

A Review on Nano Hydroxyapatite Fabrication Techniques

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ABSTRACT

Bio ceramics that exhibit positive interactions with human tissues can be used in various biomedical applications. A strong bone bonding is triggered by the natural and synthetic bio ceramics when used as implants. One of the most common calcium phosphate bio ceramic used as biomaterial is hydroxyapatite. The structure and composition of hydroxyapatite analogous to the inorganic portion of bone and teeth, thus it has endless biomedical applications. As a result, demand for hydroxyapatite nanoparticles has risen over the last decade. Several synthesis methods are followed to prepare nano HAp such as dry, wet, high temperature and combination techniques. Synthesis from biogenic sources like bone waste, egg shell and exoskeleton of marine animals are also in practice. Various preparation methods results HAp nanoparticles of various morphologies, crystallite size and phases. Also the processing factors in different synthesis methodologies influence the physical, chemical, and biological properties of hydroxyapatite. In this review several fabrication techniques of nano HAp are discussed.

KEYWORDS: Hydroxyapatite, implants, nanoparticles

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INTRODUCTION

Diseases such as arthritis, tumors and trauma may cause skeletal defects that require surgery to remove or repair the missing bone. Orthopedists repair bone loss areas by using autogenous bone graft, allogenic graft, and bone graft implants. These surgical implants used to repair the diseased bone in conjunction or in isolation. The right selection of biomaterials for the proposed application is critical in order to ensure that the implant has the intended function, lifespan and overall performance. Biomaterials consisting of metals, alloys and polymers were used in the 1970s. Drawbacks of these materials were rectified by the implementation of ceramic bio material. Therefore, attention was drawn on these materials to detect their bone incorporation properties.

Ceramics are inorganic solids made of either metal or non-metal compounds. Generally it is prepared by heating succeeded by cooling. Compared to other biomaterials,

ceramics are hard to shear plastically because they have covalent and ionic bonding as well as a minimal number of slip planes.[1]. In addition, these ceramics show a great biological affinity to the hostile environment when they are introduced into the body. Ceramics that are specially developed for the replacement and restoration of diseased and broken organs are named as 'bio ceramics.' [2].

Calcium phosphate based ceramics and its phases

The proof for the presence of calcium phosphate in bone tissue was recorded in 1769 which brought a turning point in the medical history. From then, scientists are attracted by synthetic calcium phosphate bio ceramics to put back the damaged bones. Each calcium phosphate differs from others by its composition, indicating the variations in their synthesis methods and origin. Details about the different calcium phosphate phases are given in the following table.

Chemical Name	Acronym	Formula	Ca/p ratio
Amorphous Calcium Phosphate	(ACP)	$\text{Ca}_3(\text{PO}_4)_2$	1.3-1.5
Mono Calcium Phosphate	Mono Hydrate(MCPM) Anhydrous (MCPA)	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ $\text{Ca}(\text{H}_2\text{PO}_4)_2$	0.5
Di Calcium Phosphate	Di Hydrate(DCPD) Anhydrous (DCPA)	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ CaHPO_4	1
Octa Calcium Phosphate	(OCP)	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$	1.33
Tri Calcium Phosphate	(α -TCP) (β -TCP)	$\alpha\text{-Ca}_3(\text{PO}_4)_2$ $\beta\text{-Ca}_3(\text{PO}_4)_2$	1.5
Hydroxyapatite	(HAp)	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	1.67
Tetra Calcium Phosphate	(TTCP)	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	2

Table 1 Various calcium phosphate phases with Ca/P ratios

Hydroxyapatite

Human and animal hard tissues contain organic and inorganic components. The main inorganic component of hard tissue is 'hydroxyapatite', which is a biologically active ceramic. A powerful chemical bond is formed between natural bone and hydroxyapatite implants; thereby it stimulates bone formation and assists bone development. At the interface between bone and bio active HAp implants, the bonding strength is greater than other bio inert implants, so that cracking occurs in implants or bones and not at the interface. Also the high gradient Young's modulus of the coupling zone neutralize the difference of Young's modulus between bone and implant[3] that enable the effective load transfer between them.

Bio active calcium phosphate-based ceramics otherwise known as hydroxyapatite is used as bone and teeth implant material because its crystal structure and chemical properties of resemble to the inorganic portion of bone and bony tissues of mammals. So hydroxyapatite (HAp) gained more attention by the researchers. The phrase "apatite" represents a group of phosphate minerals with the general formula $M_{10}(XO_4)_6Z_2$. Here M^{2+} indicates metallic elements. XO_4^{3-} and Z-groups are anions. Each apatite's special name differs based on M, X and Z. For hydroxyapatite, the terms M, X and Z are replaced by calcium (Ca^{2+}), phosphorus (P^{5+}) and hydroxyl (OH^-) ions respectively and the chemical formula for this apatite is $Ca_5(PO_4)_3(OH)$. But in order to indicate the two entities of the unit cell, the formula is commonly scripted as $Ca_{10}(PO_4)_6(OH)_2$. Weight percentage of each HAp component is 39% calcium, 18.5% phosphorous and 3.38% hydroxyl ions. In hydroxyapatite the calcium and phosphorous atomic ratio is 1.67.

Preparing hydroxyapatite in nanoscale results in a greater area-to-volume ratio and provides stronger interaction with bones. It is also possible to increase the porosity and shape of the material at the atomic scale, along with the functional surface required for the best interaction with the bone [4]. HAp with improved biological and mechanical properties is obtained by doping it with trace quantities of doping elements found in natural bones. Synthetic nano HAp is developed in many routes. The most desirable techniques currently followed by researchers are precipitation and sol-gel processes. The following paragraphs address various methods of synthesis.

