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Multifunctional NaYF$_4$:Yb, Er@mSiO$_2$@Fe$_3$O$_4$-PEG Nanoparticles for UCL/MR Bioimaging and Magnetically Targeted Drug Delivery

Bei Liu $^{a,b}$, Chunxia Li, $^{a,*}$ Ping’an Ma $^{a}$, Yinyin Chen $^{a,b}$, Yuanxin Zhang $^{a}$, Zhiyao Hou $^{a}$, Shanshan Huang $^{a}$ and Jun Lin $^{a,*}$

A low toxic multifunctional nanoplatfrom integrating both multimodal diagnosis methods and antitumor therapy is highly desirable for assuring the antitumor efficiency. In this work, we show a convenient and adjustable synthesis of multifunctional nanoparticles NaYF$_4$:Yb, Er@mSiO$_2$@Fe$_3$O$_4$-PEG (MFNPs) based on different sizes of upconversion nanoparticles (UCNPs). With strong up-conversion fluorescence offered by UCNPs, superparamagnetism properties attributed to Fe$_3$O$_4$ nanoparticles and porous structure come from the mesoporous SiO$_2$ shell, the as-obtained MFNPs can not only be utilized as a contrast agent for dual modal upconversion luminescence (UCL)/magnetic resonance (MR) bio-imaging, but also achieve an effective magnetically targeted antitumor chemo-therapy both in vitro and in vivo. Furthermore, the UCL intensity UCNPs and the magnetic properties of Fe$_3$O$_4$ in the MFNPs were carefully balanced. Silica coating and further PEG modifying can improve the hydrophilicity and biocompatibility of the as-synthesized MFNPs, which were testified by the in vitro long-time bio-distributions. Those results revealed that the UCNPs based magnetically targeted drug carrier system we synthesized here has great promise in the future multimodal bio-imaging and targeted therapy of cancer.

1. Introduction

In the past few years, Ln$^{3+}$-doped upconversion nanoparticles (UCNPs) as a rising new star have provoked more and more attention in biomedical fields, which originate from their captivating merits such as high chemical stability, low autofluorescence, high signal-to-noise ratio, detection sensitivity, penetration depth and good biocompatibility.$^{1,9}$ In particular, spurred by the great progress in the controlled synthesis of uniform UCNPs and followed surface modification, UCNPs-based nanocomposites that combine with other functional nanoparticles such as super-paramagnetic Fe$_3$O$_4$, ultrasmall Au$^{14,15}$ and CuS$^{16}$ in one hybrid system can act as multifunctional “theranostic” platforms and provide new possibility for the synergistic diagnosis and therapy of disease. Among various nanomaterials, the nanocomposites combining with upconversion luminescence and magnetic properties as well as porous structures are a class of novel carriers for drug delivery and multimodal bioimagings.$^{17}$ On the one hand, such nanomaterials can offer dual modal bioimagings including upconversion luminescence (UCL) imaging and magnetic resonance imaging (MRI), which may simultaneously achieve high spatial resolution and penetration depth for clinical diagnosis.$^{18-24}$ On the other hand, the composite nanoparticles containing super-paramagnetic Fe$_3$O$_4$ are a class of promising magnetic targeted “smart” drug delivery system, which is one of effective route to ameliorate the major hurdles in current chemotherapy such as the low accumulation concentration of therapeutic drugs at targeted location, the toxic side effects of drugs to the healthy cells and tissues as well as unspecific uptake of normal cells.$^{25-28}$ As such, it is of great importance to integrate UCNPs with Fe$_3$O$_4$ and porous structure to enhance the tumor-targeted drug delivery with elevated local dosages and multimodal bioimagings.

Porous SiO$_2$ is considered as one of the most promising porous structures for drug delivery applications, as it generally possesses good bio-compatibility and ease of surface functionalization that provides reservoirs for loading various antitumor drug molecules and active sites for linking other biological or targeted molecules by covalent association.$^{29-31}$ Therefore, taking use of mesoporous silica as intermediate layer to combine UCNPs with Fe$_3$O$_4$ can help us establish UCNPs-based magnetically targeted drug delivery systems. Furthermore, the intermediate mesoporous silica shell could not only solve the problems of lattice mismatch and chemical dissimilarity between those two kinds of nanoparticles, but also somehow protect UCNPs from the luminescence quenching by Fe$_3$O$_4$.

