Supporting Information

Construction of iridium(III)-complex-loaded MOF nanoplatform mediated with a dual-responsive polycationic polymer for photodynamic therapy and cell imaging

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Author Contributions

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1. Experimental section

1.1 Reagents and chemicals

All chemicals used were commercially available organic reagents and were of analytical grade. Unless otherwise specified, reagents were used as received without further purification. Deionized water was used throughout the experiments. Zirconium (IV) chloride ($ZrCl_4$), 2-aminoterephthalic acid, 2-(N,N-diethylamino)ethyl acrylate (DEAEA) and 2',7'-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma-Aldrich (Shanghai, China). Glycidyl methacrylate (GMA) was procured from Shanghai Adamas Reagent Co., Ltd (Shanghai, China). Trimethyl orthoformate, pyridinium-ptoluenesulfonate, and neopentyl glycol (NPG) were purchased from Shanghai Aladdin BioChem Technology Co., Ltd (Shanghai, China). 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used in the cell cytotoxicity assay (Dojindo, Japan). All other reagents were obtained from Tianjin Chemical Reagent Co. (Tianjin, China).

1.2 Synthesis of UiO-66-MOF

NH_2-UiO-66 was synthesized according to the solvothermal method.[1] Briefly, 2-amino-benzenedicarboxylic acid (0.248 g, 1.372 mmol), and $ZrCl_4$ (0.320 g, 1.372 mmol) were dissolved in 80 mL DMF under sonication. Then, the mixture was transferred into a Teflon-lined stainless steel autoclave (120 mL) for a homogeneous reaction. 1.236 mL water (68.7 mmol, 50 equivalent to $ZrCl_4$) was added into the solution under stirring. The solution was heated to 120 °C for 24 h affording UiO-66 nanoparticles, which were collected by centrifugation (4500 rpm, 15 min) and washed by dispersion-centrifugation cycles with mixture of DMF (x1) and methanol (x3). The obtained crystal was redispersed in methanol for 24 h, and then rinsed with methanol, followed by drying under reduced pressure at 80 °C for 24 h.

1.3 Synthesis of 2-(5,5-dimethyl-1,3-dioxan-2-yl) ethyl acrylate (DMDEA)

Monomer of DMDEA was prepared following our previously reported procedures.[2] Briefly, trimethyl orthoformate (21.2 g, 200 mmol) was dissolved in a DCM (50 mL) solution containing pyridinium-p-toluenesulfonate (0.506 g, 2 mmol) and NPG (15.2 g, 200 mmol) under continuous stirring for 12 h at 30 °C. The crude product was

collected by extraction with 10% NaOH aqueous solution (three times), and the organic layer was dried over anhydrous K_2CO_3. Then, the DCM solution was evaporated to afford colorless liquid under reduced pressure. 2-Methoxy-5,5-dimethyl-1,3-dioxane (3.0 g, 25 mmol), 2-hydroxyethyl acrylate (3.8 g, 32 mmol), pyridunium-p-toluenesulfonate (0.125 g, 0.5 mmol), and 4-methoxyphenol (0.1 g, 0.8 mmol) were added to toluene (25 mL), and agitated at 120 °C overnight. The solution was cooled, extracted with 10% NaOH aqueous solution (three times), and the combined organic layer was then dried over the anhydrous K_2CO_3. After drying, toluene was removed by vacuum, the residue was purified through a simple Al_2O_3 column elution with petroleum ether/ethyl acetate (v/v = 10:1) to yield colorless oil (3.6 g, yield: 52%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.42-6.49 (q, 1H, $\underline{CH_2}CH$), 6.13-6.22 (q, 1H, $CH_2\underline{CH}$), 5.84-5.88 (q, 1H, $\underline{CH_2}CH$), 5.37 (s, 1H, HCO_3), 4.34-4.38 (t, 2H, $CO_2\underline{CH_2}CH_2O$), 3.88-3.91 (t, 2H, $CO_2CH_2\underline{CH_2}O$), 3.75-3.79 (d, 2H, $CO_2CH_2CH_2O$), 3.39-3.43 (d, 2H, $CO_2CH_2CH_2O$), 0.99 (s, 6H, $C(CH_3)_2$).

