Waste to Wealth: An approach to HAp synthesis by different methods.

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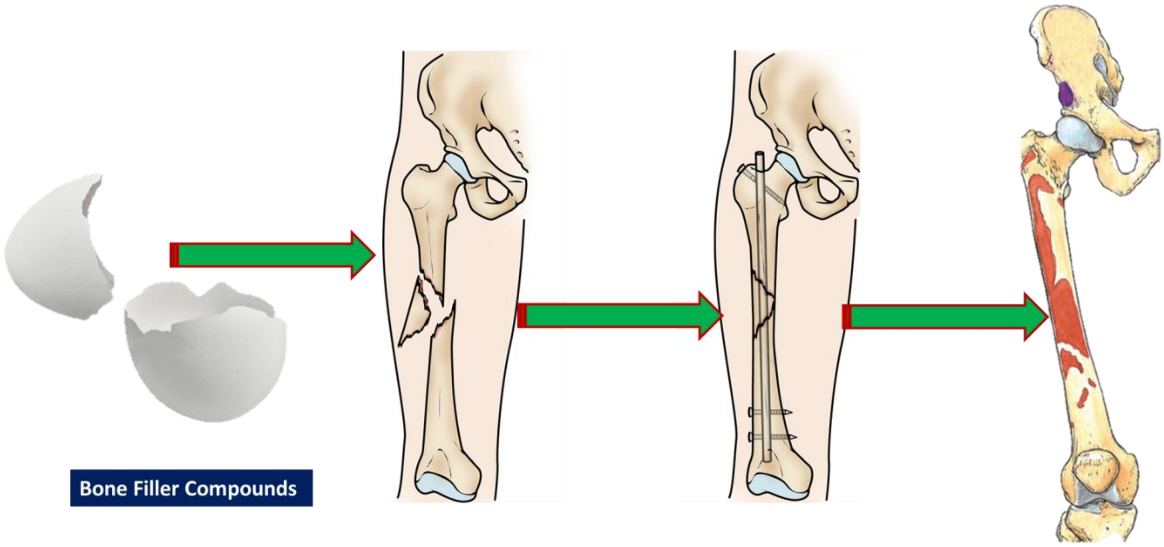
Abstract-Waste materials are an important source for the recovery and extraction of various valuable compounds. Transforming these wastes into valuable compounds require various techniques and approaches. It is the requirement to design various functional bioactive substitute materials that are able to survive the harsh and diverse conditions within the human body. Biomaterials are gaining increased importance due to their applicability to ageing population and treating diseases. Biomaterials acts to restore, repair or to replace any tissue that has been damaged in the body thus increasing the life expectancy. Hydroxyapatite (HAp) is one of the biomaterials obtained from the natural waste materials. It is universally used in biomedical because of its bioactivity, biocompatibility, remarkable oestoconduction property, etc. HAp is receiving importance in orthopaedic implants and also in dental materials. This review outlines the various methods of extraction of HAp from various natural sources like marine, aquatic, mammalian, shell, plant and algae. The Ca/P, crystallinity, particle shape, size, morphology and clinical studies are also discussed.



Keywords: *Hydroxyapatite, Natural Product Chemistry, Bio-waste, Bio-implants, Natural Resources*

1. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) has very similar composition as of bone mineral due to its thermodynamically stable and crystalline structure[1]. It can bind to bone without disturbing the bone response by any kind of local or systemic toxicity & inflammation by foreign body[2]. Due its succession rates its universally used in biomedical applications mainly in the fields of orthopaedic, odontology, and also used as coating material for metallic implants as it is having one of the good bio-compatibility, Bioactivity, Non-immunogenicity properties [3].

Some studies showed the ability of HAp for absorption which has attracted a great interest in the purification of wastewater and takeoff various heavy metals from aqueous solution for having factors like low cost and high stability, under the conditions like oxidizing & reducing and also due to its high removal capacity and low water solubility. HAp can be chemically extracted from various natural sources. There are various methods which can be used to synthesize natural as well as synthetic HAp. When compared with synthetic HAp, natural HAp is non-stoichiometric as it contains element traces like Na^{+} , Zn^{2+}, Si^{2+} , F^{-}, Mg^{2+}, K^{+} and CO^{3-} , thus resembling to the human bone chemical composition[4,5].

As the structure of HAp indicates its absorption ability and bioactivity, it is key to study various physical parameters such as phase purity, thermal stability, particle size and shape for obtaining pure and high quality and better performance HAp for variety of applications in biomedical field [5]. Because of its importance, HAp has came out to be a biomaterial of great interest specifically in biomedical field such as tissue engineering, dental, damaged bone replacement, along with this it also has other applications like ion conductors, gas sensor and as an adsorbent in volume chromatography [6].

HAp can also be synthesised by various methods like Dry methods (solid-state and mechanochemical), wet methods (chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, and sono-chemical), and also high temperature process (Combustion & prolysis) [1]. Since Long Years Ago, some biologically naturally derived substances like Bovine Bones, Oyster Shells, Egg Shells, Corals, Fish Bones, were used for extraction of HAp.

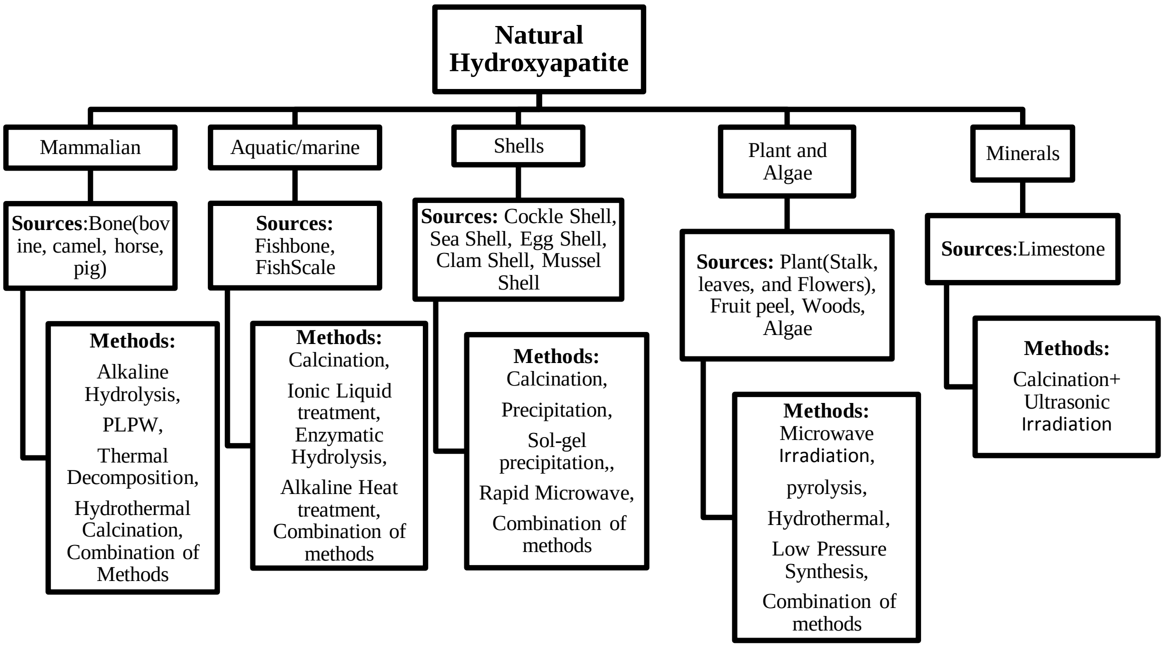


Fig.1. Summary of processes for synthesising natural HAp [7].

