Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2021

Electronic Supplementary Information

NIR-triggered upconversion nanoparticles@thermo-sensitive liposome hybrid theranostic nanoplatform for controlled drug delivery

Yibin Yu [a], Yida Huang [b], Wanqian Feng [c], Mei Yang [b], Baiqi Shao [a,d*], Jingjing Li [d,**], Fangfu Ye [a,e,***,]

[a] Wenzhou Institute, University of the Chinese Academy of Sciences, Wenzhou 325001, China

[b] Institute of Advanced Materials for Nano-bio Applications, Wenzhou Medical University, Wenzhou 325027, China

[c] Scientific Research Center, Wenzhou Medical University, Wenzhou 325035, China

[d] State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

[e] Beijing National Laboratory for Condensed Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China

Materials and methods

Materials

Anhydrous lanthanide chlorides, namely YCl_3 (99.9%), $YbCl_3$ (99.9%), $ErCl_3$ (99.9%), were gained from Sigma-Aldrich (USA). Oleic acid (OA, 90%) and 1-octadecene (ODE, 90%) were also offered by Sigma-Aldrich (USA). Mannitol, poly(vinyl pyrrolidone) (PVP, M_W = 58 kDa), $Bi(NO_3)_3 \cdot 5H_2O$, $NaBH_4$, and tetraethyl orthosilicate (TEOS) were supplied by Aladdin (China). 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) were all provided by Avanti Polar Lipids (USA). Cholesterol, genistein, hexadecyl trimethyl ammonium bromide (CTAB), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC), and N-hydroxysulfosuccinimide (sulfo-NHS) were attained from Aladdin (China). Hyaluronic acid (HA, M_W = 44 kDa) was afforded by Freda Biopharm (China), and penetratin (PNT) was gained from GL Biochem (China). BCA protein assay kit was purchased from Beyotime (China). Dulbecco's modified Eagle's medium/nutrient mixture F12 (DMEM/F12), fetal bovine serum (FBS), and penicillin–streptomycin were attained from Gibco (USA). Cell Counting Kit-8 (CCK-8) was supplied by Dojindo (Japan). Calcein-AM and propidium iodide (PI) were afford by Sigma-Aldrich (USA). 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) was offered by Beyotime (China). All other reagents were used without further purification.

Characterization

We employed a Talos F200S transmission electron microscope (FEI, USA) to observe the morphology of the upconversion nanocomposites. The elemental analysis was conducted by high-angle annular dark-field scanning TEM-energy dispersive X-ray spectroscopy (HAADF-STEM-EDS). Particle size and distribution of the nanoparticles were acquired by dynamic light scattering (DLS) using a ZetasizerNano 90 (Malvern, UK). X-ray diffraction patterns were obtained by D8 X-ray diffractometer (Bruker, Germany) and the angular range (2θ) covered was between 10° and 70°. FTIR spectra were collected by an IFS55 spectrophotometer (Bruker, Germany). UCL spectra were scanned on a FluoroMax fluorescence spectrometer (Horiba, Japan).

Synthesis of UCNPs

UCNPs were synthesized as reported earlier with slight modification.[1, 2] Briefly, 1 mmol of anhydrous $LnCl_3$ (Y: Yb: Er =78:20:2) were dissolved by 18 mL of ODE and 6 mL of OA at 160 °C

for 40 min and then cooled down to 50 °C. Afterward, 12 mL of methanol solution containing NH_4F (4.0 mmol) and NaOH (2.5 mmol) was added and the system kept stirring for 2 h to evaporate methanol. Subsequently, under an argon atmosphere, the solution was heated at 300 °C for 1 h and then cooled naturally. The dispersions were precipitated by ethanol, collected by centrifugation at 8000 rpm for 10 min, rinsed several times with ethanol, and redispersed in cyclohexane.

OA-capped UCNPs were transferred into distilled water through an acid-induced ligand removal process.[3] 5 mL of as-obtained lipophilic UCNPs dripped into 5 mL of ethanol and centrifuged at 13,000 rpm for 10 min. UCNPs pellet was transferred to the aqueous solution by adding 5 mL of HCl (2 M) and 5 mL of ethanol. Subsequently, UCNPs were sonicated, rinsed several times with ethanol, and dispersed in 5 mL of distilled water for further study.

Fabrication of UCNPs@Bi@SiO_2

UCNPs@Bi and UCNPs@Bi@SiO_2 were prepared as reported earlier by our group.[4] The as-acquired UCNPs dripped into mannitol solution. PVP K30 and $Bi(NO_3)_3 \cdot 5H_2O$ were added and the system was under magnetic stirring for 1 h. Subsequently, $NaBH_4$ was added rapidly into the dispersion and then stirred overnight. The dispersion was centrifuged, rinsed, and redispersed in distilled water. The preparation process of UCNPs@Bi nanocomposites was under light exclusion.

To obtain UCNPs@Bi@SiO_2, UCNPs@Bi nanocomposites were blended with PVP K30 under magnetic stirring for 1 h, added with ethanol, and sonicated for 20 min. After stirring for another 2 h, the system was added with ammonium and sonicated again for 20 min. TEOS was dispersed in ethanol and added dropwise to the system. Subsequently, the system maintained stirring for 12 h, rinsed with ethanol, and dispersed in distilled water.

