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Doxorubicin conjugated $NaYF_4:Yb^{3+}/Tm^{3+}$ nanoparticles for therapy and sensing of drug delivery by luminescence resonance energy transfer

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ABSTRACT

In this study, we report an anticancer drug delivery system based on doxorubicin (DOX)-conjugated $NaYF_4:Yb^{3+}/Tm^{3+}$ nanoparticles. The as-synthesized nanoparticles consist of uniform spherical nanoparticles with an average diameter of 25 nm. The drug delivery system demonstrates the ability to release DOX by cleavage of the hydrazone bond in mildly acidic environments. The spectra overlap between emission of donor $NaYF_4:Yb^{3+}/Tm^{3+}$ nanoparticles at 452 nm ($^1D_2 \rightarrow {}^3F_4$) and 477 nm ($^1G_4 \rightarrow {}^3H_6$) and the broad absorbance of acceptor DOX centered at around 480 nm enables energy transfer to occur between the nanoparticles and DOX. The quenching and recovery of the up-conversion luminescence of $NaYF_4:Yb^{3+}/Tm^{3+}$ by DOX due to luminescence resonance energy transfer (LRET) mechanism are applied as optical probe to confirm the DOX conjunction and monitor the release of DOX. The DOX-conjugated $NaYF_4:Yb^{3+}/Tm^{3+}$ nanoparticles exhibit an obvious cytotoxic effect on SKOV3 ovarian cancer cells *via* MTT assay. Meanwhile, the endocytosis process of DOX-conjugated $NaYF_4:Yb^{3+}/Tm^{3+}$ nanoparticles by SKVO3 cells was demonstrated through confocal laser scanning microscopy (CLSM), flow cytometry and ICP-OES. Such drug delivery system, which combines pH-triggered drug-release and up-converting nanoparticles-based LRET property, has excellent potential applications in cancer therapy and smart imaging.

1. Introduction

Currently there is an explosion of research interest in the application of nanoparticles as carriers for anticancer drug delivery system (DDS) [1–3]. An ideal DDS should not only protect the drug from biological degradation before reaching the target organs or cells, but also provide a sustained release in a controlled manner by external stimuli in the physiological condition [4–6]. To realize this goal, inorganic nanoparticles-based DDS have received considerable attention due to their large surface area to load drugs, relatively long plasma half-time, high stability in physiological environment [7–11]. Furthermore, the nanoparticles with dimensions between 25 and 75 nm can exploit the enhanced permeability and retention (EPR) effect of tumor vasculature to release highly toxic drugs within tumors, but reducing the side effects on normal tissue [12,13]. Therefore, a series of functionalized nanoparticles conjugated with anticancer drug for drug delivery applications have been reported in recent years. Gibson et al. studied the paclitaxel conjugated gold nanoparticles using hexaethylene glycol as a linker [14]. Savla et al. reported quantum dot conjugated doxorubicin (DOX) by acid-cleavable hydrazone linkage for the chemotherapy of ovarian cancer [15]. Chen et al. synthesized DOX chemically bonded to Fe_3O_4 with a polyethylene glycol functionalized porous silica shell for drug delivery [16].

Fluorescence Resonance Energy Transfer (FRET) is a non-radiative energy transfer process in which the energy is transferred from an excited state donor to a proximal ground state acceptor [17–23]. Traditional FRET based detection systems use quantum dots and organic dyes as the donor, which absorb high energy photon through down-conversion process. However, these down-conversion donors suffer from some drawbacks, including low light penetration depth, strong background fluorescence, possible severe photodamage of living organisms and high bleaching rate [24]. In order to tackle this problem, the lanthanide doped up-conversion nanoparticles (UCNPs) have been received tremendous attention and invested as the energy donor. The up-conversion fluorescent nanoparticles can convert longer wavelength radiation (near infrared, NIR) to shorter wavelength fluorescence (UV or

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visible light) *via* a two-photon or multiphoton mechanism, which have been used for biological labeling, imaging and sensing [25–34]. Compared with conventional down-conversion fluorescent labels which require higher-energy UV or visible excitation wavelength, up-conversion fluorescent materials have many conceivable advantages including sharp absorption and emission lines, greater tissues penetration, weak auto-fluorescence, high signal-to-noise ratio [35–42]. When lanthanide doped up-conversion nanoparticles are invested as the energy donor, the nonradiative energy transfer from a lanthanide donor to an appropriate acceptor has been called luminescence resonance energy transfer (LRET) because emission from lanthanides is not fluorescence [43,44]. So far Yb^{3+}/Er^{3+} (or Yb^{3+}/Tm^{3+}) co-doped hexagonal-phase (β-phase) $NaYF_4$ has been considered to be the most efficient NIR-to-visible UC materials [45].