To our knowledge, previous studies that combined Fe$_3$O$_4$, mesoporous silica with UCNPs by two-step sol-gel process$^{11,32}$...
involved multistep and complicated procedures. In addition, the UCNPs were deposited on the surface of Fe₂O₃ or mesoporous silica by calcination, which would lead to the irreversible particle aggregations and uneven size distribution. In order to address these problems, here we design a more facile methodology to synthesize hybrid nanostructure NaYF₄:Yb, Er@mSiO₂@Fe₂O₃-PEG (MFNPs) that can simultaneously combine UCL, MRI and magnetically targeted drug delivery in one system. UCNPs with different sizes were directly encapsulated with the mesoporous silica through a phase-transfer-assisted surfactant-templating coating process. Then ultrasmall Fe₂O₃ nanocrystals were decorated onto the surface of mesoporous silica shell by nucleophilic substitution reaction. In order to achieve the enough bright upconversion luminescence intensity while maintain the magnetic performance of Fe₂O₃, optimal reaction conditions were conducted carefully by changing the size of UCNPs and the amounts of Fe₂O₃ immobilized on the surface of UCNPs@mSiO₂. The capacity of loading anticancer drug doxorubicin (DOX), in vitro and in vivo biocompatibility, in vivo magnetically targeted tumor therapy efficacy and biodistribution as well as in vitro UCL and MR imaging of the as-synthesized MFNPs were also investigated in detail.

2. Experimental

2.1 Materials

All the chemical reagents were directly used without further purification. The rare earth oxides Y₂O₃, Yb₂O₃ and Er₂O₃ (99.999 %) were purchased from Science and Technology Parent Company of Changchun Institute of Applied Chemistry. Sodium fluoride (NaF), Oleic acid (OA), Cetyltrimethylammonium bromide (CTAB), Tetraethylorthosilicate (TEOS), Ammonium nitrate (NH₄NO₃), Sodium hydroxide (NaOH), Ammonium fluoride (NH₄F), Ammonium hydroxide (NH₄OH), Citrate acid (C₆H₄O₇), n-hexane and Cyclohexane were all purchased from Beijing Chemical Reagent Company. Octadecene (ODE), 3-Aminopropyltriethoxysilane (APTES), 2-Bromo-2-methyl-propiionic acid (BMPA) and Ferric acetylacetonate (Fe(acac)₃) were prepared from Aldrich. PEG succinimidyl carbonate (PEG-SC) was purchased from Beijing Kaizheng Bio-technology Company (China). Doxorubicin hydrochloride (DOX) was purchased from Nanjing Duodian Chemical Limited Company (China). The YCl₃, YbCl₃, and ErCl₃ solutions were prepared by dissolving the corresponding rare-earth oxides in hydrochloric acid aqueous solution followed by removal of the hydrochloric acid through evaporation.

2.2 Synthesis of NaYF₄:Yb, Er (UCNPs) and Fe₂O₃ Nanocrystals

Three different sizes of NaYF₄:20% Yb, 2% Er nanoparticles were fabricated according to the method reported previously with a little modification of the reaction solvents, temperature and time (20 nm core: 6 mL OA/15 mL ODE at 300 °C for 1 h; 33 nm core: 6 mL OA/15 mL ODE at 320 °C for 2 h; 55 nm core: 3 mL OA/17 mL ODE at 320 °C for 2 h). Oleic acid (OA) modified Fe₂O₃ nanocrystals were successfully synthesized following a previously reported synthesis method with a little modification. The synthesis process was described as follows: Firstly, 0.353 g of Fe(acac)₃, 1.5 mL of OA, 1.5 mL of OM and 10 mL of benzyl alcohol were mixed totally before transferred into a Teflon bottle (50 mL), sealed and maintained at 180 °C for 10 h. As the autoclave cooled naturally, excess ethanol was added and isolated by centrifugation. Then the surfactants OA molecules modified on the surface of Fe₂O₃ nanocrystals were replaced by BMPA in order to attach Fe₂O₃ nanocrystals on the surface of mesoporous silica. The reaction for ligand exchange is as follows: a mixture of Fe₂O₃ nanocrystals (about 15 mg), BMPA (0.5 g) and citrate acid (0.05 g) was dispersed in 16 mL of chloroform/DMF (v/v=1:1) solution and kept stirring overnight at 30 °C. The resulted BMPA-capped Fe₂O₃ nanocrystals were obtained by centrifugation and redispersed in 10 mL ethanol for further use.

