Electronic Supplementary Material (ESI) for RSC Advances

Supporting information

TWEEN coated $NaYF_4$:Yb,Er/ $NaYF_4$ core/shell upconversion nanoparticles for bioimaging and drug delivery.

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Experimental details

Materials:

$LnCl_3 \cdot xH_2O$ (Ln=Y, Yb, Er; x ≈ 5, 99.90%), ammonium fluoride (NH_4F, 96%), 1-octadecylen (ODE) and oleic acid (OA) were purchased from Alfa Aesar Reagent Company. TWEEN serials (TWEEN 20, 40, 60 and 80) were all supplied by Sigma-Aldrich Reagent Company. Doxorubicin (DOX) was purchased from Beijing HuaFeng United Technology CO., Ltd. NaOH, ethanol, methanol and cyclohexane were obtained from Beijing Chemical Reagent Company. All chemicals were of analytical and used without further purification.

Characterization:

Powder X-ray diffraction (XRD) measurement was performed with a Japan Rigaku D/max-2500 diffractometer with $Cu_{k\alpha}$ radiation (λ=1.5418 Å). The transmission electron microscope (TEM) images were obtained from an FEI Tecnai G^2 20 S-TWIN instrument operated at 200 kV. Fourier Transform infrared (FT-IR) spectra were measured on a Fourier transform Bruker EQUINOX55 spectrometer using the KBr pellet technique. The upconversion luminescent properties were studied using a Horiba Jobin Yvon FluoroLog3 spectrometer equipped with a 980 nm CW laser as excitation. The daylight and luminescent photographs were taken with a Nikon D3100 digital camera. The quantification of cell viability was determined using an ELISA microplate reader (Spectra Max M2, USA) at an optical absorbance of 450 nm. The *in vitro* optical cell imaging was taken under 980 nm NIR laser excitation using an inverted fluorescence microscope (Olympus IX71). The UV-vis absorption was performed on a spectrophotometer (TU-1901, Persee).

Synthesis of $NaYF_4$:Yb,Er and core/shell structured $NaYF_4$:Yb,Er/$NaYF_4$ UCNPs:

The synthesis of core precursor $NaYF_4$:Yb,Er (18, 2 mol %) UCNPs was carried out following a literature protocol with a slight modifications.[1] In a typical procedure, 0.8 mmol of YCl_3, 0.18 mmol of $YbCl_3$ and 0.02 mmol of $ErCl_3$ were mixed in a 50 mL flask and completely dissolved in 2 mL of methanol with the assistance of sonication. Then, 6 mL of oleic acid and 15 mL of 1-octadecene (ODE) were added

and the mixture was stirred for 10 minutes to form a homogeneous solution. After slowly adding 5 mL of methanol solution containing NaOH (2.5 mmol) and NH_4F (4 mmol) into the flask, the solution was heated at 100 °C to evaporate methanol, degassed and then maintained at 300 °C for 1 h under argon protection. After cooling to room temperature, 10 mL of the medium solution was first collected as the core precursors for the later fabrication of core-shell $NaYF_4$:Yb,Er/$NaYF_4$ UCNPs and the residual solution was disposed with ethanol to precipitate the nanoparticles, which were then collected after centrifugation and washed with ethanol for three times.

For the synthesis of core/shell structured $NaYF_4$:Yb,Er/$NaYF_4$ UCNPs, 1 mmol of YCl_3, 2.5 mmol of NaOH and 4 mmol of NH_4F were dissolved in 5 mL of methanol and slowly added into the collected solution mentioned above, which had the core precursor. After a homogeneous solution was formed under stirring at room temperature, the solution was heated to 100 °C and stirred for 30 min to remove methanol. Then, the system was degassed and maintained at 330 °C for 1 h under argon protection. The core/shell UCNPs were obtained by centrifugation and washed for three times with ethanol when the system cooled to room temperature.

TWEEN modification of core/shell UCNPs:

In a typical experiment, 50-100 μL of TWEEN 80 was added into a 25 mL flask containing 5 mg of core/shell structured $NaYF_4$:Yb,Er/$NaYF_4$ UCNPs and 2 mL of $CHCl_3$, and the solution was stirred for 1 h at room temperature. Then, 10 mL of deionized water were poured in the flask and the dispersion was kept in a 70 °C water bath for 3 h. During this period, the $CHCl_3$ was evaporated and these UCNPs were gradually transferred into the hydrophilic system. The TWEEN coated UCNPs (TWEEN-UCNPs) were obtained by centrifugation. It is worth noting that using other TWEEN series (TWEEN 20，TWEEN 40 and TWEEN 60) for coating UCNPs could get similar results with TWEEN 80.