Synthesis routes of hydroxyapatite

Typically, synthetic hydroxyapatite synthesis techniques can be classified as dry, wet and high-temperature processes. These methods grow particles of varying morphologies, phases and sizes[5]. The following flow chart explains different methods under each major category.

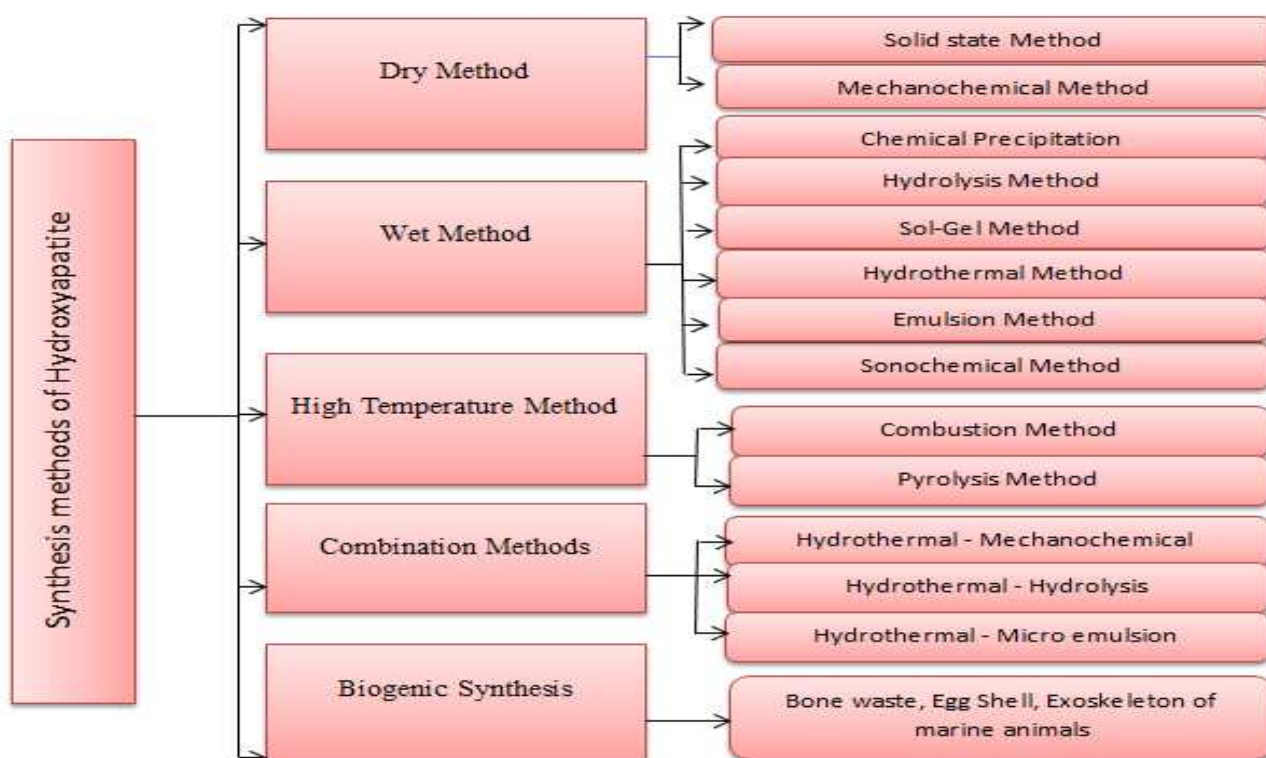


Fig 1: Fabrication Methods of synthetic Hydroxyapatite

Dry synthesis Method

In the dry approach, the raw materials are taken in dry form and combined together to synthesise HAp. No precise and regulated conditions required[6] and it is an ideal method for lump powder processing.

A. Solid State Method

In this process, heat is used to decompose the solid reactants, resulting in new solids and gases. It is a facile and profitable approach. Sumit Pramanik et al.[7] synthesized HAp with hexagonal structure through the solid state

reaction method. Calcium-deficient hydroxyapatite and monetite phases have been identified using this technique. Hartatiek et al.[8] manufactured BCP/aluminum composite by solid state sintering technique using $Ca(OH)_2$ as the base material collected from calcite stone. The sintering temperature suited to this synthesis was $1200^{\circ}C$, which triggered a phase shift from hydroxyapatite to tricalcium phosphate. Sumit Pramanik et al.[9] utilized solid state sintering process to create high strength HAp. Samples crushed and resintered at $1250^{\circ}C$ showed improved mechanical properties and good biocompatibility. The

density of the prepared sample was influenced by the pelletization pressure and thus improved the properties like Young's modulus, compressive strength, bending strength and tensile strength. Arkin et al. [10] obtained high purity hydroxyapatite at a calcinating temperature of 1300°C. The calcium and phosphate precursors used for this synthesis were calcium carbonate and ammonium dihydrogen phosphate respectively.

B. Mechanochemical Method

Mechanochemical synthesis is a synthesis based on mechanical reactions. Chemical reactions are triggered by compression, friction or grinding. By using a mechanochemical process, Sharifah Adzila et al. [11] examined the influence of milling rate and rotational speed in the fabrication of hydroxyapatite nanoparticles under dry conditions. Various rotational speeds such as 170rpm, 270rpm, 370rpm and various milling periods such as 15hrs, 30hrs, and 60hrs were followed. No major differences obtained in the powder properties by extended milling time. Yet increased rotational speed resulted in decreased particle size and agglomeration. Sang-Hoon Rhee [12] has selected two calcium base ingredients, calcium pyrophosphate and calcium carbonate for the preparation of pure HAp by means of mechanochemical process and blended in acetone and water respectively. The findings revealed that high crystalline particles could be produced in water mixtures followed by heat treatment. High-purity HAp nano powder was manufactured by Yeong et al. [13] through a mechanochemical technique. Calcium oxide and calcium hydrogen phosphate were used as the calcium and phosphorous reactants, respectively. It was mechanically activated by high-energy shaker mills. After 20 hours of milling, nanoparticles with an average size of 25nm formed.