2.3 Synthesis of UCNPs@mSiO₂@Fe₂O₃-PEG Nano-composites

In a typical synthesis for UCNPs@mSiO₂@Fe₂O₃-PEG (MFNPs), first of all, the UCNPs@mSiO₂ nanoparticles were obtained as follows: the oleic acid capped UCNPs (22nm, about 20 mg) and CTAB (0.1 g) were mixed in 30 mL distilled water to form a transparent solution through stirring before the addition of 150 µL of NaOH solution (2 M) and 200 µL of TEOS. UCNPs@mSiO₂ was formed after the as-obtained mixture was maintained at 70 °C for 1 h under vigorous magnetic stirring. The pore-generating template CTAB was further removed by refluxing the above UCNPs@mSiO₂ in the NH₄NO₃ ethanol solution at 60 °C for 2 h. Then 200 µL of APTEs was added into 20 mL of UCNPs@mSiO₂ ethanol solution under stirring overnight in order to functionalize UCNPs@mSiO₂ nanospheres with amine groups. Thirdly, in order to obtain UCNPs@mSiO₂@Fe₂O₃ nanocomposites, the BMPA-capped Fe₂O₃ nanocrystals were mixed with 15 mL of the amine-functionalized UCNPs@mSiO₂ ethanol solution under magnetic stirring overnight. After centrifugation, the UCNPs@mSiO₂@Fe₂O₃ nanoparticles were obtained. Finally, PEG-stabilized UCNPs@mSiO₂@Fe₂O₃ (MFNPs) were prepared by mixing the as-obtained UCNPs@mSiO₂@Fe₂O₃ with PEG-SC (20 mg) overnight to induce covalent bonding between residual amine groups of UCNPs@mSiO₂ and PEG-SC. After removal of unreacted PEG-SC by centrifugation, the resulting MFNPs were dispersed in water.

2.4 In Vitro Magnetic Resonance Imaging

In vitro MR imaging experiment of MFNPs was performed in a 0.5 T MRI magnet (Shanghai Niumai Corporation Ration NM120-Analyst). MFNPs with different Fe concentrations (determined by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES)) were dispersed in water for the tests. The obtained relaxation time T₂ values were recorded and plotted as 1/T₂ versus molar concentration of Fe (mM).

2.5 In Vitro Cytotoxicity of the MFNPs Nanocomposites

A typical cell assay was carried out to investigate the in vitro cytotoxicity of MFNPs. Briefly, HeLa cells were planted out in a 96-well plate (6000 cells per well) and incubated in 5% CO₂ at 37 °C overnight. After the cells attached to the wells, MFNPs solutions with concentrations of 1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500 µg/mL respectively, were added into wells and incubated for another 24 h. Then MTT solution (20 µL, 5
mg/mL) was added to each well and further incubated in 5% CO2 at 37°C for 4 h. After that, 150 µL of DMSO were added to each well after carefully removing the original culture medium. The absorbance of the suspension was recorded by a microplate reader at the wavelength of 490 nm.

2.6 In Vitro Drug Storage and Release

In vitro drug storage of MFNPs was carried out by mixing the MFNPs (about 10 mg) with 2 mL DOX solution (1 mg/mL) under magnetic stirring overnight at 37°C. The obtained DOX loaded MFNPs were collected by centrifugation and denoted as MFNPs-DOX. The amount of the DOX that loaded into the MFNPs was determined by UV-Vis spectral measurement. Then the as-obtained MFNPs-DOX were dispersed in 2 mL of pH = 7.0 and 5.0 phosphoric acidic buffer solutions (PBS) at 37°C under magnetic stirring, respectively. At predetermined time intervals, 2 mL of fresh PBS was replaced for the original PBS in order to determine the amount of the released DOX by UV-Vis spectral measurement.

2.7 In Vitro Cytotoxicity of the DOX Loaded MFNPs

HeLa cells were seeded in a 96-well plate (6000 cells per well) and incubated (37°C, 5% CO2) overnight. Free DOX, MFNPs-DOX and pure MFNPs with different concentrations were added to the wells of 96-well plate respectively and the cells were further incubated for another 24 h. The concentrations of DOX were 0.78125, 1.5625, 3.125, 6.25, 12.5, and 25 µg/mL, respectively, while the corresponding concentrations of pure MFNPs were 15.63, 31.25, 62.5, 125, 250, and 500 µg/mL, respectively. The following steps were same with the previously mentioned MTT cell assay and the final fraction surviving of HeLa cells was measured by a microplate reader at the wavelength of 490 nm.

