Size Controlled Synthesis of One - dimensional Single Crystalline Hydroxyapatite Nanorods by Hydrothermal Method

Sneha M, Meenakshi Sundaram N*, Kandaswamy A

Department of Biomedical Engineering, PSG College of Technology, India

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Abstract Hydroxyapatite (HAp) nanorods were widely used in biomedical fields such as multifunctional drug delivery systems, bone tissue damages and for dental applications. Here, rod shaped HAp nanoparticles with uniform morphology and controllable size were successfully synthesized without any templates by hydrothermal method. Powder X-Ray diffractometer (XRD), Optical Micrographs (OM), Nitrogen adsorption and desorption studies (BET), Field Emission Scanning Electron Microscope (FE-SEM) and Transmission Electron Microscope (TEM) were used to characterize the structure and composition of the prepared HAp samples. The biocompatibility of the prepared nanorods was tested against MG 63 human osteosarcoma cell lines using MTT assay. The results confirmed that the synthesized HAp nanorods are biocompatible, high purity in nature and high aspect ratio with mean width of 10 nm and mean length of 100 nm. Thus the prepared hydroxyapatite nanorods can be used as drug carrying vehicles for hard tissue repair, bone regeneration, bone tissue engineering applications and as strength-enhancing additives for the preparation of biocompatible nanocomposites with improved mechanical properties.

Keywords Hydroxyapatite, Rods, Hydrothermal, Osteosarcoma, Tissue Engineering

1. Introduction

Calcium phosphates with Ca/P ratio between 1.5-2.0 belongs to the group of bioactive inorganics. It is classified into three main compounds namely, tetraphosphate ($\text{Ca}_4\text{P}_2\text{O}_9$), hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and tricalciumphosphate ($\text{Ca}_3(\text{PO}_4)_2$). Among these, hydroxyapatite is the most stable form of calcium phosphate and major component of bones and teeth [1]. HAp are highly suitable for applications in nanomedicine especially in the area of bone reconstruction and cancer treatment [2]. It has

the ability to form chemical bonding with bone tissue during in vivo conditions and to conduct bone tissue growth on the implanted materials [3]. The important properties such as bioactivity, biocompatibility, stability and mechanical properties of HAp are determined by its crystal shape, size, composition and structure [4]. The synthesis of HAp with controlled particle size and shape is important in order to improve the contact and stability at the natural bone interface [5]. There are many methods developed for synthesizing of hydroxyapatite. Some of them include co-precipitation, sol-gel method, hydrothermal route and electrochemical deposition [4,6,7], solid state reaction [8], and physical methods [9]. HAp nanorods were prepared using different organic surfactant or templates in order to manipulate crystal growth [10- 17].

Coelho et al prepared HAp nanorods using CTAB(cetyltrimethylammonium bromide) as a surfactant and obtained diameter between 20 and 50 nm [18]. According to Sun et al HAp nanorods with diameter 8-15 nm and length 25-50 nm was achieved by reverse microemulsion method [19]. Salarian et al prepared HAp particles in the presence of CTAB and PEG (poly ethylene glycol) with uniform, rod-like morphology with diameter of 50-80 nm, average aspect ratio of 6-20 [20]. Wei et al prepared fibrinogen fibrils and fibril networks in the absence of thrombin and used as fibrous template for the growth and nucleation of hydroxyapatite crystals [21]. Wei et al also reported the development of HAp nanoparticles onto super water-soluble carbon nanotube-protein hybrid nanofibers to improve its mechanical properties and biocompatibility of composite materials [22]. Su et al developed 3D biaxially orientated polymer nanofibers and bone morphogenetic protein 2 to facilitate the biomineralization of Hydroxyapatite [23].

In this work, we focused on the synthesis of HAp nanorods that resemble bone minerals with narrow size distribution. Nanorods were prepared without the presence of templates and their morphology and crystallinity were characterized by various techniques. The biocompatibility

test using MG 63 osteosarcoma cell lines confirmed that the synthesized nanorods are biocompatible in nature. Therefore, the HAp nanorods can be used for various biomedical applications.

2. Materials and Methods

2.1. Materials

Calcium nitrate and di-ammonium hydrogen phosphate were purchased from Himedia. Ammonia solution was purchased from Merck. All chemicals were analytical grade and used as received without further purification.

2.2. Preparation of Hydroxyapatite Nanorods

Calcium nitrate solution (1 mol/l) and Di-ammonium hydrogen phosphate solution (0.6 mol/l) was added under stirring for one hour. Ammonia solution was added till the solution reaches pH 10 and white precipitate was formed. It was hydrothermally treated at 180°C for 6 hrs duration under nitrogen atmosphere and washed with distilled water several times and dried at 70°C, overnight. The prepared samples were sintered at 900°C for 2 hrs and stored in an air tight container for further analysis. Figure 1 shows the flow chart of HAp nanorods formation in detail.

