Synthesis of Lenvatinib-Loaded Upconversion@Polydopamine Nanocomposites for Upconversion Luminescence Imaging-Guided Chemo-Photothermal Synergistic Therapy of Anaplastic Thyroid Cancer

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Contents

1. Additional Experimental Section

1.1 Materials and Regents

Rare-earth oxides including erbium oxide (Er_2O_3, 99.99%), titanium trioxide (Tm_2O_3, 99.99%), ytterbium oxide (Yb_2O_3, 99.99%), yttrium oxide (Y_2O_3, 99.99%) and neodymium oxide (Nd_2O_3, 99.99%) were obtained from Alfa Aesar. (Ward Hill, USA). The Er_2O_3, Tm_2O_3, Yb_2O_3, Y_2O_3 and Nd_2O_3 were reacted with excess hydrochloric acid (50% v/v) to form rare-earth chloride compounds, respectively. Oleic acid (OA, 90%), 1-Octadecene (ODE, 90%), cyclohexane, Igepal CO-520 and ammonium hydroxide were obtained from Sigma-Aldrich Co. (St Louis, USA). Dopamine hydrochloride and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were received from Beijing Dingguo Biotechnology Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO) was purchased from Beijing Chemical Reagents Company (Beijing, China). Lenvatinib and Margital were obtained from Changchun Baijin Biotechnology Company. (Changchun, China). Fetal bovine serum (FBS), RMPI-1640 medium, penicillin-streptomycin Solution (100×) and trypsin solution (0.25%) were obtained from Gibco. (New York, USA). Calcein acetoxymethyl ester (Calcein-AM) and propidium iodide (PI) were purchased from Dalian Meilun Biotechnology Ltd. (Dalian, China). C643 cell lines were purchased from Shanghai Cell Bank, CAS (Shanghai, China). The other reagents were obtained from Beijing Chemical Reagents Company (Beijing, China). NOD-SCID mice (female) were purchased from Vital River Company (Beijing, China). All chemicals were analytical grade and used as received without further purification. Milli-Q water (18.2 MΩ cm)

was used in all experiments.

1.2 Characterization

Transmission electron microscope (TEM) images were recorded by a Hitachi H-600 electron microscope with an acceleration voltage of 100 kV (Hitachi Ltd., Tokyo, Japan). The UV-vis spectra of samples were measured by TU-1901 double light beam ultraviolet obvious spectrophotometer (Purkinje General Co., Beijing, China). The MTT assay was measured using a Versamax microplate reader (Bio-Tek Instruments, Inc., USA). The upconversion luminescence (UCL) spectra were recorded on an F-4500 spectrophotometer (Hitachi Co., Japan) equipped with a commercial continuous-wave (CW) NIR laser (BWT Beijing Ltd., China) as exciting wavelength. The UCL imaging of cells was performed with a reconstructive Ti-S fluorescent microscope (Nikon Co., Japan) equipped with a 980 nm CW NIR laser (BWT Beijing Ltd., China). The UCL imaging of tumors were recorded by a home-made in vivo UCL imaging system which included a M2590 (GenieTM Nano Cameras, Teledyne DALSA, Waterloo, Canada) and a 808 nm/980 nm CW NIR laser. All UCL images were analyzed by Image J software.

1.3 Synthesis of $NaErF_4:Tm^{3+}@NaYbF_4@NaYF_4:Nd^{3+}$ UCNP

The $NaErF_4:Tm^{3+}$ UCNP was synthesized by a previously reported method with a little modification.[S1] Basically, Er_2O_3 and Tm_2O_3 were reacted with excess HCl, dried by heating and dissolved in water. The obtained $ErCl_3$ (1 mmol, 1.99 mL) and $TmCl_3$ (0.1 mmol, 0.1 mL) were injected into a 100 mL flask, mixed well and dried by heating. Then, 6 mL oleic acid and 15 mL 1-octadecene were added into the flask,

and the mixture was heated to 150 ℃ under argon atmosphere for 30 min. After cooled down to room temperature, 10 mL methanol solution containing NH_4F (4 mmol) and NaOH (2.5 mmol) was added into the mixture, and continuously stirred for 60 minutes and gradually heated to 70℃ to remove methanol. Subsequently, the solution was heated to 300 ℃ and maintained at this temperature under an argon flow with vigorous stirring for 60 min. After cooled down to room temperature, the solution was collected by centrifugation (10000 rpm, 10 min) and washed with ethanol (10 mL, three times). Finally, the as-prapared $NaErF_4:Tm^{3+}$ UCNP was redispersed in 10 mL cyclohexane.

For the Synthesis of $NaErF_4:Tm^{3+}@NaYbF_4$ UCNP, the obtained $YbCl_3$ (1 mmol, 1 mL) was injected into a 100 mL flask, mixed well and dried by heating. Then 4 mL oleic acid and 16 mL 1-octadecene were added and the mixture was heated to 150℃ under argon atmosphere for 30 min. After cooled down to 80 ℃, 1 mmol as-prepared $NaErF_4:Tm^{3+}$ UCNP was added into the mixture, and stirred for 30 min to remove cyclohexane. After cooled down to 50℃, 10 mL methanol solution containing NH_4F (1.375 mmol) and NaOH (1.25 mmol) was added and stirred for 60 min. Then the temperature of the solution was slowly increased to 280℃ under argon atmosphere and maintained at this temperature for 60 min. After cooled down to room temperature, the solution was collected by centrifugation (10000 rpm, 10 min) and washed with ethanol (10 mL, three times). The as-prapared $NaErF_4:Tm^{3+}@NaYbF_4$ UCNP was redispersed in 10 mL cyclohexane.

