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Synthesis of nano-hydroxyapatite and its rapid mediated surface functionalization by silane coupling agent

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ABSTRACT

In this work, hydroxyapatite (HA) nanorods were synthesized by simple one step wet precipitation method followed by their rapid surface functionalization via aminopropyltriethoxysilane (APTS) to give modified (HA-APTS) product. Functionalized hydroxyapatite (HA-APTS) holds amino groups on their surface that can be further functionalized with other bioactive molecules. The extent of functionalization of HA was studied under three different processing conditions; at room temperature, at 80 °C and under microwave condition (600 W). Three different temperatures have been use for the purpose of comparison between the functionalized products so that we can judge that whether there is any effect of temperature on the final products. In the last we conclude that temperature has no effect. So microwave condition is best to carried out the functionalization in just 5 min.

1. Introduction

Owing to its multifunctional characteristics hydroxyapatite (HA) has great potential in tissue engineering and biomedical field for instance in targeted drug delivery [1], maintaining control release of drugs [2,3], bone regeneration [4], as bone replacement material [5], coating on artificial metal and composite implants [6,7], bone augmentation [8], as a scaffold, dental filling material [9], biosensor and in magnetic resonance imaging (MRI) [4,5]. Calcium phosphate base inorganic nanoparticles have number of advantages due to ease of synthesis, controllable physiochemical properties, bioactivity, feasibility for handling, biocompatibility and strong anticorrosive properties in physiological environment [10].

Synthetic HA can be used temporarily or permanently as bone replacement material in case of trauma or disease condition of bones where it can regenerate the bone properties up to greater extent [1, 11,12]. Historically metal implants having high stiffness and strength were used as bone replacement material but the main issue with metal implants is the stress shielding due to the difference in the stiffness of natural bone and metal artificial implants [13]. Afterward metal implants were replaced by polymer base composites that have stiffness and viscoelastic properties matching with bones [14–16]. But again problem with both metals and polymer base composites are their biocompatibility, they are not feasible in the human physiological environment. To solve this problem metals/polymer and HA composites were synthesized and coated with HA to make them biocompatible [17]. However due to low interaction between metals/polymers with HA mechanical properties of the composite are constrained. To attain the better connection, HA was modified with different functional groups at their surface [18–20]. Various literature reports that silane coupling agent has been used as intermediate link between HA and polymers or metals for biomedical applications [21]. The functional groups of silane coupling agents can be bond both with organic and inorganic materials and due to this unique property these are used for surface modification of large number of materials [22,23].

Herein for the first time we report the modification of HA nanorods via silane coupling agent in a short time of just 5 min by an efficient, facile and rapid microwave method. Despite HA having been modified by various techniques using silane coupling agent, most of literature reports high temperature and long duration for functionalization. In this work HA were functionalized under three different processing conditions; at room temperature, at 80 °C and under microwaves condition (600 W) to compare the effect of processing temperature. The functionalization of HA at room temperature is green and economical but long reaction time (12 h) impedes its application while in the present work rapid functionalization of HA has been carried out in short time of just 5 min under microwave condition [21]. HA-APTS was further modified by succinic anhydride to obtained highly dispersed homogeneous (HA-APTS-SA) spherical particles with negatively charged

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surface that can be functionalized with other bioactive molecules which are beneficial from biomedical point of view. Cell viability study was carried out and results show that all the functionalized products are biocompatible. Systematic study has led to the conclusion that temperature has no effect so microwave condition is best to carried out the functionalization in just 5 min. This study has significant scope for further conjugation of nano-hydroxyapatite with various morphology with other polymers or biological ligands that are useful for various biomedical applications.

2. Experimental procedures

2.1. Materials

Various chemicals used for the synthesis of nano-HA include calcium nitrate $Ca(NO_3)_2\cdots 4H_2O$ (UniChem) (99% pure) as Ca^{2+}, di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (AppliChem) (99% pure) as PO_4^{3-} and ammonia solution as OH^- sources. APTS (Sigma Aldrich) for HA functionalization. Osteoblast cell, Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin and streptomycin for cell viability evaluation.

2.2. Preparation method

2.2.1. Synthesis of HA nano-rods

Molar solutions of the precursor were prepared to form the ideal ratio of Ca/P 1.67. Both precursor's salt diammonium hydrogen phosphate (0.3 M) and calcium nitrate (0.5 M) salts were separately dissolved in 250 mL distilled water. The pH of both salt solutions was adjusted up to pH 11 by adding ammonium hydroxide (BDH) and stirring for 1 h. With a dropping rate of 2 mL per minute pH was adjusted. Diammonium hydrogen phosphate salt solution was added drop to drop to the calcium nitrate solution. When both solutions were mixed completely then the mixtures were stirred for 24 h at 80 °C. Then white milky solution was filtered and washed with deionized water several times to remove excess of ammonia. The white cake of precipitate obtained at filter paper was dried at 90 °C in oven then calcined at 500 °C for 3 h. The white harder material obtained was grinded in pestle and mortar to obtained nano-HA powder.

