TRENDS IN THE DEVELOPMENT OF HYDROXYAPATITE FROM NATURAL SOURCES FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

Hydroxyapatite (HAp), a member of the apatite family is similar in structural and chemical composition to the natural bone. HAp is bioactive and biocompatible and has been used for several biomedical applications. Synthesizing HAp from natural sources can contribute significantly towards natural resource management, health care, waste utilization amongst others. Therefore, in this summarized review, we highlight the classification of HAp, discuss the sources and synthesis of hydroxyapatite, enumerate some applications of hydroxyapatite and touch on polymeric scaffolding materials based on naturally synthesized hydroxyapatite. The overall idea of this article is to summarize recent studies on this subject and link it to the waste management potentials of natural biowastes for the synthesis and biomedical applications of HAp.

Keywords: hydroxyapatite, biowastes, biocompatible, bioactive, scaffolds, polymers, waste valorization, natural sources, implants, apatite.

1 INTRODUCTION

Hydroxyapatite (HAp) with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is one of the most versatile classes of biomaterials available today. Its chemical composition in terms of calcium–phosphate (Ca/P) ratio and other organic matter bears very close resemblance to that of the human bone [1]. This calcium phosphate salt is the chief inorganic mineral component of the human bone, enamel, and dentine [1], [2]. HAp is a calcium phosphate (CaP) which is the nomenclature of a family of minerals containing calcium cations (Ca^{2+}), orthophosphate (PO_4^{3-}), metaphosphate (PO^-_3), or pyrophosphate ($P_2O_7^{4-}$) anions, and sometimes hydrogen (H^+) or hydroxide (OH^-) ions. Consequently, hydroxyapatites are bioactive, biocompatible and bioresorbable materials that can be successfully adapted for biomedical applications. They have the characteristics to synergize with biological systems, with the potential to produce devices for assessment, treatment, supplementation, and/or substitution of ailing bodily tissues or organs. Hydroxyapatites have increasingly gained notable attention owing to their relevance in addressing the medical needs of ageing people (for instance, treating osteoporotic patients), and management of defective hard and soft tissues. HAp finds

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extensive use in the areas of treating rickets diseases, autografts and allografts, promotion of rapid bone growth and union, drug delivery, etc. [3]. The trend in the development of hydroxyapatite from natural sources now saves the environment from solid waste pollution which may likely lead to land degradation [2], [4], and the effective utilization of these natural sources in HAp synthesis through environmentally friendly processes, now creates value addition from hitherto waste products [5]–[18]. HAp has been successfully devised as grafts for hard and soft tissues, and for drug transport systems. Furthermore, HAp acts to rejuvenate, damaged tissues through bio-integration with the site and location of the diseased part, which helps to lengthen the life expectancy of the patient [19]. The excellent resemblance in the biochemical configuration of HAp with that of the human bone minerals makes them very useful in orthopaedics and odontology, and their ability to maintain a thermodynamically stable crystalline state within their host tissue can be attributed to their interfacial stability and good adhesion potentials which rapidly leads to osseointegration [20]. Literature studies have reported and shown that HAp does not result in any inflammatory, toxic, or carcinogenic reactions to the host tissues surrounding it [1], [2], [5]–[18], [21], [22]. More so, it promotes osteoblast proliferation, osteointegration and forms a direct bond between an implant and the newly formed bone tissue when used in maxillofacial orthopaedic and dental applications [23]. Currently, hydroxyapatite is also being used to carry out drug delivery in human systems, as therapeutic agents etc. [24], [25]. From these, it has been established that HAp has enjoyed an extensive use in the biomedical fields, predominantly in dentistry, disorderliness and deformities of the bone and joints, and as implant coatings.