For the synthesis of $NaErF_4:Tm^{3+}@NaYbF_4@NaYF_4:Nd^{3+}$ UCNP, the growth process of $NaYF_4$: 7% Nd outer shell was the same as that for the $NaErF_4:Tm^{3+}@NaYbF_4$ shell, except that 1 mmol YCl_3 and 1 mol $NdCl_3$ were first dried and mixed with oleic acid and 1-octadecene. The purified $NaErF_4:Tm^{3+}@NaYbF_4@NaYF_4:Nd^{3+}$ UCNP was also redispersed in cyclohexane for further experiments.

1.4 Calculation of the Photothermal Conversion Efficiency

Following Roper's report [S2], the photothermal conversion efficiency (η) was calculated according to the following equation

$$\eta = \frac{hs(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10)^{(-A_{808})}}$$

where h is the heat transfer coefficient, s is the surface area of the container, T_{max} is the maximum steady-state temperature of the sample solution, T_{surr} is the ambient surrounding temperature, I is the laser power, and A_{808} is the absorbance of the sample solution at 808 nm. Q_{dis} represents the heat dissipation from the light absorbed by the water and container, which is measured independently. To calculate hs, another equation is introduced

$$\mathrm{hs} = \frac{mC_{water}}{\tau_s}$$

where m is the mass of sample, C_{water} is the heat capacity of water (4.2 J g^{-1} k^{-1}), and τ_s is the sample system time constant.

1.5 Release of LEN on UCNP@PDA

To evaluate the releasing of LEN with or without laser irradiation, UCNP@PDA@LEN was sealed in a dialysis bag with 8000-14000 molecular weight cut-off and submerged into 3 mL PBS (pH 5.0) with gentle shaking. At different time points, the dialysis buffer containing LEN was collected and replaced with fresh PBS with an equal volume. The absorption value of free LEN was recorded by UV-visible spectroscopy. The amount of free LEN was calculated by the standard curve of LEN. For 808 nm NIR-laser triggered LEN release, 1 mg UCNP@PDA@LEN were resuspended in 1 mL PBS (pH 5.0) with gentle shaking. At predetermined time intervals, the solutions were irradiated with an 808 nm NIR-laser (1.8 W cm^{-2}) for 10 min. The dialysis buffer was collected for measuring the releasing amount of LEN before and after irradiation with 808 nm NIR-laser, respectively.

1.6 In Vivo Imaging

The in vivo UCL imaging was performed at the indicated time points of post-irradiation on a home-made in vivo imaging system equipped with 980 nm laser.

1.7 In Vivo Toxicology Analysis

For histology analysis, 200 μL NaCl solutions (0.9 wt%) containing 5 mg mL^{-1} UCNP@PDA and 5 mg mL^{-1} UCNP@PDA@LEN were injected intravenously into the C643 tumor-bearing mice through the tail vein, respectively. After injection for 24 h and 30 days, the blood samples were taken from the orbit of mice and sent for routine blood examination. Then the main organs of C643 tumor-bearing mice including heart, liver, spleen, lung and kidney were collected, fixed with 4%

polyformaldehyde and then sectioned. The hematoxylin and eosin (H&E) staining of organs was performed and observed through microscope.

