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Synthesis of different sized and porous hydroxyapatite nanorods without organic modifiers and their 5-fluorouracil release performance

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ABSTRACT

Porous biocompatible hydroxyapatite (HAP) nanorods of various sizes were synthesized by the combination of chemical precipitation and hydrothermal method without the use of organic modifiers. The HAP nanorod samples were characterized by powder X-ray diffraction, transmission electron microscopy, and N_2 adsorption/desorption techniques. HAP nanorods with average diameters and average lengths ranging from 8.5 to 26.6 nm and from 23.1 to 49.7 nm, respectively, could be controllably synthesized via these methods. Low autoclaving temperature and high pH value favored the formation of relatively small HAP nanorods. The TEM images showed that the nanorods possessed porous structures with average pore diameters ranging from 1.6 to 2.7 nm. These HAP nanoparticles effectively prolonged the release time of 5-fluorouracil up to 24 h. The as-synthesized HAP nanorods displayed no cytotoxicity to bone marrow stem cells at low HAP concentration, indicating that these nanorod materials could serve as potential carriers for novel drug release systems.

1. Introduction

Nanosized hydroxyapatite [$Ca_5(PO_4)_3(OH)$, HAP] is the major phosphate mineral constituent of bones and teeth. It has gained more and more attention in the field of bone reparation due to its similarity to natural bone, biocompatibility, and osteoconductibility. Several methods, such as sol–gel [1,2], hydrothermal [3], chemical precipitation [4,5], chemical precipitation/hydrothermal [6,7], and microemulsion [8], have been used to synthesize HAP nanoparticles. Among these synthesis methods, the combination of chemical precipitation and hydrothermal method can be used to synthesize well-crystallized HAP nanoparticles [6,7].

The morphology and size of HAP nanoparticles significantly affect their strength, toxicity to cells, osseointegration, bioresorption, and other properties in practical applications [9–12]. To control the morphology and size of HAP nanoparticles, organic modifiers are conventionally used in the synthesis process, such as trisodium citrate, Tween 20, polyethylene glycol [6], citric acid, sodium dodecyl sulfate, sodium dodecylbenzene sulfonate [7], ethylenediamine tetraacetic acid (EDTA) [9], ethylene glycol [13], polyvinylalcohol [14], and cetyltrimethylammonium bromide [8,14,15]. However, FTIR spectra of the washed HAP samples synthesized using trisodiumcitrate, Tween 20, citric acid, sodium dodecyl sulfate, and sodium dodecylbenzene sulfonate as modifiers revealed that these organic modifiers are difficult to remove by washing with water [6,7]. The remaining organic modifiers unavoidably affect the interaction between HAP and human tissue. Morphology- and size-controlled synthesis of pure HAP nanoparticles without the use of organic modifiers is worth investigating.

Recently, controlled drug release from polymer and inorganic carriers has attracted much researcher interest [16]. 5-Fluorouracil, a highly effective anticancer drug, has been widely used in the clinical treatment of numerous types of cancers including colorectal, stomach, liver, pancreas, neck, rectum, and breast cancers [17]. Unfortunately, 5-fluorouracil can induce a strong rise in metabolism and its biological half-life is rather short, lying in the range of 10–20 min [17]. In order to maintain the concentration level of 5-fluorouracil over a sufficient period of time, the drug is often administered repeatedly or else to increase the amount of the drug dosage, either of which could induce serious side effects in the human body. Embedding 5-fluorouracil in a nanoporous carrier could potentially act as a time-release vehicle to maintain a constant concentration level of the drug, and at the same time help to alleviate its adverse side effects. Loading of 5-fluorouracil has been examined in a number of studies of nanoporous materials, including alginate-chitosan/montmorillonite nanocomposites [17], polylactic-co-glycolic acid-based microparticles [18], magnetic glass ceramics [19], montmorillonite [20], polymerized-chitosan coated Fe_3O_4 nanoparticles [21], diepoxy-terminated poly(ethylene glycol)s/aliphatic polyamines [22], and chitosan hydrogel membrane [23]. However, these polymer and inorganic carriers are not well-suited for bioabsorbtion within human body.

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HAP has been widely used as a surface coating for artificial hip joints in orthopedic surgery. A recent literature review of its biological safety concluded that nanoscale HAP exhibited excellent osseous and biological compatibility according to the ISO 10993 standard and displayed no cytotoxicity [24]. Nanoscale HAP materials with needle, rod, and plate morphologies demonstrated an overall low toxicity in NR8383 cells and primary macrophages [25]. However, it was recently reported that the cytotoxicity of nanoscale HAP with MDA-MB-231 cells increased with decreasing the grain size, since cellular inflammation is generally enhanced by endocytosis as the particle size decreases on the order of 100 nm [26]. Although the cytotoxicity of nanoscale HAP is still debatable, its potential is high as a possible alternative to other drug carrier materials from the perspective of its biocompatibility and low cytotoxicity.

In the present work, pure HAP nanorods of different particle size and porous structure were synthesized by the combination of hydrothermal and chemical precipitation methods without using organic modifiers. The effect of several experimental parameters on the evolution of porous HAP nanorods was investigated, namely, pH, reaction temperature and time. The time-release properties of 5-fluorouracil from porous HAP nanorods were also investigated within a simulated body fluid. The results showed that porous HAP nanorods effectively prolonged the 5-fluorouracil release time. The cytotoxicity of HAP nanorods of various sizes on bone marrow stem cells was also investigated.

