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A Multifunctional Nanocrystalline $\text{CaF}_2:\text{Tm}, \text{Yb}@m\text{SiO}_2$ System for Dual-Triggered and Optically Monitored Doxorubicin Delivery

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Abstract

Daunting challenges in investigating the controlled release of drugs in complicated intracellular microenvironments demand the development of stimuli-responsive drug delivery systems. Here, a nanoparticle system, $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$, made of a mesoporous silica (mSiO_2) nanosphere with $\text{CaF}_2\text{:Tm,Yb}$ upconversion nanoparticles (UCNPs) is developed, filling its mesopores and with its surface-modified with polyacrylic acid for binding the anticancer drug molecules (doxorubicin, DOX). The unique design of $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ enables us to trigger the drug release by two mechanisms. One is the pH-triggered mechanism, where drug molecules are preferentially released from the nanoparticles at acidic conditions unique for the intracellular environment of cancer cells compared to normal cells. Another is the 808 nm near infrared (NIR)-triggered mechanism, where 808 nm NIR induces the heating of the nanoparticles to weaken the electrostatic interaction between drug molecules and nanoparticles. In addition, luminescence resonance energy transfer occurs from the UCNPs (the energy donor) to the DOX drug (the energy acceptor) in the presence of 980 nm NIR irradiation, allowing us to monitor the drug release by detecting the vanishing blue emission from the UCNPs. This study demonstrates a new multifunctional nanosystem for dual-triggered and optically monitored drug delivery, which will facilitate the rational design of personalized cancer therapy.

1. Introduction

Over the past decades, a variety of drug delivery systems (DDSs), such as liposomes, micelles, nanogels, and inorganic nanoparticles, have been developed for cancer diagnosis and therapy.^[1] Inorganic nanoparticles, especially mesoporous silica nanoparticles (MSNs), have been considered as one class of potential DDS owing to their chemical stability, tunable microstructures, large surface area, high biocompatibility, and the ease of functionalization.^[2] Recent studies confirmed that nanoparticles such as MSNs may present other profoundly powerful cellular effects. Their interaction with cells may result in the formation of a gap, facilitating the particles to pass cross endothelial barrier to reach the cells.^[3] To improve their therapeutic efficacy while minimizing negative effects reported (i.e., drug prereleasing etc.), the design of smart MSNs nanocarriers with on-demand drug release functionality after being uptaken by cancer cells is being endeavored.

The external or internal stimuli to trigger drug release behavior of MSNs have been explored extensively.^[4] Generally, the pH value of intracellular compartments, such as lysosomes and endosomes, is 4.5–6.5, and the average extracellular pH value in cancerous tissues is ≈ 6.8 . Thus, cancerous tissues are more acidic than normal tissues ($\text{pH} \approx 7.4$).^[5] The microenvironment with such varied pH values has been utilized for pH-triggered MSNs with promoted selectivity and efficacy of chemotherapy. Meanwhile, external light triggers are even more advantageous owing to their excellent controllability, and they may power the current DDSs with remote and positive manipulation of the doses in a timely manner. A variety of investigations on light-responsive MSNs delivery systems have been carried out recently.^[6] However, most of light-stimulated DDS utilizes ultraviolet (UV) light, of which the wavelength is less than 400 nm, to stimulate the drug release. UV light has low tissue penetration depths and can harm living tissues and organs, restraining its applications in triggering DDS mainly at in vitro and in vivo level. The near-infrared light (NIR, $\lambda = 700\text{--}$

1100 nm), so-called “phototherapeutic window”, is harmless to natural tissue with high penetration depth. NIR irradiation has been utilized to not only stimulate the drug release, but also to promote the chemotherapy efficiency.^[7] Therefore, the NIR-triggered release kinetics of anticancer drug has been recognized as one iconic feature for advanced drug delivery protocols. Moreover, NIR-triggered MSNs-based drug delivery system, which offers controlled drug release spatially and temporally, is highly demanded in cancer therapy, in addition to pH-triggered DDSs.