2. Additional Figures S1-S11.

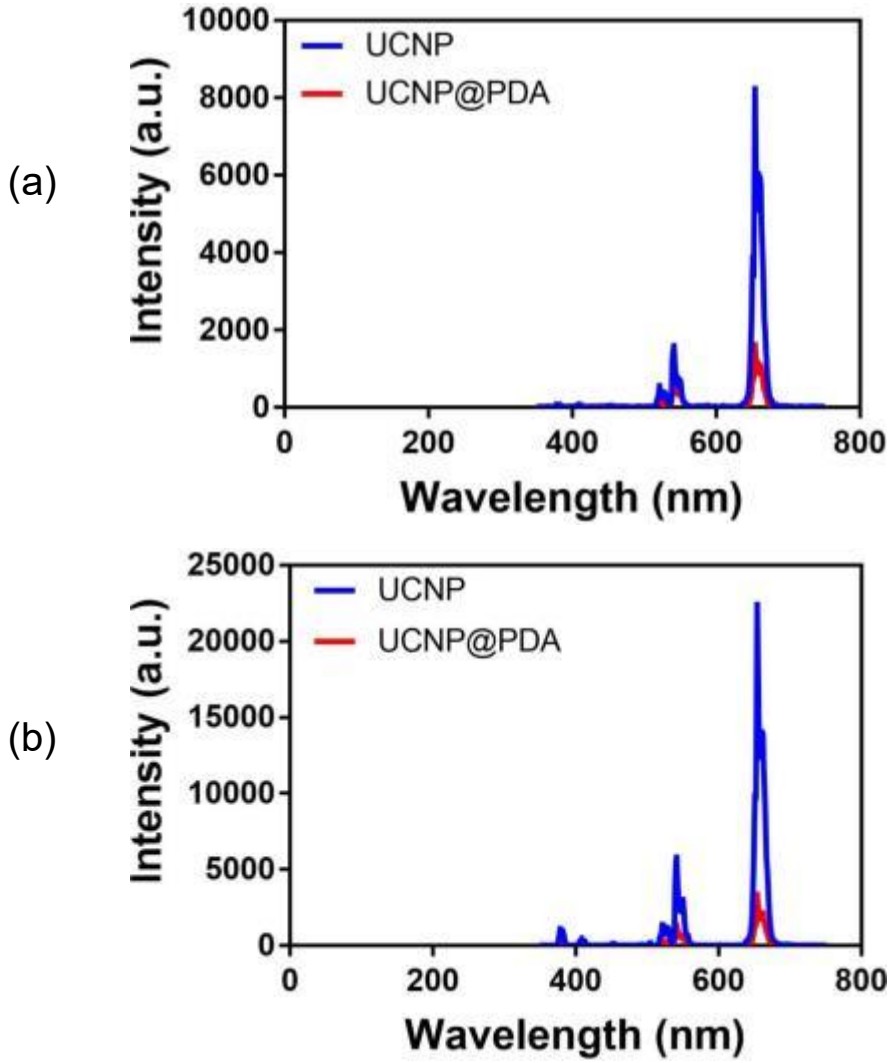

Fig. S1. The UCL spectra of UCNP and UCNP@PDA under (a) 808 nm and (b) 980 nm laser irradiation.

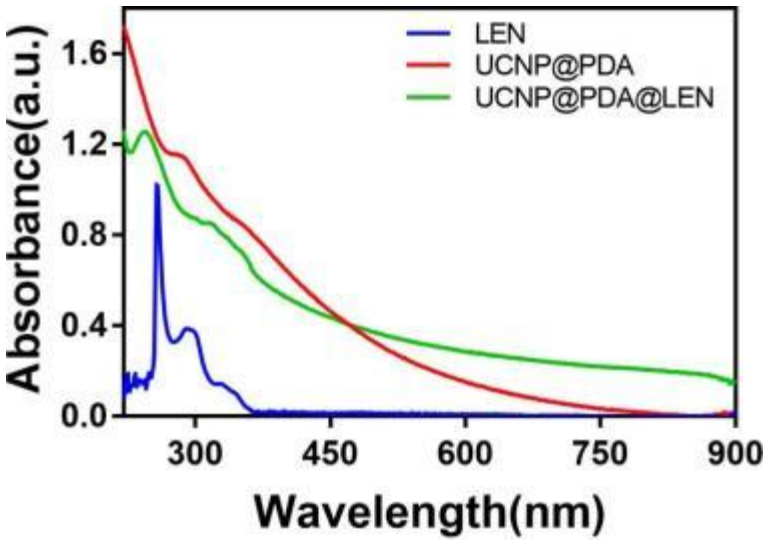

Fig. S2. The UV-Visible spectra of LEN, UCNP@PDA and UCNP@PDA@LEN, respectively.

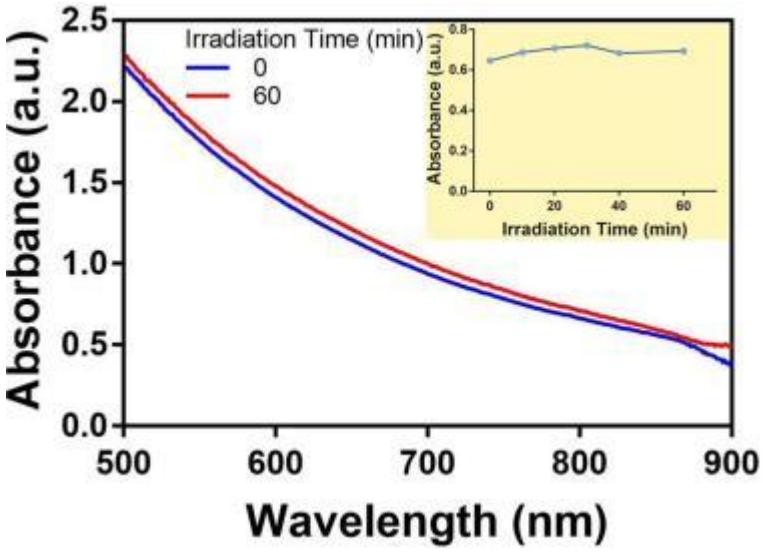

Fig. S3. The UV-Visble spectra of UCNP@PDA solution (5 mg mL^{-1}) before and after irradiated by 808 nm NIR-laser for 60 min (1.8 W cm^{-2}), respectively. The inset is the absorbance of UCNP@PDA at 808 nm as a function of 808 nm NIR-laser irradiation time.

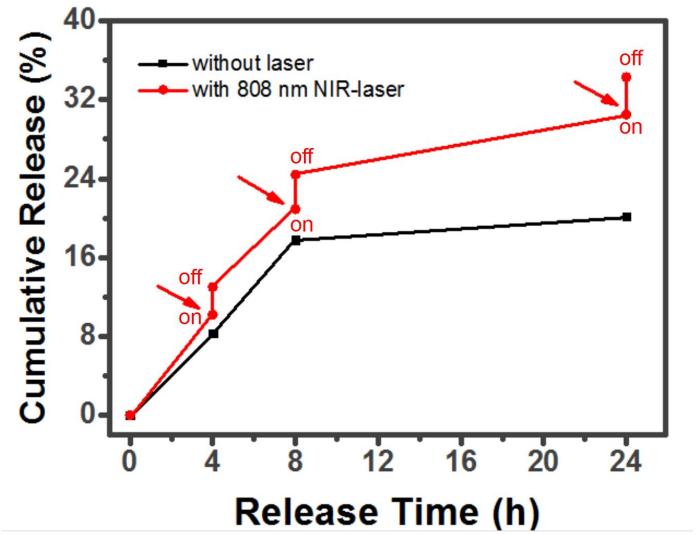

Fig. S4. Release profiles of LEN from UCNP@PDA @LEN at pH 5.0 with or without 808 nm NIR-laser irradiation (1.8 W cm^{-2}) at predetermined time points indicated by arrows.