Wet Method

Wet methods deals with chemical reactions in aqueous solutions using precursors under appropriate laboratory conditions. The benefit of this approach is that it controls the average size of the nano particle and its morphology.

A. Precipitation Method

Chemical precipitation is the method of transforming the solution into a solid by converting the material into an insoluble shape or by converting the solution into a supersaturated one. The key benefit of this approach is the processing of a significant quantity of nanoparticles at a fair rate. Azade Yelten-Yilmaz & Suat Yilmaz [14] varied three parameters namely addition rate of acid, reaction rate and temperature of heat treatment to prepare hydroxyapatite by precipitation technique. XRD results showed that HAp nano powders formed at 1250°C heat treatment had sharp, narrow peaks indicating high crystallinity. HAp heat treated powders at 950 °C possessed inferior mechanical properties than HAp heat treated powders at 1250 °C. Sudip Mondal et al. [15] prepared well dispersed stoichiometric, spherical hydroxyapatite nanoparticle using chemical precipitation process. The mean surface area of the obtained sample was 78.415 m²g⁻¹. Also the pore volume and pore size of the sample were 0.4797 cc g⁻¹ and 24.468 nm respectively. Rodri'guez-Lugo et al. [16] synthesized nanoHAp by wet precipitation technique with various pH solutions [9,10,11] and different sintering temperatures (300°C, 500°C, 700°C and 900°C). Spherical particles of 30-50nm size were observed at pH 9, flakes like particles of 150nm identified at pH 10, and mixtures of rod and flake particles received at pH 11. In addition, it has been shown that an increasing

sintering temperature contributes to a raise in crystalline size from 20 to 56 nm. Al-Qasas and Rohani [17] analysed the influence of temperature and reaction addition rate on crystallinity, mean particle size and morphology. At 85°C and fast reactant addition rate condition, less agglomerated large crystals with high crystallinity were produced. With slow reactant addition rate at 25°C, well defined crystals were produced.

B. Hydrolysis Method

Hydrolysis is a mechanism of water ionisation that occurs in the diffusion of hydrogen and hydroxide ions. This technique can yield high purity HAp, but it needs a long processing period.

Sinitsyna et al. [18] studied the morphology changes and production rate of α -Ca₃(PO₄)₂ hydrolysis by varying temperature. Temperature has significant impact on the hydrolysis rate that alters the morphology of the product. Plate-like intersecting HAp crystals of maximum size 1 μ m - 2 μ m were produced at a temperature of 40°C after 24 hours of hydrolysis. When boiled the suspension, needle like morphology was received. Atsushi Nakahira et al. [19] observed the effect of the solvent on hydroxyapatite formation from the hydrolysis of α -TCP. Delayed transformation was recorded for ethanol solvent. Different morphologies were obtained for different solvents like ethanol, 1-butanol, 1-octanol, 1-hexanol. Moo-Chin Wang et al. [20] prepared HAp nanoparticle by the hydrolysis of dicalcium phosphate dehydrate in NaOH solution. The XRD results showed that as-dried powders retained HAp structure in an alkaline solution atmosphere ranging from 0.1M NaOH (aq) to 5M NaOH (aq) at temperature 348 K for an hour. But in 10M NaOH (aq), complete phase transformation happened to octa calcium phosphate.

C. Sol-Gel Method

This technique is a wet chemical procedure that utilizes colloidal particles or chemical solution to generate a compound network (gel). This process permit a better control of the entire reactions during nanoparticle synthesis. Highly, pure, crystalline and reactive ceramic particulates can be prepared by this technique.

Using calcium nitrate tetra hydrate and phosphorous pentoxide as starting materials, Yusriha Mohd Yusoff et al. [21] synthesized pure hydroxyapatite nanoparticles by considering the parameters such as stirring rate, ageing time and sintering temperature. It was inferred from the findings that the optimum parameters for this process were 500 rpm (stirring rate), 1 hour (ageing time) and 600°C (sintering temperature). Rapid sol-gel process to fabricate hydroxyapatite without ageing and one hour drying was reported by Basamet al. [22]. Pure hydroxyapatite was developed at 400 °C calcination temperature and pH at 7.5, but at the same calcination temperature and pH at 5.5, biphasic mixtures of HAp/ β -tricalcium phosphate was formed. Particles developed by this novel rapid process had smaller crystalline sizes and a larger specific surface area (SSA) that could lead to enhanced bioactivity. CHEN Chun Yu et al. [23] developed highly effective microwave assisted sol-gel method to prepare hydroxyapatite nanoparticles. Samples prepared in different experimental conditions like two solvents (water and ethanol), three temperatures (25°C, 40°C, 60°C) and two microwave irradiation conditions (on & off). High-pure crystalline phase particles found when ethanol was used as solvent. Raise in reaction temperature

increased the size of nano particle. But the morphology and structure of the prepared samples were not changed by microwave irradiation.

D. Hydrothermal Method

Hydrothermal synthesis may be regarded as a precipitation reaction. Here the ageing process is carried out within an autoclave or inside a pressure vessel at temperature above the boiling point of water. The reaction between calcium and phosphate solution is triggered by the high temperature and pressure.