On the other side, insufficient or excessive drug dosage locally at the tumor site is one of the main culprits to the unsuccessful cancer chemotherapy.^[8] Toward this end, the real-time tracking of antitumor drug release from DDSs is equally important but technically challenging. Among the current diagnostic approaches, positron emission tomography is one of the general protocols for detecting drugs comprising a radionuclide (i.e., ^{18}F , ^{11}C , and ^{15}O) within their molecular structure.^[9] Unfortunately, few drugs may meet such harsh preconditions at present. Another possible approach for tracking drug release is to develop a photoimaging strategy, which is probably the most successful approach so far. A variety of MSN systems functionalized with fluorescence compounds have been investigated for tracking the anticancer drug release behavior.^[10] Lanthanide-doped (Ln^{3+} -doped) upconversion (UC) nanomaterials possess various expected physicochemical features, such as long-lived luminescence, sharp emission bandwidth, high resistance to photobleaching, and relatively low toxicity.^[11] When excited with 980 nm laser, Tm/Yb codoped nanoparticles (Yb^{3+} ions are used as the sensitizers in the UC process) exhibit upconverted luminescence covering UV, visible, and NIR regions,^[12] enabling them ideal for biological applications, and thus they can be used to functionalize MSNs.

Generally, the current photoluminescent MSN materials consist of a luminescent core such as UC nanoparticles (UCNPs) for multimodal bioimaging and a mesoporous silica shell for drug storage.^[13] The UC luminescent nanoparticles (the core) are conventionally synthesized using the hydrothermal process, or high temperature decomposition approach. The core-shell structure is then formed following a sol-gel encapsulation process. This approach usually involves cumbersome synthesis procedures with complicated surface chemical modification. More importantly, the drug-loading efficiency is likely to be restrained at relatively low level due to the limited thickness of mesoporous silica shell for drug storage. Recently, in addition to the core-shell structured MSNs, induction of luminescent functional factors within the mesopores can maintain the original host mesostructures and inspire a promising protocol to synthesizing well-defined functional MSNs.^[14] A series of functional groups, such as organic, ionic, and photoactive molecules, have been incorporated into ordered mesoporous materials.^[15] Owing to the relatively weak chemical stability of such functional factors in the complex in vivo physiological environment, the direct in situ growth of light-responsive nanocrystals, rather than just molecules or ions, into ordered mesoporous MSNs may offer remarkable advancement to the drug delivery platforms to achieve the real-time control of drug pharmacokinetics.

Toward this end, a new type of photoluminescent MSNs filled with CaF_2 : Yb,Tm UCNPs (CaF_2 :Yb,Tm@mSiO₂ nanoparticles) was designed and synthesized via a chemical assisted sol-gel growth method. Ultrasmall CaF_2 :Yb,Tm nanocrystals were successfully

incorporated within the mesopores present in MSNs. With the aid of surface functionalization via polyacrylic acid (PAA) molecules, the drug (doxorubicin, DOX) loading properties and dual-stimuli (pH/NIR laser) triggered release behavior of the particles were systematically investigated (Scheme 1). The relationship between the drug release kinetics and the photoluminescent phenomenon was uncovered. In addition, the therapeutic efficacy of the nanoparticles was also investigated via an in vitro study using MCF-7 human breast cancer cells. This study has thus been anticipated to inspire a new type of multifunctional nanosystem for dual-triggered and optically monitored drug delivery.