2.8 Cellular Uptake and Upconversion Luminescence Imaging of MFNPs

Cellular uptake degree of MFNPs was determined by flow cytometry method as follows: HeLa cells were seeded in 6-well culture plates (3×10^4 cells per well) and incubated (37°C, 5% CO2) overnight. Then the cells were treated with MFNPs-DOX for 0 min, 30 min, 2 h and 4 h respectively. After reaching the predetermined time, the cells were prepared consecutively by trypsinization, washing with PBS three times, and filtration through 35 mm nylon mesh. Finally FACSCalibur flow cytometer (BD Biosciences) was used to test the cellular uptake degree of MFNPs. The upconversion luminescence imaging of MFNPs was also carried out as follows: HeLa cells were seeded in 6-well culture plates (3×10^5 cells per well) and incubated (37°C, 5% CO2) overnight. MFNPs solution (2 mL, 200 µg/mL) was added into each well and incubated for 0.5 h and 4 h, respectively. After that, the cells were carefully washed with PBS three times, fixed with 2.5% formaldehyde for 10 min and washed again with PBS three times. The final UCL was watched by the up-conversion luminescence microscopy (UCLM), which was rebuilt on an inverted fluorescence microscope (Nikon Ti-S) and an external CW 980 nm diode laser.

2.9 In Vivo Biocompatibility of MFNPs

Female Kunming mice (25-35 g) were purchased from Center of Experimental Animals, Jilin University (Changchun, China), and the animal experiments agreed well with the criterions of The National Regulation of China for Care and Use of Laboratory Animals. Ten healthy mice were randomly divided into two groups, one group was treated with 0.2 mL of MFNPs (40 mg/kg) while the other was treated with 0.2 mL of saline as control. The weights of the mice were measured every two days. In order to further investigate the biocompatibility of MFNPs, after 12 days treatment, the mean organs of mice, such as liver, spleen, heart, lung, and kidney, were removed and fixed in 4% paraformaldehyde solution for histological examination. The serum of mice collected from both the treated group and control groups were also sent for the chemistry tests analysis. Briefly, about 0.8 mL of blood from each mouse was collected by quickly removing the eyeball from the socket. The serum was retrieved by centrifugation.

2.10 In Vivo Magnetic Targeting Effect of MFNPs

Eight healthy female Kunming mice (25-35 g) were chosen for the following test. The tumors were established by subcutaneous injection of H22 cells (murine hepatocarcinoma cell lines) in the left axilla of mice. After the tumors grew 5 days to reach the size of around 100-200 mm³, the tumor-bearing mice were injected with MFNPs (12 mg Y3+/kg) by tail vein injection. Then the treated mice were randomly divided into two groups (n = 4, each group): one group was managed to attach a -magnet at the left axilla of each mouse (right on the tumor), while the other group kept magnet away. After two hours, all the mice were sacrificed and the tumors were collected and solubilized by HNO3 and H2O2 (v:v=1:2) at 70°C for ICP-AES measurement of Y3+.

2.11 In Vivo Targeted Antitumor Efficacy of DOX Loaded MFNPs

The 24 tumor-bearing mice were randomly divided into four groups (n = 6, each group), treated with free DOX, MFNPs-DOX without magnetic field (labeled as MFNPs-DOX), MFNPs-DOX with an external magnet right outside the tumor (labeled as MFNPs-DOX+MR) and saline as control by tail vein injection respectively. We injected the above materials by two times on day 1 and 5 and the injected DOX dose was 5.0 mg/kg body weight at each time points. The body weights and tumor volumes were monitored every two days. After 12 days, the tumors were dissected and weighed to evaluate the therapeutic efficacy. The tumor volume was calculated by: Tumor volume (V) = length×width^2/2. Relative tumor volume was calculated as V/V₀ (V₀ was the corresponding tumor volume before the treatment). Tumor growth inhibition rate was determined as (C-T)/C×100 (C was the average tumor weight of the control group and T is the average tumor weight of each treated group).

2.12 Biodistribution of Nanoparticles in Major Organs of Mice

The 16 healthy female Kunming mice weighted about 20-25 g were treated with MFNPs (12 mg Y3+/kg) by tail vein injection...
and then randomly divided into four groups (n = 4, each group), euthanized at four different time points (1 h, 12 h, 2 day, and 7 day) individually. Major organs such as heart, liver, spleen, lung and kidney were carefully collected, weighed and finally treated with HNO$_3$ and H$_2$O$_2$ (v:v=1:2) at 70 °C. The final solutions were sent to test the concentrations of Y$^{3+}$ ion by ICP-AES.