2. Main text

2.1. Mammalian Source

2.1.1. Bovine Bone

Bovine Bone has approximately 65–70% hydroxyapatite and 30–35% organic compounds (on the basis of dry weight). In natural bovine bone collagen is main organic material present which is approx. 95%. Chondroitin sulphate, keratin sulphate and lipids (e.g. phospholipids, triglycerides, fatty acids, and cholesterol) are some of the organic compounds which are in smaller amounts present in bovine bone [8].

Some methods like - Thermal, subcritical water and alkaline hydrothermal are used for decomposition, dissolution or hydrolysis of the organic matter available in the bovine bones, respectively.

Preparation of Bovine Bone Powder:

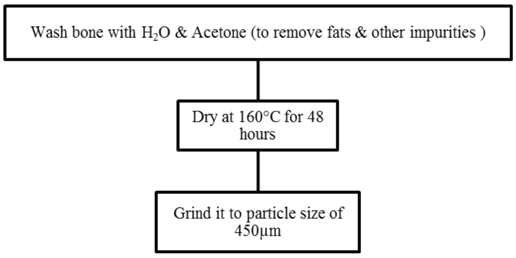


Fig.2. Preparation of Bovine Bone Powder [9]

I. Alkaline Hydrolysis

We can do alkaline hydrolysis by using autoclave and also by microwaves for keratin. As, keratin and collagen have fibrous shapes and are similar proteins. So for the alkaline hydrolysis process Autoclave was used for the hydrolysis of collagen and various other organic matters which are present in the bovine Bones.

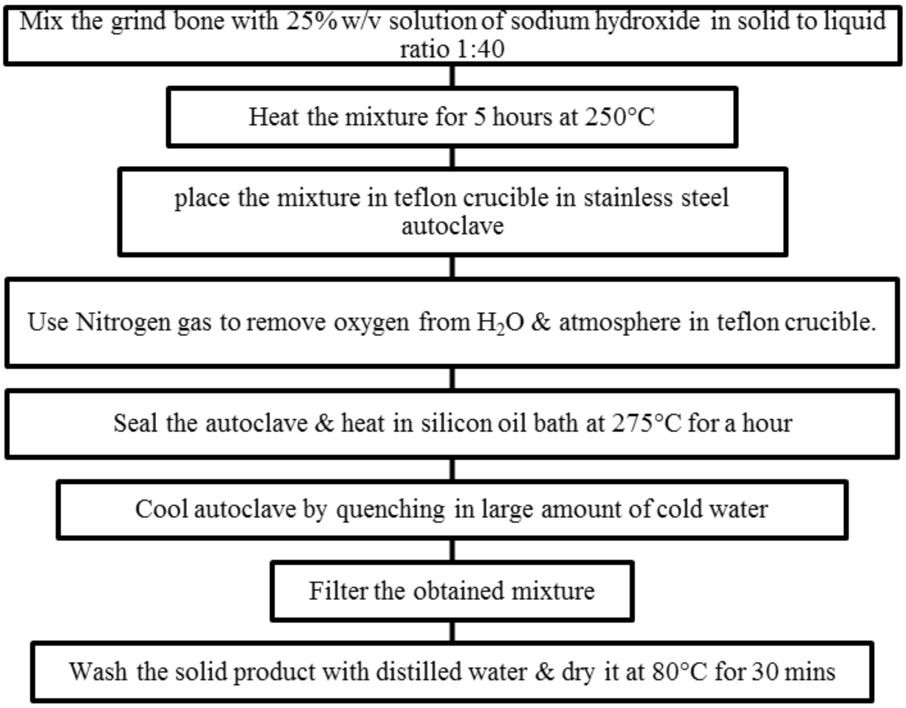


Fig.3. Block diagram for HAp synthesis from Bovine Bone by Alkaline Hydrolysis method [10, 11]

II. Pressurised Low polarity water Extracting Process (PLPW)

Pressurised low polarity water process is an extraction process that requires hot water under pressure. Usually, the temperature required is below the critical value of water i.e. 379°C but above 100°C. The increase in temperature of water causes change in polarity, surface tension and viscosity. This also causes reduction in dielectric constant of water with the increasing temperature polarity of water decreases. Thus, this process is applicable for extraction of various organic compounds.

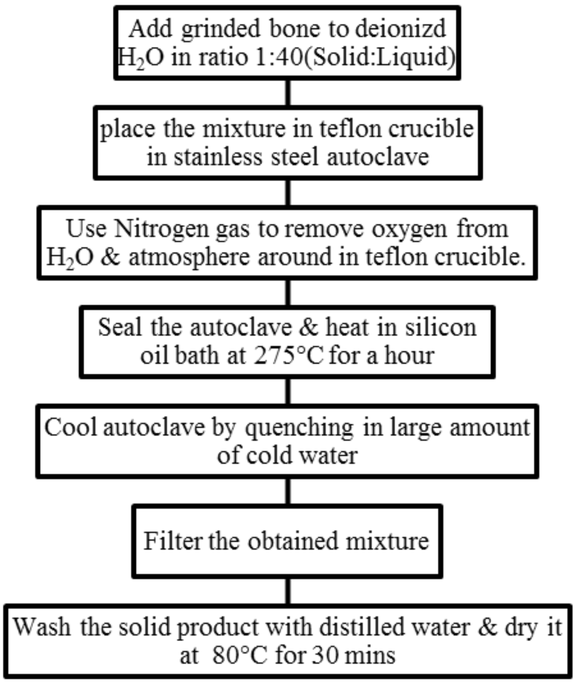


Fig.4. Block diagram for HAp synthesis from Bovine Bone by PLPW method [12]

III. Thermal Decomposition

Thermal decomposition is the traditional processes used in synthesis of organic matter from various bio wastes. In this method, the bone is heated directly for the decomposition of collagen and other organic matter are eliminated by the process of calcinations.