Preparation of UCNPs@Bi@SiO_2@GE HP-lips

We utilized thin film hydration combined with extrusion technique to prepare the blank liposomes.[5] Briefly, chloroform solutions of lipids (DPPC:DOPE:Chol=55:45:5, molar ratio) were blended and evaporated under high vacuum, in order to form a thin lipid film. The thin film was hydrated using MES buffer under magnetic stirring for 30 min. Afterward, the crude dispersion was homogenized by an ultrasonic homogenizer for 5 min, and then repeatedly extruded through

a 100 nm polycarbonate membrane 11 times. The as-prepared uniform liposomes in nanoscale were freeze-dried for further study.

A similar protocol was adopted to fabricate UCNPs@Bi@SiO_2 and GE co-loaded liposomes (UCNPs@Bi@SiO_2@GE lips). Lipophilic GE was dissolved together with the lipids, and 5 mL of UCNPs@Bi@SiO_2 solution was applied to hydrate the lipid film to fabricate co-loaded liposomes. Free UCNPs@Bi@SiO_2 and GE were removed by centrifugation. The amount of free GE was determined by high performance liquid chromatography (HPLC) at 262 nm to calculate entrapment efficiency (EE, %) and drug loading (DL, %), as follows:

$$\mathrm{EE\%} = \frac{(total\ amount\ of\ genistein - free\ genistein)}{total\ amount\ of\ genistein} \times 100 \quad (1)$$

$$\mathrm{DL\%} = \frac{(total\ amount\ of\ genistein - free\ genistein)}{total\ amount\ of\ lipid} \times 100 \quad (2)$$

For the fabrication of theranostic nanoplatform based on UCNPs@Bi@SiO_2 and liposomes with retina-targeted ability (UCNPs@Bi@SiO_2@GE HP-lips), HA and PNT were conjugated to DOPE of the liposomes by amidation, as reported earlier with slight modification.[6] Briefly, HA, EDC and sulfo-NHS were dissolved in MES buffer at a molar ratio of 1:10:5, and the blended solution was stirred at room temperature for 2 h. Subsequently, the liposomes were added to the solution and reacted at 37 °C for 12 h. The unreacted reagents were removed by high-speed centrifugation with distilled water.

The conjugation of HA was quantified by the CTAB turbidimetric method.[7] Briefly, the supernatants containing HA were incubated with sodium acetate buffer at 37 °C for 10 min. Afterward, 10 mM CTAB solution was added to the mixture, and the absorbance was recorded at 570 nm. The amount of conjugated HA was calculated as follows:

$$\mathrm{HA\%} = \frac{(total\ amount\ of\ HA - free\ HA)}{total\ amount\ of\ HA} \times 100 \quad (3)$$

PNT was conjugated to the liposomes by a similar method. The conjugation of PNT was quantified using a BCA protein assay kit (Beyotime, China) and calculated as follows:

$$\mathrm{PNT\%} = \frac{(total\ amount\ of\ PNT - free\ PNT)}{total\ amount\ of\ PNT} \times 100 \quad (4)$$

Photothermal conversion features

The photothermal conversion profiles of UCNPs@Bi@SiO_2 and UCNPs@Bi@SiO_2 lips upon NIR laser exposure (808 nm, 500 mW/cm^2) were depicted using a thermocouple sensor. To investigate the photothermal stability of UCNPs@Bi@SiO_2 and UCNPs@Bi@SiO_2 lips, we recorded the temperature every 20 s using a thermocouple sensor for 5 cycles of heating and cooling. IR thermal photos were taken using a thermal imager (E5-XT, FLIR, USA).

In vitro drug release study of UCNPs@Bi@SiO_2@GE HP-lips upon NIR irradiation was performed as reported earlier with slight modification.[8] Briefly, 1 mL of UCNPs@Bi@SiO_2@GE HP-lips were placed into dialysis bags (8-14 K MWCO), and the release medium was 50 mL PBS (pH 7.4, containing 0.5% Tween 80) at 35 ± 0.5 °C with a constant speed (100 rpm). After 20 min, the dialysis bags would be taken out from the shaker incubator, placed under magnetic stirring, and exposed to NIR laser (808 nm, 650 mW/cm^2) for 20 min. Periodically, 1 mL of the mediums containing genistein were taken out and replaced with 1 mL of preheated fresh medium. The released genistein was quantified by HPLC. The control trial was performed in the shaker incubator without NIR exposure. In vitro drug release study of GE lips at same NIR irradiation was also conducted.

Cell culture

Human retinal pigment epithelial cells (ARPE-19, American Type Culture Collection) were incubated in DMEM/F12 medium enriched with 10% FBS and 1% streptomycin-penicillin solution at 37 °C, 5% CO_2.

Cytotoxicity Study

Cell viability was evaluated using a CCK-8 assay kit (Dojindo, Japan). ARPE-19 cells (1×10^4) were seeded in each well of a 96-well plate. After 24 h of incubation, ARPE-19 cells were co-cultured with different formulations, including blank lips, blank HP-lips, UCNPs, UCNPs@Bi@SiO_2, UCNPs@Bi@SiO_2 lips, GE solution, GE lips, and UCNPs@Bi@SiO_2@GE HP-lips, for 24 h and 48 h, separately. Subsequently, ARPE-19 cells were cultured with 10 µL of CCK-8 reagent for 1.5 h and the absorbance was read at 450 nm. Cell viability was expressed as follows:

$$\text{Cell viability}\% = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \times 100 \qquad (5)$$

where $A_{control}$ and A_{sample} are the absorbances at 450 nm in absence and presence of samples, respectively. A_{blank} is the absorbance at 450 nm of the culture medium.