Drug storage and release

The drug storage and in vitro release experiments were performed according to our previous report.[2] Typically, the loading of DOX on the TWEEN-UCNPs was carried out by impregnating 25 mg of TWEEN-UCNPs in a 5 mL solution of DOX in

phosphate buffer solution (PBS, pH = 7.4) under stirring for 24 h at room temperature. For the DOX loading saturation experiment, different concentrations (50 ~ 500 μM) of DOX were mixed with TWEEN-UCNPs for 24 h at room temperature. The DOX attached TWEEN-UCNPs (DOX@TWEEN-UCNPs) were collected by centrifugation at 14000 rpm for 10 minutes washed with PBS for 3 times and then dried at 60 °C for 8 h. The DOX loading amount was determined by the absorbance change of the characteristic DOX absorption peak at 480 nm between the original and final DOX solution under UV-vis measurement.

To assess the *in vitro* drug release property, 20 mg of dried DOX@TWEEN-UCNPs and 20 mL of PBS (pH = 5.0 or 7.4) were mixed in a round flask. The flask was placed in a 37 °C water bath and continuously stirred. At certain time intervals, 4 mL of the release medium was taken out to determine the released drug concentration under UV-vis measurement and then was returned to the original release medium.

In vitro cell viability tests:

The *in vitro* cell viability of TWEEN-UCNPs at various concentrations (6.25, 12.5, 25, 50, 100 and 200 μg/mL) were determined using a cell counting kit-8 (CCK-8, Dojindo Laboratories in Japan) assay in the human lung cancer cells (A-549). The cells were seeded into a 96-well plate (about 1000 cells/well) and incubated at 37 °C and 5% CO_2 for 24 h. Then, the TWEEN-UCNPs dispersed in DMEM were added to the wells and these cells were subsequently incubated for another 24 h at 37 °C. The wells without TWEEN-UCNPs were regarded as control. After that, 100 μL/well of CCK-8/DMEM solution (volume ratio = 1:10) was added, the plate was put into incubator for additional 2 h and the absorbance was measured using SpectraMax M2 (MDC，USA) at 450 nm. The complete assay was performed six times, and the results were averaged. The cell viability was calculated as the ratio of the sample well absorbance to the control well absorbance and expressed as a percentage.

For DOX cell killing ability estimation, A-549 cells seeded in the 96-well plate for 24h were incubated with series concentrations of free DOX or DOX@TWEEN-UCNPs for 1 h and then changed to CCK-8/DMEM solution (volume

ratio = 1:10) after washing. After further 2 h incubation, the absorbance was measured to determine the cell viability.

Cell imaging:

Human cervix adenocarcinoma cell lines (HeLa) were incubated for 24 h on quartz-bottom dishes. Then the cells were incubated with TWEEN-UCNPs (50 μg/mL, dispersed in DMEM) for 2 h (37 °C under 5% CO_2). The unbound nanoparticles were washed out with PBS. After that, the cells incubated with TWEEN-UCNPs were imaged in bright field and under NIR excitation using the inverted fluorescence microscope (Olympus IX71) equipped with a 980 nm NIR laser.

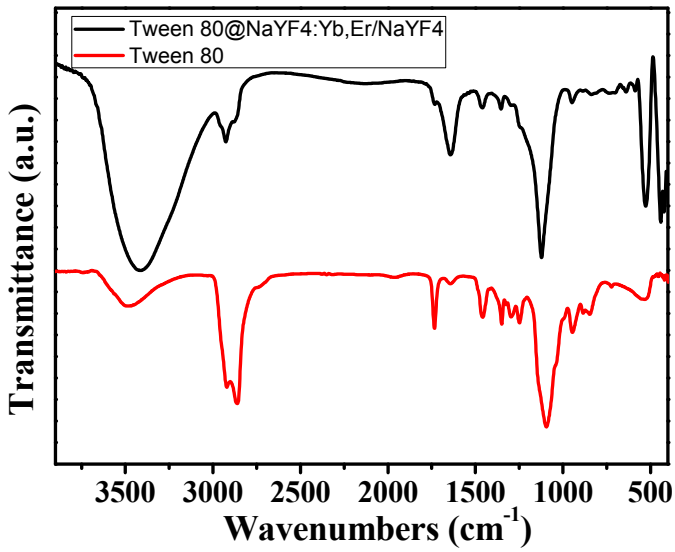
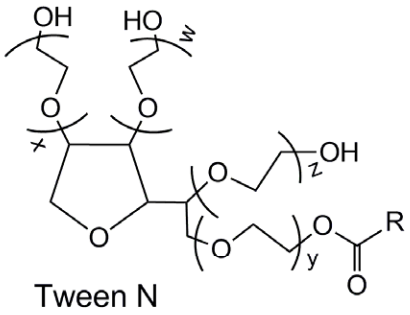


