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Fish scale derived hydroxyapatite scaffold for bone tissue engineering

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ABSTRACT

The tailored mechanical characteristics of bioceramic scaffold are very important for the bone tissue engineering. Functional hydroxyapatite (HAp) synthesized from fish scale (*Labio rohita* & *Catla catla*) is explored as scaffold materials. HAp scaffold exhibits better mechanical properties such as hardness (~1.08 GPa), compressive strength (~0.8 GPa) and tensile strength (~187 MPa) with adequate porosity (~35%) compared to commercial and synthetic body fluid derived HAp. The in vitro assays (MTT, LDH and Trypan blue) with MG63 osteoblast cell lines reveal that fish scale derived HAp materials is non-toxic and bioactive. The in vivo histological analysis of implanted HAp scaffold shows the osteo-conductive characteristics. The findings are significant with respect to cytotoxicity, osteo-conductivity and mechanical stability during bio-mineralization of traumatized bone tissues.

1. Introduction

Implanted biomaterials may not always deliver an ideal or successful outcome in the presence of some unreceptive environment in human body. Generally, any implant scaffold materials should be biocompatible and biologically active [1]. Normal traumatized tissues gradually grow up and repair itself after injury to replace the damaged ones with regeneration of new tissues. However, in cases of accidental or severe injury, sometimes the normal healing process is irreparable. The repairing can be made with the help of tissue engineering with novel biomaterials. The approach for tissue engineering is to use structural support to facilitate healing of the injured parts of the body. Live cells of bone tissue can be implanted or 'seeded' into an artificial matrix capable of supporting three dimensional tissue formations which is called scaffold. An interconnected porous structure of scaffold facilitates in cell colonization and ensuing formation of new tissue which renders cell proliferations and differentiation in the bone matrix. Different techniques are employed for manufacturing porous scaffold materials for tissue engineering. The most common techniques are computer aided Rapid Prototyping (RP), injection or compression moulding, gel-casting, compacting, 3D-printing and electrophoretic deposition etc. [2–5]. Computer aided RP and 3D-printing are the advanced process technology for suitable scaffold development [6–8]. In this experimental study, hydroxyapatite (HAp) biomaterial from different sources is used to develop porous scaffold through gel-casting/sponge replica route, where very small polymer foams are cut into desired shapes and immersed in 2 wt.% soluble starch mixed gel solution. The gel cast samples are dried in a humidifier at 70° ± 2 °C with 50% relative humidity. The HAp material mixed with 2 wt.% soluble starch granules is compacted for scaffold development. Injection mould press is utilized to develop small fillers. Finally, all the developed scaffold and filler samples are examined for its mechanical properties like tensile strength, compressive strength [9], hardness and apparent porosity. The biological response with the materials and tissue in-growth has been investigated by Nath et al. [10] and Abu Bakar et al. [11]. The survivability of the prostheses depends on the quality of biomaterials and its mechano-biological properties.

In general, living organisms can treat artificial implants as bio-toxic/bio-inert or as bio-active/bio-resorbable materials [12]. Bio-toxic (e.g. alloys containing cadmium, vanadium, lead and other toxic elements) materials released into the body in toxic concentration level stimulate the formation of antibodies that may cause immune reactions ranging from simple allergies, inflammation to septic rejection leading to severe health consequences. It is caused for pathological changes, atrophy, or infection of living tissue. Bio-inert [12] (e.g., zirconia, alumina, carbon and titanium) and bio-tolerant (e.g., polymethylmethacrylate (PMMA), titanium and Co-Cr alloy) materials neither release any toxic constituents nor show any positive interaction with living tissues [13], whereas composite biomaterials based on polylactide foams coated by bio glass particles is reported as bioactive, resorbable scaffolds in bone tissue engineering by Boccacciniet al [13].

In the present work, biological response behaviour of natural resourced HAp material with osteoblast MG 63 and RAW 264.7 cell lines are investigated. The cytotoxicity and cellular attachment on different resource HAp scaffold surface is also studied. In vitro studies such as cellular adhesion, proliferation and differentiation of osteoblast cell lines are carried out to check the bioaffinity of HAp material. The in

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Table 1
Mechanical characterization of developed scaffold.

Testing	Type of machine used	Working variables	Number of specimen tested	Standard used
Hardness	MXT-70, Matsuzawa, Japan	Load: 0.5 Kgf	5 × 4	ASTM E384 [Ref. 187]
Tensile	Tinius olsen	Load cell: 100 KN rate: 10 mm/min	10 × 3	ASTM C1366-04 [Ref. 3]
Compressive	Tinius olsen	Rate: 1.5 mm/min	10 × 3	ASTM C 1424-99 [Ref. 4]

vivo response of HAp biomaterial is also studied with albino rabbit model. Small HAp scaffolds are prepared as fillers are implanted into femur bone region of rabbit model and histological study is performed. MG63 and RAW 264.7 cell lines are considered for biocompatibility, cell proliferation and cytotoxicity assay. Osteoblast like MG63 cell line (procured from NCCS Pune, India) - being the precursor of bone cells is selected for this purpose. The RAW macrophage cell lines show good cytotoxic effect over foreign particles. RAW 264.7 (procured from NCCS Pune, India) macrophage cell lines is taken for comparative study on cytotoxic effect over different resource HAp materials viz. commercial hydroxyapatite (Com.-HAp) powder, fish scale synthesized hydroxyapatite (FS-HAp) and synthetic body fluid synthesized hydroxyapatite (SBF-HAp) powder.

