Electronic Supporting Information

An acid-triggered BODIPY-based photosensitizer for enhanced photodynamic antibacterial efficacy

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Experimental

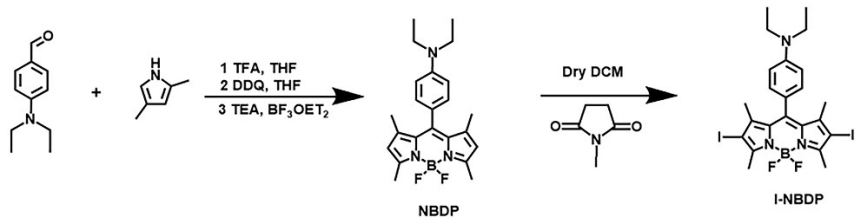
Materials

4-Diethylaminobenzaldehyde, 2,4-dimethylpyrrole, trifluoroacetic acid (TFA), 2,3-dichloro-5,6-dicyano-p-benzoquinone, triethylamine, boron trifluoride ether, *N*-iodosuccinimide, oligo (ethylene glycol) methyl ether methyl methacrylate (OEGMA), azobisisobutyronitrile (AIBN), 1,3-dipheny-lisobenzofuran (DBPF), basic alumina, diethylaminoethyl methacrylate (DEAEMA), 1,3-diphenylisobenzofuran, SYTO9, pyridine iodide (PI), glutaraldehyde, fluorescent labeled concanavalin (FITC-CONA) were purchased from Shanghai Titan Scientific Co., Ltd. All organic solvents were purchased from Aladdin. Dichloromethane (DCM) and triethylamine (TEA) were dried over CaH_2, and tetrahydrofuran (THF) were dried by refluxing over sodium shavings.

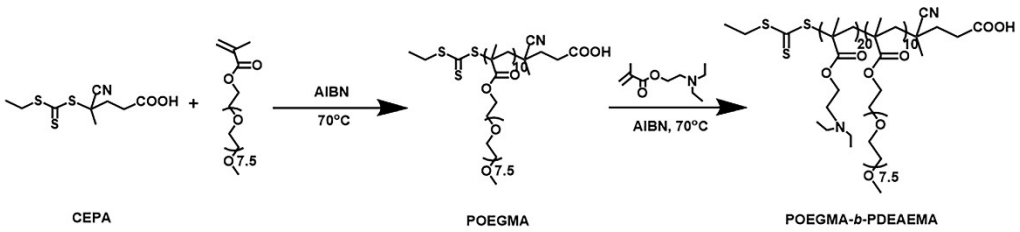
Characterization

^1H NMR spectra in chloroform-*d* were recorded with a Bruker AV400 Spectrometer with tetramethylsilane as the internal standard. The UV-*vis* spectra were measured using a Thermo Scientific Evolution 220 spectrophotometer. Fluorescence spectra were recorded with a Lumina fluorescence spectrometer. Dynamic light scattering (DLS) was performed on a Beckman Coulter Delasa Nano C particle analyzer. The images of transmission electron microscope (TEM) were determined by a JEOL JEM1400 transmission electron microscope. The live and death staining diagrams were recorded with a Nikon A1R confocal laser scanning microscope (CLSM).

Synthesis and Methods



Scheme S1. Synthesis of I-NBDP.



Scheme S2. Synthesis of POEGMA-*b*-PDEAEMA.

1. Synthesis of NBDP.

4-Diethylaminobenzaldehyde (0.53 g, 3 mmol) and 2,4-dimethylpyrrole (0.57 g, 6 mmol) were dissolved in THF (100 mL). TFA (1 mL) was added to the mixture and then degassed with nitrogen. The resulting mixture was stirred at room temperature overnight. Then 2,3-dichloro-5,6-dicyano-p-benzoquinone (0.68 g, 3 mmol) dissolved in THF (50 mL) was added and stirred for one hour. Under ice bath conditions, after adding TEA (10 mL) for half an hour, boron trifluoride ether (10 mL) was slowly added dropwise, and the mixture was stirred overnight at room temperature. Then the solution was evaporated to dryness by a rotary evaporator to remove THF. The product was dissolved in DCM (100 mL) and separated from the saturated saline solution (100 mL×3). The DCM phase was evaporated to dryness by a rotary evaporator and placed in a vacuum oven for drying. The pure product was obtained by column chromatography using hexanes/DCM (2/1, v/v) for elution. NBDP as an orange solid with 50% yield was obtained. The final product was characterized by ^{1}H NMR as shown in **Figure S1**.

