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Synthesis of organic derived hydroxyapatite scaffold from pig bone waste for tissue engineering applications

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ABSTRACT

Micro porous hydroxyapatite (HAp) had drawn great attention in the field of tissue engineering due to their numerous applications such as tissue regeneration, dental, drug delivery, and adsorption and desorption of substances etc. The chemical synthesis of HAp is often faced with the high cost of starting materials and often lacks the presence of beneficial ions which can promote biological reactions. This paper examined a novel application of pig bone waste for the synthesis of HAp via thermal annealing between 600 and 1000 °C. Thus synthesized powder was characterized by X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FT-IR), Thermogravimetric analysis (TGA), Scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX) and Transmission electron microscope (TEM). XRD results revealed the main characteristic peaks of single phase HAp powder, while the presence of various functional groups such as PO_4^{3-} , CO_3^{2-} and OH^- corresponding to HAp were observed by FT-IR analysis. The elemental composition of as-synthesized HAp powder as observed by EDX showed the presence of Ca and P in addition to some beneficial metals such as Na, K, Mg and Si which plays vital roles in biological applications. SEM and TEM observation confirmed the microscopic structure of the as-synthesized HAp to be rod-like morphology with 38–52 nm in length. Porous HAp scaffold up to 65% porosity could be prepared using ammonium bicarbonate as pores forming agent. Therefore, bio-waste such as pig bones can be utilized in the synthesis of porous hydroxyapatite scaffold which can serve as an alternative for the usual conventional chemical method.

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1. Introduction

Hydroxyapatite (HAp) with a chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is a major mineral constituent of vertebrate bones and teeth with an excellent bioactivity and biocompatibility which has necessitated its high demand in various fields of applications [1,2]. HAp can enhance new bone in growth through osteoconduction mechanism without resulting to any systemic toxicity or inflammation [1,3,4]. As a result of its importance, HAp has become a material of great interest particularly in biomedical applications such as tissue engineering [5,6], dental [7], replacement for damaged bones [8] as well as in other applications such as ion conductors [9], gas sensor [10] and as an adsorbent in column chromatography [11]. Biological structures have always been a source of inspiration

for solving technical challenges in architecture, mechanical engineering, or materials science [12]. Research into the ability of bone for the fabrication of biomedical material as substitute for damaged part or as fillers for implant is on the increase. Bone typically consists of 30–35% organic materials and 60–65% mineral phases [12]. Several chemical methods (such as mechanochemical reaction, wet chemical precipitation, hydrothermal conversion, sol-gel, emulsion etc.) are available for the synthesis of HAp, but most of these methods are very expensive to carry out and often lack the presence of some beneficial trace elements [13–15]. Xenogenous bones such as bovine, pig, sheep or fish contain trace amount of beneficial cations such as Na^+ , K^+ , Zn^{2+} , Si^{4+} , Mg^{2+} , Ba^{2+} and anions such as F^- , CO_3^{2-} etc which could play vital roles in tissue engineering and other biochemical reactions [13]. For instance, report has it that the presence of Na^+ and Mg^{2+} ions along side with HAp play an important role in the development of teeth and bone, whereas, their absence could cause bone loss and fragility [13].

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Porous HAp has been applied for cell loading, drug releasing media, chromatography analysis and most extensively for hard tissue scaffolds [16]. Since, porous HAp is more resorbable and osteoconductive than dense HAp, there is an increasing interest in the development of synthetic porous HAp for bone defect replacement [17]. Some of the criteria in the selection of scaffold for bone regeneration, include bioactivity (ability to bond to bone), osteogenic (stimulation of bone growth), biocompatible (induce minimal toxic or immune response), resorb safely and effectively in the body, similar mechanical properties to bone (such as load absorption), ability to shape to a wide range of defect geometries and meet all regulatory requirements for clinical application [18,19]. Scaffold should provide sufficient mechanical strength to support itself until the new bone tissue is completely formed into the pores [20]. Although increased porosity and pore size facilitate bone in growth, it however causes structural deteriorations of the scaffold, which results in low mechanical strength [20,21]. Thus, there is a limit on the porosity of scaffold in order not to destroy its mechanical properties [21]. Li et al. prepared porous HAp scaffold by approaching a dual phase mixing technique of HAp and polymethylmethacrylate (PMMA) slurry [22]. Naphthalene particles were used to enhance the porosity (>50%) of such scaffold. It was observed that majority of pores were located within the range of 200–300 μm with 50% porosity with an average compressive strength ~ 8.9 MPa. Similarly, Tadic et al. synthesized carbonated porous HAp by mixing polyvinyl alcohol fibers (PVA) and sodium chloride which resulted in the formation of interconnected porous HAp with pore diameter in the range of 250–400 μm [23].

Owing to the growing importance of HAp powder for different applications, it is imperative therefore to seek for cheaper and faster method for the synthesis of non-toxic HAp that will mimic the natural bone in human body. The quality and type of agricultural waste generated vary from one country to the other, but there is generally an increase in the amount of waste generation across the globe due to increase in population. When the concentration of waste of any kind in the environment is in excess, it constitutes environmental pollution and becomes critical for human, animals and vegetation. The way out therefore is to seek for proper ways to recycle these agro-waste by converting them to useful materials.