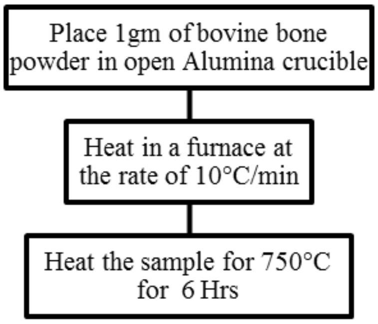
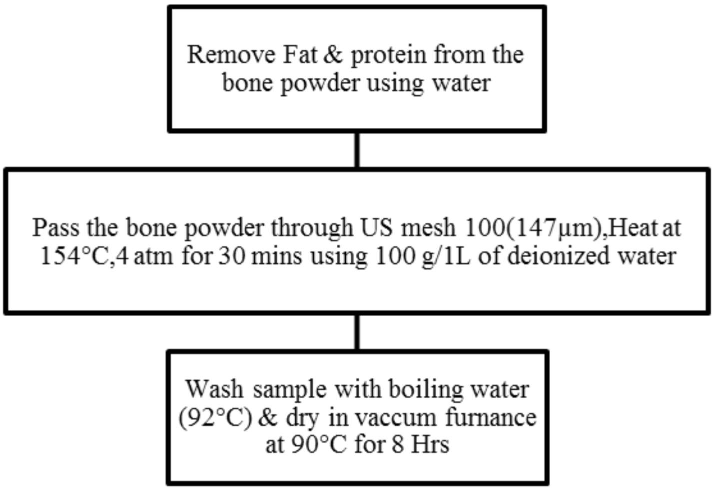


Fig.5. Block diagram for HAp synthesis from Bovine Bone by Thermal decomposition method [9]

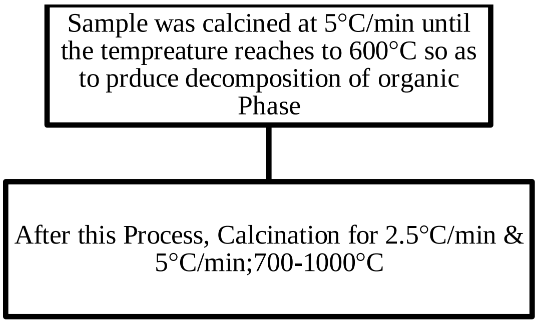
IV. Hydrothermal Calcination

Flakes of bovine bone which were taken from the central part of femur bone are used to grind and make the bovine bone powder while the soft fat tissues were separated manually. After this step fluids in the bone marrow were removed by boiling at high pressure in autoclave in water for about 40 mins for three cycles to obtain organic compounds. Then they were dried by vacuum drying to denaturalize the proteins and enhance milling procedure. And they were grinded to make small fine bovine bone powder

Process I- Hydrothermal process & vacuum dry



Process II - organic decomposition & calcinations



Process III - Cooling

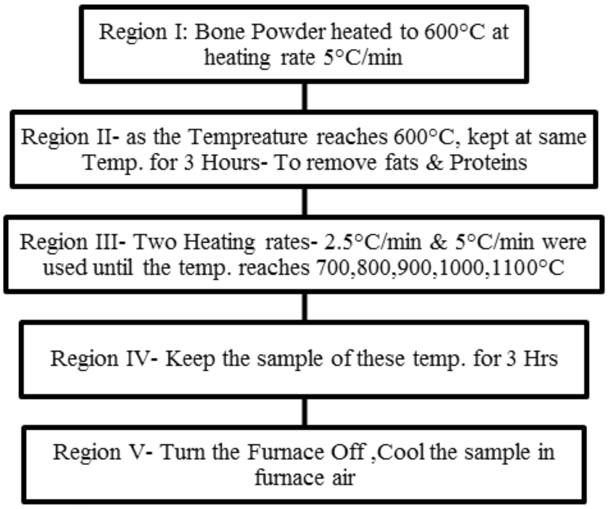


Fig.6. Block diagram for HAp synthesis From Bovine Bone by Hydrothermal calcination method [12]

Table 1: Summary of methods used for extraction of HAp from mammalian sources.

Method	Ca/ P ratio	Crystalline Phases	Particle Size	Shape	Yield	Reference(s)
Alkaline Hydrolysis	1.86	HAp	Nanosize	Nanoflakes	61%	[9]
PLPW	1.56	HAp	Nanosize	Nanoflakes	69%	[9]
Thermal Decomposition	1.65	HAp(300nm)	Nanosize	Nanorod	66%	[9]
Hydrothermal Calcination	1.62	Hap dehydroxylate	-	Irregular(700°C) Semi-Spherical(800°C)	-	[13]

The Ca/P ratio of the extracted HAp from the bovine bone ranges from 1.56 to 1.86 depending upon the method used. The Ca/P value from alkaline hydrolysis is high compared to other methods. All the values obtained lies within the range which is acceptable for hydroxyapatite.

Morphological studies from bovine bone shows that HAp extracted particles have irregular shape while some have rods, flakes like shape it can be concluded that there is no relationship between the morphology of HAp with the extraction process. Greater results can be achieved by combining one or more methods.

Above observations stated that relationship between the size of HAp and extraction method is not relatable to each other. Use of milling made HAp particles into nanosize which is almost equal to the size of human HAp particles which benefited us by giving ultrafine structures with higher surface activity [14].

The crystalline structure obtained from the thermal process is much better that other three processes. The HAp obtained through PLPW process has relatively good crysallinity, whereas the other two processes produce HAp with comparatively less crystallinity. Also the peak of XRD for the HAp extracted from thermal process produces sharp spectra indicating good crystallinity.

2.2. AQUATIC/MARINE

2.2.1. FISH SCALE

I. Calcination-Thermal Treatment

HAp from fish scale waste through alkaline heat treatment method is commonly employed method. The synthesized HAp powder is used for selenium adsorption from aqueous solution and its bioactivity is unknown. Similarly, a scientist [15], synthesized the HAp from tilapia fish scale using enzymatic hydrolysis method and showed that the synthesized HAp powder also contain low concentration of peptides. Further, HAp synthesized using enzymatic hydrolysis or alkaline heat treatment uses expensive chemicals.

And the processes are difficult to scale up. Calcination method is an inexpensive thermal treatment method to synthesize HAp from fish scale. It is important to note that the trace elements like magnesium (Mg) and strontium (Sr) present in the natural sources play an important role in bone remodelling process [14].This method is useful in evaluating these trace elements in the thermally synthesized Hap.

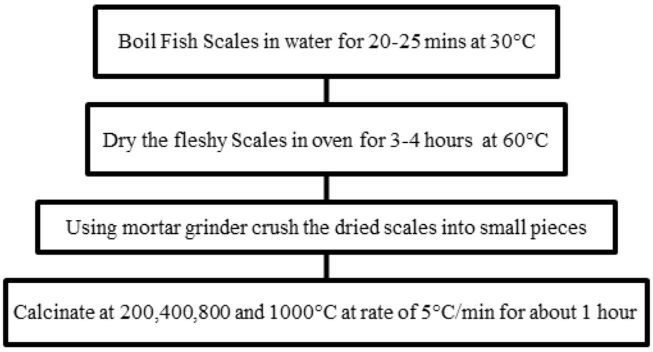


Fig.7. Block diagram for HAp synthesis From Fish Scale by Calcination-Thermal method [16]

II. Ionic Liquid Treatment

Fish scales are an alternative source of natural material that can be used for the synthesis of HAp. The preparation and biocompatibility of HAp from fresh water source was found that this type of HAp synthesized promotes cellular attachment and proliferation [17].Moreover, such type of conversion of raw materials into HAp, thermal treatment with basic $CaCl_2$ is required to be employed to obtain HAp. Such a thermal treatment led to a HA/β-TCP biphasic material and could change the physicochemical and biological activities of the original materials [18]. This method employs the synthesis of the HAp from the ionic liquid treatment without the high temperature treatment. This HAp is further characterized by several analytical techniques to determine the composition and micro structural features and further tests for biological activity.