1.4 Synthesis of BP-PDM-PG

PD-PDM-PG was synthesized by ATRP reaction first. Briefly, glycidyl methacrylate (GMA) (0.51 g, 3.6 mmol), 2-(N,N-diethylamino)ethyl acrylate (DEAEA) (1.33 g, 7.2 mmol) and 2-(5,5-dimethyl-1,3-dioxan-2-yl)ethyl acrylate (DMDEA) (0.83g, 3.6 mmol) were dissolved in 15 mL of distilled DMF in a three-necked flask. Then, bipyridyl (0.03 g, 0.2 mmol), 2-bromoisobutyryl bromide (0.02 g, 0.1 mmol) and CuBr (0.02 g, 0.1 mmol) were sequentially added into the reaction system. The reaction proceeded with constant stirring at 80 °C for 24 h under dry nitrogen. Subsequently, the reaction was quenched with an ice bath and diluted with THF. The reaction suspension was purified through a neutral Al_2O_3 column using THF as an eluent. The solvent was evaporated and the product was obtained through precipitation twice in cold n-hexane.

(6-(2-Aminoethyl)amino-6-deoxy)-b-cyclodextrin (CDen) was prepared following previously reported procedures.[3] PD-PDM-PG (0.20 g) and CDen (1.66 g) were dissolved in distilled DMF (30 mL) and reacted at 70 °C for 24 h. Subsequently, ethylenediamine was added (10 mL) to the flask and stirred at 70 °C for an additional 24 h with the aim of complete ring-opening reaction of the epoxide groups of PG.

Once cooled, 4-bromomethylphenylboronic acid pinacol ester (0.80 g) was added dropwise into the solution and continuously stirred at 25 °C for 24 h. The resulting crude product of BP-PDM-PG was dialyzed (Spectra/Por RC, cut-off 12000) against deionized water using a dialysis bag with a molecular weight cut-off of 3,500 Da for 3 days and lyophilized to obtain BP-PDM-PG (0.28 g, yield: 85%).

1.5 ^{1}H-NMR characterization

The ^{1}H-NMR spectra of DMDEA and BP-PDM-PG were recorded on a 400 MHz Bruker Avance-400 spectrometer (400 MHz, Bruker, Freemont, CA) using $CDCl_3$ as solvent.

1.6 Preparation of Ir@MOF/P NPs

For fabrication of Ir@MOF, 1 mL MOF solution (5 mg/mL) was dispersed in Ir(III) complex in methanol (1 mL, 0.5 mg/mL) and then mixed under ultrasonication for 2 hours at 25 °C to form Ir@MOF constructs. The as-prepared Ir@MOF (1 mL) was added to the dropwise BP-PDM-PG solution (1 mL, 1 mg/mL), and stirred at 25 °C for 1.5 hours to obtain Ir@MOF/P NPs, which was sealed and stored at 4 °C.

1.7 DLS measurements

Size and polydispersity index (PDI) of MOF NPs and Ir@MOF/P NPs were determined by dynamic light scattering (DLS; Zetasizer Nano, Malvern Southborough, MA) at 25 °C. MOF NPs were prepared in methanol solution and Ir@MOF/P NPs were prepared in DI water as mentioned above.

1.8 Ir(III) complex-loading and *in vitro* triggered release assay

The Ir(III) complex loading content (LC) was measured using a UV-vis spectrophotometer (Persee, TU-1900, Beijing) and computed as LC (%) = (wt of Ir(III) complex loaded in nanoparticles)/(wt of nanoparticles)×100%.

UV-vis was used to detect the cumulative release of the Ir(III) complex from Ir@MOF/P NPs triggered by dayight. KH_2PO_4 buffer solution (0.1 M) was adjusted to pH = 5.4 and 7.4 with NaOH solution (1 M) to simulate the internal environment of

tumor cells and normal tissues, respectively. Then, the Ir@MOF/P NPs were transferred into a dialysis bag (cut-off molecular weight 3500 Da), which was immersed in 15 mL of the above two buffer solutions and shaken continuously at 100 rpm under 37 ^{o}C. The Ir@MOF/P NPs were illuminated by daylight (10 mW cm^{-2}) at time zero. At predetermined time intervals, 3 mL of release dialysate was taken out and replenished with fresh dialysate of equal volume. Finally, the absorbency of released Ir(III) complex was determined using the UV-vis spectrometer. In both buffers, Ir@MOF/P NPs not triggered by daylight were used as controls.

