Poly ethylene glycol/fish scale-derived hydroxyapatite composite porous scaffold for bone tissue engineering

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Abstract. In the present study, hydroxyapatite (HAp) is synthesized from fish scale of Labeo rohita for development of composite bone scaffold. In order to determine suitable calcination temperature for the synthesis of HAp, thermo gravimetric analysis (TGA) of the collected fish scale has been performed. It is found that no degradation of mass occurs beyond 800°C indicating the calcination temperature of fish scales for HAp synthesis. The fish scales are alkali treated and calcined at 1000°C. The calcined powders are characterized by X-ray diffraction and Fourier transform infrared spectroscopy which confirms formation of HAp. TGA of the synthesized HAp has been carried out to investigate the phase changes and its thermal stability. Porous composite bone scaffold is developed using the synthesized HAp and poly ethylene glycol through solvent casting technique. The developed scaffold is tested for its mechanical strength by uniaxial compression testing and its morphology is studied by scanning electron microscopy. The scaffold manifest desired mechanical performance with a compressive strength of 4.93 MPa and interconnected porosity with a maximum pore diameter of 167 microns making it suitable for growth of bone cells. The developed scaffold can have possible applications in bone tissue engineering and treatment of segmental bone defects.

1. Introduction
The main health issues occurring these days are tissue or organ failure as a result of accidents or any other congenital disease or damage. One of the major objectives of tissue engineering is to regenerate tissues through biomimetic fabrication of scaffolds that serves as temporary or permanent artificial extracellular matrices (ECM) to accommodate cells and support 3D tissue regeneration[1]. Materials derived from calcium phosphate had gained utmost importance in recent times for the development of bone scaffold. In this context, Hydroxyapatite (HAp) [Ca_{10}(PO_4)_6(OH)_2] is attracting everyone’s attention because of its beneficial properties like non-toxicity, osteoconductivity and biocompatibility. It is one of the major constituents of bone and teeth [2]. Moreover, it finds promising application in prosthetic implants, coating of implants and several biomedical applications. Extraction of HAp can be carried out either naturally or through chemical synthesis. But the major problem with the chemically synthesized HAp is that it shows poor behavior in bio-compatibility, porosity and biodegradability [3]. Thus research in present times is focusing on naturally extracted HAp. In India, millions of tons of by-products like fish scale, scrub shells, tortoise shells and egg shells are produced annually and disposed to the environment. Unfortunately, most of these by-products do have beneficial ingredients which can be harnessed for preparing useful products. Fish is one of the major ingredients of the food in the coastal area and north-east region and fish scales are discarded as a waste. Thus, fish scales (FS) can
be assessed in production of bio-material derivatives. The current research stresses upon synthesizing hydroxyapatite from fish scales which is further extended to fabricate porous scaffold and explore its possible applicability in the field of biomaterials.

2. Materials and Method

2.1. Synthesis of Hydroxyapatite (HAp)
Fish scale of *Labeo rohita* was collected from local market of Barak valley and washed thoroughly with water to ensure the removal of undesired debris attached to the scales. The fish scales were then dried at room temperature and de-proteinized using alkali treatment with 1(N) NaOH solution to remove the organic substances. After de-proteinization, the scales were washed with distilled water and dried in a hot air oven at 40°C for 12 hours. The dried scale was calcined at 1000°C for 3h to obtain HAp ceramic.

2.2. Preparation of composite bone scaffold
The synthesized HAp was grinded and mixed with Poly ethylene glycol (PEG) 600 in the ratio of 2:3 for development of HAp-polymer composite scaffold using solvent casting technique. Insoluble starch was used as porogen to induce porosity in the scaffold. The developed scaffold was sintered at 1200°C for a period of 3 h.

3. Results and Discussion

3.1. Characterization of synthesized HAp

3.1.1. TG Analysis of collected fish scale and synthesized HAp. TGA of collected fish scale and derived HAp has been carried out to study the phase transformation and to determine the weight loss with respect to temperatures as shown in figure 1(a) and (b). The analysis was carried out in static air with a heating rate of 10°C/min. The thorough washing of the fish scales cannot remove the organic compounds from the fish scales which degrade at different temperature ranges. As represented in figure 1(a), the first degradation occurs between 100-300°C corresponding to weakly entrapped water. The major weight loss is observed between 300-400°C corresponding to degradation of collagen, connective tissue, proteins etc. The final degradation occurs between 500-700°C conforming degradation of guanine and other organic matters [4]. Thereafter no further degradation was observed beyond 800°C confirming the suitable calcination temperature.
The result of TGA analysis of the synthesized HAp as shown in figure 1(b), indicate almost no degradation between 100-400°C which confirms the removal of entrapped water. It is due to the calcination of HAp at 1000°C which eliminates moisture from the synthesized HAp. The major weight loss is observed between the temperature ranges from 400-600°C corresponding to the removal of organic substances. The synthesized HAp shows almost no degradation above 800°C which confronts higher thermal stability beyond this range. The overall degradation was observed to be around 2%.

3.1.2. XRD Analysis. XRD patterns of the calcined powder from fish scales are shown in figure 2. Identification of the phases was realized by comparing the experimental XRD pattern to standards compiled by the International Centre for Diffraction Data (ICDD) using the cards 09-0432 for hexagonal HAp structure. HAp was the only phase found in the patterns.

![Figure 2. XRD spectra of synthesized HAp.](image)

Well-resolved characteristic peak of highest intensity for HAp was obtained at 2θ value of 32.19° corresponding to 112 plane. The phase formed was pure and matches well with standard pattern. The standard corresponding plane for HAp (viz. 002, 210, 112, 300, 221, 311, 400, 222, 213, 321, 410, 004, 402, 323, 511, 513) are well observed in case of the synthesized powder [4].

3.1.3. FTIR Analysis. The FTIR analysis of synthesized powder is shown in figure 3. The peaks at 1456.25608, 1533.40872, 1635.63597 and 1647.20886 corresponds to the carbonate ion (CO$_3^{2-}$), peaks at 3502.72986, 3545.16381 and 3566.38078 corresponds to the hydroxyl ion (OH$^-$) and peaks at 559.35664, 597.93296, 960.550368 and 1022.27248 correspond to the phosphate ions (PO$_4^{3-}$). The presence of carbonate ion, hydroxyl ion and phosphate ions confirms the formation of HAp [5] [6].
3.2. Characterization of scaffold

3.2.1. Compressive strength. The compression test of the scaffold was carried out using Instron 5569 with a crosshead speed of 1 mm/min. The composite scaffold shows a compressive strength of 4.93 MPa as shown in figure 4 which can be effectively utilized in the repair of bone tissue [7]. It is due to uniform dispersion of HAp particles in PEG that results in high compressive strength.

3.2.2. SEM analysis. SEM micrographs of the fabricated scaffold are shown in figure 5. The micrographs reveal interconnected porous structure with considerable pore diameter of more than 100 microns which makes it a suitable candidate for biomedical applications [8].
4. Conclusions

Fish scale is an important raw material for the synthesis of hydroxyapatite. Synthesis of HAp from fish scales of *Labeo rohita* is confirmed through XRD and FTIR analysis. The synthesized HAp shows high thermal stability beyond 800°C. Composite scaffold developed using the HAp and polyethylene glycol shows considerable mechanical strength and interconnected porous structure having pore diameter of more than 100 microns. The developed scaffold may find promising application in tissue engineering and treatment of bone fractures.

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