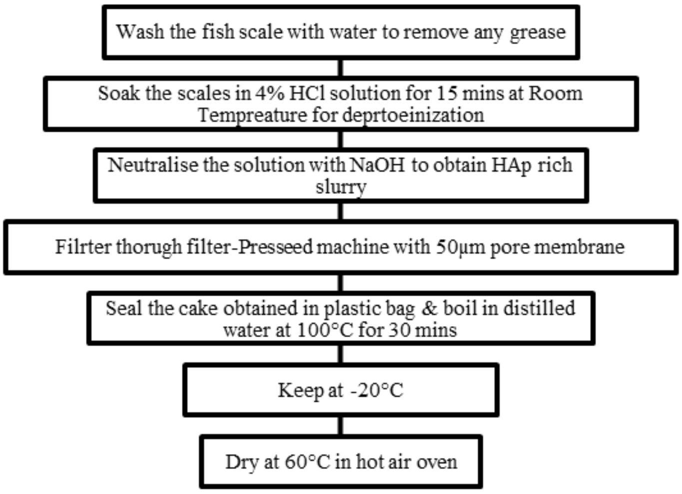


Fig.8. Block diagram for HAp synthesis From Fish Scale by Ionic Liquid method [19]

III. ENZYMATIC HYDROLYSIS

It is clear that fish scale contains various valuable organic as well as inorganic compounds which mainly contain collagen and HAp which are commercially valuable for manufacturing of functional foods, biomedical products and cosmetics [20-22].

There are various methods to synthetically produce HAp, such as microwave irradiation, chemical precipitation and radio frequency thermal plasma. Using biological waste for extraction of HAp is a biologically safe (i.e., no chemicals are required) and a potentially lucrative process, especially when come to the global demand for hydroxyapatite bio ceramics [23]. Thus enzymatic hydrolysis is the method used for the extraction of HAp from fish scales.

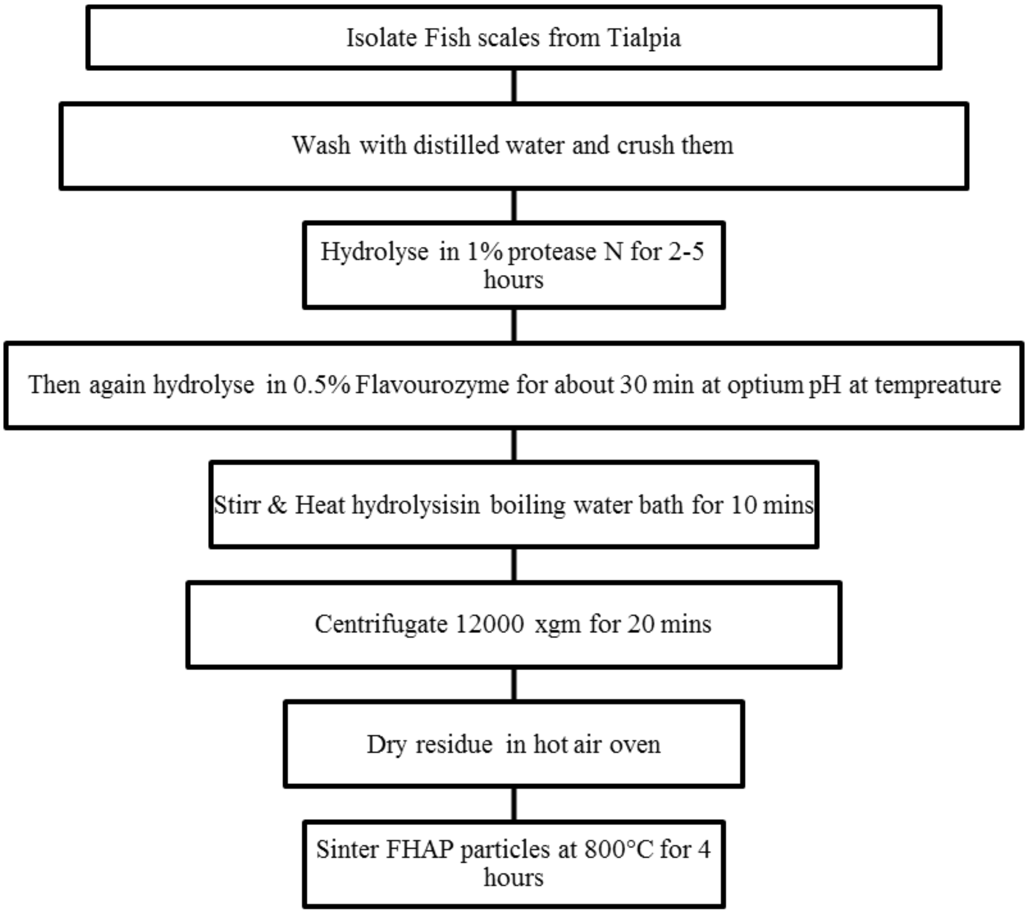


Fig.9. Block diagram for HAp synthesis From Fish Scale by Enzymatic Hydrolysis method [15]

IV. ALKALINE HEAT TREATMENT

The main aim is to extract HAp from unexplored bio wastes obtained from fishes, considering these wastes as potential source. The extracted HAp was characterized to ascertain its suitability for tissue construct development. Fish scales are mainly composed of collagen, connective tissue proteins, water and other types of proteins [24]. They also contain a variety of calcium phosphate salts due to their intense biological response to difference in osmolality and ionic inequality in physiological environment. The preparation of HAp from fish scale demonstrated that a mixture of b-TCP and HAp is obtained upon direct sintering of fish scale, while the pre-treatment of fish scale with basic $CaCl_2$ and its calcinations at 800°C for 1 hour (alkaline heat treatment method) produces pure HAp crystal.[25,26]

