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Synthesis of hollow and mesoporous structured $NaYF_4$:Yb,Er upconversion luminescent nanoparticles for targeted drug delivery

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Abstract: In this paper, we demonstrated a one-step template-free strategy to fabricate a hollow mesoporous structured $NaYF_4$:Yb,Er nanoparticles with excellent upconversion luminescence. Folic acid (FA), a commonly used cancer-targeting agent, was conjugated on the surface of the nanoparticles based on the presence of free amine groups, which were labeled as $NaYF_4$:Yb,Er-FA HMUCNPs. The properties were extensively studied, which indicated the obtained samples showed a typical hollow mesoporous structure and excellent upconversion luminescence that were useful for cell imaging and drug delivery. The L929 cells viability, hemolysis assay and coagulation test demonstrated good biocompatibility of the samples. The anti-cancer drug doxorubicin hydrochloride (DOX) storage/release properties were demonstrated to be pH-responsive, in which, the drug release might be beneficial at the reduced pH for targeted release and controlled therapy. Moreover, it was found that DOX-loaded $NaYF_4$:Yb,Er-FA HMUCNPs exhibited greater cytotoxicity to KB cells than free DOX due to the specific cell uptake by KB cells via folate receptor-mediate endocytosis. Therefore, the multifunctional nanoparticles combining upconversion luminescent property and hollow mesoporous structure have potential for simultaneous targeted anti-cancer drug delivery and cell imaging.

Keywords: template-free; hollow mesoporous structure; up-conversion luminescence; $NaYF_4$; targeted drug delivery; rare earths

The design and synthesis of multifunctional nanomedical platforms that integrate with different properties into a single nanosystem provide unparalleled opportunity for simultaneous diagnosis and therapy of diseases[1]. In particular, the construction of hollow and mesoporous structured multifunctional nanocomposites composed of rare-earth doping upconversion nanoparticles (UCNPs) has become a research hotspot in the forefront of materials science[2–5]. First of all, the high specific surface, cavity volumes and spherical morphology of hollow mesoporous nanoparticles (HMNPs) can be used as an effective nanocarrier in drug delivery and photodynamic therapy[5]. Secondly, the HMNPs can be easily modified and functionalized with specific ligand molecules to improve biocompatibility and targeting property[6,7]. As we know, folic acid (FA) has been widely used as a ligand for the selective targeting and delivery of drugs into tumor cells because the folate receptors (FRs) are frequently over-expressed in a wide variety of tumor cells but highly restricted in most normal tissues[8,9]. When FA-conjugates bind with FRs, they could transport into the cell via FR-mediated endocytosis[10]. Thirdly, the lanthanides based up-conversion luminescent materials can convert low-energy (near-infrared, NIR) to higher energy radiation (UV or visible light) via multiple absorption or energy transfer, which suggest the potential application in biomedical areas[11–14].

Many efforts have been devoted to use up-conversion luminescent materials in biological labeling, sensing, and imaging[15–17]. In comparison with conventional down-conversion fluorescent materials (such as organic dyes and QDs), up-conversion luminescent labels possess many merits including great tissue penetration, weak autofluorescence background, high signal-to-noise ratio, and absence of photodamage to live organisms[18–20]. Among all upconversion materials, ytterbium and erbium codoped sodium yttrium fluoride ($NaYF_4$:Yb^{3+},Er^{3+}) is considered to be the most efficient NIR-to-visible upconversion phosphors[21]. Therefore, designing a nanocarrier that simultaneously possesses hollow/mesoporous structure and upconversion luminescent property is undoubtedly of great importance for targeted drug delivery.

So far a lot of efforts have been devoted to the development of different methods for designing and fabrica-

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tion of HMNPs with different components and shell structures[3,22–24]. The general strategy for the synthesis of hollow spheres is a template-assisted process in which coating of templates with nanocrystals and followed by removal of the template. However, the yields of the hollow products are low, or the shells are not intact, which might lead to poor mechanical performance[25]. Recently, the sacrificial template-directed chemical transformation method based on the Kirkendall effect has been demonstrated to be an effective approach to the desired hollow spheres of inorganic materials[26,27]. During the reaction, the resultant shell forms around the surface of the sacrificial template and takes the shape of the template. Despite these endeavors, it still remains a severe challenge to fabricate size-tunable porous nano-structures with various components and functions by a simple solution approach. Therefore, it is urgently needed to explore a controllable and template-free one-pot synthesis method to fabricate HMNPs with defined shape, size, and composition.