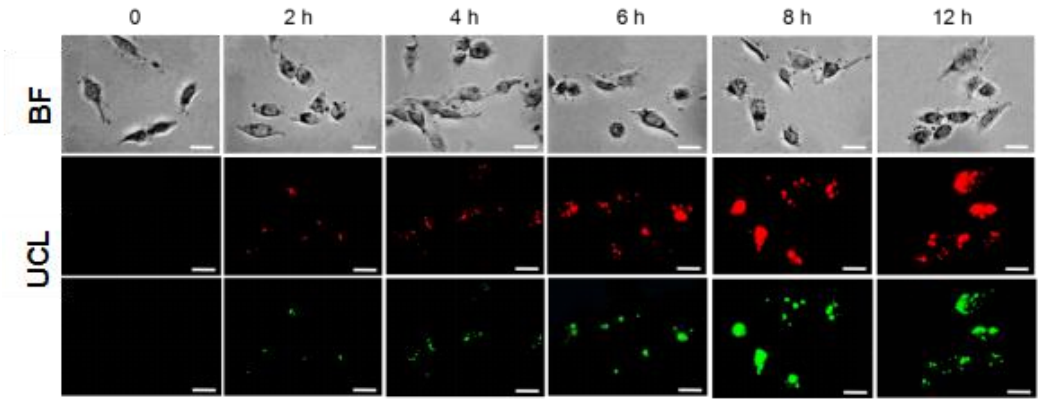

Fig. S5. The UCL images of C643 cells after co-cultured with UCNP@PDA at different times. Scale bars: 20 μm.

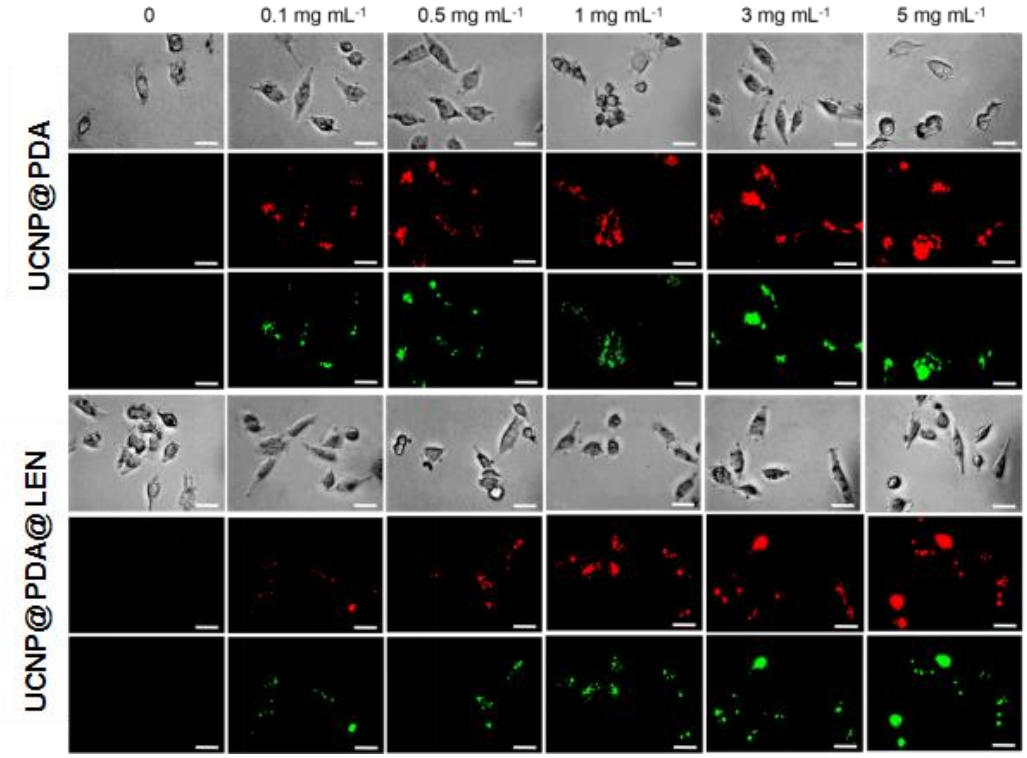

Fig. S6. The UCL images of C643 cells after incubated with various concentrations of (a) UCNP@PDA and (b) UCNP@PDA@LEN, respectively. Scale bars: 20 μm.