2. Synthesis of I-NBDP.

NBDP (0.2 g, 0.5 mmol) and *N*-iodosuccinimide (0.455 g, 2.0 mmol) were dissolved in DCM (100 mL) and then degassed with nitrogen. The solution was stirred at room temperature overnight. After that, the solution was separated from the saturated saline phase (100 mL×3), and the DCM phase was taken. The DCM phase was evaporated to dryness by a rotary evaporator and placed in a vacuum oven for drying. The pure product was obtained by column chromatography using hexanes/DCM (4/1, v/v) for

elution. I-NBDP as a red solid with 31% yield was obtained. The final product was characterized by ^{1}H NMR as shown in **Figure S2**.

3. Synthesis of POEGMA.

OEGMA (500 mg, 15 eq), AIBN (1.8 mg, 1/6 eq), CEPA (16.8 mg, 1 eq) and THF (1 mL) were added to the long-necked glass polymerization tube. The mixture was deoxygenated with nitrogen through at least three freeze-pump-thaw cycles. After that, the polymerization tube was sealed with a torch under vacuum. After reacting at 70 ℃ for 24 h, it was quenched in liquid nitrogen. The mixture was precipitated in ice ether (50 mL×3), and the product was dried in vacuum oven. The final product was characterized by ^{1}H NMR as shown in **Figure S3**.

4. Synthesis of POEGMA-*b*-PDEAEMA.

POEGMA (200 mg，1 eq), AIBN (1.09 mg，1/6 eq), DEAEMA (150 mg, 20 eq) and THF (1 mL) were added to the long-necked glass polymerization tube. This next synthesis step is the same as that of POEGMA. The final product was characterized by ^{1}H NMR as shown in **Figure S4**.

5. Preparation of **IBPAAs** assemblies.

I-NBDP (1 mg) and POEGMA-*b*-PDEAEMA (15 mg) were dissolved in THF (1 mL). The mixed solution was slowly dropped into 4 mL phosphate buffer solution (PBS). After being stirred for half an hour, the solution was transferred to a dialysis bag (MWCO = 5000 Da) and dialyzed in PBS solution for 3 days. The obtained **IBPAAs** assemblies was stored at 4 °C for further use.

6. $^1O_2$ detection *in vitro*.

The sensitive $^1O_2$ trapping agent DPBF was utilized to examine the generation of $^1O_2$ of **IBPAAs** assemblies. The DBPF solution (1 mg/mL) was added into PBS solution of different pH containing the **IBPAAs** assemblies (1×10^{-5} M). After that, the mixture

was irradiated with 550 nm laser, and UV-*vis* spectra were measured at 428 nm at different times.

7. Antibacterial activity *in vitro*.

*S. aureu*s and *E. coli* are used as typical representatives of gram-positive bacteria and gram-negative bacteria. Firstly, the bacteria and **IBPAAs** were added to a 96-well plate at different pH values. They were divided into PBS group and **IBPAAs** group, and each group was divided into dark group and light group. They were placed in a horizontal shaker at 37 °C for 1 h, and then the light group was irradiated with 550 nm laser (0.2 W/cm^2) for 5 min (*S. aureu*s) and 10 min (*E. coli*). Then, all groups were placed in a horizontal shaker at 37 °C and incubated overnight. Then it was evenly spread on a solid medium agar plate. After overnight, the growth of colonies on the plates was recorded. At the same time, *S. aureus* as a typical representative of bacteria was used to incubated with **IBPAAs** under the condition of pH = 5.5 for the adhesion test of bacteria, then the adhesion of **IBPAAs** and bacteria was observed with CLSM.

8. Live and dead staining assay and observation of the bacterial morphology *in vitro*.

The bacteria and **IBPAAs** were added to centrifuge tubes at different pH values and placed them in a horizontal shaker at 37 °C for 1 h. They were divided into PBS group and **IBPAAs** group, and each group was irradiated with 550 nm laser (0.2 W/cm^2) for 5 min (*S. aureu*s) and 10 min (*E. coli*). The mixture was washed with PBS for three times to obtain bacterial suspension.

Live and dead staining assay: The fluorescent dyes SYTO9 and PI were added to the above bacterial suspension in equal proportions and kept at 0 °C for 4 h. After that, the dead and live fluorescence staining of bacteria was observed with CLSM.

Observation of the bacterial morphology: 2.5% glutaraldehyde was added to the bacterial solution, and it was fixed at low temperature for 24 h. Then, ethanol aqueous solution of 30%, 50%, 70%, 80%, 90%, and 100% were added separately for

dehydration. Afterwards, the morphology of the bacteria was observed by SEM.

9. Dissipation of bacterial biofilm *in vitro*.

The MDR *S. aureus* was cultured in plates at 37 °C for 48 h to form biofilm. The biofilms were washed three times with PBS solution, and then incubated with PBS or **IBPAAs** solutions for 1 h. After that, the light groups were irradiated with 550 nm laser (0.2 W/cm^2) for 5 min and washed three times with PBS solution for further experiments.