In the context of waste valorization and its environmental impact, the utilization of HAp synthesized from natural sources is environmentally sustainable, economical, and friendly, since natural biowastes are in abundance. For instance, the bones of a bovine specie in Nigeria which are typically made up of the skeleton, head, horns, hooves, and feet constitutes the second largest part by weight (approximately 36%) of bovine during processing at the abattoir [26]. Since the bones are rich in calcium and phosphorus, a large portion of the waste bones are used as poultry feed. The left-over waste bones can be further utilized for the synthesis of natural HAp for biomedical applications, and this approach could eventually outweigh the use of bovine derived waste bones in other sectors. This can contribute significantly towards an improved economy, cleaner environment and enhanced general health. Therefore, in this summarized review, we highlight the classification of HAp, discuss the sources and synthesis of hydroxyapatite, enumerate some applications of hydroxyapatite and touch on polymeric scaffolding materials based on naturally synthesized hydroxyapatite.

1.1 Classification of HAp sources

HAp can be derived from either natural or synthetic sources, forms a strong bond with host tissues making it quite useful for bone remodelling applications [1], [2], [6]. Natural sources include all biomaterials such as animal bones, fish bones and scales, shells, minerals, and plants. The synthetic sources are achieved by mixing reagents of calcium (Ca^{2+}) and phosphates (PO_4^{3}-) precursors, then allowing conditions for nucleation and crystal growth [20]. Fig. 1 outlines a summary of the sources of hydroxyapatites. Natural hydroxyapatite is typically sourced from the discards of animal bones such as bovine [27], fish bones [18], marine shells [28], eggshells [29], wood [30], mineral sources e.g., limestone [31] amongst many other natural sources. A lot of studies have synthesized HAp from these two sources (natural and synthetic) with different characteristics. Sadat-Shojai et al. [20] reported that HAp materials can be produced either chemically or derived from natural sources. Procedures for the syntheses of HAp with intended features should be pre-planned. Moreover, a lot of tailored syntheses protocol have been established overtime, yet the formulation of HAp materials with targeted expected characteristics has remained a puzzle due to the inclusion of undesirable intermediate or by-products during synthesis. Hence research on the synthesis and characterization of HAp for biomedical applications will remain a continuous activity. Relating these to the state-of-the art, synthetic and natural sources of HAp are being considered either alone or combined for bone tissue regeneration. Currently, dopants such as strontium (Sr), iron (Fe), copper (Cu), zeolites, etc. have been suggested to improve the osteogenesis and angiogenesis ability of hydroxyapatite-derived scaffolds [17]. Unlike synthetic HAp, the natural HAp is non-stoichiometric due to the presence of characteristic trace elements such as Na^{+}, Zn^{2+}, Mg^{2+}, K^{+}, Si^{2+}, Ba^{2+}, F^{-}, and CO_3^{2-} making it comparable to the biochemical configuration of the human bone [2], [17].Worthy of note is that HAp and its production techniques have very strong correlation with its properties, particularly its calcium to phosphate (Ca/P) ratio, elemental composition, surface and structural morphology, crystallinity and phases. These properties are also a function of its processing parameters such as temperature, holding time and sieve size [18]. These parameters all have their influences on the overall characteristics and application-based properties of the synthesized HAp.