2. Results and Discussion

2.1. Characteristics of $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ Up-Conversion Nanoparticles (UCNPs)

The approach for synthesizing $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ UCNPs in this study involved the preparation of mesoporous SiO_2 nanoparticles as templates, followed by the subsequent formation of $\text{CaF}_2\text{:Tm,Yb}$ nanocrystals within the mesopores. The UCNPs synthesized present uniform spherical shape with good dispersibility in aqueous solution, and the average diameter is ≈ 65 nm (Figure 1a). At a higher magnification, a large number of dark spots with dimension of ≈ 3 nm appear homogeneously within the nanochannels and partial surface of silica particles (Figure 1b). Energy-dispersive X-ray spectroscopy spectrum and element mapping examination demonstrate the homogeneous distribution of Si, O, Ca, F, Yb, and Tm elements within mSiO_2 matrix (Figure S1, Supporting Information). Further, the x-ray diffraction (XRD) pattern of nanoparticles shows the presence of cubic CaF_2 phase (JCPDS No. 35-0816),^[16] which is evidenced by well-defined diffraction peaks at (111), (220), and (311), as well as the amorphous silicon dioxide phase.^[17] No other impurity is present (Figure 1c). The findings confirm the successful incorporation of CaF_2 nanocrystals within the MSNs. When $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ particles were excited under 980 NIR laser, three narrow-band emission peaks at ≈ 478 , 1–650, and ≈ 800 nm presented, and the particles showed a purple color (Figure 1d). The emission spectra observed correspond to the electron transitions of Tm^{3+} ions: $^1\text{G}_4 \rightarrow ^3\text{H}_6$ (≈ 478 nm), $^3\text{F}_2 \rightarrow ^3\text{H}_6$ (≈ 650 nm), and $^3\text{H}_4 \rightarrow ^3\text{H}_6$ (≈ 800 nm).^[12]

The $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ UCNPs were then modified with PAA molecules prior to drug loading. As shown in the Fourier transform infrared (FTIR) spectra, the appearance of a band located at ≈ 1712 cm^{-1} attributed to C=O groups suggests that carboxyl groups of polyacrylic acid (PAA) molecules were successfully attached onto nanoparticles. Meanwhile, the peaks presented at ≈ 2862 and ≈ 2953 cm^{-1} , which belong to C—Hx bonds, confirm the successful grafting of PAA molecules (Figure 2a).^[10b] In addition, the Zeta potential of UCNPs changed from -15.6 to -23.3 mV owing to the PAA modification (Figure 2b). N_2 adsorption/desorption isotherms of $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ UCNPs before and after PAA modification exhibit a typical type IV isotherm for mesoporous materials. After PAA modification, the surface area of UCNPs is reduced remarkably from 460 to 5.5 $\text{m}^2 \text{g}^{-1}$, and the pore volume is decreased from 0.24 to 0.045 $\text{cm}^3 \text{g}^{-1}$ accordingly (Table 1, Figure S2, Supporting Information). The weakened nitrogen adsorption is induced by the blockage of mesopores within the UCNPs due to the attachment of PAA molecules. This phenomenon has also been reported elsewhere.^[18] Previous studies have demonstrated that

Ca^{2+} could be deposited at the particle surface after the modification with calcium compounds.^[19] In our study, Ca^{2+} ions are adsorbed on the surface of silica when $\text{CaF}_2\text{:Tm, Yb}$ nanocrystals are incorporated into mSiO_2 particles.

The CCK-8 assay using HEK293 cells and MCF-7 cells was pursued to examine the potential cytotoxicity of PAA-modified UCNPs. As shown in Figure 2c, with the increased particle concentration from 0 to $120 \mu\text{g mL}^{-1}$, the relative cell viability with respect to the blank control retained over 90% viability after incubation for 24 and 48 h. An analogous scenario was observed when examined using MCF-7 cells; all concentrations of UCNPs remained at a high relative cell viability (Figure S3, Supporting Information), reflecting that the PAA-modified UCNPs possess low cell cytotoxicity and good cytocompatibility under certain culture time and concentration range, which is in agreement with the findings reported elsewhere.^[10,22] One notable fact is that different silica surface modification protocols may induce cytotoxicity or varied biological effects.^[20]

2.2. In Vitro Drug Loading and Upconversion Photoluminescence (PL) of UCNPs

The drug-loading property is a vital parameter when considering a DDS for tumor therapy.^[21] In this study, the FTIR spectrum of PAA-modified UCNPs after DOX loading presents the absorption bands at 1617 and 1578 cm^{-1} , which are assigned to the stretching vibration of C=O groups of DOX molecules loaded (Figure 3b). More importantly, the DOX-loading efficiency of UCNPs before and after PAA modification, examined using UV-vis spectroscopy, was calculated to be $\approx 59\%$ and $\approx 92\%$, respectively (Figure 3a). DOX molecules, with positive Zeta potential of 2.2 mV, intend to adhere to PAA-modified UCNPs with more negative Zeta potential (-23.36 mV) through the electrostatic interaction.^[22] Therefore, compared to the as-prepared UCNPs, the PAA-modified UCNPs have significantly higher loading efficiency of DOX drugs, which facilitates its applications for tumor therapy.