Additionally, a qualitative evaluation of cell viability was performed by calcein-AM/PI staining. ARPE-19 cells were cultured with the same formulations as the CCK-8 assay for 24 h. Afterward, ARPE-19 cells were stained with calcein-AM/PI for 30 min, rinsed with PBS 7.4 twice and fluorescent photos were taken using a DMi8 fluorescence microscope (Leica, Germany).

Cellular uptake

Cellular uptake evaluation of the hybrid nanocomposites was carried out using an FVMPE-RS multiphoton laser scanning microscope (Olympus, Japan) equipped with a MAITAI HPDS-OL laser at 980 nm for illuminating the upconversion nanocomposites. ARPE-19 cells were seeded into glass-bottom confocal dishes (35 mm x 10 mm) and incubated at 37 °C for 24 h. UCNPs, UCNPs@Bi@SiO_2, UCNPs@Bi@SiO_2 lips, and UCNPs@Bi@SiO_2@HP-lips were dispersed in the culture medium with a concentration of 25 μg/mL and co-cultured with ARPE-19 cells for 12 h and 24 h, separately. Afterward, residual nanocomposites were removed by rinsing softly thrice with PBS. ARPE-19 cells were fixed by 4% paraformaldehyde at 37 °C for 30 min, and then stained with DAPI for 15 min. The luminescence photos were taken under a 25X water-immersion objective, and luminescence signals were monitored in the wavelength of 495-540 nm (UCL) and 460-500 nm (DAPI), separately. In the merged channel, the green UCL signal overlapped the blue fluorescence of DAPI.

Statistical analysis

Data were reported as mean ± S.D. One-way analysis of variance (ANOVA) was conducted to assess the differences among groups, using SPSS software 23.0. $p < 0.05$ was considered to be statistically significant.

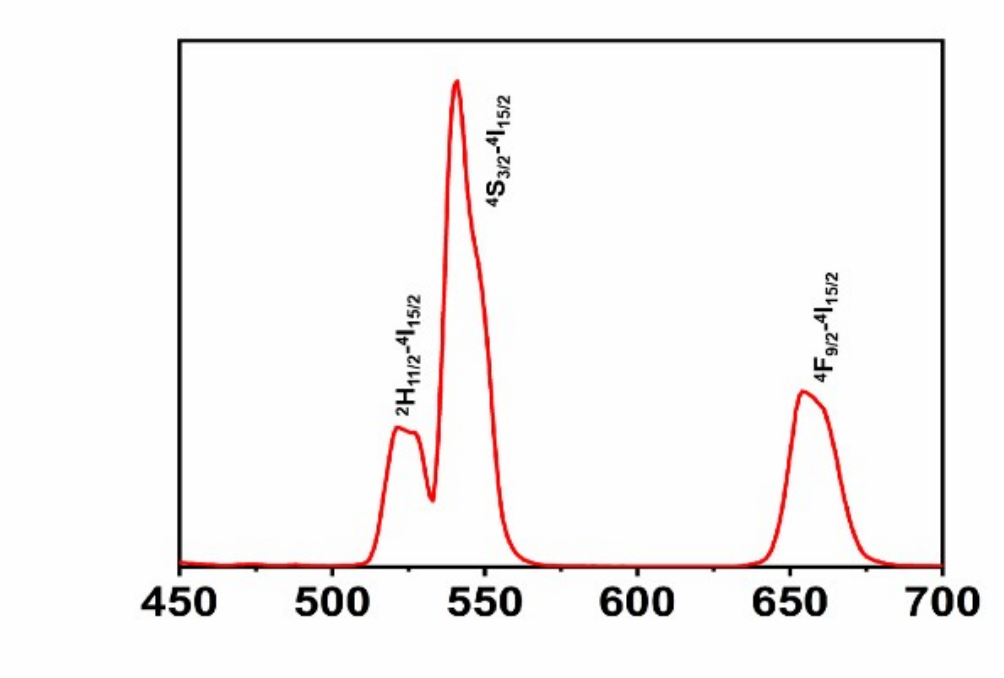

Fig. S1 Fluorescence emission spectra of UCNPs.