Figure 1. Schematic flow process chart for the synthesis of HAp nanorods

3. Characterization

The XRD patterns were recorded using Panalytical, Netherland & X'pert Powder Diffractometer. The JEOL JEM 2100 High Resolution Transmission Electron Microscope (HRTEM), Carl Zeiss Microscopy Ltd, UK, Sigma Field emission Scanning Electron Microscope (FESEM) and olympus infinity corrected optical research microscope were used to analyze the morphologies of the prepared samples. The human osteosarcoma cancer cell line (MG 63) was purchased from National Centre for Cell Science (NCCS), Pune. It was grown in Eagles Minimum Essential Medium with 10% fetal bovine serum (FBS) and the cells were incubated at 37°C, 5% CO₂ and 95% air.

3.1. Powder X-Ray Diffractometer (XRD)

The XRD data of synthesized samples were collected in the 2θ range from 15° to 80° with a scanning speed of 1.2°/min. The XRD patterns possessed a strong peak at around 32.2° corresponding to (211) planes of HAp crystalline structure. Figure 2 shows the X-ray diffraction patterns of the HAp nanorods without the presence of any other phases other than HAp. The narrow peaks represents that the prepared samples are in good agreement for crystallinity and in good agreement with standard available JCPDS (09-0432).

Figure 2. X-Ray diffraction patterns of HAp nanorods

3.2. Optical Micrographs

Figure 3 (a) and (b) shows a lower magnification view of optical micrographs for the sample treated at 900°C. Uniform sizes of HAp nanorods with average length of 20-50 nm were observed. They are monodisperse in nature due to the synthesis method by hydrothermal condition for 6 hours under nitrogen atmosphere at 180°C.

Figure 3. Optical micrographs of HAp nanorods

3.3. N₂ Adsorption-Desorption Studies (BET)

N₂ adsorption and desorption isotherm of HAp nanorods is shown in figure 4. The pore size and pore volume of the samples were obtained according to the Barrett-Joyner-Helenda (BJH) method, and the surface area was measured by the Brunauer-Emmett-Teller (BET) method. The results obtained from the BET analysis are, specific surface area = 22.397 m²/g, pore volume = 5.602 e⁻³

cc/g and pore size = 3.508 e⁻¹ nm. The negative value in the y axis is due to mesoporous structure.

Figure 4. N₂ adsorption and desorption isotherm of HAp nanorods

3.4. Field Emission Scanning Electron Microscope (FE-SEM)

Bundles of HAp nanorods with well defined, randomly oriented elongated structure were seen in figure 5. This may be due to the influence of hydrothermal treatment during synthesis procedure. FE-SEM results showed regular rod-shaped HAp nanoparticles with a mean diameter of 50 nm and mean length of 200 nm. The EDAX graph confirms the presence of calcium and phosphate in the prepared samples.

Figure 5. (a) and (b) FE-SEM images of HAp nanorods, (c) EDAX

3.5. Transmission Electron Microscope (TEM) of HAp nanorods

The TEM analysis were performed in order to obtain quantitative information regarding the exact stoichiometry of the HAp phase formed. TEM images shows that the formed HAp has characteristic nanorod which has a dimension ranges from 20 x 5 x 3 nm to 200 x 50 x 5 nm (figure 6 a-c). Most of the rods have 100 x 25 x 5 nm dimension with a aspect ratio of 1:8 (width:length). The HRTEM of HAp nanorods (figure 6 d) shows that the predominant (211) plane C with d spacing os (2.814 Å). it also shows the planes (002), (112), (300) are co-exist as mosaic structure. Figure 6 e shows the electron diffraction pattern of HAp nanorods. The bright rings corresponds to (002), (211), (112), (300) from core to the outwards. This (hkl) corresponds to characteristic and pure HAp nanorods. Figure 5 f confirms the presence of calcium and phosphate in the sample with the Ca/P ratio of 1.67.

a

b

c

d

Figure 6 a-c). TEM images of HAp nanorods d) HRTEM image e) SAED pattern f) EDAX

3.6. Invitro Cytotoxicity Study

As shown in figure 7 at the concentration of 12.5 $\mu\text{g/ml}$, the cell viability of HAp nanoparticles was 100 %. However, as the concentration increased from 50 to 200 $\mu\text{g/ml}$ the cell viability was bit lower when compared to the concentration at 12.5 $\mu\text{g/ml}$. It indicates the carbonate contents have an impact on biocompatibility of HAp nanoparticles against MG 63 cell lines. Cell viability of hydroxyapatite nanoparticles was still maintained above 75 % (figure 8) even at higher concentration 200 $\mu\text{g/ml}$, indicating that the prepared material is highly suitable for biological applications.

Figure 7. Photomicrographs (10 X) of MG 63 cells after exposure with hydroxyapatite at different concentrations for 48 hrs ((a)Control b) 12.5 µg c) 25 µg d) 50 µg e) 100 µg f) 200 µg)

Figure 8. Cell viability of HAp with MG 63 cell lines

4. Conclusions

In summary, we have obtained HAp nanorods of controlled size and length without any agglomeration and impurities by simple hydrothermal method. The samples obtained after heat-treatment at temperature in the range 900 °C, exhibit very well defined elongated and compact nanorods. This material exhibits good surface area, mesoporous and crystalline structure. The hydrothermal mediated synthesis is a simple, low cost and highly suitable

for large scale production of HAp rods and hence it can be potentially used for various biomedical applications.

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