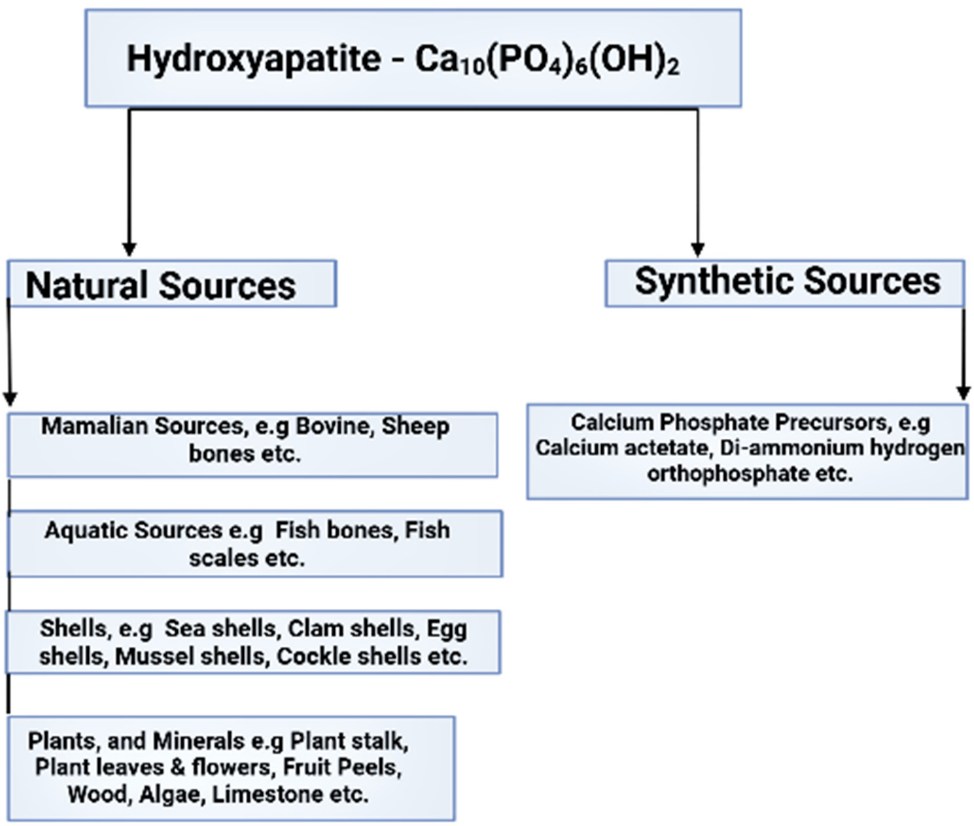

Figure 1: Sources of hydroxyapatite.

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Typically, naturally synthesized HAp often normally deviates from the stoichiometric Ca/P ratio value of HAp being 1.67 [2]. This has been attributed to the inherent presence of trace elements in the composition which imitates the apatite found in the human bone. The incidence of these trace elements is however critical for biodegradation and bio-resorption during bioactive interactions, which then leads to total bone restoration in the patient. For example, there have been instances of improved activity per osteogenesis and bio-resorption in bone restoration using rare-earth metals doped HAp [32]. Reports and reviews from Obada et al. [17] and Pu'ad et al. [33] reported that bone regeneration and formation processes were accelerated by the inclusion of trace elements in HAp.

1.2 Extraction of HAp from natural sources

The extraction of HAp from natural sources particularly with interests in animal bone and catfish bone wastes was reported by our group [2], [5], [6], [11]–[18]. The usual step was with a pre-treatment approach of the bones by washing off dirt, fatty-proteinaceous matter, and cholesterol from the surfaces of the bones/precursors under running water. Next, the bones are boiled in hot water for about 8 h or more to ensure that embedded organic matter are further removed. Some literatures have further reported the addition of solvents like acetone, etc. in the boiling water for more effective removal, while others added surfactant and alkali solutions to detach the soft tissues and break their bonds from the bones [33]. The general extraction method of HAp from natural sources is the solid-state sintering approach. Others employ combining the calcination process with other methods such as sol-gel or wet chemical precipitation processes. Overall, it is desirable to ensure that the possible inherent infections or pathogens are eliminated within the process of developing the final HAp which buttresses the need for a high processing temperature in some cases [2], [33]. Obada et al. [2] have shown that solid-state synthesis of natural sources of HAp at temperatures of 900°C with low compaction pressures of 500 Pa, can produce bio-ceramics with prominent HAp phases, and enhanced mechanical properties [2], [11]. Therefore, a focus on extracting HAp from mammalian and aquatic sources will be further reviewed in this article.

