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A Facile Approach to Upconversion Crystalline $\text{CaF}_2\text{:Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ Nanospheres for Tumor Therapy

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Abstract

A new facile approach, namely chemical-assisted sol-gel growth (CASGG), was successfully developed to induce the formation of fine $\text{CaF}_2\text{:Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals within the pore channels of mesoporous silica (mSiO_2) nanoparticles. A series of upconversion photoluminescent crystalline $\text{CaF}_2\text{:Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ nanospheres with controlled diameters from ~65 nm to ~290 nm were fabricated. All nanospheres presented sound cyto-compatibility and unique ratiometric spectral monitoring functionalities for drug release kinetics. The nanospheres with smallest dimension (UCNP-2.5, ~65nm) induced the most sustained DOX release kinetics. More importantly, the *in-vitro* study demonstrated that the DOX loaded UCNP-2.5 nanospheres presented the strongest anti-cancer efficacy to MCF-7 human breast cancer cells due to its stronger penetration ability to cell nuclei due to the size effect.

Mesoporous silica nanoparticles (MSNs) have attracted worldwide attention for drug delivery applications in the past decades, due to their favourable features such as physicochemical stability, tunable microstructure and facile surface functionalization. MSNs have been recognized as one of the most promising drug delivery systems (DDSs) for tumor diagnosis and therapy.¹⁻⁵ Recently, MSNs functionalized with photoluminescent factors have demonstrated powerful strength for the anti-cancer agent delivery with optically triggered drug releasing and release-kinetics monitoring demands.⁶⁻¹¹ A variety of silica-based fluorescence labelling materials, such as organic dyes and semiconductor quantum dots (QDs), has thus been investigated extensively.¹²⁻¹⁴ However, for organic dyes, a typical rapid photobleaching rate narrows the available detection time, while fluorescence quantum yield is low.^{15, 16} Meanwhile, some intrinsic disadvantages of QDs, such as weak chemical

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stability, potential toxicity, low signal-to-noise ratio (SNR) and intermittent fluorescence (blinking),¹⁷ also hinder the applications in *in-vitro* and *in-vivo* studies. In contrast, lanthanide (Ln^{3+}) doped upconversion (UC) nanomaterials possess superior physicochemical features, such as long-lived luminescence (from several to tens of milliseconds), sharp emission bandwidth, tunable emission, high resistance to photobleaching and low toxicity.¹⁸⁻²¹ The rare earth ions, especially Tm^{3+} ion, can convert the 980 nm excitation spectrum to the spectra with wavelengths ranging from UV to the infrared.²² Developing a photoluminescence MSNs functionalized with rare earth doped upconversion nanocrystals is expected to offer a remarkable break-through to the current research in tumor therapy.

The upconversion (UC) phenomenon has been reported in various nanoscale hosts including oxides, vanadates, phosphates and fluorides.²³ The latter has been considered as a promising candidate for hosting lanthanide ions due to their low vibrational (phonon) energies, excellent physical and photochemical stability, and low cytotoxicity for biological applications.²⁴ Recently, CaF_2 ceramic has been used as an attractive host for phosphors with interesting up/down-conversion luminescent properties.^{25, 26} CaF_2 has a typical fluorite structure, in which Ca^{2+} ions lie at the nodes in a face-centred lattice, while F^- ones lie at the centres of the octants.²⁷ The low phonon energy of CaF_2 minimizes multiphonon de-excitation probabilities. It is also known that, due to the presence of charge compensation effects, CaF_2 network promotes the formation of pairs when doped with lanthanide ions, so that an effective reduction in the interatomic distance occurs, increasing the energy transfer rates. More importantly, CaF_2 is more biocompatible than the well-documented NaYF_4 matrix since calcium is an abundant element in natural tissue and living organs.²⁸ When excited under 980 nm spectrum, $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ UCNPs (Yb^{3+} ions are used as the sensitizers in the UC process) exhibits intense upconverted luminescence spanning the UV, visible, and NIR regions, making them ideal for a slew of biological applications. Therefore, $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals have been considered as an ideal functional factor for the upconversion photoluminescent (UC PL) MSNs-based drug delivery system.