1.9 Fluorescence experiment

The fluorescence spectra of biscyclometalated Ir(III) complex and Ir@MOF/P NPs solution was measured on Varian fluorescence spectrophotometer (Varian, USA) with the excitation wavelength of 440 nm and the emission was collected from 550 to 650 nm.

1.10 Detection of ROS production

ROS production under daylight irradiation was detected using the fluorescent probe, 2',7'-Dichlorofluorescein diacetate (DCF-DA). Briefly, 0.5 mL DCF-DA (1 mM in ethanol) was added to 2 mL of NaOH (10 mM) aqueous solution and the solution was mixed at room temperature for 30 min. The hydrolysate (dichlorodihydrofluorescein, DCFH) was then neutralized with 10 mL of PBS at pH 7.4 and immediately kept on ice for further use. The above-mentioned solution (100 μL) was added to 0.9 mL of the Ir(III) complex and Ir@MOF/P NPs solution (20 μg/mL), respectively and exposed to daylight irradiation (10 mW cm^{-2}) for different time intervals. The changes in fluorescence intensity of the solution were measured at 488 nm and the emission detection range was from 500 to 650 nm.

1.11 Cell culture and imaging

HeLa cell lines were obtained from American Type Culture Collections (ATCC) and cultured in RPMI 1640 media (Thermo Fisher Scientific from Shanghai, China) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in

humidified 5% CO_2 and 95% air atmosphere incubator at 37 °C. Prior to experiments, the medium was removed and the adherent cells were washed twice with PBS to remove the remnant growth medium. The Ir@MOF/P NPs were then added to the plates with 1 mL of RPMI 1640 medium supplemented with 10% FBS (at a final concentration of 20 μg/mL). After 6 h post-incubation, the cells were washed with PBS and treated with Lyso Tracker Green DND-26 (YEASEN, Shanghai, China) to stain lysosome for 30 minutes. Then the cells were washed three times with PBS and fixed with 1 ml of 75% alcohol for 20 min and stained with 4,6-diamidino-2-phemylindole (DAPI) for 30 minutes. After replacement with PBS the fluorescence images were acquired using fluorescence microscopy. The colocalization degree of endo/lysosomes and Ir@MOF/P NPs was calculated according to the formula: Colocalization degree (100%) = the number of yellow pixels/the total number of yellow and red pixels × 100%.

1.12 Intracellular ROS detection

The intracellular ROS generation under NIR irradiation was detected using DCF-DA as an oxidation-sensitive fluorescent probe and analyzed with a confocal laser microscope system (CLMS). Briefly, HeLa cells were cultured in the 35 mm glass-bottom dishes at 37 °C. At 80% confluence, the culture medium was removed and cells were washed twice with PBS buffer and incubated with Ir@MOF/P NPs (20 μg/mL) for 6 h in the dark. Following incubation, cells were rinsed with PBS three times and ROS production under daylight irradiation for different times was detected using the fluorescent probe DCF-DA. After irradiation, the intracellular ROS was characterized by CLMS measurement. For Ir@MOF/P NPs detection, the excitation wavelength was 488 nm while the fluorescence emission range was from 550 to 650 nm; for DCF-DA detection, the excitation wavelength was 488 nm, while fluorescence emission range was from 500 to 530 nm.

1.13 Cytotoxicity assays

Using MTT-based cell viability assays, cell cytotoxicity was analyzed. Briefly, HeLa cells were seeded at a density of 5 x 10^3 cells per well. After overnight incubation, cells were treated with different concentrations of Ir@MOF/P NPs. At 4 hours of incubation, cells were exposed to daylight irradiation (10 mW cm^{-2}) for 30 min. After treatment, the cell

medium was replaced with the fresh medium and cells were further cultured for an additional 44 h. Cell viability was then determined by adding MTT solution (5 mg/mL) in fresh medium (100 μL) to each well followed by incubation for 3 h at 37°C with 5 % CO_2. Subsequently, the MTT mixture was removed and 100 μL of filtered DMSO was added into each well to dissolve all the formed crystals. Samples were agitated on a shaker for 15 min, and the absorbance at 570 nm was recorded using a microplate reader (Epoch, BioTek, Gene company Limited). The cell viability relative to the control wells was calculated from the obtained values as a percentage of control wells. Here, the treatment and the control represented the cells treated with and without irradiation, respectively. The result was presented as a mean and standard deviation obtained from samples. Cell viability of HeLa cells incubated with different concentrations of Ir@MOF/P NPs in the dark was also tested according to a similar protocol.