In this paper, we present a facile approach for the synthesis of hydroxyapatite powder from biological source of pig bones waste via thermal decomposition at different calcinations temperatures, thus reducing environmental pollution and adding values to waste materials. The as-synthesized HAp powder was characterized using various advanced techniques such as X-ray diffraction (XRD), Fourier transform infra-red Spectroscopy (FT-IR), Thermogravimetric analysis (TGA), Scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX) and Transmission electron microscope (TEM). HAp porous scaffolds were also prepared using ammonium bicarbonate as a pore forming agent for tissue engineering applications. The morphological analysis, densities, porosities of the fabricated apatite scaffold were equally evaluated and thus presented.

2. Experimentations

2.1. Synthesis of hydroxyapatite from pig bone

Cortical bones were procured from a local pig slaughter and were cleaned using hot water to remove dirty and odour. Raw bones were then subjected to de-proteinization in order to remove the presence of organic substances and collagen through external washing with a mixture of 1 M NaOH and 1 M HCl (Merck, 35%) solution by boiling for about 5–10 min at 100 $^{\circ}\text{C}$, and then the bones were washed using distilled water. This process was repeated until it yielded white and clean samples of pig bones. Before boiling, the macroscopic adhering impurities and sub-

stances, which include the ligaments and tissues that stick on the bones were shaved and removed. After boiling, the bone samples were oven dried at 100 $^{\circ}\text{C}$ over night. The dried bone samples were cut into smaller pieces and milled using a mechanical grinder to obtained fine powder. The bone powders were calcined in a muffle furnace in air at the following temperatures: 600 $^{\circ}\text{C}$, 800 $^{\circ}\text{C}$, and 1000 $^{\circ}\text{C}$ with a temperature rate of 5 $^{\circ}\text{C}/\text{min}$. The samples were cooled to room temperature by slow furnace cooling and the product forms were labeled as PBHAp - 600, PBHAp - 800 and PBHAp - 1000 $^{\circ}\text{C}$, respectively. Scaffold HAp were produced by mixing different amount of the pig bone derived HAp powder in the ratio of (100, 10, 20, 30, 40 and 50%) to ammonium bicarbonate (AMB) (0, 90, 80, 70, 60 and 50%) and pellets were made using hydraulic press at different compaction pressure. The pellets were sintered in a muffle furnace at 1000 $^{\circ}\text{C}$ to form scaffold HAp. Fig. 1 shows the flow chart for the processing of pig bone to HAp and subsequent fabrication of HAp scaffold, while a typical heating cycle used for the synthesis of the HAp powder is shown in Fig. 2.

2.2. Characterization techniques

X-ray diffraction (XRD) was recorded using X-pert PRO, PANalytical, (The Netherland) with wavelength of $\text{CuK}\alpha 1$. The diffraction patterns were collected in the scanning range of $2\theta = 10^{\circ}$ – 60° with an incremental step size of 0.02. The various functional groups present in the fabricated HAp powder were identified by Fourier Transform Infrared (FT-IR) spectroscopy (FTIR; TENSOR 27, Germany). A ratio of 1:99% of HAp powder and KBr were mixed in a mortar and pestle and then compressed at about a load of 5 tons to form pellet of 2 mm diameter. All FT-IR spectrums were recorded in the range of 400–4000 cm^{-1} with resolution 4 and 64 times scanning. Thermogravimetric analysis (TGA) was recorded using SDT Q600 V8.3 Build 101 simultaneous DSC-TGA instrument in order to evaluate the thermal stability of the as-prepared HAp powder, while scanning electron microscopy (SEM) analysis was carried out to evaluate the morphology using a Hitachi (Japan) S-3000H electron microscope with an accelerating voltage of 15 kV. Energy dispersive X-ray (EDX) analysis was also performed on the same device to provide information relating to elemental composition of the samples. The specimens were prepared using carbon tape. Transmission electron microscope (TEM) analysis was performed to further study the microscope structure of the apatite using (TEM; Tecnai 20 G2 FEI, The Netherland), while the SAED pattern were also taken. The densification of the scaffold was calculated from the equation:

$$\text{Densification} = \frac{B.D}{T.D} \times 100 \quad (1)$$

where $B.D$ and $T.D$ are bulk (sintered) and theoretical densities respectively. The $T.D$ of HAp powder was taken as 3.15 g/cm^3 . The bulk density was estimated from the equation:

$$B.D = \frac{m}{V} \quad (2)$$

where m and V are the mass in g and volume in cm^3 of the HAp scaffold respectively. The porosity of the as-prepared porous material was estimated from the equation:

$$\text{Porosity} = \left(1 - \frac{B.D}{T.D}\right) \times 100 \quad (3)$$

3. Results and discussions

3.1. XRD analysis

The phase analysis of the HAp powder from pig bones were carried out by X-ray diffraction analysis. The patterns of XRD diffrac-

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