There have been massive reports regarding the synthesis approaches for UCNP-functionalized MSNs materials.^{29, 30} In general, such MSNs consist of a luminescent core for optical imaging and a mesoporous silica shell for drug storage. The uniform-sized luminescent core is usually prepared via a hydrothermal process, or high temperature decomposition method. Subsequently, the formation of the core-shell structured composites is followed by an encapsulation process, where the luminescent core is 'wrapped' within the silica shell using a sol-gel technique.^{31, 32} For current synthesis approaches, cumbersome procedures in hydrothermal process and chemical modifications are usually involved.³³ Furthermore, the anti-cancer agent is loaded within the thin shell of mesoporous silica, inducing its relatively low drug loading capacity. Therefore, a facile approach for UCPL functionalized MSNs with integration of tunable microstructure, superior drug loading capacity and controlled releasing kinetics is highly demanded.³⁴

In this study, a new synthesis protocol, namely chemical-assisted sol-gel growth (CASGG) method, was developed for synthesizing $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystal functionalized MSNs. For this approach, the mesopore channels of MSNs were utilized as the nanoreactors for the

in-situ growth of $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals. As ultrasmall $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ crystals were formed dispersedly within the silica particles rather than occupying the major core section of the particles, and the remaining room within mesopore channels could be used as reservoirs for therapeutic agents. Three types of luminescent $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ functionalized mesoporous silica ($\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$) nanospheres with controlled dimensions were prepared. Doxorubicin (DOX), a first-line anti-cancer chemotherapeutic, was used as a drug model. The loading efficiency, releasing kinetics and its corresponding photoluminescence phenomena were systematically uncovered. Furthermore, the therapeutic functionalities of such new drug delivery platforms were explored via an *in-vitro* examination using MCF-7 human breast cancer cells.

In this study, the approach for preparing $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ nanospheres involved the synthesis of MSNs as morphology-controlling template, followed by an *in-situ* formation of $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals within the mesopores. As shown in Figure 1a-c, all UCNP present regular spherical morphology. By adjusting the ethanol/TEOS volume ratio, the mean particle diameters were controlled at ~ 65 nm for UCNP-2.5, ~ 170 nm for UCNP-4 and ~ 290 nm for UCNP-5, respectively. In addition, the good dispersibility of all samples was confirmed by SEM examination. The particle size distribution of all three samples was measured. UCNP-2.5 particles exhibited relatively narrow size distribution between 50 nm and 100 nm. In comparison, UCNP-4 and UCNP-5 samples, which were of increased mean diameters, presented relatively broad size distributions (Fig. S1a-c). The morphological and structural features of the samples were further examined via TEM. As shown in Figure 1(d-f), all three types of UCNP show monodispersed dimensions, which is consistent with SEM examination. It is also visible that tiny channels presents within the spheres. More importantly, a large quantity of 'dark' dots present in the spheres. The high-definition TEM examination confirmed that such dots are nanocrystals with dimension of ~ 5 nm. The lattice fringes of crystalline phase can be clearly observed, reflecting the incorporation of nanocrystals within the mesoporous silica spheres (Fig. S1 d-f). The XRD patterns of UCNP-2.5, UCNP-4, and UCNP-5 samples show (111), (220) and (311) peaks assigned to the cubic phase of CaF_2 ceramic (JCPDS No. 35-0816) (Fig. 1g).³⁵ A broad peak centered at $\sim 22^\circ$ shown in all three patterns, which is attributed to amorphous silica phase, presents,³⁶ and no other impurity phases are observed.

The formation of disperse ultrasmall CaF_2 nanocrystals within the well-defined mesopore of MSNs follows the "trifluoroacetate process", which consists of precursor synthesis by the reaction of a metal acetate with trifluoroacetic in aqueous solutions.³⁷ Calcium acetate and lanthanide (III) acetate hydrate are dissolved in a TFA aqueous solution, and a mixture solution of calcium and lanthanide trifluoroacetates solutions is obtained following:

$\text{Ca}(\text{CF}_3\text{COO})_2$ and $\text{Ln}(\text{CF}_3\text{COO})_3$ solution diffuses into the mesopores of MSNs under stirring, and the decomposition of $\text{Ca}(\text{CF}_3\text{COO})_2$ and $\text{Ln}(\text{CF}_3\text{COO})_3$ arises during the heating procedure, following:

Thus, the mesopores of MSNs acted as nanoreactors to form LnF_3 and CaF_2 crystal seeds in the aqueous solution. The $\text{CaF}_2:\text{Tm}^{3+}, \text{Yb}^{3+}$ crystals were formed within the mesopores in an *in situ* way during sintering process and thus crystalline $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ nanospheres were successfully achieved.

