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Direct hydrothermal synthesis of hydroxyapatite/alumina nanocomposite

Vignesh Raj Sivaperumal[a], Rajkumar Mani[b,*], Meenakshi Sundaram Nachiappan[c], Kandaswamy Arumugam[a]

[a] Department of Biomedical Engineering, PSG College of Technology, Coimbatore 641004, India
[b] Department of Physics, PSG College of Arts and Science, Coimbatore 641014, India
[c] Department of Physics, LRG Government Arts College for Women, Tiruppur 641604, India

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ABSTRACT

Hydrothermal synthesis offers numerous advantages such as: control over product homogeneity, nucleation, growth and aging. This leads to the control of particle aggregation, size uniformity and morphology over conventional and non-conventional ceramic synthesis methods. In the present work, a novel procedure for the direct synthesis of Hydroxyapatite (HAp)/alumina nanocomposite in a hydrothermal vessel with continuous stirring was investigated, where the effects of different concentration of aluminium nitrate were also studied. The optimized concentration of starting material for the characteristic formation of HAp/alumina composite (hexagonal HAp and monoclinic alumina phases) was determined by X-ray diffraction (XRD) study. The size distribution of HAp/alumina composite was found as 100 nm by electron microscopy and EDAX revealed that the concentration of alumina in the HA4 was ~10%, which is more suitable for biomedical implant. Further, the cytotoxicity study against MG 63 cells showed that HA4 was biocompatible even at higher concentration (200 μg/ml). Hence, the direct synthesis of HAp/alumina composite without the formation of any intermediate like calcium aluminate could be used as a novel approach to prepare HAp based biomedical implants.

1. Introduction

There is a huge demand on synthetic calcium phosphates, bioactive glass and metal oxides which exhibits biological properties such as biocompatibility, bioactivity, bioinertness, bioresorbability and biodegradability [1]. Synthetic hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ referred as HAp, which is one of the most frequently used calcium phosphates for bone substitutes. It has similar chemical composition with the mineral component of natural bone. However, it exhibits brittleness in pure form [2,3]. So, there is a need for highly reliable HAp ceramics which leads to the development of ceramic biocomposites with metal oxides like alumina, zirconia, titania, silica, zinc oxide and some carbon based materials [4]. The addition of metal oxides with hydroxyapatite improves the structural and morphological features and thereby enhances the thermal and mechanical properties [5,6]. Interestingly, addition of ZrO_2 andTiO_2 nanoparticles favors the apatite formation on HAp surfaces, whereas Al_2O_3 possess bioinert property [7,39]. Addition of Al_2O_3 from 5 to 30% alters the phase composition and morphological distribution of HAp.

At high temperature (> 1000 °C), HAp decomposes to β-Tri calcium Phosphate due to the interfacial reactions between HAp and alumina which lead to the formation of calcium aluminate intermediate phases [8,9]. The rise in sintering temperature and increase in weight percent of Al_2O_3 content also facilitates the decomposition of HAp [10]. However, addition of alumina nanoparticles increases the compressive strength, bending strength, flexural strength and fracture toughness of HAp material and constructively used for load bearing applications [11–16]. Moreover, alumina acts as a bioinert material without affecting the bioactivity of HAp and shows excellent compatibility against human osteoblasts cells [17,18].These observations depict that the thermal processing and addition of alumina plays significant role in the higher thermal and mechanical tolerance of HAp/Alumina composites. In addition, HAp/Alumina composites shows better biocompatibility towards bone regeneration that has been used as coatings on implants, scaffolds, bone and dental replacements [19,20]. As of now, few synthesis approaches were investigated for HAp/Alumina synthesis with different stoichiometric ratio of alumina with HAp [21–25]. Among the various approaches, hydrothermal method is considered as a desired method to facilitate the formation of one dimensional nanostructures of HAp, metal oxides and composite materials [26–29].

Herein, we report a novel method for the direct synthesis of HAp/Alumina nanocomposite at four different aluminium nitrate concentrations [HA1 (0.125%), HA2 (0.25%), HA3 (0.375%), HA4 (0.5%)] in a newly developed continuous stirring hydrothermal vessel. The

* Corresponding author.
E-mail address: rajkumar.m@psgcas.ac.in (R. Mani).

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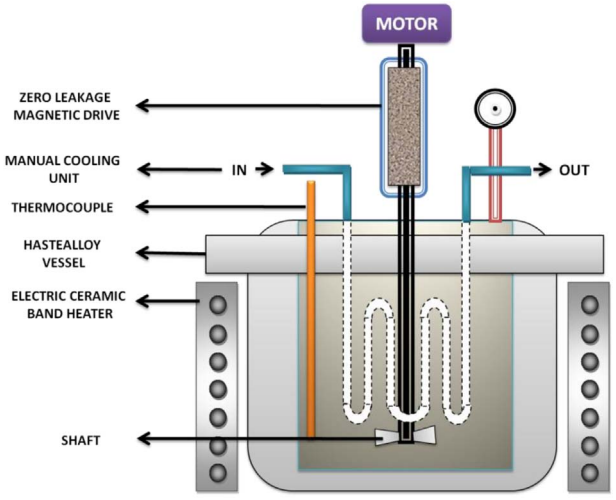

Fig. 1. Schematic representation of hydrothermal vessel.

phase composition of the synthesized materials was determined using X-Ray Diffraction (XRD). The morphological distribution and elemental composition were assessed by transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM) and X-ray energy dispersive absorption (EDAX) spectrum with elemental mapping, respectively. The hardness, Young's modulus and poissons' ratio were determined by nanointendation (Ubi 1 Scanning QuasistaticNanoindenter (TI-700; Hysitron Inc., USA)). In order to evaluate the *in vitro* biocompatibility of the prepared HAp/Alumina composite, we evaluated the viability of MG 63cells with the synthesized nanocomposite using MTT assay.