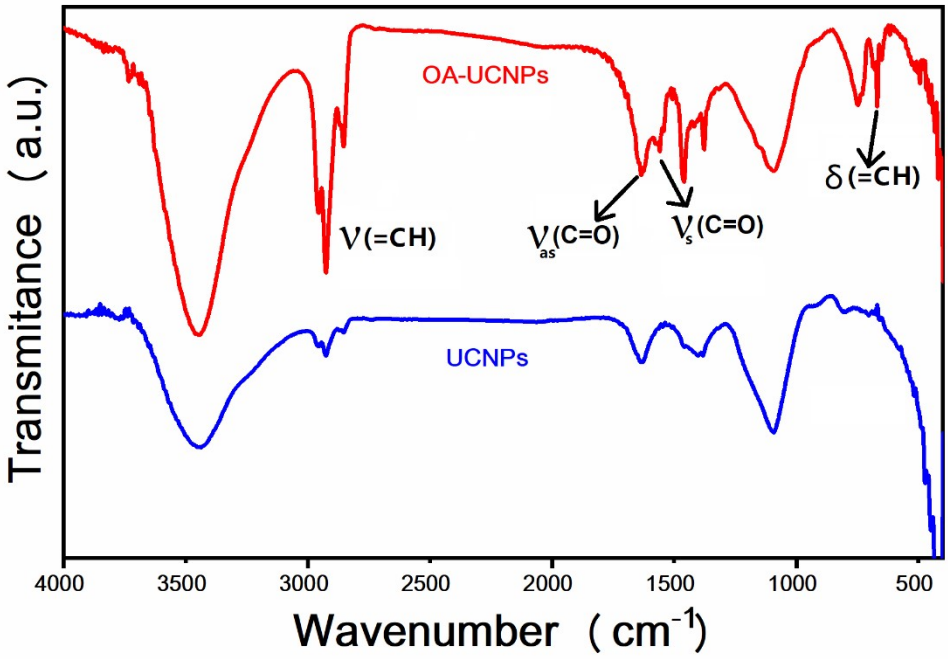

Fig. S2 FTIR spectra of OA-capped UCNPs and ligand-free UCNPs. The successful removal of OA group was proved by FTIR spectra with the smaller bands at 2924 cm^{-1} and 667 cm^{-1}, which could be ascribed to the dramatic decrease of C=H stretching and bending, respectively.

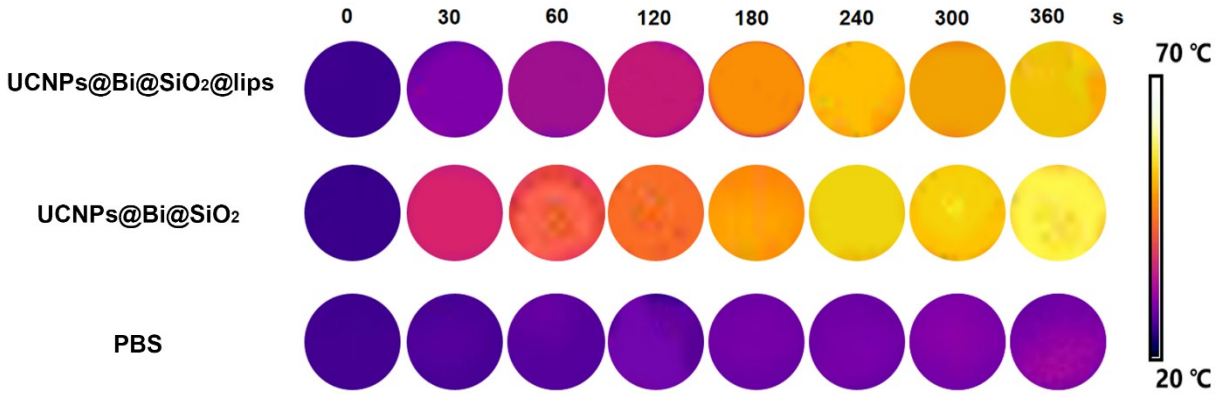

Fig. S3 IR thermal images of PBS, UCNPs@Bi@SiO_2 and UCNPs@Bi@SiO_2 lips upon CW NIR laser exposure (808 nm, 500 mW/cm^2).

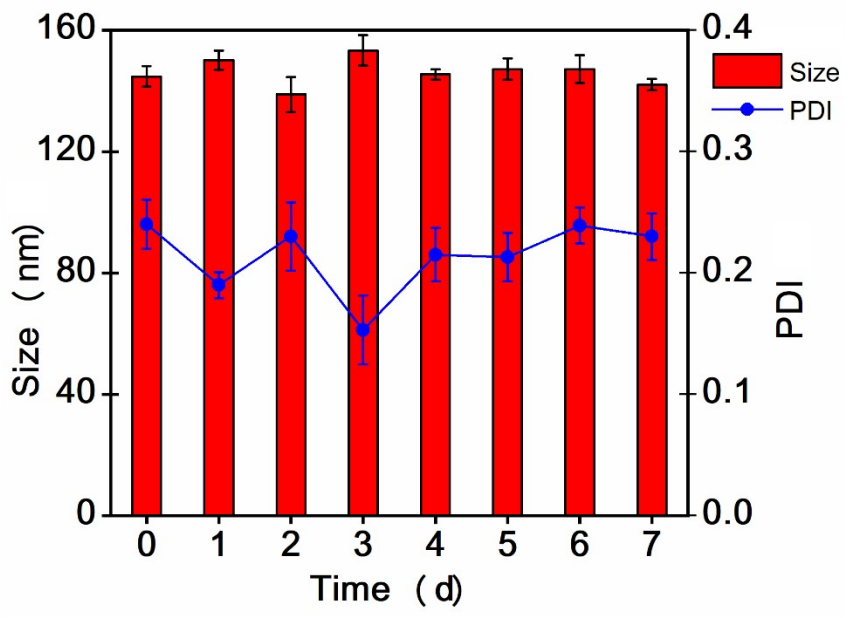

Fig. S4 Particle size and polydispersity index of UCNPs@Bi@SiO_2@lips measured for 7 d of storage at 4 °C.