The nitrogen adsorption–desorption isotherm and the textural parameters of UCNP-2.5, UCNP-4, and UCNP-5 are presented in Figure S2 and summarized Table 1, respectively. Typical type IV isotherms for mesoporous materials were observed for all three UCNPs.³⁸ The combination of a uniform mesopore size and small particle size is highly advantageous and favorable for drug delivery applications. The pore volume for UCNPs decreases from $\sim 0.24 \text{ cm}^3/\text{g}$ to $\sim 0.1 \text{ cm}^3/\text{g}$ with increased ethanol/TEOS volume ratio from 2.5 to 5, and the pore size decreases from ~ 4.3 to $\sim 2.7 \text{ nm}$ accordingly. It is noted that as the particle size increases the surface area of three UCNPs is $\sim 470 \text{ m}^2/\text{g}$, $\sim 390 \text{ m}^2/\text{g}$ and $\sim 430 \text{ m}^2/\text{g}$, respectively. The variation of surface area, pore volume and pore size is induced by the micelle aggregation of surfactant CTAB, which was reported in previous studies.^{39, 40} In addition, it was found that, under the excitation using 980 nm NIR laser, three narrow-band emission peaks at $\sim 478 \text{ nm}$, $\sim 650 \text{ nm}$ and $\sim 800 \text{ nm}$ presented for all three samples. (Fig. 1h) The emission spectra observed correspond to the electron transitions of Tm^{3+} ions: $^1\text{G}_4 \rightarrow ^3\text{H}_6$ ($\sim 478 \text{ nm}$), $^3\text{F}_2 \rightarrow ^3\text{H}_6$ ($\sim 650 \text{ nm}$) and $^3\text{H}_4 \rightarrow ^3\text{H}_6$.⁴¹ With increased particle size from UCNP-2.5 to UCNP-5, the photoluminescent intensity intends to decrease. This phenomenon is induced by the weakened crystallinity of CaF_2 nano-crystals formed within mesoporous SiO_2 nanophere.⁴² As shown in Figure 1g, with increased particle size from UCNP-2.5 to UCNP-5, the diffraction peaks intensity attributed to CaF_2 nano-crystals become decreased, and the half-height width of the peaks increases accordingly, confirming its weakened crystallinity

The cyto-compatibility has been always a crucial factor when one biomaterial is expected to be used as a drug delivery system. The Kit-8 assays were performed using 293 cells and MCF-7 human breast cancer cells to evaluate the cytotoxicity of UCNPs synthesized. As shown in Figure 1i, after culturing with 293 cells for 24 hours, the relative cell viability (to blank control) of all three UCNPs with varied concentration from 10 to 100 mg/L maintains at a high level ($>80\%$). In addition, the *in-vitro* study using MCF-7 human breast cancer cells presented a similar phenomenon, confirming that the $\text{CaF}_2:\text{Yb}^{3+}, \text{Tm}^{3+}@\text{mSiO}_2$ nanospheres synthesized do not induce negative effect to the cell proliferation. (Fig. S3)