The photoluminescent phenomena of PAA-modified UCNPs before and after DOX loading were also studied subsequently. As shown in Figure 4a, DOX drug presents a typical absorption band at $\approx 480 \text{ nm}$ measured by UV-vis spectrophotometer, which indicated a splendid spectral overlap with the emission spectra at $\approx 478 \text{ nm}$ of PAA-modified UCNPs samples under 980 nm laser excitation. In general, for an efficient luminescence resonance energy transfer (LRET) to occur, the absorption of the energy acceptor should overlap with the emission of the energy donor. In this work, a clear spectral overlap between the emission from UCNPs and the absorption band of DOX molecules has been constructed to achieve LRET. DOX drug serves as the energy acceptor, and UCNPs behave as the energy donor. UV-vis spectrophotometer was used to record the absorption spectrum of DOX drug. In consequence, the PAA-modified UCNPs samples presented the vanishing blue emission at $\approx 478 \text{ nm}$ after DOX loading procedure, and thus the color of nanoparticles changed from bluish violet to a dark red color excited by 980 nm laser (Figure 4b), as expected.

2.3. In Vitro Drug Release and Monitoring

PAA-modified UCNPs after DOX loading were immersed in phosphate-buffered saline (PBS) with varied pH values (7.4, 5.8, and 4.7) to explore its pH-triggered drug release. As

shown in Figure 5a, within the initial 5 h, $\approx 12\%$ of total drug is released when the pH value was set at 7.4. As the pH value was decreased to 5.8 and 4.7, the drug release content reached $\approx 35\%$ and $\approx 50\%$, respectively. After 10 h, while $\approx 18\%$ of total DOX loaded was released in PBS with a pH of 7.4, the nanoparticles induced $\approx 42\%$ and $\approx 65\%$ of the total drug contents to be released when the pH was set at 5.8 and 4.7, respectively. The subsequent release phenomena presented a rather sustained manner for all three conditions. The drug release kinetics exhibits a rather accelerated rate with the decreased pH value. Such fast release of DOX molecules may be caused by promoting protonation of carboxyl groups in PAA molecules with the decreased pH value, weakening the electrostatic bond between DOX and PAA molecules. Meanwhile, during the drug release progress, the FTIR peaks from 1350 to 1600 cm^{-1} , assigned to C=O bonds of DOX molecules, decreased with DOX releasing progress (Figure S4a,b, Supporting Information), confirming that more DOX was liberated from UCNPs within the same releasing period due to the use of PBS with a lower pH value. The pH-responsive drug release profile of PAA-modified $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ nanoparticles is similar to the studies reported previously.^[10b,22]

In addition to the pH-triggered drug releasing properties, the real-time tracking of drug release is equally vital. The blue emission (≈ 478 nm) intensity of DOX-loaded PAA-modified UCNPs as a function of drug-releasing progress was further investigated. As shown in Figure 5b,c, the blue emission was found to increase, while the emission at ≈ 650 nm remained barely changed. This is attributed to the weakened LRET efficiency induced by the increased distance between DOX acceptor and UCNPs donor during the drug release process in two solutions with different pH values. Meanwhile, the tendency of blue emission intensity variation corresponds well with the drug releasing behavior. For PBS with pH of 4.7, the blue emission increases in a much rapid manner. In comparison, the system with the slowest DOX-releasing kinetics in PBS with a pH of 7.4 presents a rather delayed blue emission recovery rate. The relationship between $I_{\text{blue}}/I_{\text{red}}$ intensity and the release duration is plotted in Figure 5d. Such two distinguished recovery rates of blue emission are attributed to different LRET efficiency due to different release behavior under different pH conditions. Such variation of PL intensity ratio has therefore been confirmed to effectively reflect the drug-releasing progress.