2. Experimental

2.1. Materials

Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), phosphoric acid (H_3PO_4, 85%), ammonia solution (NH_3, 25%), sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), dipotassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), calcium chloride anhydrate ($CaCl_2$), sodium sulfate anhydrous (Na_2SO_4), hydrochloric acid (HCl, 37%), and sodium hydroxide (NaOH) were purchased from China Chemical Reagent Co. 5-Fluorouracil injection (10 mL of fluorouracil injection containing 0.25 g 5-fluorouracil) was purchased from Tianjin Jinyao Aminoacid Co. All the chemicals used in the experiments were of analytical grade and used without further purification.

2.2. Synthesis of HAP nanoparticles

Aqueous solutions of calcium nitrate (1 mol L^{-1}) and phosphoric acid (0.6 mol L^{-1}) with a Ca/P molar ratio of 1.67:1 (which is the theoretical Ca/P molar ratio in HAP) were added into a three necked round bottom flask and heated at 40 °C in a water bath. An ammonia solution (25%) was added dropwise into the reaction solution under stirring over a 20 min duration to adjust the pH value to approximately 9 to 10. This reaction mixture was aged at 40 °C for 4 h under constant stirring. The resultant suspension was transferred to a teflon-lined stainless steel autoclave at 60 °C or 120 °C for 12 h. Furthermore, to investigate the effect of the aging process, after reacting at 40 °C for 4 h, the resultant suspension was maintained at room temperature (25 °C) for 24 h and then autoclaved at 100 °C for several different time periods. Subsequently, the samples were washed with deionized water until the conductivity measured less than 2 mS m^{-1}. Finally, the washed samples were dried in an oven at 100 °C for 24 h and then stored in a desiccator. The synthesis conditions of the HAP nanoparticles are collected in Table 1.

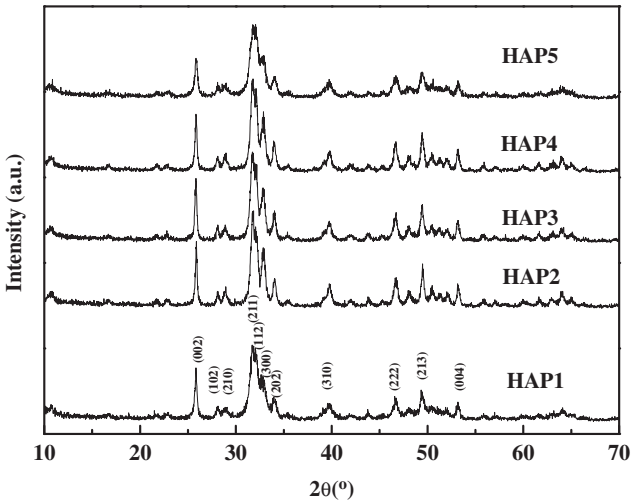

Fig. 1. XRD patterns of HAP samples.

2.3. Characterization

XRD patterns of all the samples were analyzed on a diffractometer (XRD-6100 Lab, Shimadzu) using CuKα radiation (λ = 0.154056 nm) and a scanning range from 20 to 65°.

The morphologies, particle sizes, and pore diameters of the samples were examined by transmission electron microscopy (TEM) using a JEM 2100 electron microscope operating at 200 kV. The samples for TEM measurement were prepared by dispersing a small amount of sample in ethanol and ultrasonicated for 30 min. Several drops of the resultant suspension were placed onto a copper grid coated with a layer of amorphous carbon. The average diameters, the average lengths, and the average pore diameters of the HAP nanorods were measured from the TEM images by counting at least 150 individual particles based on a weighted-average method [27,28].

Nitrogen adsorption/desorption isotherms were obtained using a NOVA 2000e physical adsorption apparatus.

2.4. 5-Fluorouracil release from HAP nanoparticles

5-Fluorouracil was loaded into HAP nanoparticles by the incipient-wetness impregnation method. Three grams of HAP sample was subjected to vacuum impregnation in a 50 mL flask for 30 min in a 5-fluorouracil aqueous solution. The 5-fluorouracil-loaded HAP sample

Table 1
Synthesis conditions, dimensions, specific surface areas, average pore diameters, and pore volumes of hydroxyapatite samples.

Samples	pH	Precipitation time at 40 °C/h	Aging time at 25 °C/h	Hydrothermal temperature/°C	Hydrothermal time/h	Average particle diameters/nm	Average particle lengths/nm	Average pore diameters[a]/nm	Specific surface areas/$m^2\ g^{-1}$	Pore volumes/$cm^3\ g^{-1}$	Average pore diameters[b]/nm
HAP1	9	4	/	60	12	8.5	23.1	1.6	85.7	0.34	12.5
HAP2	9	4	/	120	12	26.6	49.7	2.6	75.0	0.46	17.8
HAP3	10	4	/	120	12	14.6	42.2	2.0	74.0	0.29	9.6
HAP4	10	4	24	100	12	15.2	40.8	2.7	52.6	0.21	12.4
HAP5	10	4	24	100	4	11.4	30.6	2.7	67.7	0.26	3.8

[a] The average pore diameters were measured from TEM images.
[b] The average pore diameters were determined by N_2 adsorption/desorption isotherms.