3. Results and discussion

3.1 Controlled Fabrication and Characterization of MFNPs

In the present work, we detailed a simple synthetic route to produce different sizes of UCNPs-based magnetically targeted drug delivery carriers NaYF$_4$:Yb, Er@mSiO$_2$@Fe$_3$O$_4$-PEG (MFNPs). The overall fabrication procedure of MFNPs was illustrated in Scheme 1. Firstly, monodisperse NaYF$_4$:Yb, Er UCNPs with different sizes (20 nm, 33 nm and 55 nm) were successfully synthesized via a solvent thermal process by varying the reaction temperature, reaction time and the molar ratio of solvents OA/ODE (Figure 1a, d, g). The as-obtained UCNPs with different sizes have the similar properties, for example, they were all modified by hydrophobic OA molecules that can be well dispersed in a variety of nonpolar solvents, such as cyclohexane and trichloromethane. Under 980 nm laser excitation, as shown in Figure 1j and insets, the raising size of UCNPs from 20 nm to 55 nm would help to increase the green emission intensity centered at 521, 540, and 654 nm that ascribable to $^4$H$_{11/2}$$rightarrow$$^4$I$_{15/2}$, $^4$S$_{5/2}$$rightarrow$$^4$I$_{15/2}$, and $^4$F$_{9/2}$$rightarrow$$^4$I$_{15/2}$ transitions of Er$^{3+}$, respectively, mainly because of the suppressed surface quenching,$^{40-41}$ which could be of great benefits for the further bioimaging applications. Subsequently, the as-obtained UCNPs with three different sizes were directly encapsulated with the mesoporous silica through a phase-transfer-assisted surfactant-templating coating process by applying CTAB as secondary surfactant (Figure 1b, e, h). In this process, directly coating of silica shell onto different sizes of UCNPs was achieved by adjusting the initial molar ratio of UCNPs/TEOS. Then APTES was used to provide amine functional groups on UCNPs@mSiO$_2$ nanospheres which could serve as anchor points for further reactions. After that, the ultrasmall and multiple Fe$_3$O$_4$ nanocrystals were decorated onto the surface of mesoporous silica shell by nucleophilic substitution reaction. Figure 1c, f, i demonstrate that the UCNPs@mSiO$_2$@Fe$_3$O$_4$ based on different sizes of UCNPs retain their original morphology except for the uniform shaggy surface decoration with tiny Fe$_3$O$_4$ nanocrystals. Note that Fe$_3$O$_4$ nanocrystals decorated in the multifunctional nanoparticles could not only provide as T$_2$ positive agent of superparamagnetic species, but also serve for the magnetically targeted drug delivery. Finally, to make the as-synthesized nanoparticles biocompatible, PEG-SC was chosen as a hydrophilic agent to modify the nanocomposites.$^{42}$ Since all of the MFNPs based on different sizes of UCNPs were successfully fabricated and have almost the same morphology...
and phase composition, we choose to take the MFNPs based on biggest size of UCNPs as an example to do the following researches because of their improving UCL intensity. The XRD patterns of UCNPs, pure Fe$_3$O$_4$ and MFNPs were shown in Figure S1 (Supporting Information). As we can see from Figure S1a and b, the diffraction peaks of the Fe$_3$O$_4$ and UCNPs can be indexed respectively as pure cubic phase structure Fe$_3$O$_4$ (JCPDS 65-3107) and hexagonal phase structure $\beta$-NaYF$_4$ (JCPDS 16-0334). However, the obtained MFNPs (Figure S1c) were mainly ascribed to $\beta$-NaYF$_4$ structure besides an amorphous silica structure peak at $2\theta=22^\circ$, showing a very weak diffraction peaks of the Fe$_3$O$_4$ nanoparticles. This is perhaps because of the low concentration and subsize of Fe$_3$O$_4$ decorated in the MFNPs comparing with the UCNPs@mSiO$_2$ nanocomposites. The corresponding HRTEM image (inset of Figure 1i) can cover this insufficient section of the XRD pattern, which clearly shows the lattice fringes with interplanar spacing of 0.52 nm indexed to the (100) plane of $\beta$-NaYF$_4$ and 0.25 nm indexed to the (311) plane of Fe$_3$O$_4$. The elemental mapping images also show the uniformly distributed Na, Y, Si, O and Fe elements in single nanoparticle (Figure 1k), further confirming the successful linking of Fe$_3$O$_4$ on the UCNPs@mSiO$_2$ nanoparticles.

![Figure 2](image-url)  
**Figure 2** TEM images of UCNPs@mSiO$_2$@x wt% Fe$_3$O$_4$ with different Fe$_3$O$_4$ attached on the silica shell (a: x=1.2; b: x=2.4; c: x=4.8) and the corresponding upconversion emission spectra (d) under 980 nm laser excitation (blue line: x=1.2; red line: x=2.4; black line: x=4.8; the nanoparticles were dispersed in ethanol with a same UCNPs concentration of 20 mM). (e) Photos of MFNPs sample in an aqueous solution under ambient light (left), with a neighboring magnet (middle) and exposed to a 980 nm laser (right).