2.4. NIR-Triggered DOX Releasing Properties and Anticancer Efficacy of UCNPs

In addition to the pH-triggered and optical monitoring drug release functionalities, the influence of 808 nm NIR laser irradiation to DOX releasing kinetics was further investigated. The DOX release profiles of PAA-modified UCNPs with and without 808 nm NIR laser irradiation were examined in the PBS with pH = 7.4. As indicated in Figure 6a, 12.5% and 19.9% of DOX content is released within 5 and 24 h in PBS with pH of 7.4. In comparison, relatively burst drug release phenomenon was observed during 808 nm NIR irradiation. $\approx 26\%$ and $\approx 39\%$ total DOX loaded was released during 5 and 24 h, respectively. The findings indicate that the irradiation of NIR light has accelerated the release kinetics of drug contents from the nanoparticles. There are two possible reasons for 808 nm laser triggered DOX release. One is the heating effect of upconversion nanomaterials by NIR light irradiation.^[22,23] The UV-vis-NIR absorbance spectrum was utilized to examine the NIR absorbance of PAA-modified $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ nanoparticles. As shown in Figure S5

(Supporting Information), the nanoparticles exhibit broad NIR absorbance. Previous study^[22] has also demonstrated that anticancer drugs can be released in an accelerated manner from NIR-absorbable nanocarriers under NIR irradiation. The main mechanism is that the locally increased temperature of the nanoparticles induces enhanced vibration of PAA molecular chains and consequently weakens the electrostatic interaction between drug molecules and the DDS. Another factor could be that DOX molecules have weak NIR absorbance, implying that the NIR irradiation may also accelerate DOX molecular vibration and weaken the electrostatic binding.^[24] Therefore, NIR light (808 nm) can trigger and accelerate DOX release phenomenon. This unique property may facilitate the rational control of therapeutic protocol by external NIR irradiation in addition to the pH-responsive drug releasing properties.

To evaluate the antitumor efficiency of the nanoparticles via NIR irradiation, a 2D cell culture was carried out to assess the therapeutic efficacy using MCF-7 cells by CCK-8 assay. MCF-7 cells were exposed to various doses of pure DOX solution or DOX-loaded PAA-modified UCNPs with and without NIR irradiation. As shown in Figure 6b, the viabilities of MCF-7 cells incubated with free DOX and UCNPs without NIR irradiation were 53.6% and 55.5% for 24 h at the DOX concentration of $0.1 \mu\text{g mL}^{-1}$, respectively. After NIR laser irradiation ($\lambda = 808 \text{ nm}$, 1.0 W cm^{-2}) for 10 min, the particles presented enhanced in vitro anticancer efficacy. $\approx 30\%$ cell viability was induced after NIR irradiation at the equivalent DOX concentration of $0.1 \mu\text{g mL}^{-1}$. To eliminate the effect of NIR irradiation to the cellular proliferation, MCF-7 cells were exposed to different groups of blank control, PAA-modified UCNPs before and after DOX loading under the same NIR exposure for a varied period of time from 0 to 20 min. It was found that the prolonged NIR irradiation (10, 15, and 20 min) led to more cell death for DOX-loaded PAA-modified UCNPs group (Figure 6c). However, if DOX molecules were not loaded, a minor effect on cancer cell viability was observed. The findings suggested that the significantly enhanced anticancer efficiency was induced by the effective DOX release triggered by NIR light rather than the NIR irradiation itself. One notable fact is that more precise evaluation of nanomedicine efficacy can be achieved using 3D cell culture systems.^[25]

To uncover the mechanism of such enhanced killing of cancer cells, confocal laser scanning microscopy (CLSM) was used to assess cell-uptake of the particles and the subsequent intracellular DOX distribution within MCF-7 cells. Previous studies have demonstrated that SiO_2 nanoparticles may be trapped within the endosomes, lysosomes, or even interact with membrane-bound organelles such as the mitochondria and cell nucleus.^[26] In our experiment, the cells were exposed to DOX-loaded PAA-modified UCNPs with different concentrations before and after NIR irradiation. As shown in Figure 6d, remarkably low contents of DOX drug (in red color) could be observed in the cells without NIR light irradiation (808 nm), even at a high particle concentration ($0.1 \mu\text{g mL}^{-1}$). By contrast, owing to NIR irradiation, significantly enhanced DOX fluorescence was visualized around the nuclei and within cytoplasm of MCF-7 cells. It indicates that the NIR irradiation can effectively trigger intracellular release of DOX molecules from internalized nanoparticles. Additionally, the CLSM images of MCF-7 cells incubated with DOX-loaded PAA-modified UCNPs after exposure to NIR irradiation for 0–20 min was also investigated to verify the findings above. No DOX contents were detected within the cell without NIR laser irradiation

