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Control synthesis and self-assembly of calcium apatite at low temperatures

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Abstract

As members in the calcium apatite family, dicalcium phosphate dihydrate (DCPD), anhydrous dicalcium phosphate (DCPA), and hydroxyapatite (HAp) play important roles in many fields. Herein, by tuning the amount of oleic acid, octadecylamine, acetic acid and reaction conditions, hierarchically structured DCPD or DCPA nanoflowers, DCPD nanosheets, DCPA nanobelts or nanowires and HAp nanorods have been selectively synthesized via a simple, mild solution process. The phase conversion and morphological evolution of the three compounds were discussed in detail. The as-prepared samples were characterized by Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Scanning Electron Microscope and Transmission Electron Microscope. Cytotoxicity assay showed that samples own good biocompatibility.

Keywords: Calcium apatite; Morphology; Synthesis; Self-assembly; Cytotoxicity

1. Introduction

Calcium apatite (hydroxyapatite (HAp, $Ca_{10}(OH)_2(PO_4)_6$), dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$), anhydrous dicalcium phosphate (DCPA, $CaHPO_4$), monocalcium phosphate monohydrate (MCPM, $Ca(H_2PO_4)_2 \cdot H_2O$), monocalcium phosphate anhydrous (MCPA, $Ca(H_2PO_4)_2$), tricalcium phosphate (TCP, $Ca_3(PO_4)_2$), etc.) play important roles in many fields such as geology, chemistry, biology, food science, medicine, tissue engineering, etc. [1–6] Recently, the study of the synthesis and application for it has attracted widespread attention [7–10].

As one member in the calcium apatite family, HAp is well known for its biocompatible, osteoconductive, nontoxic, noninflammatory, nonimmunogenic and bioactive properties, especially the ability to form a direct chemical bond with living tissues [3,11]. DCPD also plays important roles in the medicine, tissue engineering, food industry and other fields due to its excellent biocompatible, biodegradability, low hygroscopicity, sale price, etc. [2] For example, DCPD is often found in pathological calcifications (dental calculi, crystalluria, chondrocalcinosis and urinary stones), and is proposed as an intermediate in both bone mineralization and dissolution of enamel in acids (dental erosion) [4,5,12]; in medicine, DCPD is used in calcium phosphate cements and as an intermediate for tooth remineralization [13–18]; in other applications, it is used as a flame retardant, a slow release fertilizer, glass production, as well as calcium supplement in food, feed and cereals [2,19,20]. Similar to DCPD, DCPA is also indispensable in the similar application area as DCPD, it is used as calcium phosphate cements [21–25], a polishing agent, a source of calcium and phosphate in nutritional supplements, a tabletting aid and a tooth paste component [20]. Due to the structural and componential complexity of these compounds, it is usually difficult to simultaneously tune the phases and morphologies of the calcium apatite family.

In addition, hierarchically structured materials like hybrid organic–inorganic micro/nano-flowers (organic phase: bovine serum albumin, chitosan, etc. and inorganic phase: $Cu_3(PO4)_2$, BSA/ZnO, hydroxyapatite and calcium pyrophosphate hydrate, etc.) have recently caused great enthusiasm and interest in their potential applications, including separation, catalysis, biosensing,

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diagnostic agents, energy storage, and delivery of drugs [26–32]. Lin et al. investigated flower-like HAp flowers by a low-temperature hydrothermal method [33]. However, how to develop simple strategies for effectively controlling both the morphologies and sizes of crystals and facilitating the formation of hierarchical superstructures still remains a tremendous challenge.

In the present work, DCPD, DCPA, and HAp nanocrystals with a diversity of morphologies were successfully synthesized by simple solution methods at lower-temperature. Specifically, the two new strategies for controlling hierarchically structured DCPD or DCPA nanoflowers with good biocompatibility were developed. When acetic acid and water were only used as the reaction solvent, the self-assembled DCPD nanoflows were prepared, and when adding oleic acid, octadecylamine and ethanol in above reaction system, the new hybrid organic–inorganic flows were obtained. The phase conversion and morphological evolution of final products were controlled by adjusting the amount of oleic acid, octadecylamine, acetic acid and reaction temperature.