Xing Zhang and Kenneth S. Vecchio[24] used dicalcium phosphate anhydrous (CaHPO_4 , DCPA) and calcium carbonate (CaCO_3) to produce nanohydroxyapatite through hydrothermal technique. The reactants underwent hydrothermal reaction between temperatures 120°C and 180°C . At 140°C reaction temperature, rod shaped hydroxyapatite with negligible quantity of β -tri calcium phosphate was produced. The width of the rod was approximately 200nm and its length was in several microns. Through rapid and efficient microwave hydrothermal method, Jia Chen. [25] prepared controllable size nano hydroxyapatite rods in glycine and serine amino acids presence. It was observed that amino acids greatly impede the development of nanohydroxyapatite. The effect of serine was more apparent in contrast. Samples prepared in amino acid extract showed an improvement in MC3T3-E1 cell proliferation relative to pure hydroxyapatite that enhances the biological activity. Caibao Xue et al.[26] synthesized highly crystalline hydroxyapatite nanorods with varying levels of carbonate by a convenient hydrothermal reaction technique. The length of nanorods decreased as the carbonate content increased. Also the crystallinity of CHA nanorods decreased as a result of lattice defects caused by CO_3^{2-} ion substitution.

E. Emulsion Method

An emulsion is a uniform mixture consisting of two incompletely miscible liquid, one of which is dispersed as finite globules in the other. These two phases joined together with the help of mechanical energy by an emulsifying agent. The particle size of the globules ranges from $0.24\mu\text{m}$ to $25\mu\text{m}$.

Susmita Bose et al.[27] fabricated nanocrystalline HAp powders by micro emulsion technique. The aqueous phase consisted of calcium and phosphorous precursors, while the organic phase consisted of cyclohexane. This method produced particles with a high surface area ($130\text{ m}^2/\text{g}$) and particle sizes in the 30-50 nm range. When the volume ratio of the aqueous and organic phases changed from 1:5 to 1:15, the morphology varied from needle to almost spherical. W. Y. Zhou et al.,[28] studied the Characteristics of carbonated hydroxyapatite nanospheres through nanoemulsion technique. Acetone has been shown to be an effective oil phase for the generation of nano emulsions. Primarily formed amorphous nano spheres of CHAp transformed to highly crystalline when reached 900°C calcination temperatures. Shaohong Wang et al., [29] fabricated nano HAp by reverse micro emulsion technique. Along with calcium and phosphorous precursors, they used organic solvent (cyclohexane) and surfactant (TX-100). The surfactant used sample had rod shaped morphology with diameter in the range 20-30nm and length not greater than 100nm. The temperature maintained to develop the nano HAp was 700°C and pH 11.

F. Sonochemical Method

In this technique chemical reaction is activated by powerful ultrasound radiation. Calcium and phosphorous precursor mixes, keeping the ca/p ratio and pH as a fixed value followed by ultrasonic waves. Pure, uniform, single phase HAp with less agglomeration could be generated.

Using shellfish shells as calcium source, Hartatiek et al.,[30] synthesized n-HA/CS composites through sonochemical method. The prepared sample had needle like morphology and crystalline size 11.36 to 26.59nm which could be used as bone filler. Laterally connected HAp nano rods of length 500nm and diameter 100nm were developed by M. Jevtic et al., [31] by sonochemical process. In this technique, sintering effect of micro jets caused by the collapse of bubbles resulted the linkage of nanorods. Micro structure investigation of the nano rods revealed the crystalline structure as orthorhombic. Also it was suggested that the reaction took place between cavitation bubble and liquid solution surrounding it which was the reason for the formation of highly crystalline, defect free nano rods. Lian-Hua Fu et al.[32] developed cellulose/Hydroxyapatite composite in the aqueous solution of NaOH/urea through sonochemical process. On the surface of the composite, carbonated hydroxyapatite was formed that evident by the biological response in-vitro study and it has biomedical applications. This composite exhibited good cyto compatibility and high protein adsorption (321.5 mg g^{-1}).

High Temperature Method

In this method, precursors are burned fully or partially at high temperatures. Creation of unnecessary CaP phases can be avoided in this process.

A. Combustion Method

This method is also referred to be self-propagating high temperature synthesis (SHS). Inorganic materials could be formed through exothermic combustion reactions. Highly pure nanopowders can be produced at a rapid rate.

Wijesinghe et al.,[33] converted the naturally occurring apatite into highly pure non-toxic hydroxyapatite by combustion technique. Initially the powdered material was treated with nitric acid and then combusted using urea as a fuel. It is then subjected to a hydrothermal reaction in order to obtain pure nano HAp. The sample had needle like morphology with diameter 80nm and length of 750nm. The non-toxicity was verified by cytotoxic evaluation. Dense HAp material with improved mechanical resistance was developed using a combustion method by Maria Canillas. et al.[34]. Both aqueous and oxidizing media were used for synthesising nano HAp. From diametral compression test it was evident that powders synthesized in oxidizing media exhibited high resistance (55MPa) than samples obtained from aqueous media (33MPa). Also low porosity volume and pore size were recorded for particles developed in oxidizing media. Suphatchaya Lamkhao et al.,[35] prepared HAp through microwave assisted combustion method. Less agglomerated planetary shaped particles of average size 20-50nm were produced. The samples displayed no cytotoxic effect and the antibacterial test indicated an inhibition region for 720 hours.

B. Pyrolysis Method

Meaning of 'Pyro' is fire and 'lysis' is separating. It involves the chemical decomposition of organic materials at elevated temperatures (above 430°C) in the absence of oxygen.