(Figure S6, Supporting Information). However, under NIR laser irradiation for a prolonged period of time (15 and 20 min), visible DOX fluorescence was detected in the cytoplasm. These findings confirmed that the intracellular dosage of DOX is significantly corresponding to the period of NIR irradiation delivered by the nanoparticles (Figure S6, Supporting Information), and thus NIR-triggered intracellular DOX release has been realized by our multifunctional nanoparticles with PPA-modified UCNP embedded in MSNs.

3. Conclusions

In this study, multifunctional $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ upconversion nanoparticles (UCNPs) were synthesized by a facile chemically assisted sol–gel growth method for the first time. The $\text{CaF}_2\text{:Tm,Yb}$ nanocrystals (≈ 3 nm) were successfully incorporated within the mesopores of mSiO_2 particles. After the surface modification with PAA molecules, the drug loading efficiency was remarkably enhanced due to its lower Zeta potential. When immersed in PBS solution with different pH values (7.4, 5.8, and 4.7), the UCNPs presented a significantly accelerated drug-releasing rate at a lower pH value. Additionally, the upconversion luminescence phenomenon of the nanoparticles corresponded well with the drug-releasing progress. Fast drug release behavior (in an acid environment) induced rapid recovery of blue-to-red emission and vice versa. The main mechanism is attributed to the LRET constructed between UCNPs energy donor and the DOX acceptor. Furthermore, the in vitro triggering of DOX release via 808 nm NIR irradiation has been demonstrated. As a result, the anticancer efficacy was enhanced by the NIR irradiation via realizing NIR-triggered intracellular drug release. Such nanocrystalline $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ nanoparticles have thus been expected to serve as another new multifunctional DDS with dual-triggered (pH and NIR light) and optically monitored pharmacokinetics, which may markedly facilitate the rational design of personalized therapeutic protocols.

4. Experimental Section

Synthesis of $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ Nanoparticles

In a typical procedure, 0.2 g cetyltrimethylammonium bromide (CTAB, 99%, Sigma–Aldrich Inc.), 25 mL deionized water, 5 mL ethanol, 50 μL of triethanolamine (TEA, Sinopharm Chemical Reagent) were mixed and stirred at 60 °C for 30 min. Subsequently, 2 mL tetraethylorthosilicate (TEOS, Sigma–Aldrich Inc., USA) was added into the solution under stirring for 2 h. When white precipitate appeared, the solution was cooled down to the ambient temperature, and the precipitate was collected by centrifugation. After being air-dried at 80 °C in air, the particles were further calcined at 550 °C for 5 h to remove organic additives. To incorporate $\text{CaF}_2\text{:Tm,Yb}$ nanocrystals within mSiO_2 particles, a mixed ionic solution of Ca^{2+} , Tm^{3+} , and Yb^{3+} was prepared by dissolving their corresponding acetates in deionized water, and added with trifluoroacetic acid (TFA, 99%, Sigma–Aldrich Inc.) under stirring for 24 h at 40 °C. Subsequently, 400 mg of the as-synthesized mSiO_2 particles was added into 20 mL of the as-prepared solution and immersed for 24 h under stirring at 40 °C. The particles were then collected by centrifuging, washed with deionized water gently, and air-dried at 80 °C in air for 3 h and sintered at 600 °C for 3 h. The surface modification via polyacrylic acid (PAA) molecules was carried out further before drug loading procedure.

100 mg of as-prepared particles was added into 80 mL deionized water with sonication for 30 min, and 20 mL PAA solution (50% aqueous solution) was added dropwise. After stirring for 12 h, the samples were collected and washed using deionized water to remove the excessive PAA molecules.

