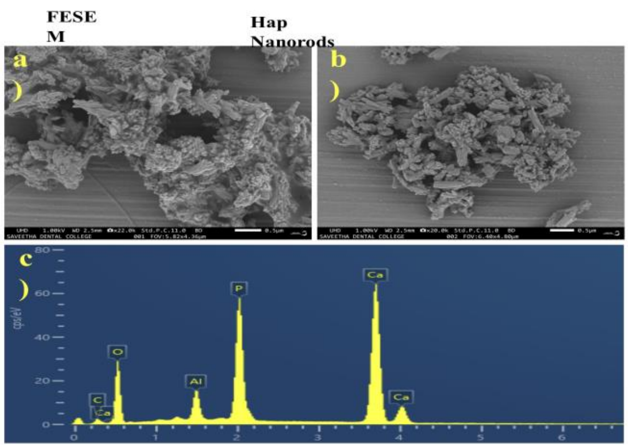

FIGURE 2: SEM imaging and EDX micrograph of Hap

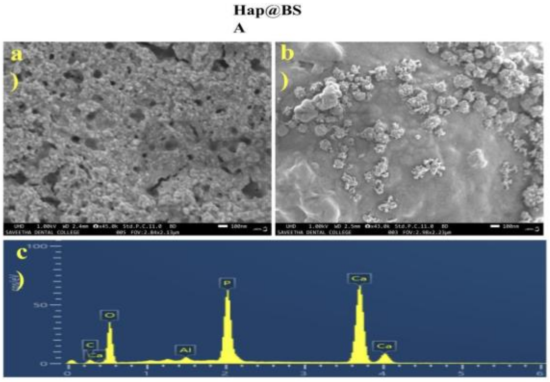

FIGURE 3: SEM imaging and EDX micrograph of Hap@BSA

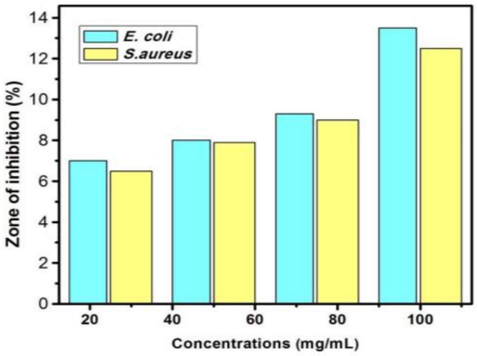

FIGURE 4: Zone of inhibition vs concentrations of E Coli and S. Aureus.

FIGURE 5: Antimicrobial activity in E.coli and S. Aureus

CONCLUSION

The primary objective of modern biomaterial research is to develop inexpensive bioactive and biocompatible materials to replace fractured bone. The current study used cuttlefish bone powder as a precursor to make hydroxyapatite nanostructures using the silicon oil bath-mediated precipitation method (HAp NS). The reaction was run over a variety of times, including 24 h at 80 C, to produce HAp nanostructures with various morphologies. FTIR and FESEM were used to characterize the produced HAp NS. It was also shown that the presence of BSA changes the form of the materials created, which influences the response. Spherical and rod-like structures, both of which are highly helpful for a range of biomedical applications, were revealed by FESEM to have two unique morphologies. Prepared nanoparticles can be used in drug delivery applications and regeneration applications due to its rod-like morphology and the presence of protein.

CONFLICT OF INTEREST

Nil.

REFERENCES

1. Anita, R. et al. (2020) ‘The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients’, American journal of cancer research, 10(8), pp. 2546–2554.
2. Chellapa, L.R. et al. (2020) ‘Biogenic Nanoselenium Synthesis and Evaluation of its antimicrobial, Antioxidant Activity and Toxicity’, Bioinspired Biomimetic and Nanobiomaterials, pp. 1–6.
3. Fujishiro, Y., Hench, L.L. and Oonishi, H. (1997) ‘Quantitative rates of in vivo bone generation for Bioglass and hydroxyapatite particles as bone graft substitute’, Journal of materials science. Materials in medicine, 8(11), pp. 649–652.
4. Ganapathy, D. et al. (2022) ‘Rarity of mucormycosis in oral squamous cell carcinoma: A clinical paradox?’, Oral oncology, 125, p. 105725.
5. Gowhari Shabgah, A. et al. (2021) ‘Interleukin-25: New perspective and state-of-the-art in cancer prognosis and treatment approaches’, Cancer medicine, 10(15), pp. 5191–5202.
6. Greish, Y.E. et al. (2005) ‘Composite formation from hydroxyapatite with sodium and potassium salts of polyphosphazene’, Journal of materials science. Materials in medicine, 16(7), pp. 613–620.
7. Guo B. et al. (2015) ‘[Inhibitory Effect of Hydroxyapatite Particles with Different Size on Malignant Melanoma A375 Cells: A Preliminary Study]’, Sheng wu yi xue gong cheng xue za zhi = Journal of biomedical engineering = Shengwu yixue gongchengxue zazhi, 32(4), pp. 832–837.
8. Kanniah, P. et al. (2020) ‘Green synthesis of multifaceted silver nanoparticles using the flower