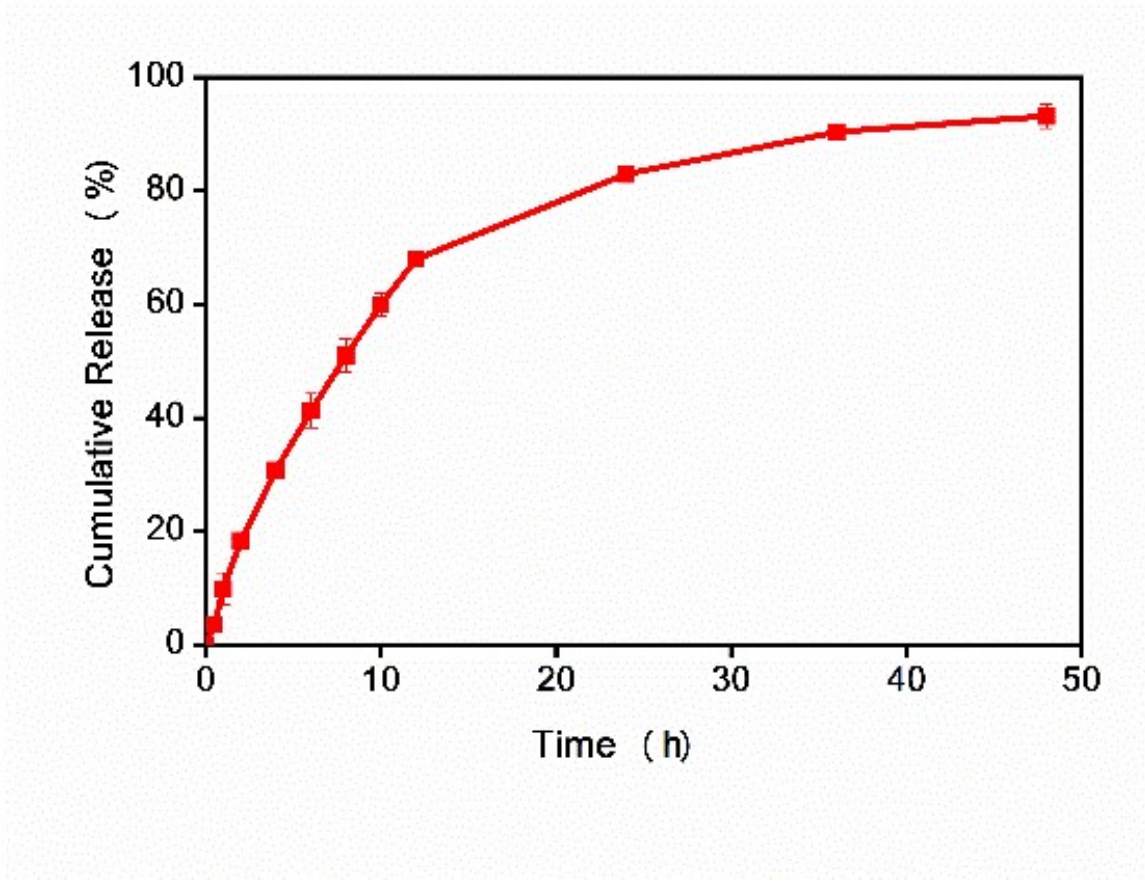

Fig. S5 In vitro GE release profiles from GE-HP lips.

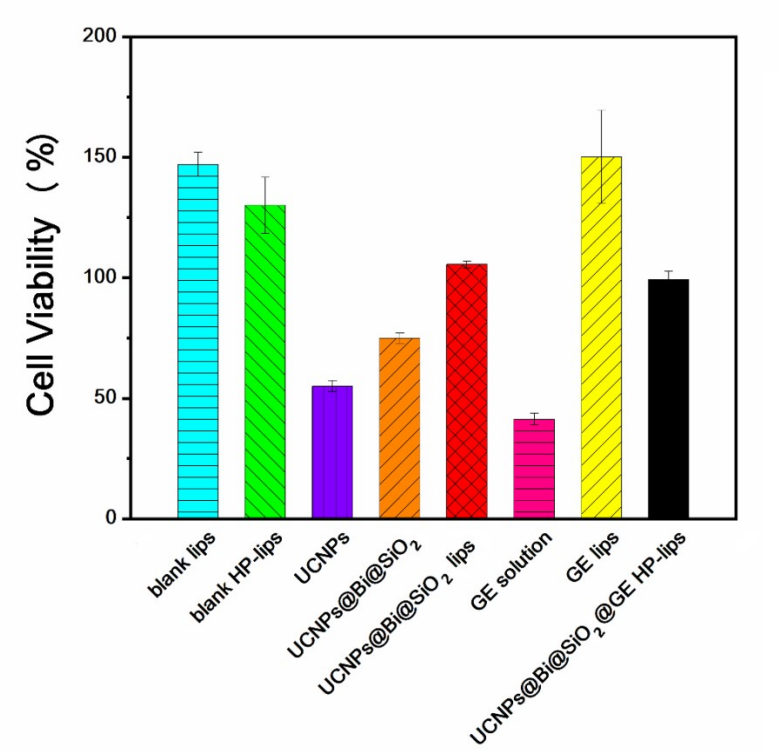

Fig. S6 Cytotoxicity of various formulations (1:2 dilution) in ARPE-19 cells for 24 h.

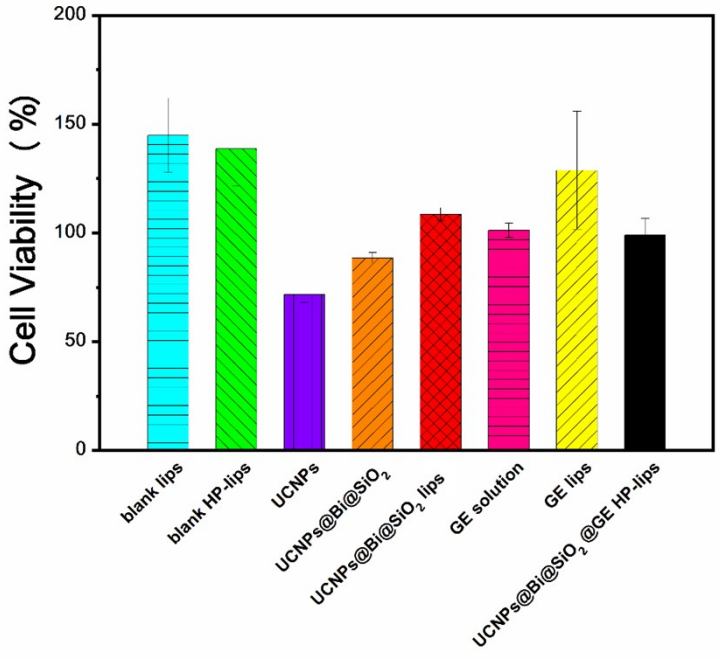

Fig. S7 Cytotoxicity of various formulations (1:4 dilution) in ARPE-19 cells for 24 h.