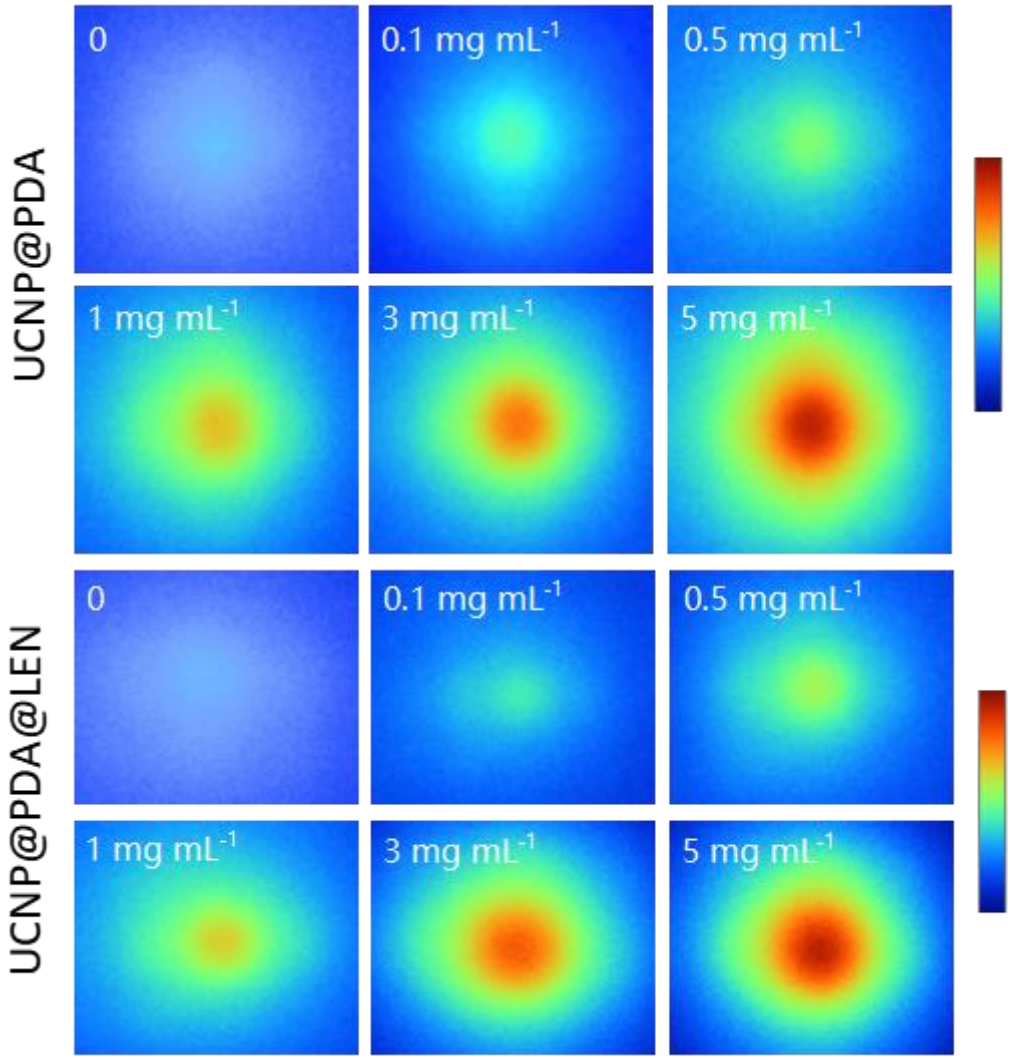

Fig. S7. The Infrared thermal images of UCNP@PDA and UCNP@PDA@LEN stained C643 cells under 808 nm NIR-laser irradiation (1.8 W cm^{-2}) for 10 min. Before NIR-laser irradiation, the C643 cells were co-cultured with different concentrations of UCNP@PDA and UCNP@PDA@LEN for 24 h, then washed with PBS, and re-cultured in fresh RMPI-1640.

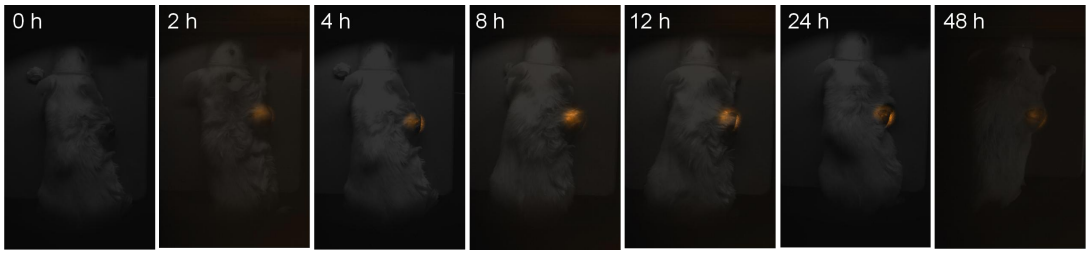

Fig. S8. The UCL images of C643 tumor-bearing mice at different time intervals (0, 4, 8, 12, 24 and 48 h) after injection of UCNP@PDA@LEN. The UCNP@PDA@LEN (5 mg mL^{-1}) were intravenously injected into C643 tumor-bearing mice through tail vein. The tumor sites were irradiated by 980 nm NIR-laser (2.5 W cm^{-2}).