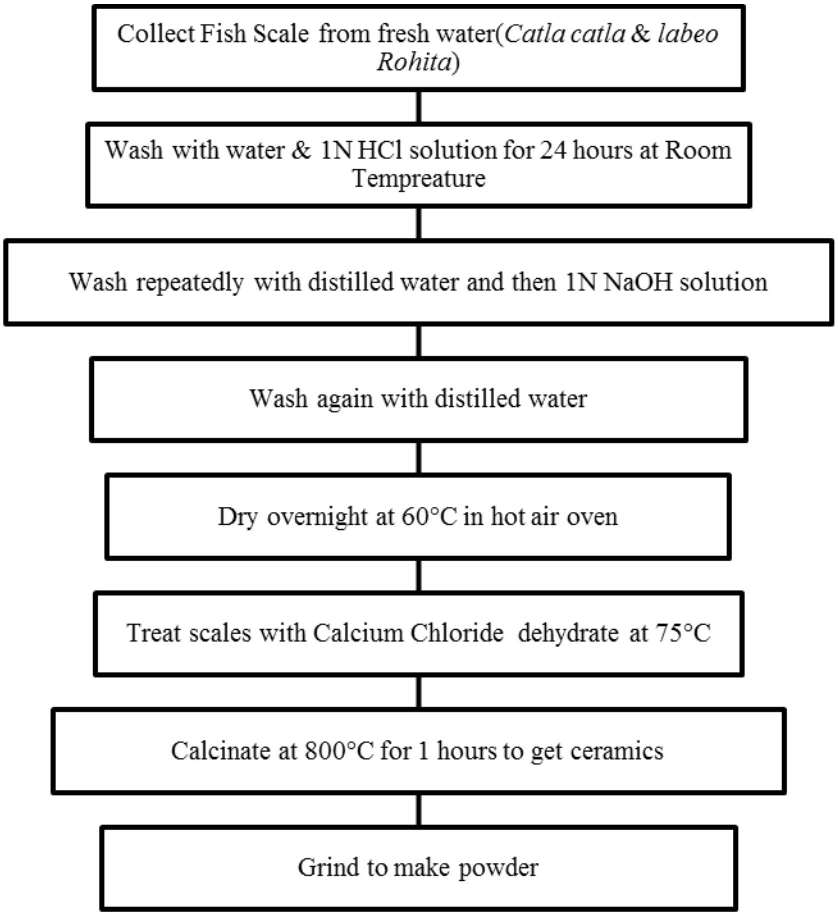


Fig.10. Block diagram for HAp synthesis From Fish Scale by Alkaline Heat Treatment method [24]

V. ALKALINE HEAT TREATMENT WITH CALCINATION

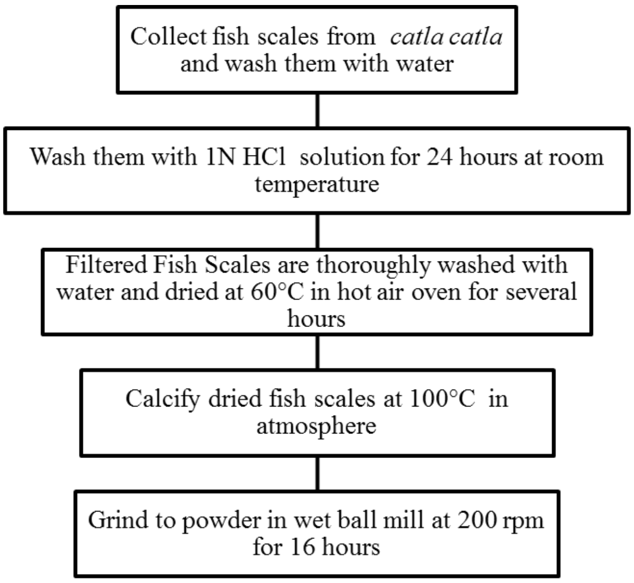


Fig.11. Block diagram for HAp synthesis From Fish Scale by Alkaline Heat Treatment with Calcination method [27-30]

Table 2: Summary of methods used for extraction of HAp from aquatic or marine Sources

Method	Ca/P ratio	Crystalline Phases	Particle Size	Shape	Reference(s)
Calcination-Thermal Treatment	1.71 (1200°C)	HAp (800 °C) HAp, β-TCP (>1000 °C)	30 nm	Irregular	[16]
Ionic Liquid Treatment	1.60	HAp	1870 nm	Vary	[19]
Enzymatic hydrolysis	1.76	HAp	719.8 nm	Irregular	[15]
Alkaline Heat Treatment	2.01	HAp	15-20mm width 100 nm length	Flat-Plate	[18]
	1.66	HAp	15-20 nm	Hexagonal	[30]
Alkaline Heat Treatment + Calcination	1.62	HAp	76.62 nm	Agglomerate	[23]

In summary, In enzymatic hydrolysis method, for hydrolysis of fish scales enzymes are used in this method while to extract pure HAp without making any changes hydrolysis (alkaline hydrolysis, enzymatic hydrolysis) and ionic pre-treatments are used. Synthesised morphology of HAp was studied under microscope the shapes were flat plates-, rod likes, or irregular shaped and produced nano-sized HAp. While elemental studies stated the presence of micro elements like Mg ions, Sr, Na were found in fish scale synthesised HAp.

As compared to calcinations method other methods like alkaline heat treatment, ionic liquid treatment, and enzymatic hydrolysis treatment produced pure HAp with less crystallinity.

2.3. SHELLS

2.3.1. EGG SHELL

I. CALINATION + wet PRECIPITATION

Some Articles have suggested using calcination & wet precipitation method in combination for egg shells. In this method nanocrystalline HAp samples can be synthesized by using various natural sources of calcium which helps to simplify the process of production [32, 33].

Nanocrystalline HAp can be synthesized using eggshell. The fact that eggshell's main content is calcium carbonate which can dissociate into carbon and calcium oxide at the temperature 900°C

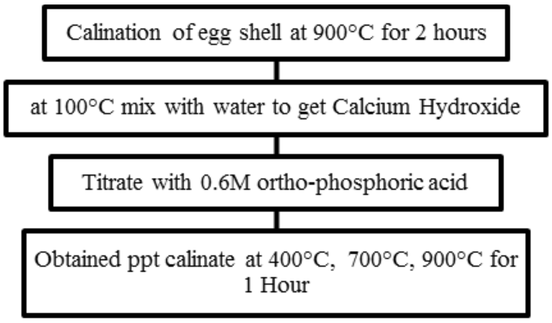


Fig.12. Block diagram for HAp synthesis From Egg shell by Calcination and wet Precipitation method [34]

II. PRECIPTATION METHOD

Synthesis of HAp from eggshell is quite effective. The main aim is to minimize the accumulation of egg waste caused by the daily consumption of egg, which is due to the daily demand of poultry product such as fast food, cakes, etc. The egg shell waste is disposed in the land that may become a reason for land degradation, thus the synthesis of HAp from egg shell helps in the utilisation of egg waste.