As reported by Barakat et al. [27], alkaline hydrothermal hydrolysis treatment was utilized to extract pure phase HAp from bovine. The extracted nano-sized HAp showed nanoflakes shape of a Ca/P ratio of 1.86. Their work further revealed that 20–25% NaOH as a pre-treatment process before calcination resulted in the development of pure Hap. Londoño-Restrepo et al. [34] employed a combination of hydrothermal and calcination process to produce a dehydroxylated crystallite phase Hap of irregular shapes at 700°C, and semi spherical shapes at 800°C. Ofudje et al. [35] extracted Hap from pig bones using the calcination protocol. The calcination temperatures were 600°C, 800°C and 1000°C. The data presented showed that pure crystalline Hap was produced with rod-like morphologies, with a Ca/P of 1.88 the highest after calcining at 1000°C. A detailed review by Pu'ad et al. [33], showed that the alkaline heat treatment method was used to produce Hap having Ca/P ratio >1.67, and irregular shaped particle sizes of 20–100 μm. Moreover, when the same method was applied in addition to the calcination process for the extraction of Hap from pig bones, Hap of irregular shape size in a range of 70–180 nm at 800°C was produced. Similarly, direct calcination process of camel bones by Jaber et al. [36] at 1000°C gave an irregular shaped particle size range of 79–97 nm, with a calculated Ca/P ratio of 1.66 which is very close to the stoichiometric Ca/P ratio. Reports by Rahavi et al. [37] showed the properties of Hap when extracted from camel and horse bones at 700°C calcination temperature. The morphologies showed irregular shaped Hap sizes of 97 nm for camel bone derived Hap, and 28 nm for horse bone derived Hap. A Ca/P ratio of 2.036 was obtained for camel bone derived

Hap, while a Ca/P ratio of 2.131 was obtained for horse bone derived Hap. In a study conducted by Obada et al. [17], the solution combustion synthesis (SCS), assisted with thermal treatment at 900°C was used for the extraction of Hap from animal bones. This protocol favoured the formation of Hap with lower crystallinity, but with increased micro hardness. Fig. 2 describes the method used for the extraction of Hap from animal bones using SCS as reported by Obada et al. [17], and all the post processes and characterization thereof.

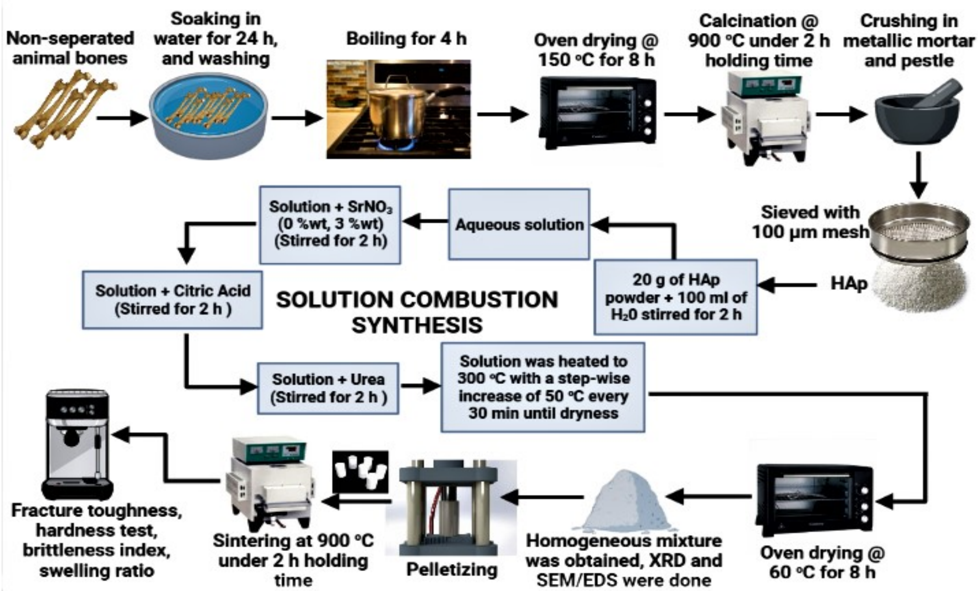

Figure 2: Schematic for the SCS protocol for the extraction and characterization of Hap [17].