2. Materials and Methods

The analytical grade $Al(NO_3)_3{\cdot}9H_2O$ was used as precursor for the preparation of alumina nanoparticles. The pH value is adjusted to 10 using 25% ammonia solution and transferred to continuously stirring assembled hydrothermal vessel (1 l capacity) as shown in Fig. 1. The vessel consists of zero leakage stirrer and manual cooling system for fast cooling process. The hydrothermal process was carried out at 100 °C for 1 h under autogenous pressure and minimal stirring rate (200 rpm). Once the process was completed, the system was cooled down to 40 °C at the rate of 5 °C/min. Then, the freshly prepared 1 M of ($CaNO_3$)·$4H_2O$ and0.6 M of di-ammnonium hydrogen phosphate $(NH_4)_2HPO_4$(pH 10) were passed into the hydrothermal vessel containing alumina slurry. The hydrothermal reaction were carried out under autogenous pressure with a stirring rate (< 60 rpm). The slurry was dried and calcined at 900 °C for 3 h.

For the effect of alumina analysis, the concentrations of Al $(NO_3)_3.9H_2O$ is varied from 0.125 to 0.5 M (such as 0.125, 0.25, 0.375, 0.5 M) was also experimented in the above mentioned process with a constant molar ratio of($CaNO_3$)·$4H_2O$ (1 M) and $(NH_4)_2HPO_4$ (0.6 M). Based on aluminium nitrate concentration, the prepared composite samples were named as HA1 (0.125 M), HA2 (0.25 M), HA3 (0.375 M), and HA4 (0.5 M). Pure HAp and Pure alumina, hydrothermally prepared at 250 °C for 5 h were used as reference material for HAp/Alumina composite.

3. Characterization

3.1. Phase Analysis

Initially, the structure and phase arrangements of the hydrothermally prepared sample such as pure HAp and HAp/alumina composite powders (HA1-HA4) were examined under X-Ray Diffraction (XRD, Xpert powder diffractometer, PAN Analytical, Netherland with Cu-kα radiation of 1.5406 Å). The obtained 2θ values were compared with standard JCPDS files of HAp and alumina to determine the characteristics phases of the sample material.

3.2. Morphological Analysis

The size and morphology of the prepared pure HAp, pure alumina and the selected composite sample was examined under Transmission Electron Microscopy (TEM, JEOL JEM 2100, Germany). Based on these studies, the suitable HAp/Alumina composite material with unique morphology was determined for further analysis.

3.3. Thermal Behavior

The Thermo Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) analysis of the selected composite powder was carried out to determine the thermal behavior of the material from room temperature to 1200 °C at a heating rate of 10 °C/min^{-1} using NETZSCH STA 449F3, Japan.

3.4. Elemental Distribution

The surface morphology and elemental composition/mapping of the selected composite material was determined using Field Emission Scanning Electron Microscopy (FESEM, SIGMA HV – Carl Zeiss With BrukerQuantax 200 – Z10 EDS Detector, United States) equipped with Energy Dispersive Absorption spectrum of X-ray (EDAX).

3.5. Nanointendation Study

For intendation study, the selected composite sample was made into green pellet in the dimensions of 120 mm diameter and 2 mm thickness using hydraulic pellet press under an applied pressure of 12 MPa. A green pellet of pure hydroxyapatite prepared under the same condition was used as reference. The mechanical activity for the green pellets of the prepared HAp and HA4 sample was determined using Ubi 1 Scanning QuasistaticNanoindenter (TI-700; Hysitron Inc., USA. Berkovich pyramidal indenter was used to create intendation for a constant load of 600 μN. with constant loading and unloading times as 5 s at a rate of 200 nm s^{-1}. The hardness, H, of the material (Eq. (1)) and the elastic modulus (Er) (Eq. (2)) were measured using the formula:

$$H = P_{max}/A \tag{1}$$

$$E_r = \frac{\sqrt{\pi}}{2\sqrt{A}}\cdot S \tag{2}$$

where

P_{max} refers to the maximum load applied, A, the projected area and S, the slope of the curve.

3.6. In Vitro Biocompatibility Study

The *in vitro* biocompatibility of the prepared composite sample was determined using MTT assay. The MG-63 human Osteosarcoma (Osteoblasts like) cell line was purchased from National Centre for Cell Science (NCCS), Pune. The MG 63 cell line was cultured in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) and maintained at 37 °C, 5% CO_2, 95% air and 100% relative humidity. Viable cells were extracted, counted and diluted in the order of 1×10^5 cells/ml with medium containing 5% FBS. The cells were transferred to 96 well plates with each well containing 10,000 cells and allowed for attachment under the conditions of 37 °C, 5% CO_2, 95% air and 100% relative humidity. After 24 h, the cells were treated with five different concentrations (12.5, 25, 75, 100 and 200 μg/ml) of the composite material. Cell culture without addition of nanomaterial is treated as