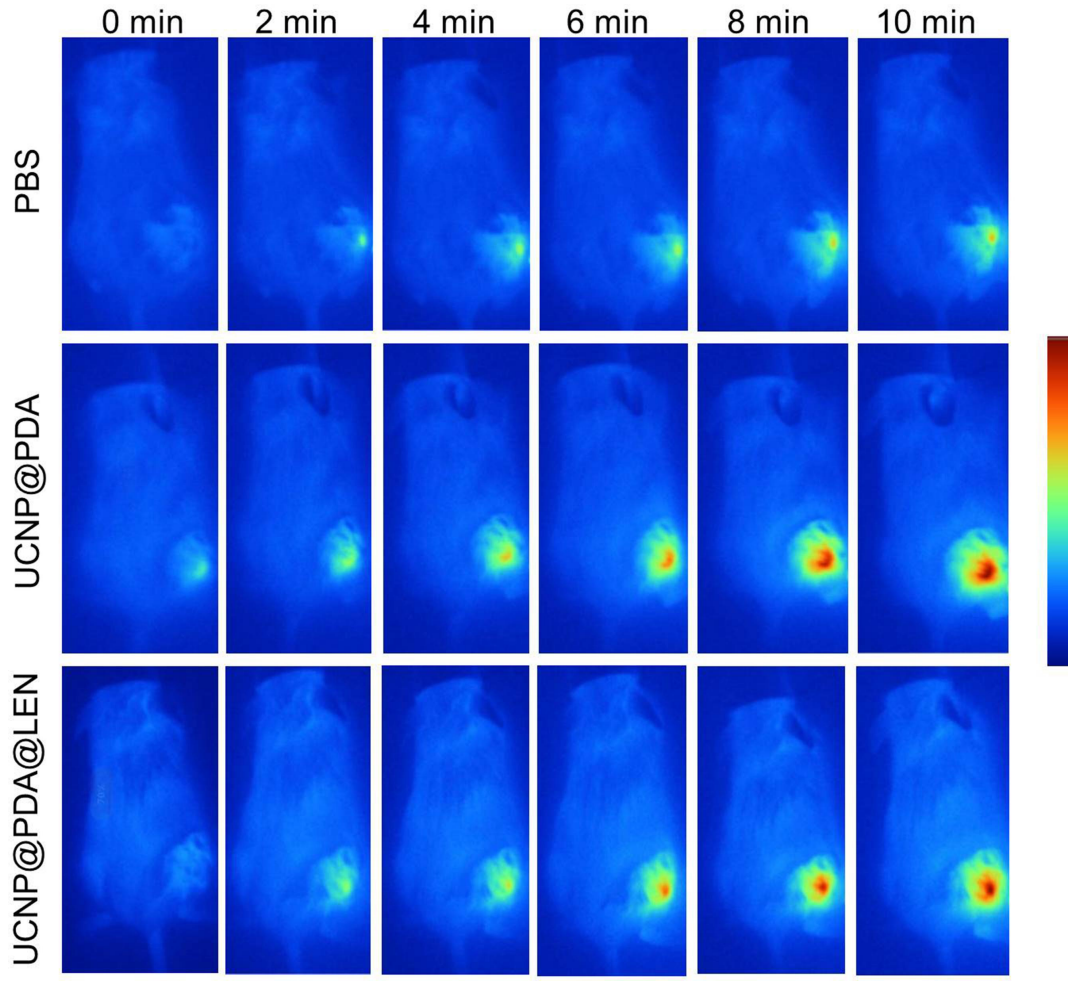

Fig. S9. The Infrared thermal images of tumor sites of C643 tumor-bearing mice after 8 h intravenous injection of PBS, UCNP@PDA and UCNP@PDA@LEN. The mice were irradiated by 808 nm NIR-laser irradiation (1.8 W cm^{-2}) for different time points (0, 2, 4, 6, 8 and10 min).

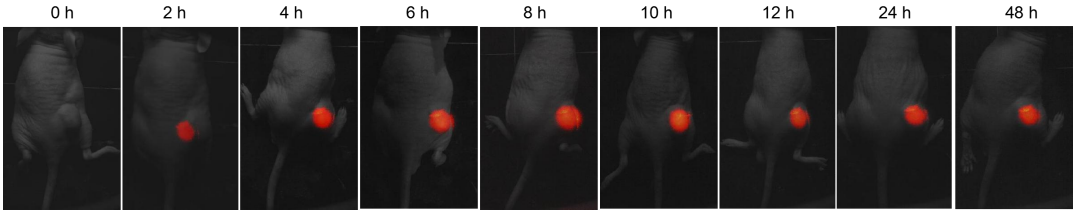

Fig. S10. The thermal images of C643 tumor-bearing mice after intravenous injection of UCNP@PDA at different time intervals (0, 2, 4, 6, 8, 10, 12, 24 and 48 h post-injection) under the 808 nm NIR-laser irradiation (1.8 W cm^{-2}) for 10 min.

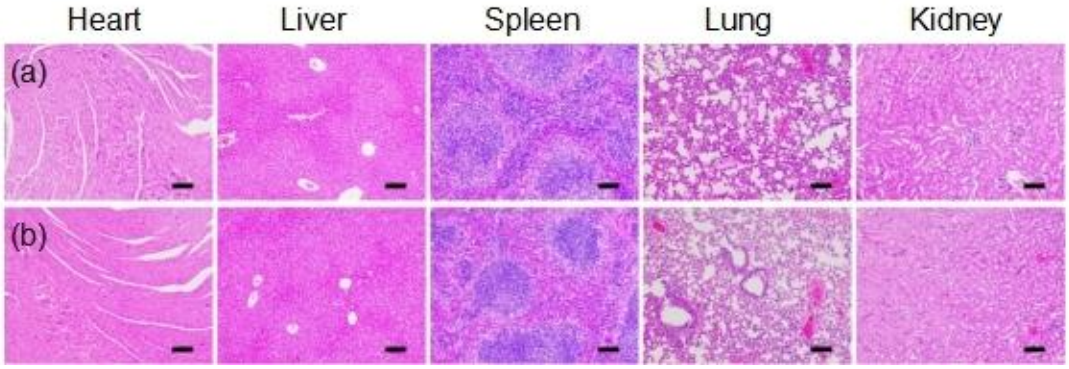

Fig. S11. Histological analysis of (a) healthy mouse (PBS) and (b) UCNP@PDA@LEN treated mouse after 30 days post-injection, respectively. Scale bars:100 μm.

