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Mesoporous hydroxyapatite nanoparticles hydrothermally synthesized in aqueous solution with hexametaphosphate and tea polyphenols

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ABSTRACT

Mesoporous hydroxyapatite (HA) nanoparticles with high surface area have been widely investigated for drug delivery. Herein we report a facile and simple strategy for the preparation of such materials using hexametaphosphate salt as inorganic phosphorus source. In the hydrothermal processing, hexametaphosphate plays an important role in the formation of mesoporous structure. The as-prepared mesoporous HA nanoparticles can be candidates for pH-responsive anticancer drug delivery by using doxorubicin (Dox) as a model drug. Furthermore, modification of these mesoporous HA nanoparticles using tea polyphenols is attempted. The presence of tea polyphenols in the HA synthesis processing result in mesoporous HA nanoparticles with tailored morphology and properties, making them more pH-sensitive for drug delivery. Both hexametaphosphate and tea polyphenols can be potential chemical sources in synthesizing mesoporous HA.

1. Introduction

Hydroxyapatite [HA, $Ca_{10(}PO_4)_6(OH)_2$] is known of the main inorganic constituent of human skeleton. On account of its osteoconductivity, biocompatibility and bioactivity, HA has been widely used in biomedical fields [1]. Recently, much attention has been attracted by mesoporous HA nanoparticles as drug delivery system [2,3].

Up to now, templating method is the most well-known technique to synthesize mesoporous HA nanoparticles. A series of surfactants, including Pluronic P123, Cetyltrimethylammonium Bromide (CTAB), Pluronic F127, and mono-alkyl phosphate (MAP) have been used as templates for the synthesis of mesoporous HA nanoparticles [4–6]. In the processing, the self-assembly of inorganic HA phases and surfactants followed by template removal can produce mesoporous structure. As an alternative, physical attempt especially microwave assisted hydrothermal processing has been considered as a template-free solution to prepare mesoporous HA nanoparticles [7]. Some typical systems were summarized in Table 1. However, on viewing these literatures, the phosphate source used in these HA synthesis is just orthophosphate.

Zhu's group has reported the possibility using organic polyphosphate such as 5′-triphosphate disodium salt (ATP) and adenosine 5-diphosphate disodium salt (ADP) as phosphorus source combined with a microwave-assisted hydrothermal method to prepare amorphous calcium phosphate (ACP) mesoporous microspheres [12,13]. Unfortunately, their system is limited to the high cost of phosphorus source; and mesoporous HA is hard to be synthesized with both ATP and ADP working as stabilizer of ACP. Inspired by their concept, a facile and simple strategy for the rapid synthesis mesoporous HA nanoparticles based on sodium hexametaphosphate ($Na_6P_6O_{18}$) as phosphorus source has been reported by our group [14]. As a continuing effort of studying this system, the as-prepared mesoporous HA nanoparticles are characterized and evaluated as pH-responsive anticancer drug carriers using doxorubicin (Dox) as a model drug in present work. In addition, tea polyphenols are used in the hydrothermal reaction as an attempt to make the mesoporous HA nanoparticles more promising for Dox delivery and medical applications.

2. Experimental

2.1. Materials

Tea polyphenols were purchased from Yuanye Co., Ltd. (Shanghai). Other chemicals used in present work were purchased from Aladdin Chemistry Co., Ltd. All chemicals were of analytical grade and used as received without further purification.

2.2. Preparation of mesoporous HA nanoparticles

In a typical experiment, 0.51 g of $Na_6P_6O_{18}$ was dissolved in 300 mL of deionized water with magnetic stirring at room temperature. Then,

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Table 1
List of some typical systems for preparing mesoporous HA.

No.	Template	Reference
1	CTAB	[4]
2	CTAB, F127,P123	[5]
3	MAP	[6]
4	1,3,5-Rimethy benzene (TMB) + CTAB	[8]
5	1-Dodecanethiol (C_{12}-SH) + CTAB	[9]
0.5		
Template-free		
6	Microwave	[7]
7	Microwave + Ultrasound	[10]
8	Gas–Liquid	[11]

0.37 g of $Ca(OH)_2$ was added to the above solution to form a suspension. After magnetic stirring for 10 min, the resulting mixture was hydrothermally heated in an autoclave at 120 °C for 4 h. The precipitates were centrifuged, washed with deionized water and dried in an oven of 60 °C. The final product was named as Ca-P6. Tea polyphenols modified powders were prepared through the same way except adding 0.01 g of tea polyphenols to the suspension before hydrothermal treatment. The as-collected powders were denoted to Ca-P6-TP.

2.3. Characterization

The as-prepared samples were characterized using X-ray powder diffraction (XRD, D/MAX2500, Rigaku). For all samples, data was collected at 2 theta angles ranging from 10 to 60° at a scan speed of 1° per minute. Fourier transform infrared spectroscopy (FTIR, PROTÉGÉ 460, Nicolet) was applied for chemical analysis of powders. The transmittance of each sample was recorded with 16 scans with resolution of 4 cm^{-1} between 4000 and 400 cm^{-1}. The morphology of as prepared samples was characterized using scanning electron microscopy (SEM, SUPRA 55, Zeiss) and transmission electron microscopy (TEM, JEM-2100, JEOL). Brunauer–Emmett–Teller (BET) specific surface area and pore size distribution (Autosorb-iQ2-MP, Quantachrome) was used to study the surface area and pore size distribution of powders. The zeta potentials of powders at pH 4–8 were determined using a zeta potential analyser (ZEN3600, Malvern).