Figure S1. FTIR spectra (a) TWEEN 80 coated UCNPs and (b) pure TWEEN 80

There are some characteristic absorption bands which could confirm the presence of TWEEN 80 on the surface of nanoparticles. The bands centered at 2938 and 2869 cm^{-1} are associated with the asymmetric (ν_{as}) and symmetric (ν_s) stretching vibrations of methylene (-CH_2), respectively. The band at 1730 cm^{-1} originates from the C=O stretching of the ester group. The strong band around 3462 cm^{-1} can be attributed to the O-H stretching vibrations.



x+y+z+w=20. N=20,40,60 and 80

Tween 20: R= $C_{11}H_{23}$; Tween 40: R= $C_{15}H_{31}$;
Tween 60: R= $C_{17}H_{35}$; Tween 80: R= $C_7H_{14}CH{=}CHC_8H_{17}$

Figure S2. Chemical structure of TWEEN series compounds. Depending on the type of their fatty-acid tail, they are commercially named as TWEEN 20, TWEEN 40, TWEEN 60 and TWEEN 80.

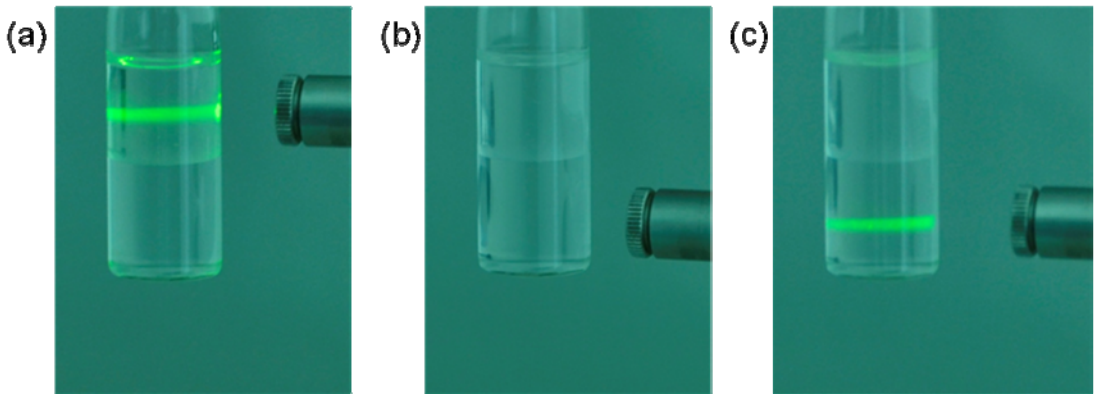


Figure S3. Photographs of phase transfer of UCNPs from cyclohexane to water upon addition of TWEEN 80. The top layer in a, b and c is cyclohexane, while the bottom layer in each image is water containing TWEEN 80.

Before shaking the bottle, as shown in Figure S3a and S3b, the OA capped UCNPs only dispersed in cyclohexane layer. While after gently shaking the bottle for several time, the OA capped UCNPs could be transferred from cyclohexane to water that contained TWEEN 80. (Figure S3c) This result further proves that TWEEN could draw the hydrophobic UCNPs into water.

References:

1 R. A. Jalil and Y. Zhang, *Biomaterials*, 2008, **29**, 4122–4128.

2 G. Tian, Z. J. Gu, L. J. Zhou, W. Y. Yin, X. X. Liu, L. Yan, S. Jin, W. L. Ren, G. M. Xing, S. J. Li and Y. L. Zhao, *Adv. Mater.*, 2012, **24**, 1226–1231.