The loading efficiency is an important parameter when considering a drug delivery system for tumor therapeutic applications.⁴³ In this work, UCNPs were loaded with DOX molecules via an immersion approach. The UV-Vis spectrum of DOX solution presents a characteristic peak at $\sim 480 \text{ nm}$. After loading procedure, the UCNPs were suspended in deionized water, and the UV-Vis spectra for all three particles presented the same characteristic peak, suggesting the successful drug loading in UCNPs. (Fig 2a) The DOX loading efficiency was determined by measuring the variation of Absorbance at 480 nm of DOX aqueous solution

before and after the loading process. UCNP-2.5 nanospheres present the highest DOX loading efficiency ($\sim 59\%$) (Fig. 2b), and its suspension shows a dark red color (the insets of Fig. 1a). In comparison, the loading efficiency of UCNP-4 and UCNP-5 spheres is $\sim 34\%$ and $\sim 43\%$, respectively. The successful loading of DOX molecules was confirmed by FTIR analysis based on the representative characteristic peaks of C-O bands between 1200 and 1600 cm^{-1} attributed to DOX molecules (Fig. S4a). It was also found that the intensity of representative characteristic peaks of C-O bands decreases from UCNP-2.5, UCNP-5 to UCNP-4 in sequence (Fig. S4b), that is consistent with the loading efficiency quantified by UV-vis analysis (Fig. 2b). The UCNP-2.5 exhibit significantly enhanced drug loading efficiency due to the enlarged pore volume and increased surface area. Such phenomenon has also been reported in the previous studies on other MSNs-based drug delivery system.^{3, 44}

For a typical upconversion-based luminescence resonance energy transfer (LRET) system, the absorption wavelength of the energy acceptor overlaps with the upconversion emission of UCNPs. In our study, an excellent spectral overlap between the absorption band of DOX molecules (~ 480 nm) and the emission spectra from the higher-lying energy orbital of UCNPs was constructed (Fig. S5), providing an important prerequisite for LRET effect between UCNPs and DOX drug. DOX molecules have thus played a role as the energy acceptor. Due to the construction of such LRET process, UCNPs with varied DOX loading efficiency induce the changed intensity ratio between blue spectrum (I_{480}) and red spectrum (I_{650}). The upconversion emission properties of UCNP-2.5, UCNP-4 and UCNP-5 spheres after DOX loading were examined under excitation using 980 nm spectrums. It was found that, after the loading procedure, the spectrum at ~ 480 nm decreased remarkably while the spectrum at 650 nm barely changed, as expected. In consequence, the color of UCNP particles varied from a purple to dark red (The inset of Fig 2c). Additionally, the increased DOX loading efficiency induced a steady decrease in $I_{\text{blue}}/I_{\text{red}}$ ratio. UCNP-2.5 spheres showed the lowest ratio after DOX loading due to its highest drug loading efficiency, while UCNP-4 sample presented the highest ratio value, which is consistent to the findings above (Fig. 2d).

The drug releasing properties of UCNPs were examined via immersion in phosphate buffered saline (PBS) aqueous solution. In general, accompanied by fluid diffusion into the pores of UCNPs, DOX molecules are liberated and diffuse into the fluid by a diffusion-controlled mechanism.⁴⁴ As shown in Figure 3a, DOX loaded UCNPs with different ethanol/TEOS volume ratios (2.5, 4, and 5) exhibit dramatically different releasing phenomena. Within the initial 10 hours, $\sim 50\%$ of total DOX loaded is released from UCNP-4 particles, and $\sim 70\%$ is released after immersion for 80 h. In contrast, the drug releasing behavior shows a sustained manner for UCNP-2.5 and UCNP-5 samples. Only $\sim 30\%$ of DOX drug is liberated from the UCNP-2.5 within the initial 10 h, and as low as $\sim 45\%$ of the total drug load is released after 80 h. The drug release kinetics of UCNP-5 particles presents at a moderate degree. Therefore, it is confirmed that DOX-loaded UCNP-2.5 particles present the highest drug loading capacity with the most sustained release kinetics. A possible reason is that the high amount of DOX may be loaded in the inner mesopores, to some extent, which blocks the mesopores. More over, the solvents had

difficulty penetrating into the pore channels, and thus prevented drug transportation from leaching out of the carriers, which further decreased the release kinetics of DOX.^{44, 46}

The variation of its corresponding upconversion photoluminescence phenomenon during drug release progress was further examined. It was found that, accompanying with DOX releasing, the intensity of blue emission that was initially quenched by DOX molecules gradually recovered, while the red emission intensity remained barely changed (Fig. S6). The recovered blue emission is induced by the reduced LRET effect caused by the increased distance between UCNP's energy donor and DOX molecules (the energy receptor) along with its releasing process. The relationship between $I_{\text{blue}}/I_{\text{red}}$ intensity and the releasing time is summarized in Figure 3b. For UCNP-4 samples, the blue emission recovers in a rapid fashion due to its fast drug release kinetics. In contrast, the nanospheres with most sustained drug releasing kinetics (UCNP-2.5) present a delayed blue emission recovering rate. This photoluminescence variation has thus been confirmed to effectively reflect the drug releasing phenomenon.