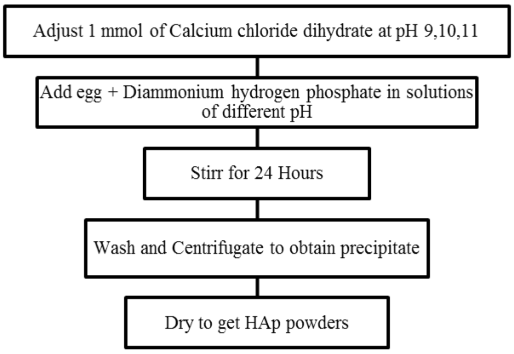


Fig.13. Block diagram for HAp synthesis From Egg shell by Precipitation method [35]

2.3.2. COCKLE SHELL

III. Sol-Gel + Precipitation Method

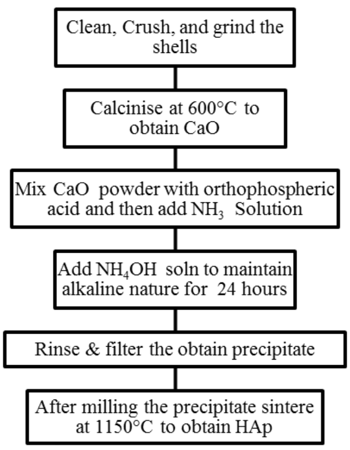


Fig.14. Block diagram for HAp synthesis From Cockle shell by Sol-gel Precipitation method [36]

2.3.3. MUSSEL SHELL

IV. Rapid Microwave Method

Mussel shell is one of the waste products produced in large-scale by fish industry in various countries which becomes the reason for landfills. In many western countries, mussel shells are produced in large quantity annually, which causes environmental stress like odours while treatment and transportation and high element concentration in the landfills. From the total weight of the mussel, the shells constitute about 55% and consist of 95-99% aragonite (a carbonate mineral, one of the three most common naturally occurring crystal forms of calcium carbonate, $CaCO_3$) [37].

Thus, mussel shells prove to be one of the useful sources to produce HAp by developing fast and simple method.

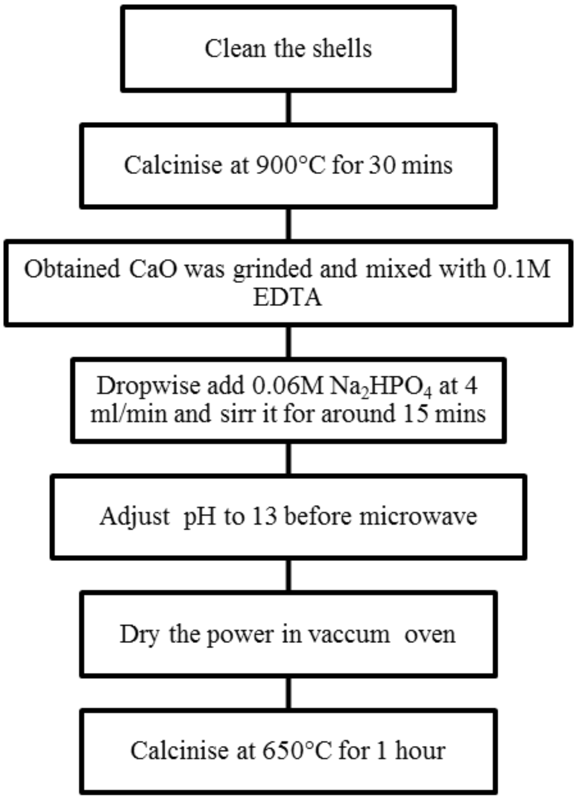


Fig.15. Block diagram for HAp synthesis From Mussle shell by Rapid Microwave method [38]

Table 3: Summary of methods used for extraction of HAp from shell sources

Method	Ca/P ratio	Crystalline Phases	Particle Size	Shape	Reference(s)
Calcination + wet precipitation	1.7-2.1	HAp	30nm	Globules	[34]
Precipitation	1.425	HAp	5 nm diameter 50-100 nm	Needle like	[35]

			length		
Sol-gel Precipitation	< 1.68	HAp	4.03-10.4 µm	Spherical	[36]
Rapid Microwave	1.65	HAp	30-70nm	Rod-Like	[38]

From Table.3: The Ca/P produced by the calcination+precipitation method and precipitation method is 1.7-2.1 (which is very close to the stoichiometric ratio of HAp) and 1.425 (much lower than the stoichiometric ratio of HAp) respectively. In terms of crystallinity, the HAp extracted from the process of calcination + precipitation has high crystallinity. But the HAp produced from the process of precipitation from eggshell shows low crystallinity when compared with calcination + precipitation. The increase in crystallinity of HAp in calcination + precipitation than precipitation is due to increased heat.

2.4. PLANTS AND ALGAE

2.4.1. Red ALGAE

I. Low Pressure Synthesis

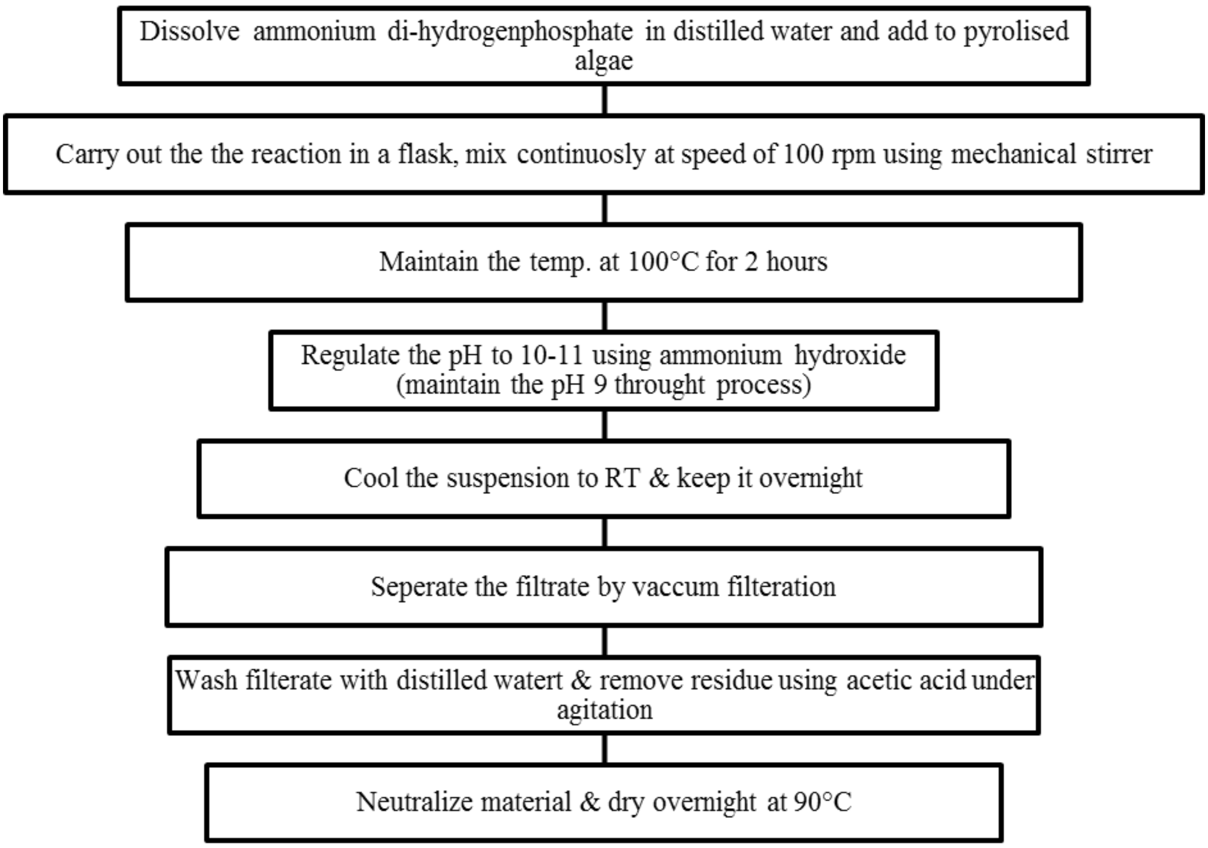


Fig.16. Block diagram for HAp synthesis From Red Algae by Low Pressure method [39]