2.4. In vitro bioactivity study

The bioactivity of as-prepared samples was studied in vitro by soaking material samples into 37 °C simulated body fluid (SBF) for up to 3 weeks with SBF refreshed every other day [15]. The composition of used SBF is listed in Table 3. The weight change of incubated samples was measured every week. When the experiment was done, the samples were collected by centrifugation and subsequently examined using SEM.

2.5. In vitro cytocompatibility study

The cytocompatibility of as-prepared samples was tested using MC3T3-E1 osteoblast cells. In brief, powders of Ca-P6, Ca-P6-TP at 10 mg was immersed in 10 mL cell culture medium (1000 μg/mL) for 48 h. MC3T3-E1 cells were seeded to the bottom surface of each culture well at the density of 5000 cells/well. Subsequently, the culture medium that prepared with materials was added into the wells with 200 μL per well. Regular HA synthesized via the reaction of $Ca(OH)_2$ and NaH_2PO_4 at pH 10 was used as control group. Cell density was measured using MTT assay for 1 day and 7 days. Briefly, the medium were removed and 20 μL of a 5 mg/mL solution of MTT (Solarbio) was added to each well followed by incubation for 4 h at 37 °C. The formed formazan crystals were then dissolved by addition of 150 μL DMSO per well for 10 min at 37 °C under shaking. The optical density (OD) at 490 nm was recorded on a microplate reader to show the relative density of cells.

2.6. Anti-bacterial attachment test

The anti-bacterial attachment ability of Ca-P6, Ca-P6-TP was tested using *E. coli* [16]. Regular HA was used as a control. LB broth containing 1% NaCl were used as the medium for *E. coli*. 100 μL of bacteria was taken from the stock and added to 10 mL LB broth for 12 h incubation at 37 °C. Sterilized Ca-P6, Ca-P6-TP, and HA pellets were added to tubes with 10 mL of PBS and 100 μL of *E. coli* suspension. Pellet was prepared by pressing 0.2 g of powders in a mold with a diameter of 15 mm. The tubes were incubated for 12 h at 37 °C. After that the samples were picked up using forceps and washed using PBS to remove unattached bacterial. Pellets were then moved to new tubes with 3 mL of PBS in each. The attached bacterial were removed from sample surface via vortex. The suspension of bacterial was diluted and plated. The formed bacterial colonies after 24 h incubation were counted and compared.

2.7. In vitro Dox loading and release

The powder (10 mg) of mesoporous HA nanoparticles was immersed into 10 mL of Dox (0.1 mg/mL) aqueous phosphate buffer

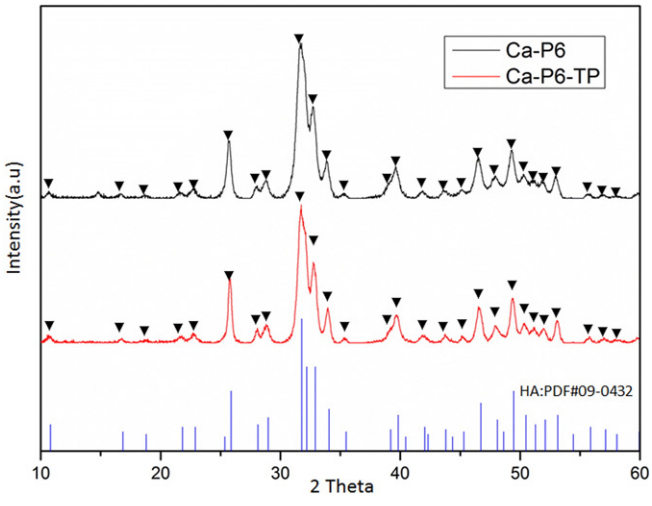

Fig. 1. XRD patterns of as prepared Ca-P6 and Ca-P6-TP, both matches HA patterns in JADE database.

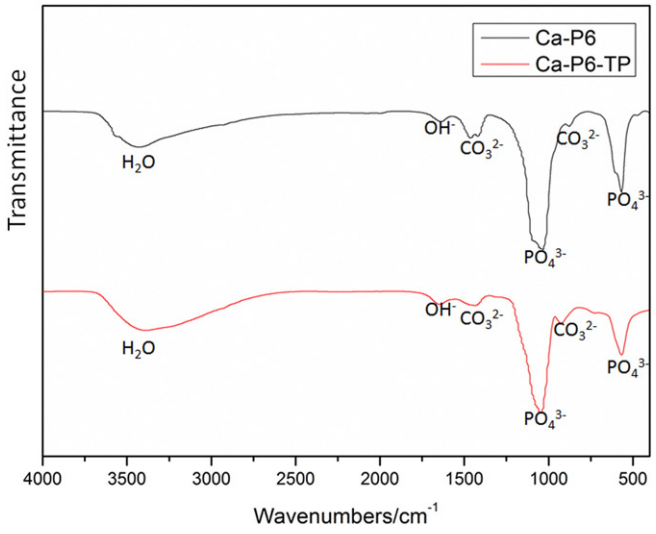

Fig. 2. FTIR spectra of Ca-P6 and Ca-P6-TP.