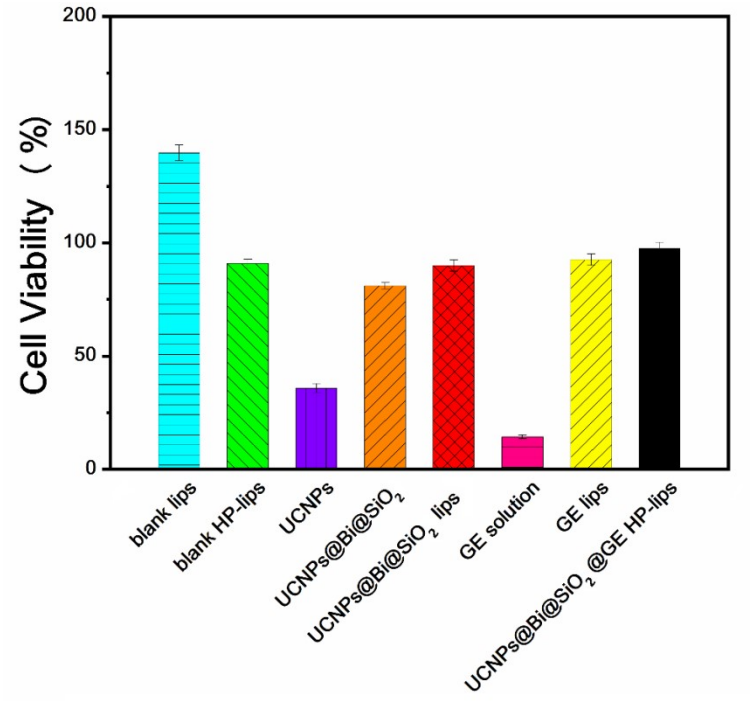

Fig. S8 Cytotoxicity of various undiluted formulations in ARPE-19 cells for 48 h.

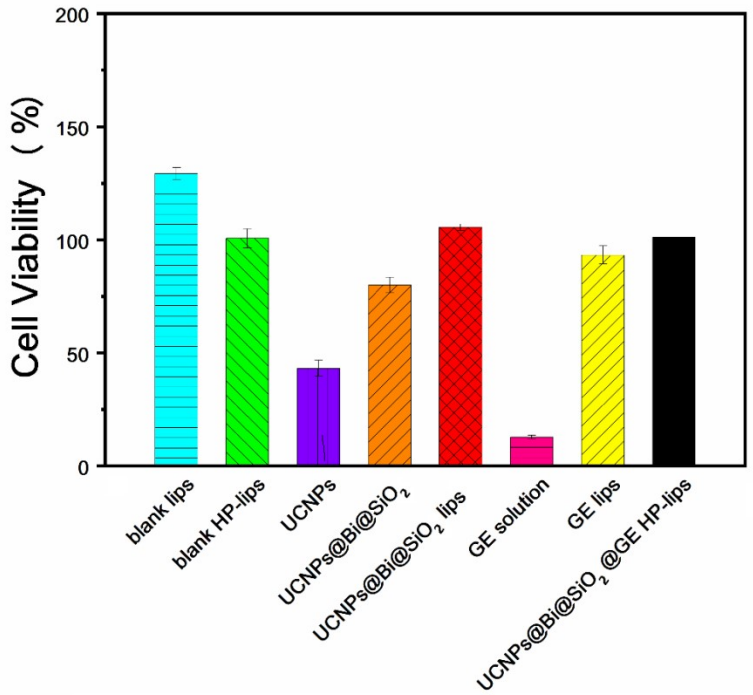

Fig. S9 Cytotoxicity of various formulations (1:2 dilution) in ARPE-19 cells for 48 h.