In addition, because of the nonspecific absorbency of Fe$_3$O$_4$ nanocrystals$^{43}$, the UCL intensity of UCNPs could be significantly quenched after decorating the Fe$_3$O$_4$ nanoparticles on UCNPs@mSiO$_2$. In order to meet the various demands of magnetic and optical properties simultaneously, we take a serious of experiments to adjust the amount of Fe$_3$O$_4$ nanoparticles in MFNPs. By decreasing the dosage of Fe$_3$O$_4$ reactants in the system, the amount of Fe$_3$O$_4$ nanoparticles attached on the silica shell decreased obviously (Figure 2a-c) while the luminescence intensity of MFNPs with the same UCNPs concentration (20 mM) increased clearly (Figure 2d). Therefore, suitable amounts of Fe$_3$O$_4$ nanoparticles could be attached on the UCNPs@mSiO$_2$ nanocomposites to get enough bright luminescence intensity, while remain the magnetic performance of Fe$_3$O$_4$ nanocrystals in MFNPs. According to this baseline, the UCNPs@mSiO$_2$@2.4%Fe$_3$O$_4$ nanoparticles in Figure 3b were chosen for the following experiments, which have both a proper luminescence intensity to satisfy the bioimaging applications and strong magnetic performance for magnetic targeted drug delivery (Figure 2e). In this way, the as-synthesized MFNPs could perfectly satisfy the anticipations for further bioapplications.

The N$_2$ adsorption/desorption isotherm in Figure S2 of the Supporting Information shows a type-IV isotherm and the corresponding pore size distributions of MFNPs nanospheres was very uniform with an average size of 2.4 nm. Moreover, it could be determined that the Brunauer–Emmet–Teller (BET) surface area of the as-obtained MFNPs was about 458 m$^2$/g and the total pore volume was 0.45 cm$^3$/g. All those results indicate that the mesoporous silica shell of MFNPs was capable of loading sufficient amount of drugs for drug delivery applications.

### 3.2 In Vitro and in Vivo Toxicology Studies of MFNPs

![Figure 3](image-url)  
**Figure 3** Systematic analysis of toxicity for MFNPs (40 mg kg$^{-1}$) on healthy Kunming mice. (a) Hematoxylin and eosin (H&E) stained images of major organs for control group and MFNPs group. (b) Changes in body weights of mice after two doses intravenous injection in MFNPs group and control group. (c) Blood analysis data for mice of control group and MFNPs group respectively.

In *vitro/in vivo* toxicology of MFNPs is a very important prerequisite for a potential anti-tumor drug carrier. Firstly, *in vitro* cytotoxicity of the MFNPs was tested by a standard MTT assay on HeLa cells. As shown in Figure S3a of the Supporting Information, HeLa cells were not impaired with a concentration of as high as 500 µg/mL after the 24 h culture. Moreover, after incubation of 24 h with MFNPs, the fluorescence microscopy images of HeLa cells were in good condition and began to
proliferate (Figure S3b, c), which means that the biocompatibility of the MFNPs could satisfy the requirements as potential drug carriers for biological applications.

In vivo toxicology studies on Kunming mice were also carried out to further make sure that the MFNPs were suitable for further bio-applications. We can conclude that all the mice treated by tail vein injection with the MFNPs (40 mg/kg) were survived in good condition from the following experimentation results: (i) The gross anatomy and pathomorphology examinations (Figure 3a) show that the main organs, including heart, liver, spleen, lung and kidney were healthy with no obvious sign of organ damage from both the control group and MFNPs group; (ii) The body weights of mice in MFNPs group increased normally without any differences with the control group (Figure 3b); (iii) The biochemical and hematological analyses of blood on the treated mice show that all the tested biochemical parameters, including aspartate aminotransferase (AST)/alanine aminotransferase (ALT), creatine kinase (CK), blood urea nitrogen (BUN) and creatinine, were in the normal range without any dysfunctions (Figure 3c). From all the above results, we could conclude that the MFNPs have a negligible side effect for further in vivo biomedical applications and could act as a biocompatible drug delivery to restrain tumors.

3.3 DOX encapsulated in MFNPs for chemotherapy

To be a drug delivery carrier, the drug loading and controlled release ability of MFNPs should be carefully studied. Firstly, the antitumor chemotherapeutic drug-doxorubicin (DOX) was chosen to be loaded into the mesoporous SiO$_2$ shell of MFNPs as the model drug. The drug release percentage from DOX-loaded MFNPs complexes were evaluated at 37 °C in PBS solution (pH = 7.0 & 5.0). As shown in Figure 4, the in vitro DOX release profiles were obviously pH-dependent: only 35% of DOX was released after 74 h at pH 7.0, while 68% of DOX released when pH decreased to 5.0. This tendency was ascribed to the increased hydrophilicity of DOX resulting from the promoted protonation of amino group when the pH was lower. Since the extracellular pH of solid tumors presents a much acidic microenvironment than the normal tissues, this DOX release tendency was beneficial as a drug carrier for targeted tumor drug release. In this way, the as-synthesized MFNPs could be used as a potential drug carrier for pH dependent anti-tumor drug release.