Efficient delivery of DOX molecules into cancer cells with increased intracellular drug concentration can inhibit the cell proliferation and ultimately lead to the cell death.⁴⁷ In this study, the cell viability incubated with three types of UCNP's was analyzed by the Cell Counting Kit-8 assay using MCF-7 human breast cancer cells. Free DOX, UCNP-2.5-DOX, UCNP-4-DOX, and UCNP-5-DOX at different DOX dosages were cultured for 24, 48, and 72 hours. As shown in Figure 3c-e, all UCNP's samples after DOX loading show significant inhibition effect to the cell proliferation, and as expected, the inhibition effect becomes more significant as drug concentrations and incubation durations increase. It is noted that no significant difference in cytotoxicities between UCNP-4-DOX and UCNP-5-DOX samples after incubation for 24 hours with MCF cells, and both of them show weakened cellular toxicity to MCF cells. However, the relative cell viability (to blank control) of UCNP-2.5-DOX samples presents the remarkable enhancement of cellular inhibition. Only ~20% cells remained viable after culturing for 24 hours, even at as low DOX concentration as 0.3 ~ 0.5 $\mu\text{g/mL}$. The cytotoxicity difference between UCNP-2.5-DOX, UCNP-4-DOX and UCNP-5-DOX samples is accumulatively distinctive after incubation for 48 h and 72 h. The prolonged incubation leads to decreased cell viabilities, and more cells are killed at increased drug concentrations. Overall, the UCNP-2.5-DOX sample presents the strongest *in-vitro* anti-cancer efficacy, and the efficacy is weakened in sequence from UCNP-2.5-DOX, UCNP-5-DOX and UCNP-4-DOX samples. (Fig. S7)

Such increasingly amplified cytotoxicity induced by UCNP's was generally thought to result from the size effect-mediated endocytosis and the sustained intracellular release of DOX molecules. The examination of the endocytosis and intracellular transportation of nanospheres is compulsory to understanding the varied anti-cancer efficacy achieved. To this end, we evaluated the intracellular DOX delivery with UCNP's using MCF breast cancer cells by measuring their characteristic red fluorescence using confocal laser scanning microscope (CLSM). The confocal images show that most of the red fluorescence emitting from UCNP-4 and UCNP-5 are evenly distributed surrounding the cytoplasm region with less fraction entered into the nucleus. In contrast, for the confocal images of MCF cells pretreated with DOX loaded UCNP-2.5 samples, majority of the red fluorescence present

within the cellular nucleus. The findings demonstrate that the UCNP-2.5 nanospheres promote the delivery of DOX to cell nuclei after cell internalization. To further verify the nuclear delivery behavior of UCNP-2.5 nanospheres, Fluorescein isothiocyanate (FITC, Nanjing Search Biotech Co., Ltd) labeled UCNP-2.5, UCNP-4 and UCNP-5 nanospheres without DOX loaded were incubated in MCF cells, and the intracellular distribution were studied. After internalization by MCF cells, high concentration of UCNP-2.5 nanospheres is distributed in both the nucleus and cytoplasm. In contrast, the UCNP-4 and UCNP-5 nanospheres mainly present in the cytoplasm with low contents of nanospheres shown in the nucleus. (Fig S8) Therefore, all nanospheres were successfully internalized by tumor cells, and drug release mainly occurred within the cells. One notable fact is that certain degree of drug content may also be internalized via a diffusion mechanism due to the ineluctable spontaneous DOX releasing from the nanospheres synthesized in this work. In addition, the findings have provided a direct evidence of the effective nuclear DOX delivery of UCNP-2.5 nanospheres synthesized. Various studies have reported that the dimension of drug delivery particles is an important parameter may play a vital role in influencing the rate of cellular uptaking effect.^{45, 48} The nuclear delivery of antitumor drugs is of great potential which is expected to kill tumor cell with enhanced efficacy.^{49, 50} Therefore, the $\text{CaF}_2:\text{Yb}^{3+}, \text{TM}^{3+}@\text{SiO}_2$ nanospheres synthesized in the study are anticipated to be highly advantageous for anti-cancer therapy with enhanced efficiency.