2.2.2. Functionalization of HA nanorods

a) Functionalization at room temperature

Nano-HA 0.36 g was sonicated into 400 mL ethanol for 4 h to get homogeneous solution. The mixture was then transferred to a beaker and under continues stirring then APTS (10 mL) was added to it. To hydrolyze the alkoxysilane groups of APTS to silanol groups 2 mL of deionized water was added into the above mixture. After stirring for 12 h the mixture was centrifuged at 10,000 rpm to get the product and washed 3–4 times with water then with methanol.

b) Functionalization at 80 °C

The same procedure was followed up for the functionalization HA nanorods as above but in this case after the addition of APTS and 2 mL of deionized water the mixture was stirred at 80 °C for 12 h. After stirring for 12 h at 80 °C the mixture was centrifuged at 10,000 rpm to get the product and washed 3–4 times with water then with methanol.

c) Functionalization under microwave conditions

In this case for the functionalization HA nanorods same procedure was used but after the addition of APTS and 2 mL of deionized water to the mixture, the suspension was exposed to microwave radiations for 5 min at 600 W. After that the mixture was centrifuged at 10,000 rpm to get the product and washed 3–4 times with water then with methanol to avoid the existence of byproducts.

d) Carboxylation of HA-APTS by succinic anhydride

0.3 g of HA-APTS was dispersed in 100 mL of dimethylsulfoxide. 2.5 g of succinic anhydride was dissolved in 25 mL of dimethylsulfoxide under vigorous magnetic stirring. Then both the suspensions were mixed and vigorously stirred for 24 h. After that the mixture was centrifuged at 10,000 rpm to get the product and washed 3–4 times with water then with methanol to avoid the existence of byproducts and (HA-APTS-SA) was obtained in purified form.

e) Cell viability evaluation

Osteoblast cells were cultured in tissue culture flasks (25 cm) in a Dulbecco's modified Eagle's medium (5 mL) containing 10% fetal bovine serum, with penicillin and streptomycin (100 U/mL) in an incubator and 5% CO_2 at 37 °C. The cells were plated in a complete medium overnight with 96 well plates. The medium was replaced by fresh medium containing samples with a concentration range of 50–200 μg/mL. The viability of the cells was evaluated via an MTT assay after 24 h of incubation. The plates were read using a microplate reader at 570 nm. The mean and standard deviation for the triplicate wells for each sample were reported. The viability of the cells was also evaluated by visual observation of the morphology of cell using inverted phase contrast microscope with a magnification of 200× for each sample.

2.2.3. Materials characterization

The final product obtained after washing was dried and calcined. The fine powder was characterized by X-ray diffraction (XRD) for crystal structure and purity analysis using STOE diffractometer at room temperature in scanning range of 20° to 80° 2θ with step size of 0.04° and step time of 1 s. Fourier transforms infrared (FTIR, Perkin Elmer 100 series) with scan region of 4000–400 cm^{-1} at resolution of 4 cm^{-1} was performed for compositional and different functional groups analysis. For FTIR analysis KBR method was used and sample was prepared by compaction of few crystal of HA powder with KBr powder in hydraulic press under the pressure of 5 MPa to form compact and transparent pellets. As prepared pallets were analyzed for functional groups determination in powder samples.

TEM (Transmission electron microscope) FEI Tecnai T20 and SEM (Scanning electron microscope) (JEOL JSM6490A, Japan) 20 kV with a working distance optimized for imaging and large spot size were used for powder morphological (size and shape) evaluation. HRTEM F30 with 300 kV was used to study the interplaner distance. For SEM samples were prepared by dissolving of 1 mg HA powder in 1 mL deionized water for 1 h. After sonication a drop of this dispersion with help of dropper was poured on a piece of glass slide and dried. The glass slide was then gold coated by gold sputtering in Argon (Ar) gas atmosphere. The Ar gas ionized in cold cathode discharge and accelerates the ions at 1–30 kV, these ions strike the gold target and impart momentum for dislodging the atom from the target. These free atoms of the gold target are then deposited on the sample placed on glass slide. Then glass slide was fixed on stub and analyzed by SEM. For HRTEM and TEM the samples were prepared by adding 1 mg sample to 2 mL ethanol and were sonicated for 15 min to get dispersion. One drop from this dispersion was then put on cupper grid and was analyzed for morphology and crystal structure analysis.

3. Results and discussion

In this work we have functionalized HA nanorods by APTS that have amines group at their surface. To confirm the presence of the amino group and its susceptibility to other functional groups, HA-APTS was further functionalized by succinic anhydride to form negatively charged nanoparticles. The three products HA, HA-APTS and HA-APTS-SA were characterized using possible available characterization techniques.