2. Experimental

2.1. Materials

Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$), sodium phosphate tribasic dodecahydrate ($Na_3PO_4 \cdot 12H_2O$), ethanol, oleic acid, and acetic acid were obtained from Beijing Chemical Reagents Company (Beijing, PR China). BHK-21 cells (baby hamster kidney cells) were obtained from Biok&KM Co. Ltd. (Jiangsu, China). Roswell Park Memorial Institute medium 1640 (RPMI 1640) and fetal bovine serum (FBS) were obtained from Hyclone (Logan, UT, USA). The Cell Counting Kit-8 (CCK-8) was provided by Keygen Biological Technology Development Ltd. Co. (KGA317, Nanjing, China). 5(6)-carboxyflourescein diacetate (CFDA) was obtained from Sigma (USA). All chemicals were of analytical grade, and used as received without any further purification.

2.2. Preparation of DCPD and DCPA crystals

The calcium apatite powders were prepared by hydrothermal method as following: in a 40 ml PTFE autoclave, ethanol (15 ml), oleic acid (0, 1, 2, 3, or 4 ml), acetic acid (0, 1, 2, 3, or 4 ml), and octadecylamine (0 or 0.5 g) mixed together under agitation. Then 7 ml $Ca(NO_3)_2$ aqueous solution (0.2 M) and 7 ml Na_3PO_4 aqueous solution (0.2 M) were quickly added in turn. The above mixture was stirred for 10 min, and treated at 25 °C for 48 h or 60 °C for 30 h or 100 °C for 10 h. The obtained deposits were washed by ethanol for three times. The final samples were obtained after oven-drying at 30 °C for 24 h.

2.3. Sample characterization

The crystal structure and phase purities of the as-prepared samples were verified by means of X-ray diffraction (XRD) spectrometer (model: Rigaku D/max 2500PC). Phase identification was achieved by comparing the diffraction patterns with Joint Committee on Powder Diffraction Standards card (JCPDS). Fourier transform infrared spectroscopy (FT-IR, model: Nicolet 560) were recorded in the wave number range of 4000–400 cm^{-1} to characterize the functional groups on the samples. The size and morphology of the final samples were characterized by an LEO 1530 scanning electron microscope (SEM) and a Hitachi H-1200 transmission electron microscope (TEM).

2.4. Cytotoxicity assay of DCPD and DCPA crystals

BHK-21 cells were cultured in the RPMI-1640 medium supplemented with 10% FBS in a CO_2 (5%) incubator at 37 °C. DCPD and DCPA crystals synthesized in the octadecylamine, oleic acid, and acetic acid system were chosen to investigate their biocompatibility. A stock aqueous solution of 0.25 mg/ml DCPD or DCPA crystals were added into RPMI-1640 medium supplemented 10% FBS to the concentration 320 μg/ml, respectively. After incubation at 37 °C for 24 h, centrifuge the mixture and the supernatant collected was used as the first extract. Subsequently, the first extract was serially diluted with RPMI-1640 medium supplemented 10% FBS to prepare different concentration extracts (20, 40, 80, 160 μg/ml). Then these extracts were filtered through 0.22 μm filter membranes under sterilized conditions.

First, 3×10^3 cells/well were seeded into the 96-well plates in a CO_2 (5%) incubator at 37 °C for 24 h. Then the culture medium was replaced by prepared extracts. Cells without samples extracts were used as the control groups. The wells with culture medium were used as the blank groups. After incubation for 24 h, culture medium was removed and 10 μl CCK-8 plus 100 μl RPMI-1640 supplemented 10% FBS was added into each well. The culture plates were incubated for additional 2.5 h. The absorbance was measured at 450 nm using a microplate reader (Power Wave XS2, Gene Company, USA). The cell viability was calculated as follows: Cell viability (%) $= [OD_{test} - OD_{blank}]/[OD_{control} - OD_{blank}] \times 100\%$. All samples were done in quintuplicate to get average data.

The morphology of cells cultured with sample extracts was observed using 5(6)-carboxyfluorescein diacetate (CFDA) (Sigma) which could label live cell. BHK-21 cells were seeded on the 24-well plates at the density of 9×10^3 cells/well at 37 °C for 24 h. Then culture medium was changed into samples extracts. After incubation for 48 h, removed supernatant and added 20 μl CDFA with 100 ul RPMI-1640 medium supplemented 10% FBS into wells to stain for 30 min. Finally cell morphology can be visualized in an inverted microscope (Nikon Eclipse TE2000-U, Japan).

3. Results and discussion

3.1. XRD measurement

Powder XRD patterns of the samples obtained at different conditions were shown in Fig. 1. Fig. 1A showed the XRD pattern of the samples obtained at 25 °C for 48 h. By comparing the diffraction patterns with JCPDS, the obtained patterns matched well with the standard diffraction data of DCPD [JCPDS