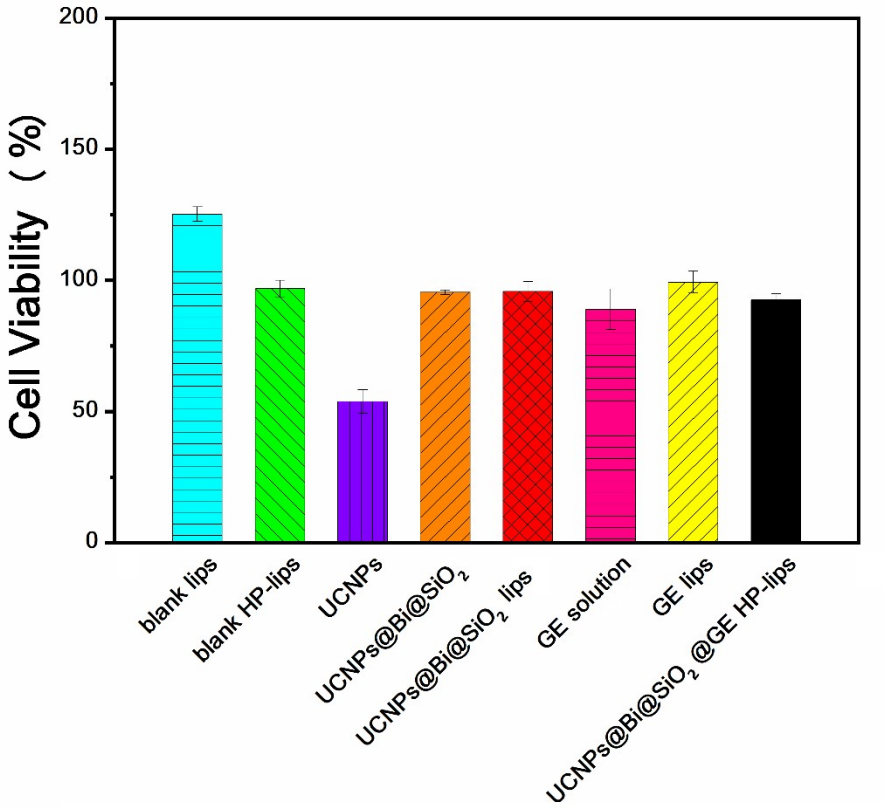

Fig. S10 Cytotoxicity of various formulations (1:4 dilution) in ARPE-19 cells for 48 h.

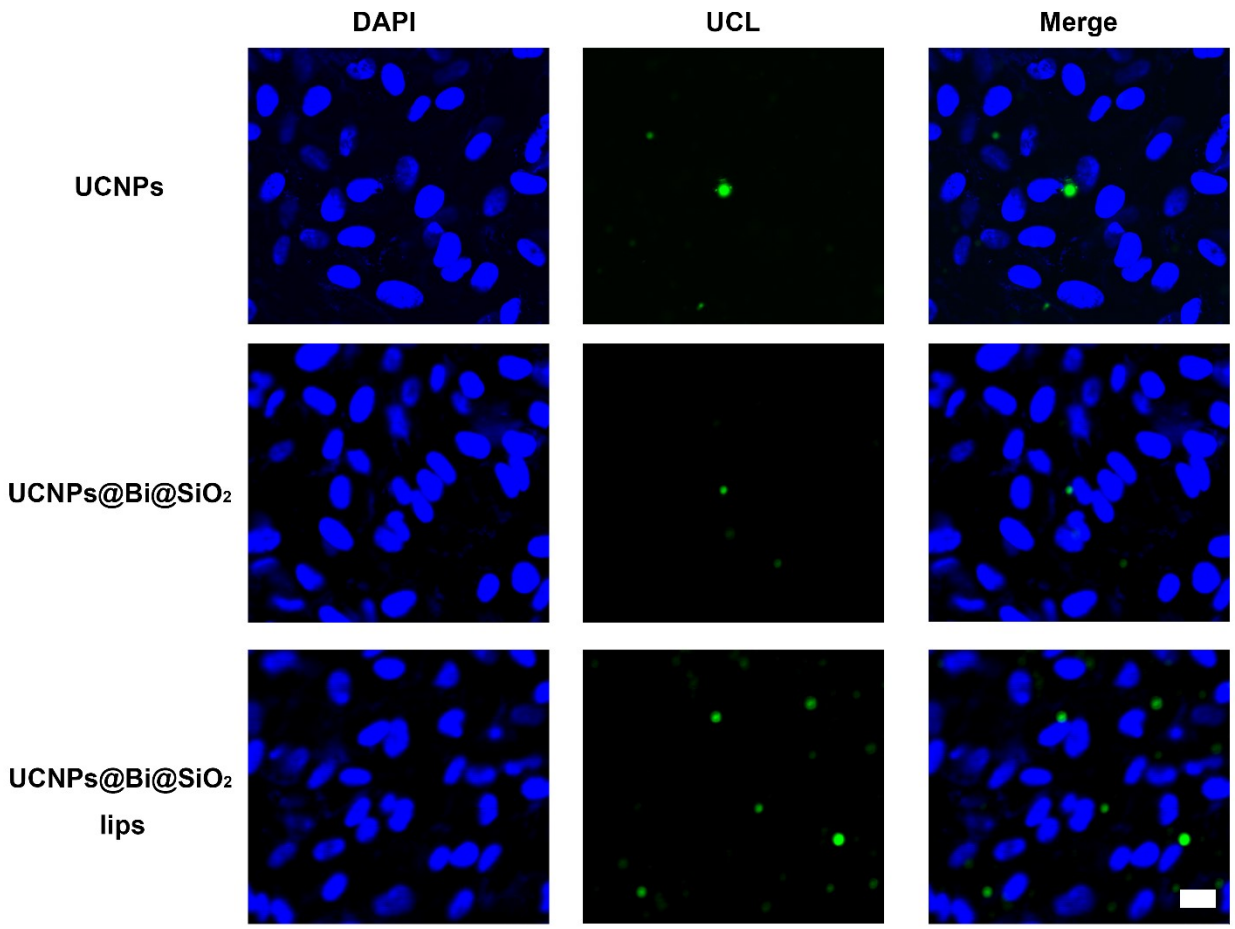

Fig. S11 Multiphoton laser scanning microscopy observation of ARPE-19 cells upon 980 nm NIR laser exposure incubated with UCNPs, UCNPs@Bi@SiO_2 and UCNPs@Bi@SiO_2 lips for 3 h. Scale bar: 20 μm.