As fish is consumed widely globally, there is a consequent increase in biowaste generation in the form of fish bones and scales. In our group, we have employed same pre-treatment method as used for animal bones to remove all unwanted debris from fish bones. Since fish bones and scales are rich in calcium, phosphate and carbonate, a lot of studies have engineered the extraction of Hap from these sources [13], [38]. Pal et al. [39] subjected *calcarifier* bones to temperatures from 200°C–1200°C, at an interval of 200°C for a holding time of 1 h. At higher sintering temperature of 1200°C, it was found that the Ca/P ratio of the sample was 1.62, a value lesser that the Ca/P ratio of stochiometric Hap by reason of the formation of tricalcium phosphate. Venkatesan et al. [40] extracted Hap from *Thunnus Obesus* fish using a combination of alkaline hydrolysis (AH) and calcination (CN) methods in the process. The tuna fish bone was subjected to heat treatment at 900°C for a holding time of 5 h. These methods (alkaline hydrolysis and calcination) were deemed suitable for producing Hap. Nonetheless, the Hap produced through the calcination process was more crystalline comparatively. Furthermore, Venkatesan et al. [40] reported that the AH method did not change the sizes of the particles. The Ca/P ratio of Hap derived via the calcination process was 1.65, a value quite close to that of stoichiometric Hap. Mondal et al. [41] subjected fish scales to the calcination process at 1000°C, followed by a 16 h milling procedure for the synthesis of Hap with Ca/P ratio of 1.71. The morphological features of the

Hap showed spherical shapes with an average size of 76.62 nm. We show the characterization result of the synthesis of Hap from *Clarias macrocephalus* fish bones. We used a handheld X-ray fluorescence (XRF) instrument from SPECTRO xSORT Combi (Kleve, Germany), with an energy dispersive SDD detector, an Rh-anode X-ray tube, a spot time of 3 mm, and an acquisition time of 60 s to elucidate the composition of the fish bones (see Table 1). The concentration of major and trace elements was determined. The accuracy of the hXRF instrument was ensured by performing several measurements. It can be observed from Table 1 that as reported by several researchers, extraction of Hap from natural fish bones contains trace elements like Mn, Fe, Sr. Zn, etc., with calcium and phosphorus the main elements in the composition. It has been reported that mammalian bones are composed of more trace elements and ions [33]. The concentration of these trace elements in a natural source varies by reason of differences in source, species, etc.

Table 1: XRF data for the composition of *Clarias macrocephalus* fish bones.

Element	Symbol	Concentration (%)
Silicon	Si	0.52
Phosphorus	P	26.4
Sulphur	S	0.06
Calcium	Ca	47.2
Manganese	Mn	0.02
Iron	Fe	0.02
Strontium	Sr	0.07
Zinc	Zn	0.02

We complement this data by reporting the morphological features (see Fig. 3) of the extracted Hap calcined at 900°C, using a scanning electron microscope (SEM machine model no-S2500, Hitachi, Japan) equipped with an electron dispersive spectrometer. The SEM images are complemented by EDS spectra as shown in Fig. 4. It can be observed that irregular and spherical shapes with agglomerated portions can be observed in the SEM images (Fig. 3(a) and (b)). This data is in line with summarized characteristics of Hap synthesized from natural sources as shown in Tables 2 and 3.

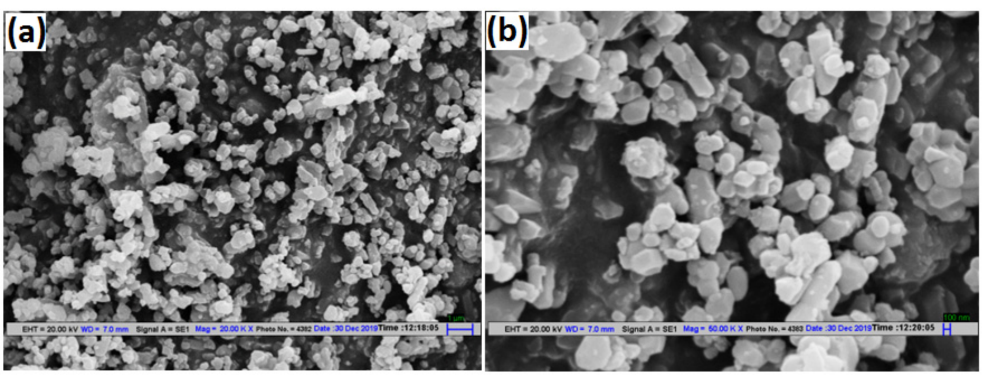

Figure 3: SEM images of *Clarias macrocephalus* fish bones. (a) Magnification of 20.00 kX; and (b) Magnification of 50.00 kX.