![Figure 4 Cumulative DOX release from MFNPs nanocomposite spheres at pH=7.0 (black line) and pH=5.0 (red line) PBS buffer.](image)

Figure 4 Cumulative DOX release from MFNPs nanocomposite spheres at pH=7.0 (black line) and pH=5.0 (red line) PBS buffer.

Although the loaded DOX could be released from the MFNPs in a pH dependent pattern, the in vitro cytotoxic effect of DOX loaded MFNPs should be further verified. Therefore, a standard MTT assay was carried out to evaluate the cytotoxic effect against HeLa cells after incubating in culture medium containing free DOX, MFNPs-DOX and pure MFNPs nanospheres for 24 h at different concentrations. As shown in Figure 5, the DOX loaded MFNPs had comparable cytotoxicity with free DOX at the same concentration of DOX, which indicated that the DOX loaded MFNPs was pharmacologically active as a potential drug carrier.

![Figure 5 In vitro cytotoxicity of free DOX, MFNPs-DOX, and pure MFNPs against HeLa cell after 24 h incubation.](image)

Figure 5 In vitro cytotoxicity of free DOX, MFNPs-DOX, and pure MFNPs against HeLa cell after 24 h incubation.

3.4 UCL/MR Imaging of MFNPs

The cell uptake process was measured by flow cytometry test. Firstly, DOX was loaded into the mesoporous SiO$_2$ shell of MFNPs so that the cell uptake degree can be detected by flow cytometry through the red fluorescence of DOX. As shown in Figure 6a, after incubation of HeLa cells with MFNPs-DOX for different times (30 min, 2 h and 4 h), the cell uptake efficiency is increasing obviously with time progressing. Therefore, MFNPs-DOX nanospheres could be gradually taken up by HeLa cells. This effective incubation is a crucial prerequisite for the bio-applications of MFNPs. Then in vitro UCL imaging of MFNPs was investigated by utilizing a modified inverted fluorescence microscopy under the excitation of 980 nm laser. As presented in Figure 6b, a weak UCL signal was seen after the incubation with HeLa cells for 30 min. As the prolonging of time, stronger UCL signal could be seen,
Figure 6 (a) Flow cytometry analysis of HeLa cells incubated with MFNPs-DOX for 0 min, 30 min, 2 h and 4 h. (b) Inverted fluorescence microscope images of HeLa cells incubated with MFNPs for 0.5 h and 4 h at 37 °C. Each series can be classified to the bright-field image, upconversion luminescent image (UCL) and the overlay of the above, respectively. indicating that more MFNPs were incubated into the HeLa cells. This phenomenon suggested that the MFNPs can be taken up by human cells gradually and act as a successful UCL signal probe for cell imaging.

Since the ultrasmall Fe₃O₄ nanocrystals were decorated onto the surface of UCNPs@mSiO₂ by nucleophilic substitution reaction, the as-synthesized nanocomposites show superparamagnetism properties, as revealed by field-dependent magnetization measurement (Figure 7a). A low magnetic saturation is also shown in Figure 7a, perhaps due to the decreased interparticle interactions and low amount of Fe₃O₄ particles on the silica shell. T₂-weighted MR images of MFNPs revealed a concentration-dependent darkening effect (Figure 7c), with a transverse relaxivity (r₂) of 28.7 mM⁻¹ S⁻¹ (Figure 7b) through a linear regression curve fitting of inverse relaxation times plotted against the Fe ion concentration. The above results reveal that the MFNPs could act as a useful T₂ contrast agent for T₂-weighted MR imaging. In this way, the obtained MFNPs can offer UCL/MR dual modal bioimaging, which could simultaneously achieve high spatial resolution and penetration depth for clinical diagnosis.²⁶

3.5 In Vivo Magnetic Targeting Effect of MFNPs

Figure 7 (a) The magnetization as a function of applied field for the as-synthesized MFNPs at 300 K. (b) Relaxation rate R₂ (1/T₂) versus different mass concentrations of MFNPs at room temperature. (c) T₂-weighted MR images of MFNPs at different [Fe] concentrations.