1.14 Statistics analysis

Significant differences in cell viability between two groups were evaluated using Student's *t*-test.

2. Figures

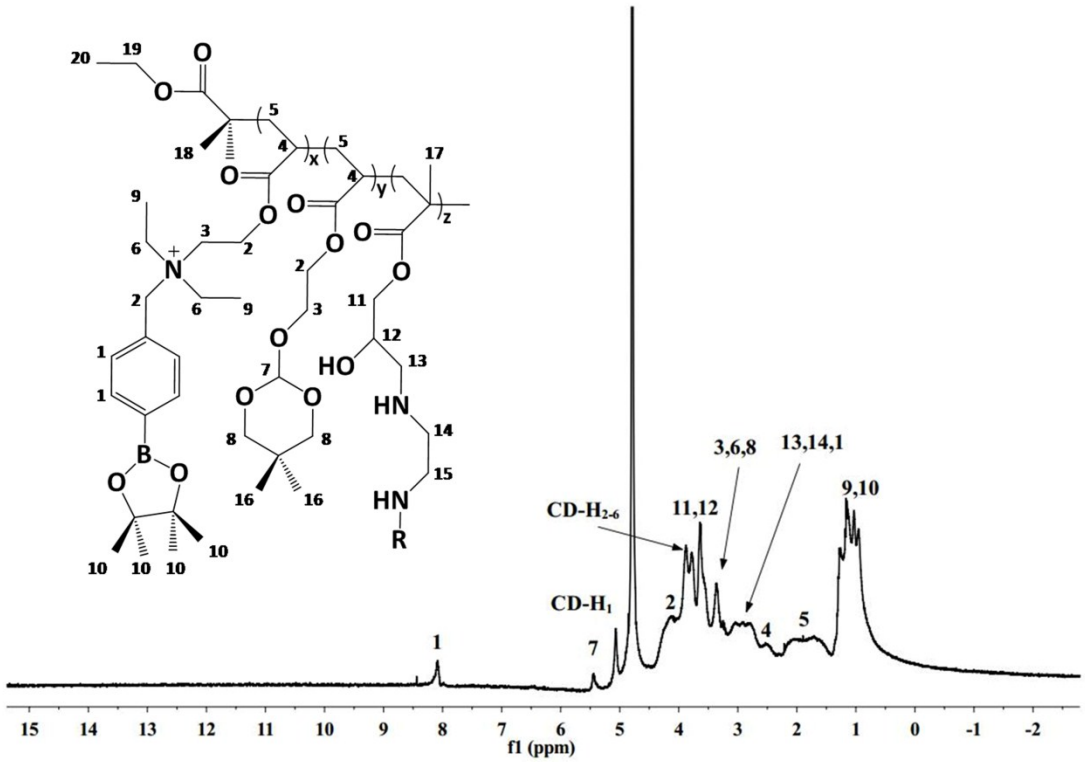

Fig. S1 ^{1}H-NMR spectrum of BP-PDM-PG.