Herein, we design and develop a multifunctional nanoparticles DDS. β-$NaYF_4$:Yb^{3+}/Tm^{3+} nanoparticles were utilized as drug carriers and optical nanoprobes. DOX, a commonly used anticancer drug, chemically conjugated to $NaYF_4$:Yb^{3+}/Tm^{3+} nanoparticles. The drug release property, cytotoxicity, and cellular uptake behavior were examined in detail. Furthermore, the LRET property between the donor $NaYF_4$:Yb^{3+}/Tm^{3+} and acceptor DOX was also investigated.

2. Experimental section

2.1. Materials

Y_2O_3 (99.99%), Yb_2O_3 (99.99%) and Tm_2O_3 (99.99%) were purchased from Science and Technology Parent Company of the Changchun Institute of Applied Chemistry. Oleic acid (OA), 1-Octadecene (ODE), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aldrich. $KMnO_4$, $NaIO_4$ hydrazine monohydrate ($N_2H_4{\bullet}H_2O$) and methanol were purchased from Beijing Chemical Regent Co., Ltd., DOX was obtained from the Nanjing Duodian Chemical Limited Company.

2.2. Synthesis of rare earth oleate complexes

A literature method for the synthesis of iron–oleate complex was adopted to prepare the rare earth oleate complexes [46]. 10 mmol of rare earth chloride $RECl_3$ (RE = 79.5%Y + 20%Yb + 0.5%Tm) and 30 mmol of sodium oleate were dissolved in a mixture solvent composed of 20 mL of ethanol, 15 mL of distilled water, and 35 mL of hexane. The resulting solution reflux at 70 °C for 4 h. The upper organic layer was separated and washed with distilled water. After being washed, rare earth oleate complexes were produced by evaporating off the remaining hexane.

2.3. Synthesis of oleic acid stabilized β-$NaYF_4$:Yb^{3+}/Tm^{3+}

Oleic acid capped β-$NaYF_4$:Yb^{3+}/Tm^{3+} were synthesized by thermal decomposition methodology according to Chen et al. [47]. In a typical procedure for the preparation of β-$NaYF_4$:Yb^{3+}/Tm^{3+}, 1 mmol of $RE(oleate)_3$ (RE = 79.5%Y + 20% Yb + 0.5%Tm), 12 mmol of NaF, 10 mL of OA and 10 mL of ODE were added to the three-neck round-bottom reaction vessel and subsequently heated to 100 °C under a vacuum with magnetic stirring for 30 min and flushed with N_2. The reaction was kept at 320 °C for 1.5 h in N_2 atmosphere under vigorous stirring. When the reaction was completed, the transparent yellowish reaction mixture was cool to 80 °C. The finally obtained $NaYF_4$:Yb^{3+}, Tm^{3+} was dispersed in 10 mL cyclohexane for further use.

2.4. Synthesis of water-soluble and carboxylic acid-functionalized β-$NaYF_4$:Yb^{3+}/Tm^{3+}

Water-soluble and carboxylic acid-functionalized β-$NaYF_4$:Yb^{3+}/Tm^{3+} ($NaYF_4$:Yb^{3+}/Tm^{3+}–COOH) nanoparticles were synthesized by Lemieux-von Rudloff oxidation method developed by Li et al. [48]. A mixture of as-prepared β-$NaYF_4$:Yb^{3+}/Tm^{3+} (0.1 g), cyclohexane (100 mL), tert-butanol (70 mL), water (10 mL) and 5 wt% K_2CO_3 aqueous solution (5 mL) were stirred at room temperature for about 20 min. Then 20 mL of Lemieux-von Rudloff reagent (5.7 mM $KMnO_4$ and 0.105 M $NaIO_4$ aqueous solution) was added dropwise into the above solution. The as-obtained mixing solution was stirred at 40 °C for 48 h. The products were separated by centrifugation and washed with deionized water, acetone, and ethanol in turn. Subsequently, the product was dispersed in hydrochloric acid (50 mL) of pH 4–5, and stirred for 30 min. At last, the oxidized product was obtained by centrifugation, washed twice with deionized water and dried under vacuum at 70 °C for 12 h.