In Vitro Cytotoxicity Assay

Cytotoxicity of PAA functionalized particles synthesized was assessed using two cell lines (Type HEK 293 cells or MCF-7 human breast cancer cells) by Cell Counting Kit-8 method (CCK-8; Dojindo, Kamimashiki-gun Kumamoto, Japan). Briefly, cells were seeded into 96-well plate and incubated with particles for 24 and 48 h. The particles concentration was ranged from 0 to 120 $\mu\text{g mL}^{-1}$. At each time point, the medium was refreshed, and 10 μL CCK-8 solution was added and incubated at 37 °C for 4 h. The cytotoxicity of particles was examined by measuring the absorbance at 450 nm using a Universal Microplate Reader (BIO-TEK Instruments, Minneapolis, MN, USA).

Drug Loading and Release

Doxorubicin (DOX), an anticancer drug, was used as a model drug. 50 mg particles were added into DOX aqueous solution (1 mg mL^{-1} , 20 mL) at ambient temperature. The mixed solution was subsequently stirred in dark for 24 h. The DOX-loaded samples were collected and washed with deionized water to remove the physically adsorbed DOX. The drug loading efficiency was calculated using UV-vis spectroscopy by subtracting the original and remaining DOX content, following: Drug loading efficiency = (weight of DOX loaded)/(weight of total DOX used) $\times$ 100%. The in vitro DOX releasing test was performed by immersing the DOX-loaded particles in 20 mL phosphate-buffered saline (PBS, HyClone Laboratories Inc., Logan, Utah) with three different pH values (7.4, 5.8, and 4.7) under stirring at 37 $\pm$ 0.1 °C. At each time interval, 10 mL buffer solution was collected and replaced with an equal volume of PBS with the same pH value adjusted. The released content of DOX in different pH environment was measured using an ultraviolet-visible (UV-vis) spectrophotometer (TU-1810, China) at a maximum wavelength (λ_{max}) of 480 nm. Three measurements were performed for each sample.

In Vitro Study for Anticancer Efficiency

The anticancer properties of the up-conversion nanoparticles functionalized with PAA molecules after the DOX loading was characterized using MCF-7 cell via Cell Counting Kit-8 method (CCK-8; Dojindo, Kamimashiki-gun Kumamoto, Japan). Briefly, 6×10^3 cells were seeded onto 96-well plate and cultured overnight. After a wash step with 6.7×10^{-3} M PBS (pH 7.4, HyClone Laboratories Inc., Logan, Utah), MCF-7 cells were incubated with free DOX or DOX loaded $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ nanoparticles at different relative concentrations of DOX (0–0.1 $\mu\text{g mL}^{-1}$) for 24 h at 37 °C in a humidified atmosphere with 5% CO_2 . The medium used was fresh dulbecco's modified eagle medium (DMEM, 100 μL). The experimental groups were irradiated with NIR spectrum ($\lambda = 808$ nm, 1 W cm^{-2} , 10 min). The laser spot was adjusted to cover each well. The cells were incubated for another 24 h at 37 °C in a humidified atmosphere with 5% CO_2 . Then the medium was refreshed with fresh culture medium, and 10 μL of CCK-8 solution was added and incubated at 37 °C for 2 h. The relative cell viability was assessed by examining the absorbance at 450 nm

using a Universal Microplate Reader (BIO-TEK Instruments, Minneapolis, MN, USA). The relative cell viability treated with culture medium, UCNPs, and DOX-loaded UCNPs at different irradiation times was measured correspondingly at a relative DOX concentration $0.01 \mu\text{g mL}^{-1}$. The NIR light used had a wavelength of 808 nm and a power density of 1 W cm^{-2} . The irradiation time varied from 0–20 min. To visualize the cell morphology during the cell culture, the cells were seeded onto 8 chamber cell culture slide (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 2×10^4 cells per chamber and treated similarly as above processing. Then the cells were fixed in 4% para formaldehyde for 15 min. After washing with PBS, MCF-7 cells were stained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich Co., St. Louis, MO, USA) for 10 min. The cells were then examined using laser scanning confocal microscopy (LSCM, Fluoview OLYMPUS, JAPAN).