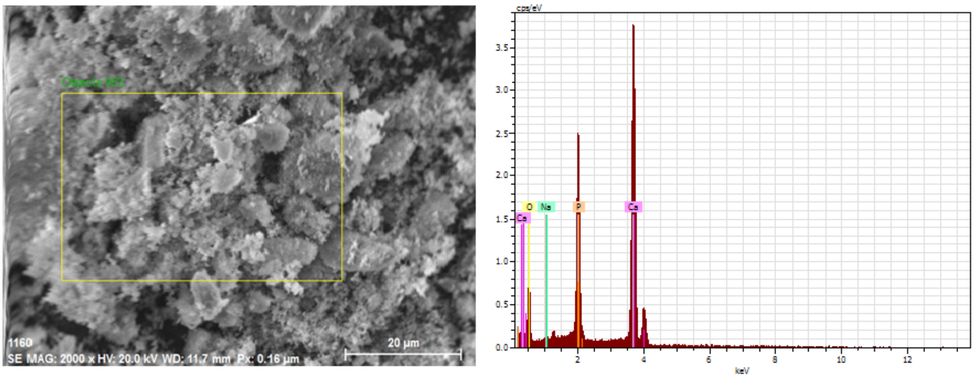

Figure 4: SEM/EDS spectra of *Clarias macrocephalus* fish bones.

Table 2: Characteristics of Hap extracted/synthesized by different protocols from natural sources (mammalian).

Methods			
Properties	Alkaline hydrolysis (AH)	Calcination ©	Combination (AH + C)
Sizes of the particles	20–100 µm	28–900 nm	70–700 nm
Morphological features	Irregular and flake-like in most cases	Irregular and rod like in most cases	Irregular, needle-like, and plate lie in most cases
Crystallinity	Low	High	High
Ca/P ratio	1.67–1.86	1.50–2.13	1.68–1.72
Trace elements	Yes (e.g. Sr, Mg, Na etc.)	Yes (e.g. Sr, Mg, Na, Al etc.)	Not reported

Table 3: Characteristics of HAp extracted/synthesized by different protocols from natural sources (fish bones and fish scales).

Methods			
Properties	Alkaline heat treatment (AHT)	Calcinati©(C)	Combination (AHT + C) **Only for fish scales*
Sizes of the particles	5–20 nm width; 17–100 nm length	5–76.2 nm	76.2 nm
Morphological features	Flat plate, rod like in most cases	Irregular, nearly spherical, agglomerate	Agglomerate
Crystallinity	Not reported	High [11]	Not reported
Ca/P ratio	1.76–2.01	1.62–1.71	1.62
Trace elements	Not reported	Mn, Fe, Sr, Na, Zn (this work)	Not reported

The EDS spectra of the *Clarias macrocephalus* fish bones corroborates the inclusion of trace elements in HAp extracted from aquatic natural sources and is in line with data obtained as shown in Table 1. The inclusion of sodium (Na) is noticed in the EDS spectra.

Tables 2 and 3 show summarized characteristics of HAp extracted/synthesized by different protocols from mammalian and aquatic sources.