Stoichiometric, homogeneous, highly crystalline nanoparticles can be produced by this technique.

Jung Sang Cho et al.,[36] revealed the addition of an organic additive Poly Ethylene Glycol (PEG) to the spray solution increased the average size of nanoparticles from several tens nm (for 0.1M PEG) to several hundreds nm (for 0.6M PEG). Particles acquired fiber-like morphology when it was post treated at 400°C, rod-like morphology at 600°C and spherical-like morphology at 1000°C. In another study, Jung Sang Cho et al.,[37] confirmed that, Spray pyrolysis was found to be the best route for processing Biphasic Calcium Phosphate. Non aggregated spherical shaped BCP nano powders were produced, when evaporated vapors subjected to the process of nucleation and growth. Widiyastuti et al.,[38] fabricated pure HAp with hollow-shape morphology through spray pyrolysis technique. Ultrasonic nebulizer atomized the mixture (calcium acetate and diammonium hydrogen phosphate) to generate droplets. The flow rate of the mixture was maintained at 1L/min and then subjected through various temperature like 500°C, 700°C, 900°C and 1000°C. HAp phase pattern appeared at higher temperature i.e., 1000°C.

C. Combination Method

Two or more different strategies may be merged to generate a collegial approach to enhance the characteristics of the end product. Mainly three combination methods are commonly used. They are Hydrothermal-Mechanochemical, Hydrothermal-Hydrolysis, and Hydrothermal-Microemulsion combination techniques. Wet mechanochemical is another name for the Hydrothermal-Mechanochemical process. Nudthakarn Kosachan et al., [39] reported the wet mechanochemical method to synthesize HAp nano particle using water and ethanol as liquid media. Single phase nanocrystalline HAp was successfully synthesized in water milled mixture. Chun-Wei Chen [40] fabricated hydroxyapatite nano crystal through mechanochemical-hydrothermal process in a short reaction time (1 hr). The specific surface area of the prepared samples decreased, when heated at 900°C for an hour. Sodium polyacrylate has been found to be the strongest HAp dispersant in water. Xiaoguo Liu et al.[41] converted α -TCP into HAp by the Hydrothermal-Hydrolysis combination technique. It was concluded that temperature and extra calcium ions were the two factors that control the macroscopic form and micro structure. Hydrothermal-Micro emulsion technique otherwise known as solvothermal technique. Dariusz smolnet al.,[42] synthesized highly biocompatible HAp nanopowder by solvothermal reaction with micro wave heating. The prepared sample had grain size 6nm and specific surface area 240 m²/g. Secondary grain growth was inhibited because of short duration synthesis process.

Synthesis from Biogenic Sources

As the mechanical and chemical synthesis methods were found to be expensive, researchers adopted biosynthesis methods for the preparation of nanoparticles. Hydroxyapatite extracted from biogenic materials is believed to be readily adopted by living organs. Egg shell, bone waste, exoskeleton of marine animals is generally used as main sources in this method.

The calcium carbonate (95-97%) present in egg shell provides the calcium source to prepare hydroxyapatite. Tabinda Rasool, [43] treated the ball milled egg shell powder

with phosphoric acid and sintered at 900°C to get highly pure HAp powders. The particle size obtained ranges from 0.15µm to 45 µm. Eric et al., prepared HAp from egg shells at higher temperature in phosphate solution. The HAp concentration could be increased by tuning the factors like phosphate solution composition, time of annealing and temperature of annealing [44]. Azis et al., developed high purity hydroxyapatite from egg shells through the formation of precipitated calcium carbonate (PCC). The sample had specific surface area 8.968 m²/g. The grain size of the obtained HAp particle was 35-54nm [45]. When sucrose was used as template in the production of hydroxyapatite from egg shells, it resulted morphology changes, reduced particle size, increased porosity and specific surface. [46]. Vijay H. Ingole et al., [47] prepared SSHAp (solid state reaction HAp) from recycled egg shell bio waste. The prepared non-toxic, osteogenic and bio active SSHAp graft has potential applications in bone therapy. Hydroxyapatite prepared using natural bone waste showed enhanced bioactivity and metabolic activity relative to chemically synthetic HAp. Fariborz et al. [48] noted the biocompatibility and progressed mechanical properties and biocompatibility of hydroxyapatite prepared using pigeon bones. At elevated sintering temperature, the upgraded compressive strength obtained was 3.7 GPa and hardness 47.57 MPa. Sudip Mondal [49] proved biocompatible osteoconductive bone fillers of HAp ceramic had potential application in bone tissue engineering. Fish bones were used to prepare this HAp ceramic bone fillers. Edwin A. Ofudje et al. fabricated hydroxyapatite scaffold with 55% porosity and pore diameter 10-15µm through thermal annealing using pig bone as raw material that could be used for tissue engineering applications. Ammonium bi carbonate was used as pores forming agent [50]. Shells and bones of sea urchin were utilized to prepare HAp through chemical reaction by Mancilla-Sanchez et al. They concluded that the optimum period for HAp production was 18 hours [51]. Birendra Nath Bhattacharjee et al. [52] derived HAp and CHApapatite powder from crab shells, and the sample showed biocompatibility with osteoblast cells. The average crystalline size of apatite nanoparticles was 24.4 nm and the range of particle size was found to be 100-300 nm. Amin Shavandi et al., obtained rod shaped nano hydroxyapatite of 30-70nm long from waste mussel shell through micro wave irradiation technique. In this process EDTA was used as chelating agent [53].