Characterization

The particle morphology was characterized by field-emission scanning electron microscopy (FESEM, Hitachi SU-70, Japan) operated at 3 kV and high-resolution transmission electron microscopy (HRTEM, Tecnai F20, FEI, USA) operated at 200 kV. The phase and crystal structure were examined using an X-ray diffraction instrument (XRD, X'Pert PRO MPD, The Netherlands) operated at 40 mA and 40 kV using Cu K α radiation. The scanning range was set at $20^\circ < 2\theta < 60^\circ$ with a step size of 0.167° . The FTIR spectra of the samples were recorded using a PerkinElmer 580B infrared spectrophotometer on KBr pellets (Tensor 27, Bruker, Germany) in the frequency range of $4000\text{--}400 \text{ cm}^{-1}$ with a resolution of 4 cm^{-1} . The upconversion photoluminescence spectra were recorded via a fluorescence spectrophotometer (PL, FLSP920, Edinburgh) under excitation by 980 nm laser at ambient temperature. To minimize experimental accidental uncertainties, the spectra collection and sample positions were maintained in identical conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

a) SEM image (the inset shows the UCNPs dispersions in aqueous solution and particle size distribution), b) TEM images, c) X-ray diffraction patterns, and d) Photoluminescence spectrum of crystalline $\text{CaF}_2:\text{Tm}, \text{Yb}@m\text{SiO}_2$ nanospheres (the inset shows the optical image of nanospheres under excitation by 980 nm laser and the scale bar is 1 mm).

Figure 2.

a) FTIR spectra of the $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ nanospheres before and after PAA modification; b) Zeta potential of UCNPs with or without PAA modification; c) relative cell viability of HEK293 cells incubated with PAA functionalized nanospheres of varied concentrations for 24 and 48 h.

Figure 3.
a) DOX loading efficiency of UCNPs before and after PAA modification, b) FTIR spectra and enlarged characteristic peaks of PAA modified UCNPs before and after DOX loading.

Figure 4.

a) The emission spectrum of PAA-modified UCNP and the UV-vis absorption spectrum of DOX; b) The UC PL emission spectra of PAA modified nanoparticles before and after DOX loading under the excitation by 980 nm laser (the insets show the optical images captured by digital camera).

Figure 5.

a) Cumulative DOX release profiles of nanoparticles in the medium with different pH values. The change of upconversion emission spectra of nanoparticles as a function of drug release time in the medium with pH of b) 7.4 and c) 4.7; d) the relationship between the intensity ratio of blue and red emission spectra (I_{blue}/I_{red}) and the DOX release time in medium with a different pH value.

Figure 6.

a) DOX release profile of PAA modified nanoparticles in the neutral PBS solution (pH = 7.4) with and without NIR laser irradiation. b) Relative cell viability (to blank control) of free DOX and DOX loaded PAA modified nanoparticles with or without the NIR irradiation ($\lambda = 808 \text{ nm}$, 1 W cm^{-2} , 10 min). c) Cell viability of MCF-7 cells exposed to different time (0–20 min) of laser irradiation (NIR 808 nm, 1 W cm^{-2}) at DOX concentration of $0.01 \mu\text{g mL}^{-1}$. d) LSCM images of MCF-7 cell incubated with DOX loaded PAA modified nanoparticles at different concentration (0– $0.1 \mu\text{g mL}^{-1}$) for 24 h and then irradiation with

NIR. For each panel, images from left to right show the MCF-7 cell nuclei stained by DAPI (blue), DOX fluorescence in cells (red), and overlay of both images (Scale bar: 20 μm).

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Scheme 1.
Schematic illustration of $\text{CaF}_2\text{:Tm,Yb@mSiO}_2$ nanospheres for DOX delivery.

Table 1

Properties of UCNPs before and after PAA functionalization.

	Surface area [m ² g ⁻¹]	Pore volume [cm ³ g ⁻¹]	Pore size [nm]
UCNPs	460	0.24	4.4
PAA modified UCNPs	5.5	0.045	2.9