2 APPLICABILITY OF HYDROXYAPATITE IN BIOMEDICINE AND POLYMERIC SCAFFOLDING MATERIaLS

HAp has stirred incredible interest in the field of biomedicine for various applications, noticeable among them is the drug delivery ability of HAp in providing antibiotics to hard tissues. Bi et al. [42] studied the fabrication and characterization of hydroxyapatite/sodium alginate/chitosan (HA/SA/CS) composite for drug delivery. The result of the study revealed that the structure of the developed composite improved the quality of the loaded drugs. Furthermore, HAp was used to coat metal inserts with a view to improving their biocompatibility. De Oliveira et al. [43] investigated the response of a nano-HAp coated implant via gene expression. The analyses they conducted showed that the use of nano-HAp coated implants can promote new bone formation in diabetic animals which is considered as a better alternative to the use of machined or double etched surfaces. Another industrial applicability of HAp is in the production of solid scaffolds for tissue engineering. Ergul et al. [44] used a 3D printer to fabricate hydrogels based on chitosan/poly (vinyl alcohol) with the inclusion of synthesized hydroxyapatite scaffolds for hard-tissue engineering. The study concluded that through the addition of HAp to scaffolds production, there is a promising innovation for bone tissue engineering.

In our group, we have attempted to use Polylactic acid (PLA) reinforced with HAp extracted from catfish bones to synthesize a biodegradable polymer that can be useful for biomedical applications. PLA is a biodegradable thermoplastic aliphatic polyester which is easily processed using a variety of protocols and has been used for an array of biomedical applications. However, because one of the key disadvantages of PLA is its low hydrophilicity, this results in a lack of cell attachment and interaction between the polymer and the surrounding tissues, as well as poor wetting qualities [45]. PLA bioactivity must be improved for it to be used in the intended bone regeneration application, considering all these features. The addition of natural HAp and trace elements like Sr [17] to PLA matrix can improve the 3D structures' hydrophilicity, osteoconductivity, mineralization after implantation, and mechanical characteristics. We performed these experiments using the melt extrusion technique. The melt extrusion/hot pressing manufacturing method is a method of turning raw materials, usually containing a polymer substrate and plasticizer, into a product that has uniform shapes and densities by pushing them through a heated barrel at a controlled temperature and pressure. Fig. 5 shows a description of the methods used in the development of the natural HAp-Sr reinforced polymer and all the characterization thereof. The structural data obtained for the composite using this process reveal the incorporation of the bioactive natural derived HAp with a semicrystalline characteristic (not shown).

3 CONCLUSION

This review summarizes trends in the synthesis of HAp from natural sources such as mammalian and aquatic amongst other sources like minerals, shells, plants and algae. We highlighted the differences in characteristics of natural HAp produced from different sources in terms of crystallinity, shapes, and sizes. The methods of extraction were crucial in tailoring the characteristics of the obtained HAp. The applications of HAp for biomedical applications

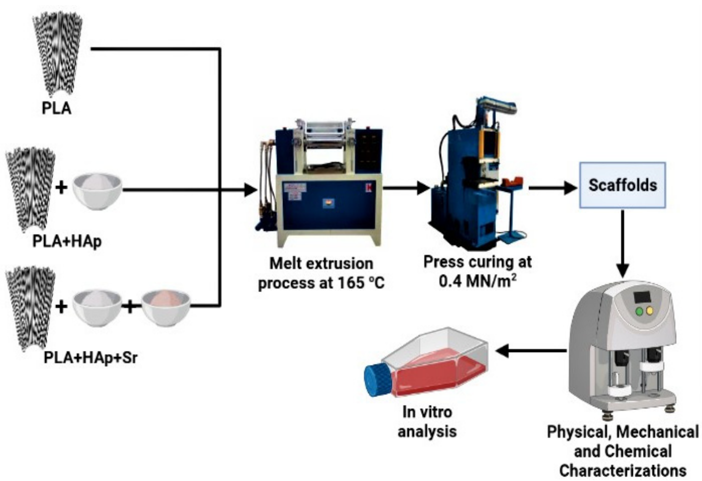

Figure 5: Methodological procedure to produce PLA-HAp-Sr scaffolds.

are ever increasing, thus a need to engineer more practical solutions like the development of natural HAp reinforced biodegradable polymers. It is therefore important to conclude that the extraction of HAp from natural sources ensures a cleaner environment and can be sustained because these natural sources can enhance the recovery of nutrients from biowastes to transform them into value added products.

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