Table 4: Summary of methods used for extraction of HAp from algae sources

Method	Ca/P ratio	Crystalline Phases	Particle Size	Shape	Reference(s)
Low Pressure Synthesis	-	HAp, CaCO3,βTCP (650 _C) HAp, βTCP, SiO2 (700 _C)	-	-	[39]

2.4.2. Moringaoleifera Leaves

I. Microwave irradiation method

The aim of the below work is to easy and rapid microwave radiation technique. The powder leaves treated with the HNO_3 to remove organic components. The irradiated leaves gives white precipitate and high purity HAp was produced.

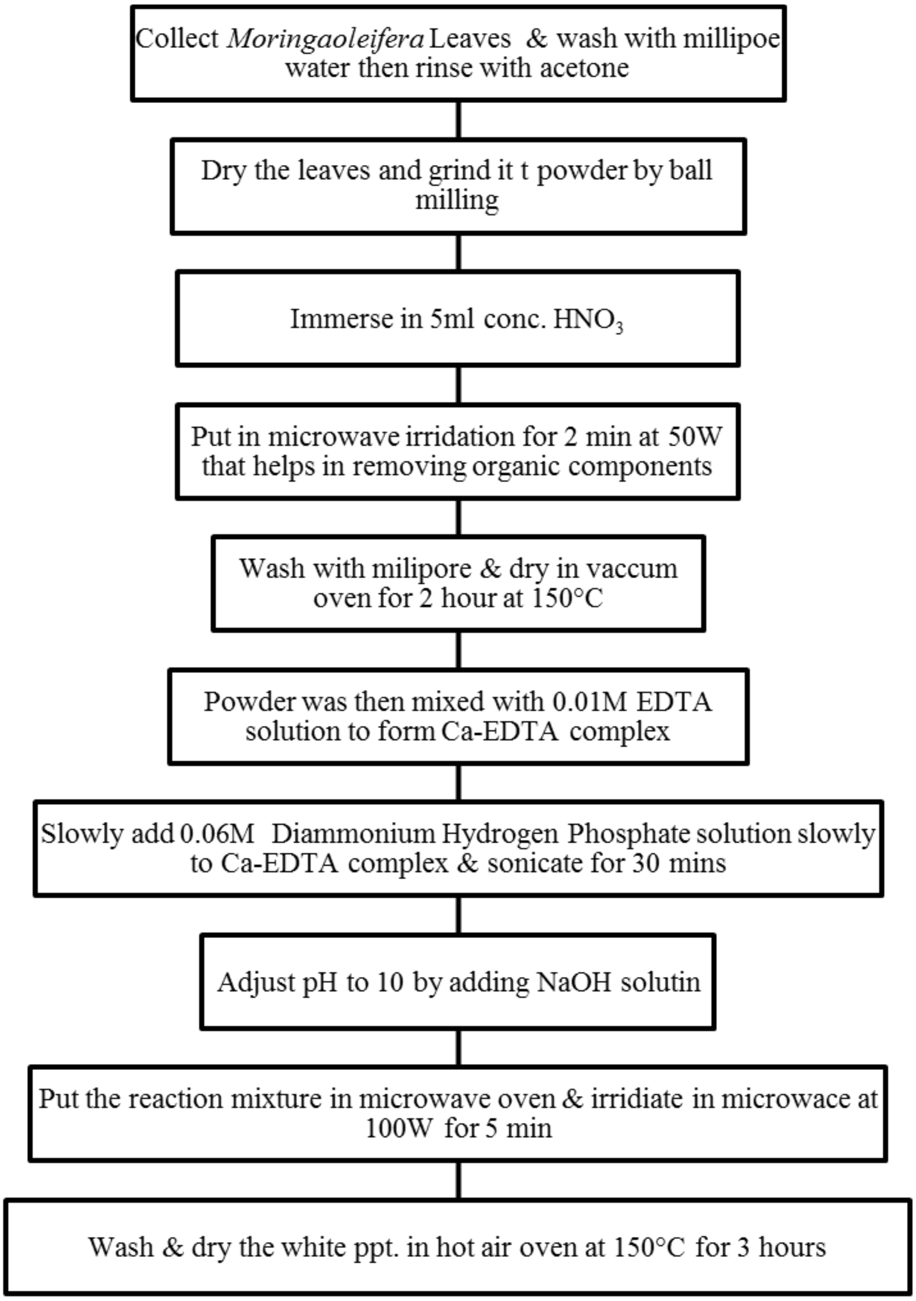


Fig.17. Block diagram for HAp synthesis From *Moringaoleifera* Leaves by Microwave irradiation method [40]

Table 5: Summary of methods used for extraction of HAp from plant sources

Method	Ca/P ratio	Crystalline Phases	Particle Size	Shape	Reference(s)
Microwave irradiation method	1.72	HAp	Nano-size	Flakes	[40]

From Above table we can say that the HAp synthesized from the leaves of Moringa Oleifera has nano sized flake-like morphology. Analysis of the HAp obtained showed Ca/P ratio of 1.72. These leaves are low cost, easily available, safe green food and waste for the continuous and rapid HAp synthesis. Using this organic food material i.e. Moringa Oleifera helps in avoiding the cost required for high quality source of calcium for preparation of HAp

2.5. MINERALS

2.5.1. LIMESTONE

I. Calcination + ultrasonic Irradiation method

Among the various calcium containing mineral sources, limestone is one of the natural source of mineral that can be used for synthesizing hydroxyapatite. Naturally, by using animal shell or skeleton, foraminifera or algae as a rich source of CaCO3, limestone can be synthesized by the method of precipitation [41].Due to its abundance in nature; limestone is very possible source of calcium used for the synthesis of HAp [42,43].