In this paper, we demonstrated a one-step and template-free method to fabricate a hollow mesoporous structured upconversion luminescent $NaYF_4$:Yb^{3+},Er^{3+} nanoparticles (labeled as $NaYF_4$:Yb,Er HMUCNPs) with controllable size by a simple hydrothermal route. The effects of various reaction conditions on the morphology and size of the as-prepared samples were investigated in detail and Ostwald ripening effect was adopted to explain the formation mechanism of the HMUCNPs. Then, FA was conjugated to the surface of the HMUCNPs via amide reaction for targeted delivery of anti-cancer drugs so as to enhance the therapeutic efficacy. Meanwhile, the up-conversion luminescence of the samples was measured under 980 nm NIR laser excitation. Doxorubicin hydrochloride (DOX), a well known anticancer drug, was used as a model drug to evaluate the loading and controlled releasing behaviors of the HMUCNPs. Furthermore, the biocompatibility, targeted drug delivery, *in vitro* cytotoxicity and the uptake behavior of the HMUCNPs were examined in detail.

1 Materials and methods

1.1 Materials

$Y(NO_3)_3·6H_2O$ (99.99%), $Yb(NO_3)_3·5H_2O$ (99.99%), $Er(NO_3)_3·6H_2O$ (99.99%) and polyethylenimine (PEI) were purchased from Xiya Chemical Technology Co., Ltd.. Folic acid (FA), (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Co., Ltd.. Doxorubicin hydrochloride (DOX) was purchased from Shanghai Hualan Chemical Limited Company. All other materials were of analytical reagent grade and double distilled water was used throughout. All starting materials were used without further purification.

1.2 Synthesis of the $NaYF_4$:Yb,Er HMUCNPs

The $NaYF_4$:Yb,Er HMUCNPs were synthesized by a one-step hydrothermal process without any sacrificial templates. In a typical experiment, 5 mL $RE(NO_3)_3$ (0.2 mol/L, RE=78 mol.%Y+20 mol.%Yb+2 mol.%Er) solution and 5 mL NaCl aqueous solution (0.2 mol/L) were mixed with 30 mL diethylene glycol (DEG) in a round-bottom reaction vessel. Subsequently, 10 mL of aqueous solution of PEI (10 wt.%) was added into the above mixture solution with a vigorous stirring for 0.5 h. Then, 5 mL $NaBF_4$ aqueous solution was added into the above mixture solution and maintained the pH about 8. After additional agitation for 1 h, the as-obtained solution was transferred into a Teflon autoclave, which was tightly sealed and maintained at 180 ºC for 6 h. As the autoclave was cooled to room temperature naturally, the resulting precipitates were separated via centrifugation, washed for three times with deionized water and ethanol, and finally dried under vacuum at 70 ºC for 12 h. Finally, the $NaYF_4$:Yb,Er HMUCNPs was obtained.

In order to reveal the formation process of the hollow spheres, hydrothermal treatment for different reaction times (0.5, 3, 6 and 12 h) was performed as parallel experiments by keeping other reaction parameters unchanged. We also used NH_4F and NaF instead of $NaBF_4$ as the fluorine ion source to carry out contrastive experiments. In addition, to confirm the effect of DEG on shape evolution, another control experiment was carried out in the absence of DEG (replaced by water), while other parameters remained identical.

1.3 Conjugation of $NaYF_4$:Yb,Er HMUCNPs with FA

FA is conjugated to the surface of the $NaYF_4$:Yb,Er HMUCNPs due to the presence of dendrigraft cationic polymer PEI. Carboxylic group of FA were activated by an EDC/NHS solution (molar ratio of FA:EDC:NHS= 1:1:2.5) for 2 h in dark[28]. Subsequently, 50 mg of $NaYF_4$:Yb,Er HMUCNPs were added and allowed to react with stirring overnight in dark at room temperature. The as-prepared products (labeled as $NaYF_4$:Yb,Er-FA HMUCNPs) were separated by centrifugation and washed for three times with deionized water and ethanol to remove the unreacted chemicals.

1.4 Characterization

The X-ray power diffraction (XRD) was performed on a D8 Focus diffractometer (Bruker) with Cu Kα radiation (λ=0.15405 nm). The morphology of the samples was inspected with an analytical scanning electron microscope (SEM, JSM-6390A). Low- and high-resolution transmission electron microscopy (TEM) was performed using FEI Tecnai G2 F20 S-Twin with a field emission gun operating at 200 kV. Images were acquired digitally