extract of Aerva lanata and evaluation of its biological and environmental applications', ChemistrySelect, 5(7), pp. 2322–2331.
9. Komlev, V.S., Barinov, S.M. and Koplik, E.V. (2002) 'A method to fabricate porous spherical hydroxyapatite granules intended for time-controlled drug release', Biomaterials, pp. 3449–3454. Available at: https://doi.org/10.1016/s0142-9612(02)00049-2.
10. Kumar, S.P. et al. (2020) 'Targeting NM23-H1-mediated Inhibition of Tumour Metastasis in Viral Hepatitis with Bioactive Compounds from Ganoderma lucidum: A Computational Study', Indian Journal of Pharmaceutical Sciences. Available at: https://doi.org/10.36468/pharmaceutical-sciences.650.
11. Lim, P.N. et al. (2021) 'Silver, silicon co-substituted hydroxyapatite modulates bacteria-cell competition for enhanced osteogenic function', Biomedical materials , 16(5). Available at: https://doi.org/10.1088/1748-605X/ac1c62.
12. Liu, T.-Y. et al. (2005) 'On the study of BSA-loaded calcium-deficient hydroxyapatite nano-carriers for controlled drug delivery', Journal of controlled release: official journal of the Controlled Release Society, 107(1), pp. 112–121.
13. Muthukrishnan, L. (2021) 'Multidrug resistant tuberculosis - Diagnostic challenges and its conquering by nanotechnology approach - An overview', Chemico-biological interactions, 337, p. 109397.
14. Ramesh Kumar, K.R. et al. (2011) 'Depth of resin penetration into enamel with 3 types of enamel conditioning methods: a confocal microscopic study', American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics, 140(4), pp. 479–485.
15. Samuel, S.R., Kuduruthullah, S., et al. (2021) 'Impact of pain, psychological-distress, SARS-CoV2 fear on adults' OHRQOL during COVID-19 pandemic', Saudi journal of biological sciences, 28(1), pp. 492–494.
16. Samuel, S.R., Mathew, M.G., et al. (2021) 'Pediatric dental emergency management and parental treatment preferences during COVID-19 pandemic as compared to 2019', Saudi journal of biological sciences, 28(4), pp. 2591–2597.
17. Sobczak-Kupiec, A. et al. (2018) 'Synthesis and characterization of ceramic - polymer composites containing bioactive synthetic hydroxyapatite for biomedical applications', Ceramics International, pp. 13630–13638. Available at: https://doi.org/10.1016/j.ceramint.2018.04.199.
18. Tagaya, M. et al. (2011) 'Nano/microstructural effect of hydroxyapatite nanocrystals on hepatocyte cell aggregation and adhesion', Macromolecular bioscience, 11(11), pp. 1586–1593.
19. Traykova, T. et al. (2006) 'Bioceramics as nanomaterials', Nanomedicine, pp. 91–106. Available at: https://doi.org/10.2217/17435889.1.1.91.
20. Wei, Q. et al. (2016) 'Study the bonding mechanism of binders on hydroxyapatite surface and mechanical properties for 3DP fabrication bone scaffolds', Journal of the mechanical behavior of biomedical materials, 57, pp. 190–200.
21. Wong, C.-T. (no date) 'Osteoconduction and osseointegration of a strontium-containing hydroxyapatite bioactive bone cement : in vitro and in vivo investigations'. Available at: https://doi.org/10.5353/th_b2994063.
22. Wu, C. et al. (2022) 'Biomechanical and osteointegration study of 3D-printed porous PEEK hydroxyapatite-coated scaffolds', Journal of biomaterials science. Polymer edition, pp. 1–14.
23. Yu, J. et al. (2014) 'Synthesis, characterization, antimicrobial activity and mechanism of a novel hydroxyapatite whisker/nano zinc oxide biomaterial', Biomedical materials , 10(1), p. 015001.
24. Zhang, B.-J. et al. (2017) 'Enhanced osteogenesis of multilayered pore-closed microsphere-immobilized hydroxyapatite scaffold via sequential delivery of osteogenic growth peptide and BMP-2', Journal of materials chemistry. B, Materials for biology and medicine, 5(41), pp. 8238–8253.