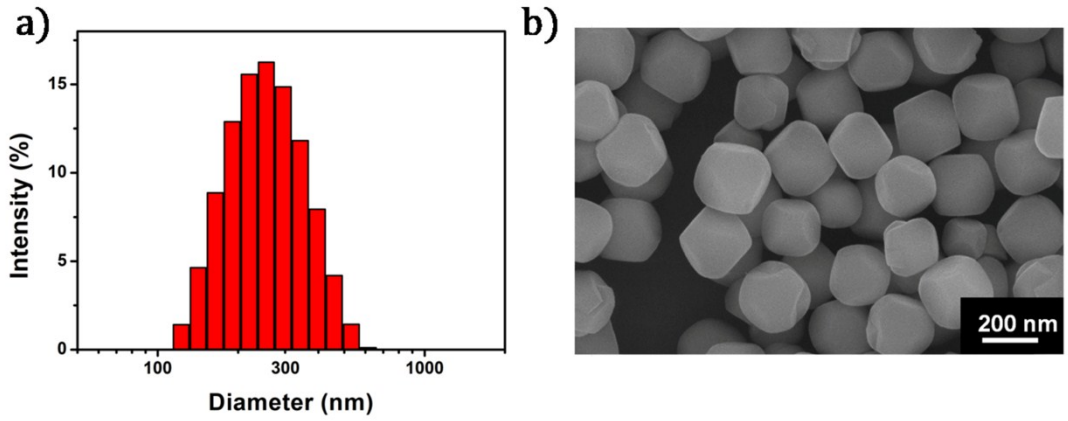

Fig. S2 (a) Size distribution and (b) SEM image of MOF NPs.

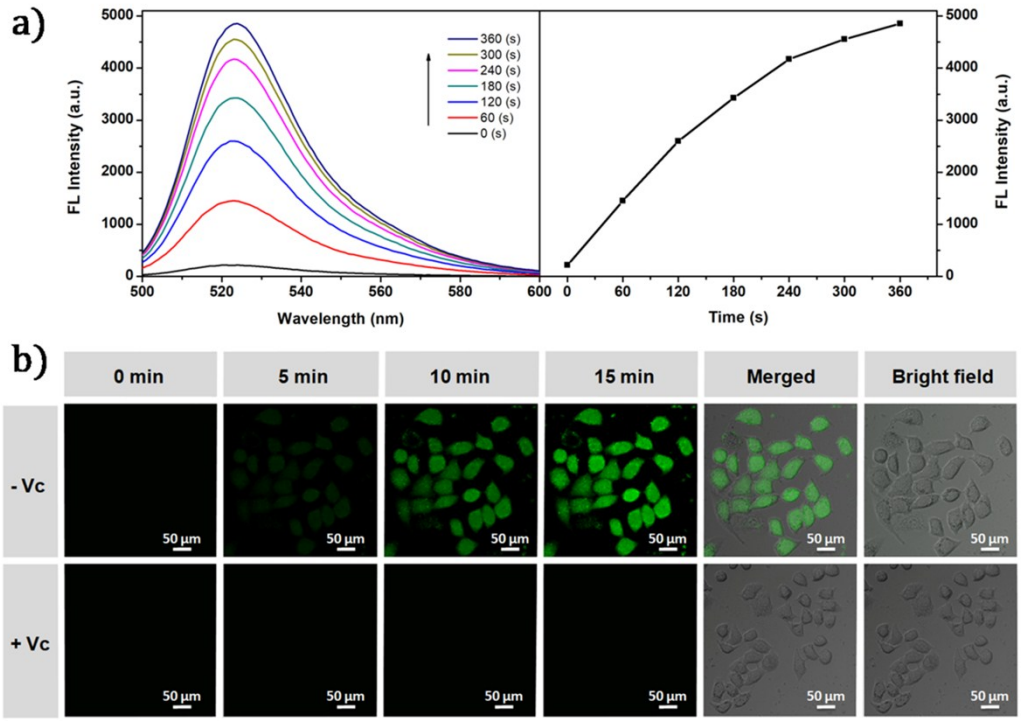

Fig. S3 (a) Fluorescence spectra of Ir@MOF/P NPs and DCF mixture in aqueous solution to detect the ROS production under varied daylight irradiation time (10 mW cm^{-2}). (b) CLSM images of HeLa cells after incubation with Ir@MOF/P NPs and DCF-DA under daylight irradiation for a different time with or without Vc. (λ_{ex} = 488 nm, λ_{em} = 525nm) Scale bars: 50 μm.

3. Reference

1. S. Dong, Q. Chen, W. Li, Z. Jiang, J. Ma and H. Gao, *J. Mater. Chem. B*, 2017, **5**, 8322-8329.
2. J. An, X. Dai, Z. Wu, Y. Zhao, Z. Lu, Q. Guo, X. Zhang and C. Li, *Biomacromolecules*, 2015, **16**, 2444-2454.
3. H. Zhu, J. An, C. Pang, S. Chen and H. Gao, *J. Mater. Chem. B*, 2018, **7**, 384-392.