Conclusion

Several synthesis methods of hydroxyapatite by chemical means and from biological sources have been analysed in this article. Each approach is beneficial and can be utilized on the basis of end product, expense, product quality, application, etc. It is hoped that the researcher would prefer a better and greener approach from the methods described here concomitantly on suitable environmental conditions. More feasible and improved synthesis methods of hydroxyapatite are still being expected to make it easily accessible worldwide.

References

- [1] W. G. Billotte. (2000). The Biomedical Engineering Handbook, 2nd ed., Vol. 1, J. D. Bronzino, Ed., CRC Press, Heidelberg; Boca Raton, FL: Springer, 1-33.
- [2] L. L. Hench & J. Wilson. (1993). "Introduction", in An Introduction to Bioceramics. Eds. World Scientific, Singapore, 1-24.

- [3] Hench, L. (1993). Bioceramics: from concept to clinic. American Ceramic Society Bulletin, 72(4), 93-98.
- [4] H. Zhou & J. Lee. (2011). Nanoscale hydroxyapatite particles for bone tissue engineering. Acta Biomater, 7, 2769–2781.
- [5] N. A. S. Mohd Pu'ad, R. H. Abdul Haq, H. Mohd Noh, H. Z. Abdullah, M. I. Idris, & T. C. Lee. (2020). Synthesis method of hydroxyapatite: A review. Materials Today: Proceedings, 29(1), 233-239.
- [6] Mehdi Sadat-Shojai, Mohammad-Taghi Khorasani, Ehsan Dinpanah-Khoshdargi, & Ahmad Jamshidi. (2013). Synthesis methods for nanosized hydroxyapatite with diverse structures. Acta Biomater, 9(8), 7591-621.
- [7] Sumit Pramanik, Avinash Kumar Agarwal, K. N. Rai, & Ashish Garg. (2007). Development of high strength hydroxyapatite by solid-state-sintering process. Ceramics International, 33, 419–426.
- [8] Hartatiek, Kurniawati, R., Yudyanto, Hidayat, N., Kurniawan, R., & Masruroh. (2019). Solid-State Sintering Synthesis of Biphasic Calcium Phosphate/Alumina Ceramic Composites and Their Mechanical Behaviors. IOP Conf. Ser. Mater. Sci. Eng. 515 012095.
- [9] Sumit Pramanik, Avinash Kumar Agarwal & Rai, K. N. (2005). Development of High Strength Hydroxyapatite for Hard Tissue Replacement Trends Biomater. Artif. Organs, 19 (1), 45-49.
- [10] V. H. Arkin, M. Lakhera, I. Manjubala, U. Narendra, & Kumar. (2015). Solid state synthesis and characterization of calcium phosphate for biomedical application. Int., J. Chem. Tech. Res, 8264–267.
- [11] Sharifah Adzila, Iis Sopyan, & Mohd. Hamdi. (2012). Mechanochemical synthesis of hydroxyapatite nanopowder: Effects of rotation speed and milling time on powder properties. Applied Mechanics and Materials Vols, 110(116), 3639-3644.
- [12] Sang-Hoon Rhee. (2002). Synthesis of hydroxyapatite via mechanochemical treatment. Biomaterials, 23(4), 1147-1152.
- [13] Bernard Yeong, Xue Junmin, & John Wang. (2001). Mechanochemical Synthesis of Hydroxyapatite from Calcium Oxide and Brushite. Journal of the American Ceramic Society, 84(2), 465 – 67.
- [14] Azade Yelten-Yilmaz, & Suat Yilmaz, (2018). Wet chemical precipitation synthesis of hydroxyapatite (HA) powders. Ceramics International, 44, 9703–9710.
- [15] Sudip Mondal, Apurba Dey & Umapada Pal. (2016). Low temperature wet-chemical synthesis of spherical hydroxyapatite nanoparticles and their in situ cytotoxicity study. Advances in Nano Research, 4, 295-307.
- [16] V. Rodriguez-Lugo, T. V. K. Karthik, D. Mendoza-Anaya, E. Rubio-Rosas, L. S. Villaseñor Cero'n, M. I. Reyes-Valderrama, & E. Salinas-Rodriguez. (2018). Wet chemical synthesis of nanocrystalline hydroxyapatite flakes: effect of pH and sintering temperature on structural and morphological properties. <http://rsos.royalsocietypublishing.org/>.
- [17] N. S. Al-Qasas & S. Rohani. (2007). Synthesis of Pure Hydroxyapatite and the Effect of Synthesis Conditions on its Yield, Crystallinity, Morphology and Mean Particle Size, Separation Science and Technology, 40, 3187–3224.
- [18] O. V. Sinitsyna, A. G. Veresov, E. S. Kovaleva, Yu. V. Kolen'ko, V. I. Putlyaev, & Yu. D. Tretyakova, (2005) Synthesis of hydroxyapatite by hydrolysis of $\alpha\text{Ca}_3(\text{PO}_4)_2$. Russian in Izvestiya Akademii Nauk. Seriya Khimicheskaya, 1, 78—85.
- [19] Atsushi Nakahira, Kiyoko Sakamoto, Shunro Yamaguchi, Kazunori Kijima, & Masayuki Okazaki. (1999). Synthesis of Hydroxyapatite by Hydrolysis of $\alpha\text{-TCP}$. Journal of the Ceramic Society of Japan, 107 (1241), 89-91.
- [20] Moo-Chin Wang, Min-Hsiung Hon, Hui-Ting Chen, Feng-Lin Yen, I-Ming Hung, Horng-Huey Ko, & Wei-Jen Shih. (2013). Process Parameters on the Crystallization and Morphology of Hydroxyapatite Powders Prepared by a Hydrolysis Method. Metallurgical and Materials Transactions A, 44, 3344–3352.
- [21] Yusriha Mohd Yusoff, Midhat Nabil Ahmad Salimi & Adilah Anuar, (2015). Preparation of Hydroxyapatite Nanoparticles by Sol-gel Method with Optimum Processing Parameters. AIP Conference Proceedings 1660, 070054.
- [22] Basam A. E. Ben-Arfa, Isabel M. Miranda Salvado, José M. F. Ferreira, & Robert C. Pullar. (2017). Novel route for rapid sol-gel synthesis of hydroxyapatite, avoiding ageing and using fast drying with a 50-fold to 200-fold reduction in process time. Materials Science and Engineering, 70, 796–804.
- [23] CHEN Chunyu, DING Xuan, LI Shouchuan, SUN Baochang, & LV Shanshan, (2019). Microwave-assisted sol-gel synthesis of hydroxyapatite nanoparticles. Journal of Beijing University of Chemical Technology (Natural Science), 46(3), 7 - 15.
- [24] Xing Zhang, Kenneth S. Vecchio, Hydrothermal synthesis of hydroxyapatite rod. (2007). Journal of Crystal Growth, 308, 133–140
- [25] Jia Chen, Jiawei Liu, Haishan Deng, Shun Yao, & Youfa Wang. (2020). Regulatory synthesis and characterization of hydroxyapatite nanocrystals by a microwave-assisted hydrothermal method. Ceramics International, 46(2), 2185-2193.
- [26] Caibao Xue, Yingzhi Chen, Yongzhuo Huang & Peizhi Zhu, (2015). Hydrothermal Synthesis and Biocompatibility Study of Highly Crystalline Carbonated Hydroxyapatite Nanorods. Nanoscale Research Letters, 10(1), 1018.
- [27] Susmita Bose & Susanta Kumar Saha. (2003). Synthesis and Characterization of Hydroxyapatite Nanopowders by Emulsion Technique, Chem. Mater, 15, 4464-4469.
- [28] W. Y. Zhou & M. Wang & W. L. Cheung & B. C. Guo & D. M. Jia. (2008). Synthesis of carbonated