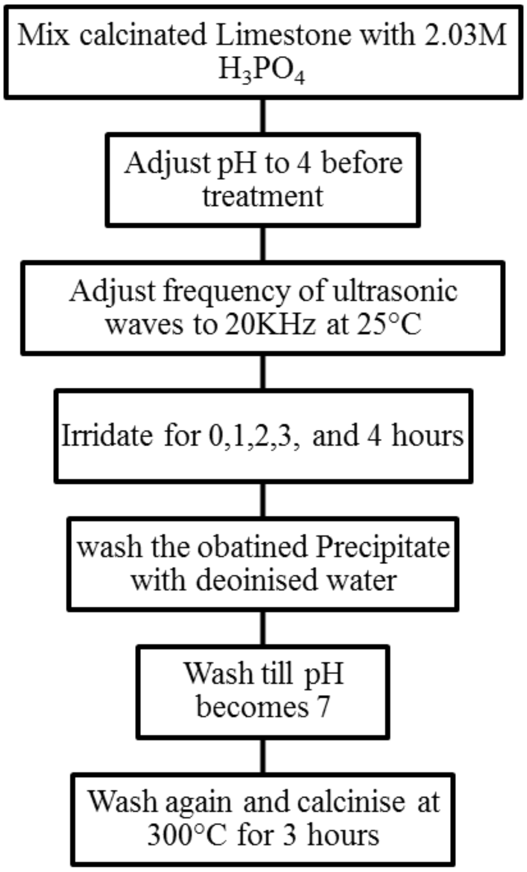


Fig.18. Block diagram for HAp synthesis From Limestone by Calcination and ultrasonic irradiation method [44, 45]

Table 6: Summary of methods used for extraction of HAp from mineral sources

Method	Ca/P ratio	Crystalline Phases	Particle Size	Shape	Reference(s)

Calcination + ultrasonic Irradiation method	-	HAp + $CaHPO_4$	7.4 nm width and 62.5nm length (3 hours)	Needle-like, Plate-like	[46]

The HAp synthesised from minerals (Limestone) by calcinations and ultrasonic irradiation combination method shows that irradiation time is directly prortional to the smaller sized needle like nanoparticles of HAp. The Ca/P ratios of HAp from limestone are non-stichiometric with an overall size of 7.4 nm width and 62.5 nm lengths.

3. CLINICAL STUDIES:

3.1. Nano-hydroxyapatite in bone defect regeneration: a histological study in rats [47]

The surgery was performed on Wistar rats (200-250g).

Randomly selected 30 animals were grouped into 5 groups depending upon the type of material used:

Group 1: Geistlich Bio-Oss granules size 0.25mm

Group 2: nano-hydroxyapatite (nano-HA) in combination with beta-TCP

Group 3: nano-HA

Group 4: nano-HA covered by the collagen membrane.

Group 5: Control group, defects without augmentation.

The treatment on every animal was the same. After anaesthesia, the animals were treated with 3% sodium pentobarbital with ketamine (60mg/kg body weight) after shaving the operation site, the skin was sterilised with iodine. The rats were later provided with local anaesthesia of 2% lignocaine. By doing a longitudinal incision of about 3cm the calvaria bone was exposed. 5mm bone defects were created under permanent saline cooling with low speed drilling. The defects were located in the calvaria bone in the middle of the saggital plane and it was completely filled with various materials of group 1-4 except for control group. With the cutaneous single interrupted suture 5/0, the wound was closed. After surgery, administer penicillin intramuscularly for 3 consecutive days to prevent infection.

By giving an overdose of sodium pentobarbital, the animals were sacrificed 8weeks post insertion of material in randomized manner. Once again the calvaria bone of each animal was exposed and for further investigation bone tissue was taken. For the analysis, a CBCT (Cone beam computed tomography) scanner was used with various exposure parameters of 90kV, 3.0mA, 17.5sec scan, 0.16 slice intervals and 0.16mm slice thickness data. For the histological examination, the specimens were taken from the test site and immersed in 4% buffered formalin for 7days at room temperature and further embedded in paraffin. About 5micro m serial longitudinal sections were stained with haematoxylin/eosin (H. E.) for recognising Masson Goldner trichome and various tissue types for differentiating the collagen, muscle and bone tissue. These histological parameters were subjected to microscopic evaluation consisting assessment of type and quality of tissue that fill the defect as well as the nature of cells that covers the bone defect surface.

Histomorphometric analysis was done by 2 independent observers and performed on composed pictures that showed the complete cavity at magnification of ×100 [48, 49].Histological and molecular-biological analyses of poly (3-hydroxybutyrate) (PHB) patches for enhancement of bone regeneration.The new bone formation was marked along with the line between old and new bone. Bone regeneration was measured and expressed as percentage of bone height of main bone in the periphery of the lesions.

Statistical analysis was made, all the data were given as means ± standard deviation. $P<0.05$ that was considered statistically significant.

3.2. Bovine Bone- PICP (procollagen type I a carboxyl procollagen) [50]

The hydroxyapatite obtained from bovine bone enhances the formation of bone significantly after a month. The PICP level increases about 99.00% when compared with the base line control. The preliminary report of hydroxyapatite shows that it can be used for the cases such as bone remodelling with a normal range of turnover. Thus, it can be said that hydroxyapatite obtained from bovine bone can be used for the prevention of bone loss and enhances the growth of bone in young adults. In cases of controllable bone loss for e.g. after biphosphonate intervention, the HAp can be administered [51-54].

3.3. Egg Shell:

Studies [55] ,revealed the comparison between eggshells and HAp where more amount of new bone was developed around the grafted HAp particles. However, Area around the synthetic HAp there were more amount of foreign particles which were multinucleated than naturally obtained HAp from egg shell. After observing the micro CTScan, egg shell HAp showed remarkable increased volume as compared to synthetic HAp [56] .While some studies were conducted on animal models which illustrated the advantages of using bio ceramic scaffolds in the procedures of bone regeneration. The chemical-physical properties are most important or the proper dimensional bone healing [57].

4. Conclusion

Various natural sources are the precursors for synthesizing HAp. The HAp produced from different sources shows different characteristics. The very frequent shape obtained was rod-like or needle-like, but shape of HAp is neither affected by the extraction method nor the source. Mostly the nanosized Hap particles were obtained. Crystallinity of HAp particles depends on the method of extraction, whereas thermal method showed increased crysallinity and the low crystallinity was observed in chemical precipitation method.

On the other side, Ca/P ratio obtained from each natural source was non-stoichiometric. In sources like mammalian and aquatic, the trace elements present may result in change in HAp ratio i.e. values which are higher than stoichiometric HAp. For the remaining sources like shell, minerals, algae, other calcium species like CaO present may increase the Ca/P value. It is very persistent to use the combination methods, other than the alkaline hydrolysis and calcination. Using combination method for extraction helps in enhancing the properties of extracted HAp.

Some of the recent methods for the extraction of HAp from various natural sources like mammalian, aquatic, shell, plants, algae, mineral source were studied. Generally, the production of

HAp from different sources shows characteristic morphology and size. Differences in the characteristics of HAp obtained depend upon the method of extraction. The HAp produced from thermal methods has high crystallinity when compared with chemical precipitation. Some of the sources that are rich in CaCO3 like shell and algae require further treatment to produce pure HAp. Either solely or in combination, many methods are used to produce high purity HAp which resembles Ca/P value close to that of the human bone. It is important to study the properties of HAp such as thermal stability, phase purity especially when it is extracted from natural sources. Using natural sources for the extraction of HAp ensures effectiveness as these natural sources allows the nutrient recovery of the waste material thus adding the value to the material.

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Author contribution statement

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Refernces

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