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Microwave-assisted facile synthesis of mono-dispersed Ba/Ho co-doped nanohydroxyapatite for potential application as binary CT imaging contrast agent

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ABSTRACT

It is appealing to develop a facile way to prepare a binary CT contrast agent with good biocompatibility and CT contrast efficiency for potential clinical applications. In this work, a novel binary CT contrast agent based on the biocompatible nano-hydroxyapatite (nHAp, the main inorganic component of human bone and teeth) was prepared facilely and rapidly through doping two kinds of CT contrast ions (Ba^{2+} and Ho^{3+}) into the nHAp host via microwave-assisted synthesis. The Ba/Ho co-doped nHAp with pure nHAp phase composition and good mono-dispersion was obtained simply with microwave heating for 30 min. The as prepared CT contrast agent is very promising as a novel binary CT contrast agent for CT imaging.

1. Introduction

Due to its high spatial resolution, deep tissue penetration, simple equipment and relatively low cost, X-ray computed tomography (CT) has been widely applied in clinical practice as one of the non-invasive imaging technologies and contributed a lot to diseases diagnosis [1–3]. To obtain clear image of the lesion site, a good CT contrast agent is essential for enhancing the contrast efficiency between different tissues to provide accurate imaging for disease diagnosis. Currently, small iodinated molecules including iopamidol, iohexol and diatrizoate are routinely used in CT imaging, but they have disadvantages such as short circulation time, high renal toxicity and risk of allergies [4]. To overcome the above drawbacks, several metal elements including Au, Bi, Ba and lanthanide with high X-ray attenuation coefficient have been used to construct a series of unitary or binary CT contrast agents for CT imaging, which exhibited better CT contrast efficiencies than clinically used iodinate-based CT contrast agents. Besides, they are easy to be loaded with tumor-targeting ligands, drugs and etc., showing a great potential for early diagnosis of diseases and thus attracting great attention in recent years [5–7].

However, the above newly developed CT contrast agents are all exogenous substances to biological environment. Their biocompatibility can be problematic when used for in vivo CT imaging. Meanwhile, Au or Bi_2S_3 nano-particles only contain a single contrast element (Au or Bi) with one K-edge value, making their contrast efficiencies difficult to be tailored to the change of operating voltage, and thus resulting in a non-optimal CT contrast efficiency when used for various patients (with different age, gender and weight) [8]. In addition, the high cost of Au nano-particles may also restrict their applications in some cases.

On the contrary, since $BaYbF_5$ or $BaHoF_5$ (binary CT contrast agents developed by Liu et al. [9] and Wang et al. [10]) is consisted of multiple elements that have different K-edge values (Ba, Yb and Ho), they can not only provide much higher CT contrast efficacy compared to iodinated agents and other unitary CT contrast agents but also maintain high X-ray attenuation at different voltages. Therefore, they can be regulated to present preferable CT contrast efficiency for different patients under different CT operating voltages, thus more suitable for clinical CT imaging. Nevertheless, their relatively sophisticated and tedious synthesis procedures may hinder their further application. As a result, a facile way is desired for synthesis of binary CT contrast agents with good biocompatibility and CT contrast efficiency.

In the past few decades, as the main inorganic component of human or animals bones and teeth, nano-hydroxyapatite (nHAp) with the characteristics of good biocompatibility, large specific area and strong ion-exchange capacity has become one of the important biocompatible carriers for bio-imaging probes [11–15]. Among the fabrication methods of nHAp-based bio-imaging probes, doping contrast ions into

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nHAp host has been reported to be an efficient way [16–18]. In particularly, owing to its homogeneous heating process, short reaction time, controllable synthesis and uniform product morphology, microwave-assisted doping strategy has attracted much attention [19–21]. However, the kind and content of the doped ions, as well as the reaction conditions would inevitably influence the mono-dispersion of the as-received probe, thus probably causing a decreased imaging sensitivity [22]. Accordingly, searching for proper imaging ions, optimal doping content and reaction parameters is crucial to obtain mono-dispersed nHAp-based imaging probes.

In this work, two kinds of CT contrast ions (Ba^{2+} and Ho^{3+}) were co-doped into nHAp host via microwave-assisted way and a novel binary CT contrast agent was then obtained facilely and rapidly. The effect of the microwave heating time and contents of doped ions on its structure, morphology and dispersion was carefully examined, and its CT contrast efficiency was also evaluated. The results showed that the Ba/Ho co-doped nHAp with pure nHAp phase composition and good mono-dispersion can be obtained easily with microwave heating for only 30 min. Moreover, the Ba/Ho co-doped nHAp exhibited a greatly improved CT contrast efficiency compared with the undoped nHAp, showing a potential as a novel binary CT contrast agent.

2. Experimental

2.1. Materials

$HoCl_3{\cdot}6H_2O$, $Ca(NO_3)_2{\cdot}4H_2O$, Na_2HPO_4, NaOH, ethanol and iohexol were purchased from Aladdin (Shanghai, China), and $Ba(NO_3)_2$ from Chengdu Kelong Chemical Reagent Company (Chengdu, China). Polylactide-*block*-monomethoxy (polyethyleneglycol) (PLA-mPEG, Mw = 8000) was purchased from Jinan Daigang Biomaterial Co., Ltd. (Shandong, China). All these chemicals were analytical grade and used without further purification. Deionized water used for all the experiments was produced from a water purification system (PCUJ-10, Chengdu Pure Technology Co., Ltd., Chengdu, China).