Conclusions

In this study, a new synthesis strategy, namely chemical-assisted sol-gel *in-situ* growth method, was successfully developed for inducing the formation of $\text{CaF}_2:\text{TM}^{3+}, \text{Yb}^{3+}$ nanocrystals within the mesopore channels of MSNs. A series of upconversion crystalline $\text{CaF}_2:\text{Yb}^{3+}, \text{TM}^{3+}@\text{mSiO}_2$ nanospheres with well-controlled dimensions (from ~65 nm to ~290 nm) and microstructures was synthesized for anti-cancer drug delivery. The main forming mechanism follows the “trifluoroacetate process”. It was found that the microstructural characteristics of UCNP can be effectively tuned via controlling the ethanol/TEOS volume ratios (2.5, 4, and 5) during the synthesis. Owing to its high surface area and pore volume, UCNP-2.5 samples synthesized presented the highest drug loading efficiency and most sustained release kinetics. The variation of $I_{\text{blue}}/I_{\text{red}}$ ratio responded well with DOX releasing progress due to luminescence resonance energy transfer (LRET) effect constructed between DOX molecules and UCNP. Fast releasing kinetics induces rapid increase of $I_{\text{blue}}/I_{\text{red}}$ ratio, and vice versa. More importantly, the *in-vitro* study using MCF-7 human breast cancer cells further demonstrated that DOX-loaded UCNP-2.5 nanospheres presented the strongest *in-vitro* anticancer efficacy due to its enhanced ability of nuclear DOX delivery induced by the size effect. Although more explorations including systematic *in-vivo* study are still ahead before its clinical trials, this study has offered another new facile avenue to synthesizing upconversion luminescent nanocargoes for tumor diagnosis and therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

SEM images of (a) UCNP-2.5 (b) UCNP-4 (c) UCNP-5 (the inserts are the corresponding UCNPs in aqueous solution); TEM images of UC nanoparticles obtained at different Ethanol/TEOS volume ratio: (d) UCNP-2.5, (e) UCNP-4, and (f) UCNP-5; (g) XRD patterns, (h) UC PL spectra under 980 nm excitation of UCNP-2.5, UCNP-4, UCNP-5. (i) Relative cell viability of 293 cells incubated with UCNP-2.5, UCNP-4, UCNP-5 with different concentrations for 24 hours.

Figure 2.
 (a) UV-Vis absorbance spectra of DOX loaded particles. (b) DOX drug loading efficiency and (c) The UC PL emission spectra of particles before and after DOX drug loading (the insets show the corresponding optical images of UCNPs captured by digital camera). (d) The variation of UC PL emission intensity of I_{blue}/I_{red} before and after DOX drug loading

Figure 3.

(a) Cumulative DOX release profiles of particles. (b) The variation of UC PL emission intensity of $I_{\text{blue}}/I_{\text{red}}$ as a function of release time for DOX loaded UCNP-4 and UCNP-2.5 samples. *In vitro* cell viability of MCF-7 human breast cancer cells treated with free DOX, UCNP-2.5-DOX, UCNP-4-DOX and UCNP-5-DOX for (c) 24 hours, (d) 48 hours, and (f) 72 hours.

Figure 4. LSCM images of MCF-7 incubated with (a) UCNP-2.5-DOX, (b) UCNP-4- DOX and (c) UCNP-5-DOX for 4 hours. 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. The scale bar: 20 μm .

Table 1

Textural Properties of particle series

Particles series	Surface Area (m ² /g)	Pore Volume (cm ⁻³ /g)	Pore Size (nm)
UCNP-2.5	~470	~0.24	~4.3
UCNP-4	~390	~0.16	~3.4
UCNP-5	~430	~0.10	~2.7