- hydroxyapatite nanospheres through nanoemulsion. *J Mater Sci: Mater Med*, 19, 103–110
- [29] ShaohongWanga, CaiWangb, ZhaoxiaHou, Hao Wang, Xiaodan Hu, Haoran Lu, ZhaoluXue, &ChangleiNiu. (2012) Reverse Microemulsion Synthesis of Hydroxyapatite Nanoparticles and Adsorption Performance Study. *Key Engineering Materials*, 512-515, 119-122.
- [30] Hartatiek, P Dwiasih, Yudyanto, N Hidayat, R Kurniawan, &Masruroh. (2019). Sonochemical Synthesis of Nano-Hydroxyapatite/Chitosan Biomaterial Composite from Shellfish and Their Characterizations. *IOP Conf. Series: Materials Science and Engineering*, 515 012050.
- [31] M. Jevtic, M. Mitric, S. S'kapin, B. Janc'ar, N. Ignjatovic, & D. Uskokovic. (2008). Crystal Structure of Hydroxyapatite Nanorods Synthesized by Sonochemical Homogeneous Precipitation, 8(7), 2217–2222.
- [32] Lian-Hua Fu, Chao Qi, Yan-Jun Liu, Wen-Tao Cao &Ming-Guo Ma. (2018). Sonochemical synthesis of cellulose/hydroxyapatite nanocomposites and their application in protein adsorption. *Scientific Reports*, 8:8292.
- [33] W. P. S. L. Wijesinghe, M. M. M. G. P. G. Mantilaka, R. M. G. Rajapakse, H. M. T. G. A. Pitawala, T. N. Premachandra, H. M. T. U. Herath, R. P. V. J. Rajapakse & K. G. UpulWijayantha. (2017). Urea-assisted synthesis of hydroxyapatite nanorods from naturally occurring impure apatite rocks for biomedical applications, *RSC Adv*, 7, 24806-24812.
- [34] Maria Canillas, Rebeca Rivero, RaúlGarcía-Carrodeguasc, Flora Barbaa Q, & Miguel Angel Rodríguez. (2017). Processing of hydroxyapatite obtained by combustion synthesis. *Ceramica Y Vidrio*, 56(5), 237-242.
- [35] SuphatchayaLamkhao, ManlikaPhaya, ChutimaJansakun, NopakarnChandet, KriangkraiThongkorn, GobwuteRujijanagul, PhuwadolBangrak&ChamnanRandorn. (2019). Synthesis of Hydroxyapatite with Antibacterial Properties Using a Microwave-Assisted Combustion Method. *Scientific Reports*, 9, 4015.
- [36] Jung Sang Cho, &Yun Chan Kang. (2008). Nano-sized hydroxyapatite powders prepared by flame spray pyrolysis. *Journal of Alloys and Compounds*, 464, 282–287.
- [37] Jung Sang Cho, You Na Ko, Hye Young Koo, &Yun Chan Kang, (2010). Synthesis of nano-sized biphasic calcium phosphate ceramicswith spherical shape by flame spray pyrolysis. *J Mater Sci: Mater Med*, 21, 1143–1149.
- [38] W. Widiyastuti, AdhiSetiawan, SugengWinardi, TantularNurtono, &HeruSetyawan. (2014). Particle formation of hydroxyapatite precursor containing two components in a spray pyrolysis process. *Front. Chem. Sci. Eng*, 8(1), 104–113.
- [39] NudthakarnKosachan, AngkhanaJaroenworuluck, SirithanJiemsirilers, SupatraJinawath, &Ron Stevens. (2017). Hydroxyapatite nanoparticles formed under a wet mechanochemical method. *J Biomed Mater Res Part B: ApplBiomater*, 105B, 679–688
- [40] Chun-Wei Chen, Charles S. Oakes, KullaiahByrappa, Richard E. Riman, Kelly Brown, Kevor S. TenHuisen and Victor F. Janas. (2004). Synthesis, characterization, and dispersion properties of hydroxyapatite prepared by mechanochemical–hydrothermal methods, *J. Mater. Chem.*, 1 4, 2425–2432.
- [41] Xiaoguo Liu, Kaili Lin and Jiang Chang. (2011). Modulation of hydroxyapatite crystals formed from atricalcium phosphate by surfactant-free hydrothermal exchange. *CrystEngComm*, 13, 1959–1965.
- [42] DariuszSmolen, Tadeusz Chudoba, Iwona Malka, Aleksandra Kedzierska, WitoldLojkowski, WojciechSwieszkowski, Krzysztof Jan Kurzydowski, MałgorzataKolodziejczyk-Mierzynska, And MałgorzataLewandowska-Szumiel, (2013). Highly biocompatible, nanocrystalline hydroxyapatite synthesized in a solvothermal process driven by high energy density microwave radiation, *Int J Nanomedicine*. 8: 653–668.
- [43] TabindaRasool, Syed Rehan Ahmed, IqraAther, Madeeha Sadia, Rashid Khan, Ali Raza Jafri, (2015)Synthesis and Characterization of Hydroxyapatite using Egg-shell, *Biomedical and Biotechnology Engineerig*, 51933.
- [44] Eric M. Rivera, Miguel Araiza, WitoldBrostow, Victor M. Castano, Diaz-Estrada, J. R., Hernandez R, Rogelio Rodriguez. J., (1999) Synthesis of hydroxyapatite from eggshells, *Materials Letters*, 41, 128–134.
- [45] Azis. Y., Adrian. M., Alfarisi., C. D., Khairat and Sri., R. M., (2018), Synthesis of hydroxyapatite nanoparticles from egg shells by sol-gel method, *IOP Conf. Series: Materials Science and Engineering*, 345, 012040, doi:10. 1088/1757-899X/345/1/012040.
- [46] Francisco José Moura, MarilzaSampaio Aguilar, CecíliaBuzatto Westin, José Brant de Campos, SuzanaBottega Peripolli, Vitor Santos Ramos, Maria Isabel Navarro, BráulioSoares Archanjo, (2020). Nanostructured Hydroxyapatite from Hen's Eggshells Using Sucrose as a Template, *Mat. Res*, 23, 6
- [47] Vijay, H., Ingole, Kamal H. Hussein, Anil A. Kashale, Ketan P. Gattu, Swapnali S. Dhanayat, ArunaVinchurkar, Jia-Yaw Chang, & Anil. Ghule. (2016). Invitro Bioactivity and Osteogenic Activity Study of Solid State Synthesized Nano-Hydroxyapatite using Recycled Eggshell Bio-waste. *Chemistry Select*, 1, 3901 – 3908.
- [48] FariborzSharifianjazi, AmirhosseinEsmaeilkhanian, Mostafa Moradi, AmirhoseinPakseresht, Mehdi ShahediAsl, Hassan Karimi-Maleh, Ho Won Jang, MohammadrezaShokouhimehr, Rajender S. Varma. (2021). Biocompatibility and mechanical properties of pigeon bone waste extracted natural nano-hydroxyapatite for bone tissue engineering, *Materials Science & Engineering B*, 264, 114950.
- [49] SudipMondal, BiswanathMondal, ApurbaDey, Sudit S. Mukhopadhyay,. (2012). Studies on Processing and Characterization of Hydroxyapatite Biomaterials from