2.2. Instruments

The microwave irradiation chamber (Sineo Master 40, Shanghai, China) was used to realize microwave heating. Transmission electron microscope (TEM) and energy dispersive X-ray (EDX) were performed with a Tecnai G2 F20 STWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI, USA). Dynamic light scattering (DLS) measurements were carried out using a Zetasizer Nano ZS (Malvern Panalytical, UK). X-ray diffraction (XRD) patterns were obtained by using an X-ray diffractometer (Empyrean, PANalytical B. V., Netherlands) with Cu Kα (λ = 1.5406 Å). Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, USA). Thermal gravimetric analysis (TGA) was carried out using a TGA/DSC 2 (Mettler-Toledo, Switzerland) to study the effect of Ba and Ho addition on thermal behavior of HAp structure, performed in N_2 atmosphere with a 10 °C/min heating rate. The CT scans were performed on a quantum FX CT imaging system (Perkin Elmer, USA).

2.3. Synthesis of undoped nHAp nanorods

In a typical experiment for the synthesis of nHAp nanorods, 23.7 mg of Na_2HPO_4 and 4.2 mg of PLA-mPEG were dissolved in 10 mL of deionized water to form Solution A, and 65.6 mg of $Ca(NO_3)_2{\cdot}4H_2O$ and 4.2 mg of PLA-mPEG were dissolved in 10 mL of deionized water to form Solution B. Then, Solution A was added into Solution B drop by drop and the pH of the mixed solution was adjusted to 10 with 1.0 mol/L NaOH. The resultant solution was transferred to a Teflon autoclave, sealed, and heated by microwave to 150 °C and maintained at this temperature for a given time. After cooling to room temperature, the product was separated by centrifugation, washed with deionized water and ethanol several times, and vacuum-dried to powder.

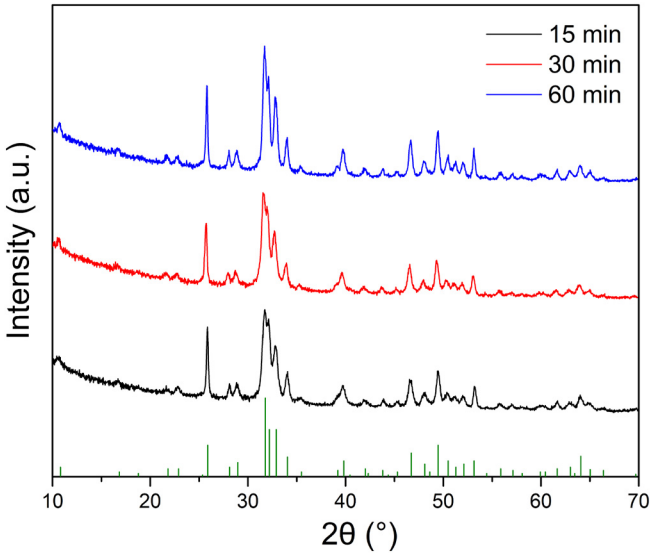

Fig. 1. XRD patterns of as-prepared undoped nHAp with different microwave heating time.

2.4. Synthesis of Ba^{2+} and Ho^{3+} co-doped nHAp nanorods

Solution B was formed by dissolving 52.5 mg of $Ca(NO_3)_2{\cdot}4H_2O$, 14.5 mg of $Ba(NO_3)_2$, 21.1 mg of $HoCl_3{\cdot}6H_2O$ and 4.2 mg of PLA-mPEG in 10 mL of deionized water. The other procedures are the same as the above.

3. Results and discussion

3.1. Effect of microwave heating time

To optimize the reaction conditions for ions doping, undoped nHAp, namely pure nHAp, was firstly synthesized by a microwave-assisted method according to the previous report [19] and the effect of the heating time were then examined. Fig. 1 showed the XRD patterns of the undoped nHAp samples obtained with microwave heating for 15, 30 and 60 min, respectively. From Fig. 1, it can be seen that all the three samples were consisted of a single phase of well-crystallized nHAp (JCPDS No.09-0432) and no other impurity phases appeared, and this indicated that well-crystalline nHAp could be obtained by microwave-assisted heating for only 15 min. Compared with the conventional heating method, the reaction time of microwave-assisted synthesis was greatly shortened (more than 10 h for the former) [23]. Although the reaction time did not affect the phase composition of nHAp, its crystallinity was affected. As shown in Fig. 1, the XRD diffraction peaks became sharper when the heating time increased from 15 min to 30 min and reached the sharpest when the time increased to 60 min, suggesting an increased crystallinity of the nHAp as the reaction time increased.

To figure out the correlation between the crystallinity and the morphology of the nHAp, the effect of the reaction time on the morphologies of the undoped nHAp samples was then investigated and their TEM images were given in Fig. 2. It is clear that the nHAp almost maintained rod-like shape, suggesting little influence of the reaction time on their morphologies and no obvious correlation existed between the crystallinity and the morphology of them. However, the nano-particles prepared with 30 min microwave heating showed the best dispersion and homogeneity in comparison with those with 15 min and 60 min reaction (Fig. 2A–C), and this was further confirmed by the DLS measurements (Fig. 2D–F). From Fig. 2D–F, it can be seen that the