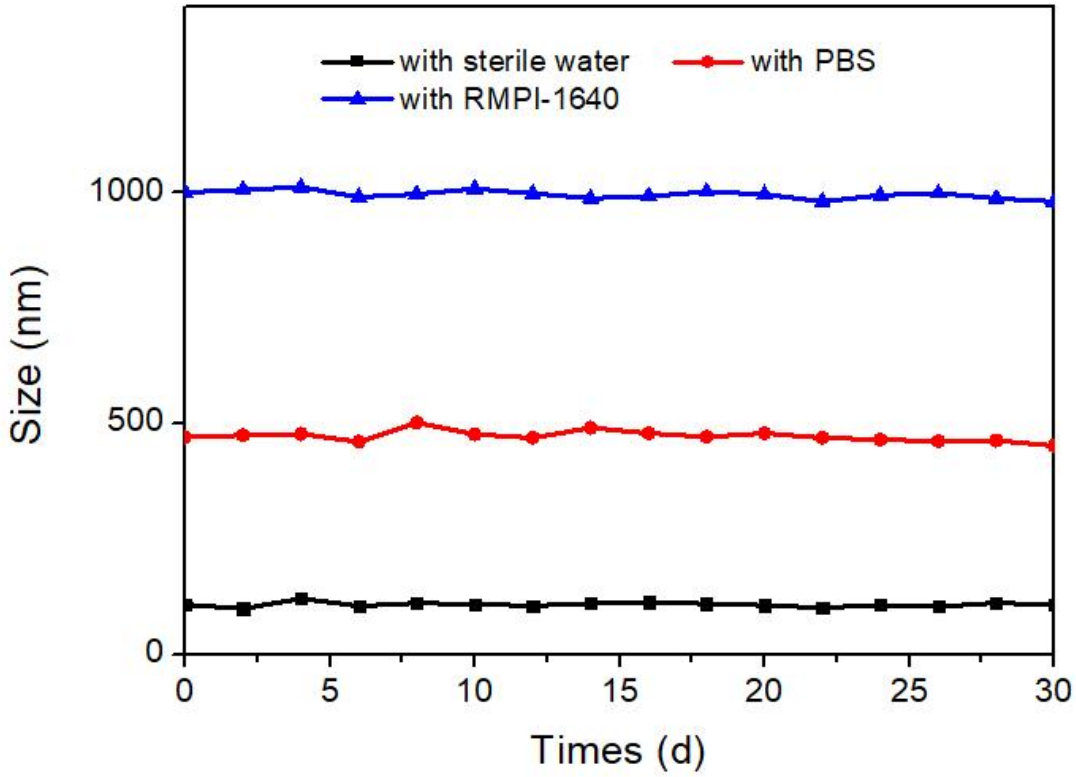

Fig. S12. The particle size change of UCNP@PDA@LEN co-cultured with sterile water, PBS, and RMPI-1640 for 30d, respectively.