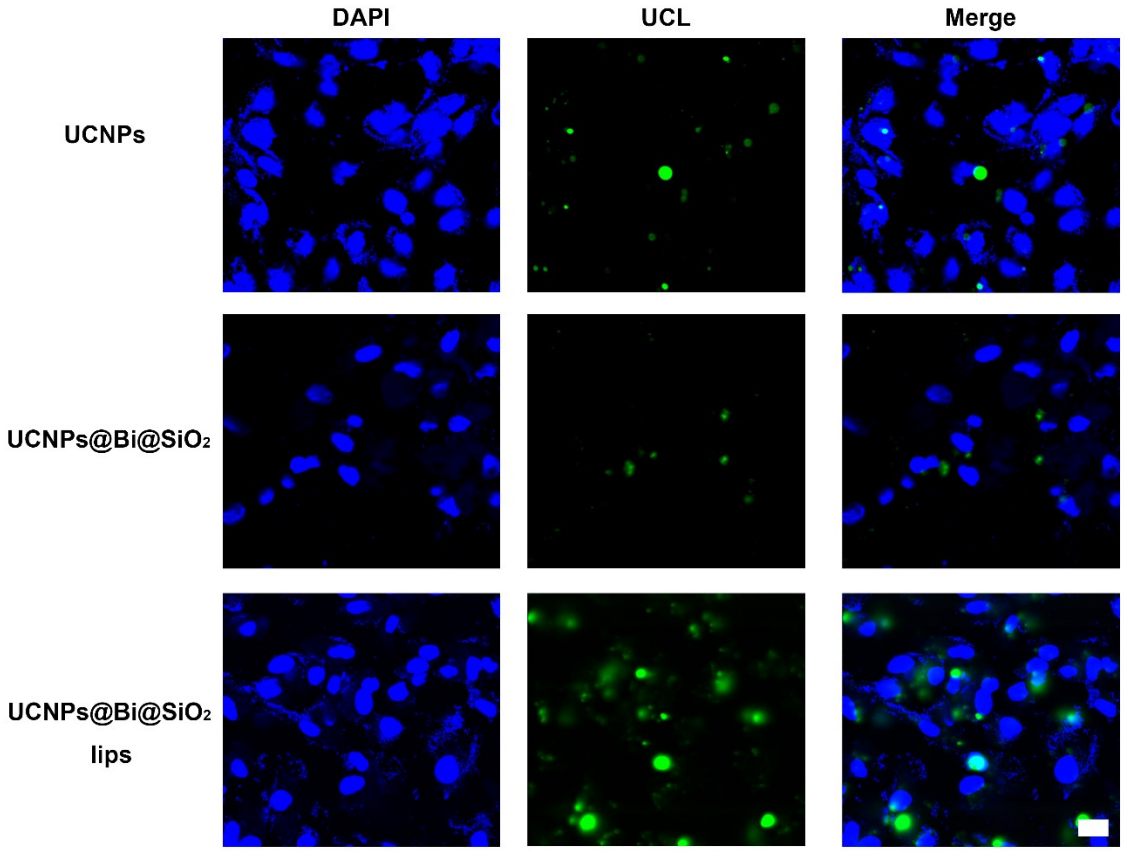

Fig. S12 Multiphoton laser scanning microscopy observation of ARPE-19 cells upon 980 nm NIR laser exposure incubated with UCNPs, UCNPs@Bi@SiO_2 and UCNPs@Bi@SiO_2 lips for 12 h. Scale bar: 20 μm.

Table S1. Entrapment efficiency and drug loading of the liposomes (n = 3).

	Encapsulation efficiency [%]	Drug Loading [%]
UCNPs@Bi@SiO_2@GE HP-lips	80.26±1.92	5.23±0.32
GE lips	80.12±1.38	6.58±0.55

Notes and references

1. L. Li, P. Hao, P. Wei, L. Fu, X. Ai, J. Zhang and J. Zhou, *Biomaterials*, 2017, **136**, 43-55.
2. C. Yao, P. Wang, X. Li, X. Hu, J. Hou, L. Wang and F. Zhang, *Adv Mater*, 2016, **28**, 9341-9348.
3. S. Chen, A. Z. Weitemier, X. Zeng, L. M. He, X. Y. Wang, Y. Q. Tao, A. J. Y. Huang, Y. Hashimotodani, M. Kano, H. Iwasaki, L. K. Parajuli, S. Okabe, D. B. L. Teh, A. H. All, I. Tsutsui-Kimura, K. F. Tanaka, X. G. Liu and T. J. McHugh, *Science*, 2018, **359**, 679-683.
4. S. Zhao, R. Tian, B. Shao, Y. Feng, S. Yuan, L. Dong, L. Zhang, K. Liu, Z. Wang and H. You, *ACS Appl Mater Interfaces*, 2019, **11**, 394-402.
5. W. L. Wan, B. Tian, Y. J. Lin, C. Korupalli, M. Y. Lu, Q. Cui, D. Wan, Y. Chang and H. W. Sung, *Nat Commun*, 2020, **11**, 534.
6. M. Tang, D. Svirskis, E. Leung, M. Kanamala, H. Wang and Z. Wu, *J Control Release*, 2019, **305**, 89-100.
7. L. Gan, J. Wang, Y. Zhao, D. Chen, C. Zhu, J. Liu and Y. Gan, *Biomaterials*, 2013, **34**, 5978-5987.
8. H. He, L. Liu, S. Zhang, M. Zheng, A. Ma, Z. Chen, H. Pan, H. Zhou, R. Liang and L. Cai, *J Control Release*, 2020, **323**, 387-397.