2.5. Synthesis of $NaYF_4$:Yb^{3+}/Tm^{3+}–DOX conjugates

The above synthesized $NaYF_4$:Yb^{3+}, Tm^{3+}–COOH was dispersed in 20 mL H_2O. 12 mg EDC, 4 mg NHS and 1 mL hydrazine monohydrate were added into the above solution and stirred overnight. The precipitates were separated by centrifugation and washed several times with deionized water and then dried under vacuum at 70 °C for 12 h. As a consequence of the above procedure, the $NaYF_4$:Yb^{3+}/Tm^{3+} nanoparticles functionalized with $-NHNH_2$ group on the surface ($NaYF_4$:Yb^{3+}, Tm^{3+}–$NHNH_2$) were obtained. Then, 20 mg of $NaYF_4$:Yb^{3+}, Tm^{3+}–$NHNH_2$ was dispersed in 5 mL of methanol, 5 mg of DOX was add to the above solution and stirred at 20 °C for 48 h. The precipitates were separated by centrifugation and washed with methanol several times until the supernatant became colorless. DOX-conjugated $NaYF_4$:Yb^{3+}/Tm^{3+}($NaYF_4$:Yb^{3+}/Tm^{3+}–DOX) nanoparticles were dried under vacuum at 20 °C. All of the supernatants were collected and diluted to 100 mL with methanol in a volumetric flask for evaluation of the drug loading efficiency by UV–vis spectroscopy. The structure of $NaYF_4$–CONHN=DOX is given in Scheme 1.

2.6. In vitro DOX release

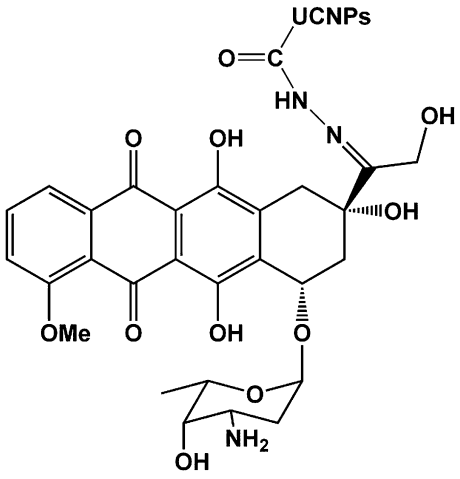
$NaYF_4$:Yb^{3+}/Tm^{3+}–DOX was dissolved in 1 mL of PBS (pH 7.4 and 5.0) at 37 °C, at selected time intervals, buffer solution was taken and replaced with fresh buffer solution. The amounts of released DOX in the supernatant solutions were measured by UV–vis spectrophotometer.

2.7. In vitro cytotoxicity of DOX-conjugated $NaYF_4$:Yb^{3+}/Tm^{3+} nanoparticles

In vitro cytotoxicity of $NaYF_4$:Yb^{3+}/Tm^{3+} nanoparticles was assayed against human SKOV3 ovarian cancer cells. Human SKOV3 ovarian cancer cells were seeded in a 96-well plate at a density of 8000 cells per well and cultured in 5% CO_2 at 37 °C for 24 h. Then free DOX and DOX-$NaYF_4$:Yb^{3+}, Tm^{3+} were added to the medium, and the cells were incubated in 5% CO_2 at 37 °C for 24 h. The concentrations of DOX were 1.5625, 3.125, 6.25, 12.5, 25 μg/mL, respectively. At the end of the incubation, 20 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution (diluted in a culture media with a final concentration of 0.8 mg/mL) was added into each cell and incubated for another 4 h. The supernatant in each well was removed. 150 μL of dimethyl sulfoxide (DMSO) was added to each well before the plate was examined using a microplate reader (Therom Multiskan MK3) at the wavelength of 490 nm. Meanwhile, cell viability was also determined using MTT assay, which was the same as the procedure for cytotoxicity assay for DOX–$NaYF_4$:Yb^{3+}, Tm^{3+} nanoparticles.

2.8. Cellular uptake of the DOX-conjugated $NaYF_4$:Yb^{3+}/Tm^{3+} nanoparticles

Cellular uptake was examined using confocal laser scanning microscope (CLSM), flow cytometry and ICP-OES. For CLSM, the SKOV3 ovarian cancer cells were seeded in 6-well culture plates (a clean cover slip was put in each well) and grown overnight as a monolayer, and were incubated with DOX-conjugated $NaYF_4$:Yb^{3+}/Tm^{3+} (20 μg equivalent DOX) at 37 °C for 30 min, 1 h and 6 h, respectively. Thereafter, the cells were rinsed with PBS three times, fixed with 2.5% formaldehyde (1 mL/well) at 37 °C for 10 min, and then rinsed with PBS three times again. In order to nucleus labeling, the nuclei were stained with Hoechst 33324 solution (from Molecular Probes, 20 μg/mL in PBS, 1 mL/well) for 10 min and then rinsed with PBS three times. The cover slips were placed on a glass microscope slide, and the samples were visualized using CLSM (FV10-ASW). For flow cytometry studies, SKOV3 ovarian cancer cells (1×10^6)



Scheme 1. The structure of $NaYF_4$–CONHN=DOX.