Different Bio Wastes, Journal of Minerals & Materials Characterization & Engineering, 11(1), 55-67

Journal of Applied Ceramic Technology · DOI: 10.1111/ijac.13086.

- [50] Edwin A. Ofudje, Archana Rajendran, Abideen I. Adeogun, Mopelola A. Idowu, Sarafadeen O. Kareem, Deepak K. Pattanayak. (2017). Synthesis of organic derived hydroxyapatite scaffold from pig bone waste for tissue engineering applications. Advanced Power Technology, 29(1), 1-8.
- [51] . Mancilla -Sanchez, C. M. Gómez -Gutiérrez, G. Guerra -Rivas, C. A. Soto -Robles, A. R. Vilchis -Nestor, E. Vargas, P. A. Luque. (2018). Obtaining hydroxyapatite from the exoskeleton and spines of the purple sea urchin *Strongylocentrotus purpuratus*, International Journal of Applied Ceramic Technology · DOI: 10.1111/ijac.13086.
- [52] Birendra Nath Bhattacharjee, † Vijay Kumar Mishra, *, †, ‡ Shyam Bahadur Rai, *, ‡ Om Parkash, † and Devendra Kumar*, †, (2019) Structure of Apatite Nanoparticles Derived from Marine Animal (Crab) Shells: An Environment-Friendly and Cost-Effective Novel Approach to Recycle Seafood Waste, ACS Omega 2019, 4, 12753–12758.
- [53] Amin Shavandi, Alaa El-Din A. Bekhit, Azam Ali, Zhifa Sun. (2014). Synthesis of nano-hydroxyapatite (nHA) from waste mussel shells using a rapid microwave method, Materials Chemistry and Physics, xxx, 1-10.