2. Materials and Methods

2.1. Processing and Characterization of Hydroxyapatite (HAp) Powder

Bio-waste material like fish scales are used for the synthesis of HAp powder. *Labeo rohita* and *Catla catla* scales are collected from fish market. Initially the scales are thoroughly cleaned with water followed by de-proteinization through external washing with 1 (N) HCl (Merck, 35%) solution (2:1, v/w, water HCl/fish scale) for 24 h at room temperature (27° ± 2 °C). Subsequently, the acetic acid treatment for removal of collagen fibres is followed [14]. The filtered fish scales are washed thoroughly with cleaned water and dried at 60 °C in a hot air oven for several hours. Dried fish scales are calcined at 1000 °C in an air atmosphere. The calcined mass is then wet ball-milled at 200 rpm in pulverized mill (Pulveristte 5, Fritsch, Germany) for 16 h to make fine HAp powders [14–16] for characterization of crystallographic structure, phase, composition and its morphology. The schematic flow chart of fish scale derived HAp (FS-HAp) powder has been previously reported by Mondal et al. [14] The commercial HAp powder (Himedia Laboratories, Mumbai, India) and other synthesized biomimetic HAp are taken for comparative studies with FS-HAp. Biomimetic HAp is synthesized by dissolving $Ca(NO_3)_2$. $4H_2O$ in synthetic body fluid (SBF) solution at 37 °C [17], while the SBF is prepared according to the chemical composition of human body fluid with various ion concentrations similar to the inorganic constituents of human blood plasma as shown in Table 1. The

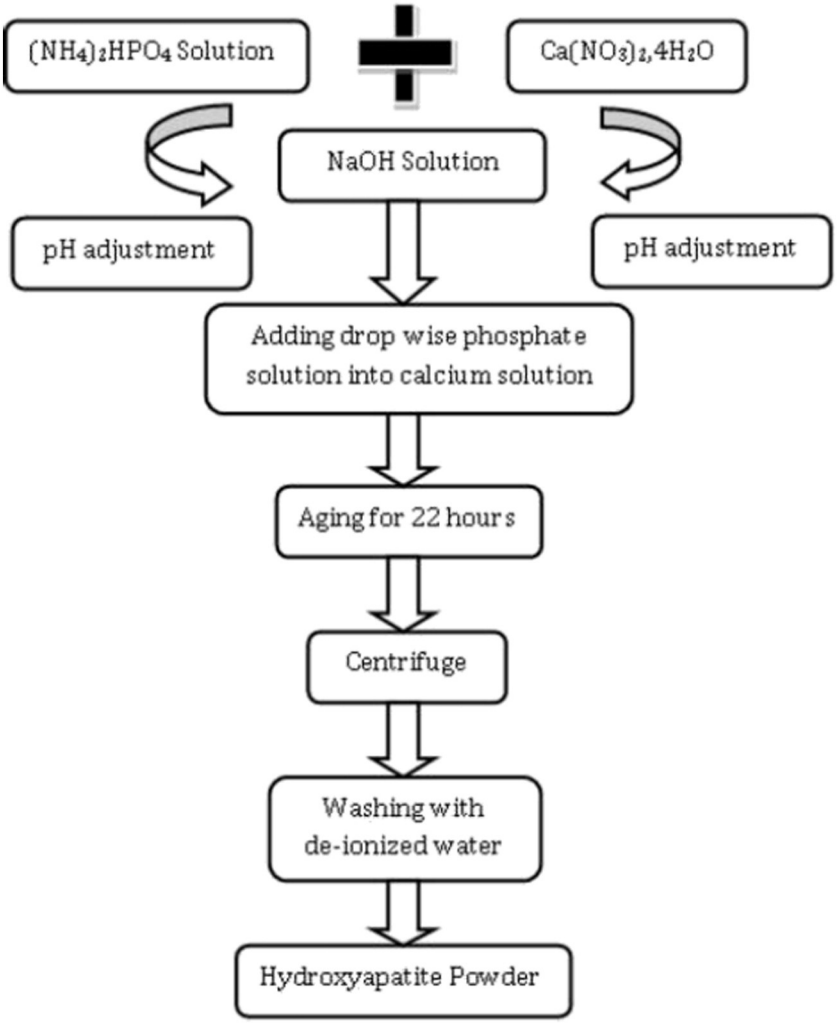

Fig. 1. The schematic representation of HAp synthesis in SBF solution.