Figure 8 Magnetically targeted accumulation of MFNPs in mice determined by ICP-AES measurement of Fe concentrations (2 h post injection) and photographs (inset) of mouse treated with magnetic tumor targeting.

The targetability of a fantastic theranostic probe is highly desirable for assuring the anti-tumor efficiency. In order to test the magnetic targeting effect of MFNPs in vivo, the tumor-bearing mice injected with MFNPs (12 mg Y³⁺/kg) with or without a magnet were sacrificed at 2 h post injection and the Y³⁺ concentrations of the tumors were shown in Figure 8. For the mice treated with the magnetic field, a small magnet was attached right on the tumor (inset in Figure 8). The Y³⁺ concentration level of the tumors treated with the magnetic field increased greatly from 1.5 µg/g of the control group to 7.0 µg/g, implying a greatly enhanced accumulation of MFNPs to the tumor comparing with the passive tumor uptake. This efficient magnetic targeting of the MFNPs can help us use an outward magnetic field to attract those nanospheres from the circulating blood to the specific region, which would be of great importance for the further anti-tumor therapy or cellular association effects of upconversion imaging with least side effects to the normal tissue.

3.6 In Vivo Targeted Antitumor Efficacy of DOX Loaded MFNPs

Since the MFNPs could accumulated effectively to the specific region in response to an outward magnetic field, if we loaded some anti-tumor drugs into the MFNPs, this as-synthesized magnetic targeted “smart” drug delivery could send the drugs to, and retention at, the required body site, which would be of
great importance to magnify the cytotoxicity to the targeted tumor and reduce the side effects to the healthy tissues. Therefore, we carried out in vivo targeted antitumor study using the Kunming mice bearing viable H22 ascites tumors under the tumor-targeted magnetic field. Twenty-four mice bearing tumors were randomly divided into four groups (n=6) and respectively treated with saline as control, free DOX, MFNPs-DOX in the absence of the magnetic field (labeled as MFNPs-DOX) and MFNPs-DOX with the magnetic field (labeled as MFNPs-DOX+MF). Each experiment mouse was intravenously injected by the tail vein on day 1 and day 5, and the body weights and tumor sizes were measured every two days. During the experiments, no mice died. The variation trends of mean body weights and mean relative tumor volumes of each group were shown in Figure 9a, b. The weights of mice (Figure 9a) increased steadily with time and the increase trends of those four groups were similar. But the mean relative tumor volumes of each group varied differently (Figure 9b), which shows that the MFNPs-DOX+MF group showed the lowest tumor growth rate than the other three groups over a course of 12 days, demonstrating that the MFNPs could be excreted gradually from the Kunming mice. It is worth to note that the Y\(^{3+}\) concentrations in spleen increased firstly as the extension of time, perhaps because the spleen is the largest organ of the immune system.

**4. Conclusions**

In summary, an effective magnetically targeted theranostic probe NaYF\(_4\):Yb, Er@mSiO\(_2@Fe_3O_4\)-PEG (MFNPs) featured with UCNPs core, mesoporous silica shell and ultrasmall Fe\(_3O_4\) nanocrystals decoration was successfully synthesized, simultaneously achieving UCL/MR dual modal bioimaging and magnetically targeted chemo-therapy. It is worth to note that the optimal reaction conditions were carefully conducted by changing the size of UCNPs and the amounts of Fe\(_3O_4\) immobilized on the surface of UCNPs@mSiO\(_2\) in order to achieve the enough bright upconversion luminescence intensity while maintain the magnetic performance of Fe\(_3O_4\). Both in vitro and in vivo tests show good bio-compatibility of the as-obtained MFNPs. Moreover, the in vitro UCL/MR bioimaging and in vivo magnetically targeted anti-tumor efficacy tests were carried out to prove the potential of MFNPs to be a highly active magnetic drug delivery.
convenient and adjustable synthesis, good biocompatibility, low cytotoxic activity, together with the in vitro UCL/MR imaging and improved in vivo magnetically targeted anti-tumor efficiency would make our as-synthesized MFNPs useful for further theranostic applications.

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Notes and references
In this work, we show a convenient and adjustable synthesis of multifunctional nanoparticles NaYF₄:Yb, Er@mSiO₂@Fe₃O₄-PEG (MFNPs) that can simultaneously combine upconversion luminescence (UCL) imaging, magnetic resonance (MR) imaging and magnetically targeted drug delivery in one platform. The integration of UCNPs, mesoporous silica and Fe₃O₄ nanocrystals that can combine the diagnostic/therapeutic functions within a single platform, may provide a more advanced way for the accurate diagnosis and magnetically targeted therapy of cancer.