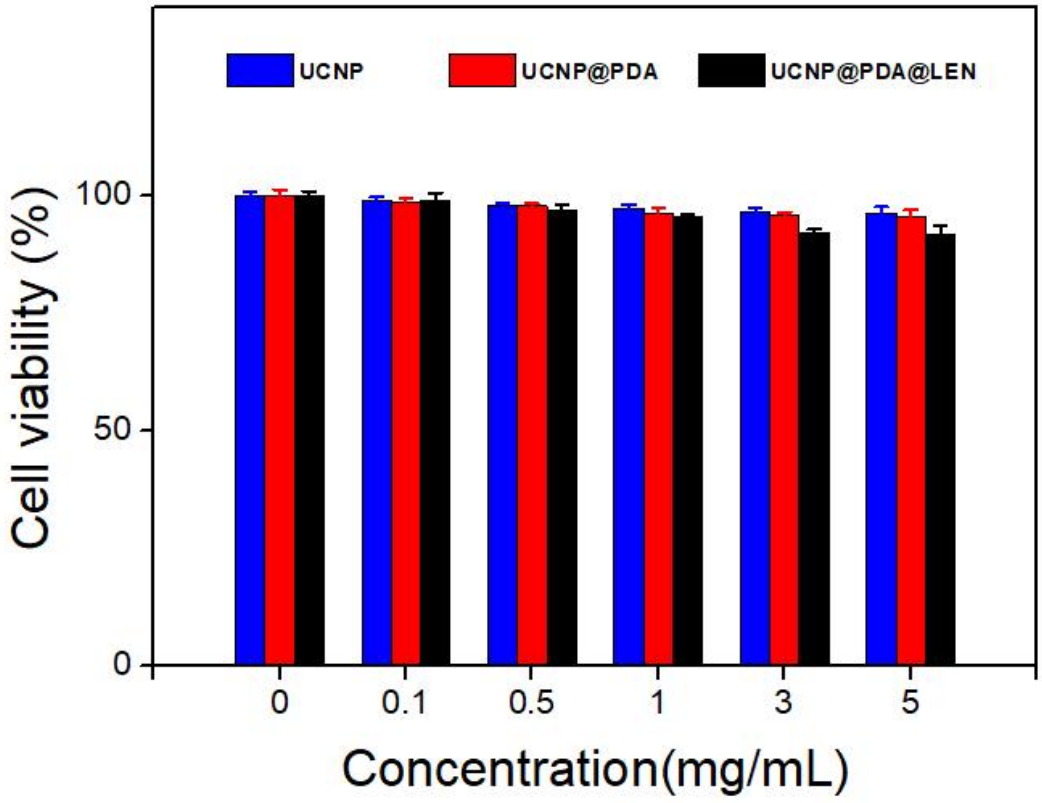

Fig. S13. The cell viabilities of normal thyroid cells after co-cultured with UCNP, UCNP@PDA and UCNP@PDA@LEN, respectively.

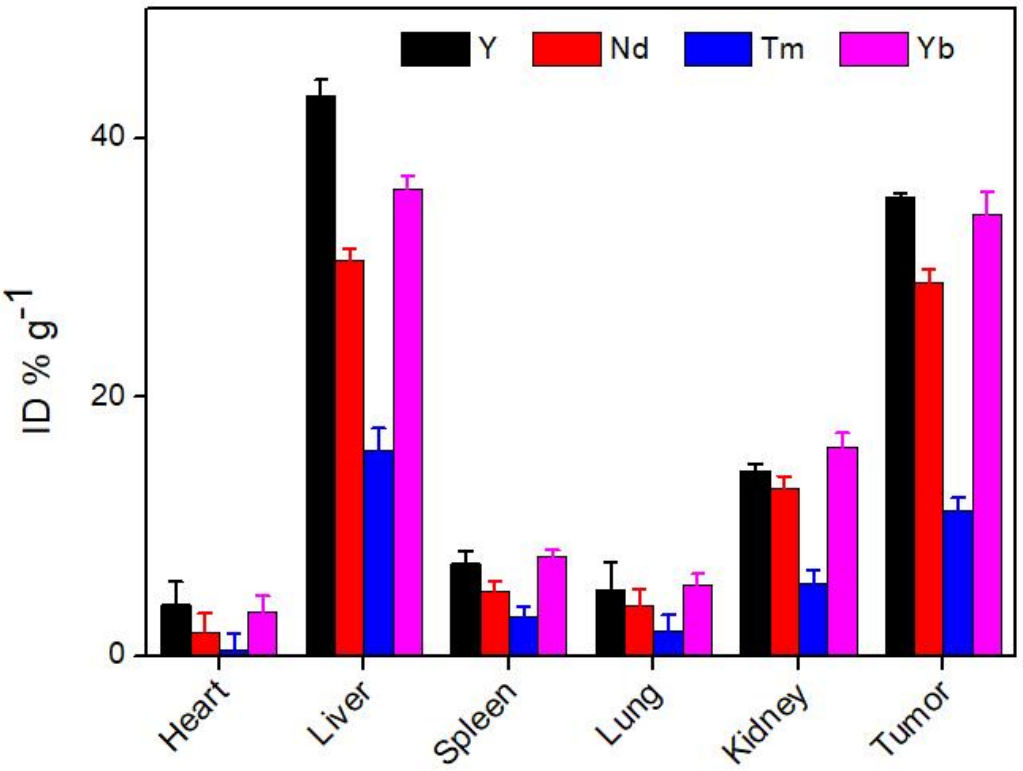

Fig. S14. ICP-AES analysis of Y, Nd, Tm and Yb element in major organs of C643 tumor-bearing mice at 8 h post injection of UCNP@PDA@LEN.

3. Additional Table S1

Table S1. Hematology analysis of health mice treated with PBS, UCNP@PDA and UCNP@PDA@LEN at 30 days post-injection.

Hematological	Units	PBS	UCNP@PDA	UCNP@PDA @lenvatinib
WBC	$x10^9/L$	2.8±0.22	3.7±0.34	1.2±0.29
LY	$x10^9/L$	2.5±0.17	2.9±0.21	0.9±0.20
GR	$x10^9/L$	0.1±1.38	0.1±1.78	0.1±1.80
RBC	$x10^{12}/L$	8.71±1.56	9.61±2.01	10.70±1.92
HGB	g/L	178±0.71	193±1.20	186±1.53
HCT	%	59.9±2.14	65.0±2.54	60.7±2.62
MCV	fL	68.8±1.25	67.7±1.68	67.1±1.39
MCH	pg	20.4±1.74	20.0±1.02	21.7±1.57
MCHC	g/L	297±7.23	295±8.19	323±8.27
PLT	$x10^9/L$	248±18.32	255±20.15	260±21.12

4. Additional References

S1. L. Lin, C. Xlb, Z. B. Hua, C. Hcb, C. Mmaab, A. Ds and C. Zwb, *Sensors and Actuators B: Chemical*, 2021, **333**.

S2. D. K. Roper, W. Ahn and M. Hoepfner, *J Phys Chem C Nanomater Interfaces*, 2007, **111**, 3636-3641.