Crystal violet staining: The crystal violet solution (10 mg/mL) was added into the above biofilm plates to stain for 30 minutes. After washing three times with PBS solution, the dissipation of bacterial biofilm was recorded. Then the biofilm stained with crystal violet was fully dissolved with ethanol, and its UV-*vis* absorption at 590 nm was measured.

CLSM study: 2.5% glutaraldehyde was added into biofilm plates and kept them at 0 °C for 1.5 h. After washing three times with PBS solution, PI and FITC-CONA were added to the biofilm plates for staining at 0 °C for 30 min. Then they were washed three times with PBS solution, and the fluorescent staining of the bacterial biofilm was observed by CLSM.

10. Cytotoxicity *in vitro*.

The cytotoxicity of the **IBPAAs** assemblies was evaluated by the standard MTT assay with L929 cells. L929 cells were cultured in DMEM medium containing 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) at 37 °C under 5% CO_2. L929 cells (25000 cells/mL, 200 μL) were seeded into a 96-well plate and cultured at 37 °C under 5% CO_2 for 24 h. After that, the culture medium was replaced with different concentrations of **IBPAAs** dissolved in the culture medium, and cultured at 37 °C for 24 h. After the medium was aspirated, the MTT solution (5 mg/mL, 200 μL) was added into and cultured for 4 h. Then, the above solution was replaced by 150 μL of DMSO

to dissolve the formazan crystals, and its absorption at 492 nm was measured by a spectrophotometric microplate reader.

11. Antibacterial activity *in vivo*.

All animal experimental procedures were performed in accordance with Chinese legislation on the Use and Care of Research Animals (Document No. 55, 2001), and institutional guidelines for the Care and Use of laboratory animals established by the East China University of Science and Technology Animal Studies Committee. Female Kunming mice around 6 weeks old were raised in a pathogen-free 24 °C environment. The dorsal hair of the anesthetized mice was shaved, and the wounds about 1 cm in diameter were made. Then the wounds were treated with *S. aureus* suspension (10^8 CFU) to induce bacterial infection of the mice wounds and this time was defined as day 0. The mice were divided into PBS group and **IBPAAs** group (3 mice in each group) for different treatments with PBS or **IBPAAs** solutions (100 μL, 10 μg/mL), and then the light groups were irradiated with 550 nm laser (0.2 W/cm^2) for 10 min. The size of the wounds was photographed and recorded at regular intervals.

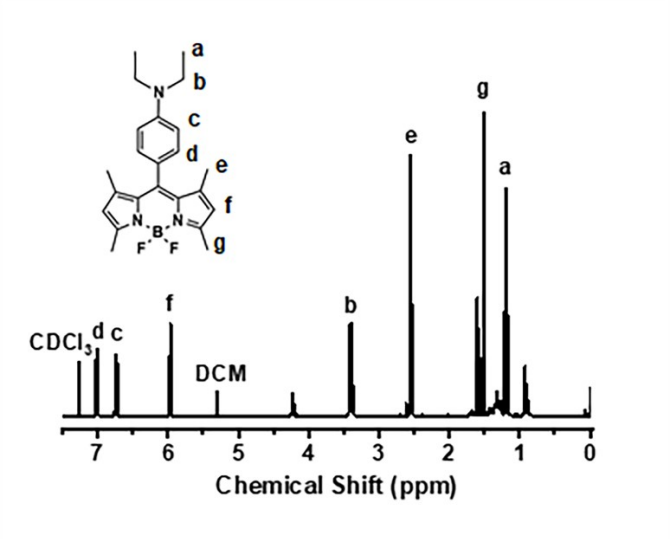


Figure S1. [1]H NMR spectrum of NBDP.

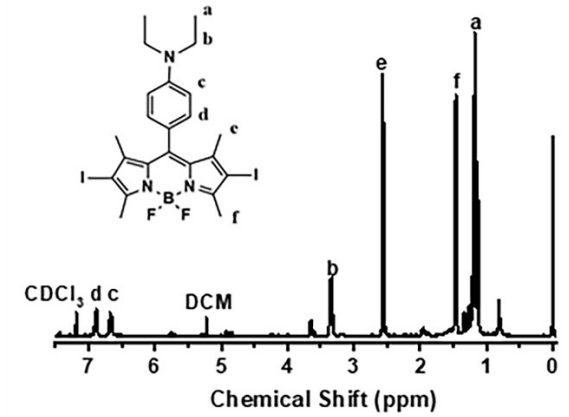


Figure S2. [1]H NMR spectrum of I-NBDP.

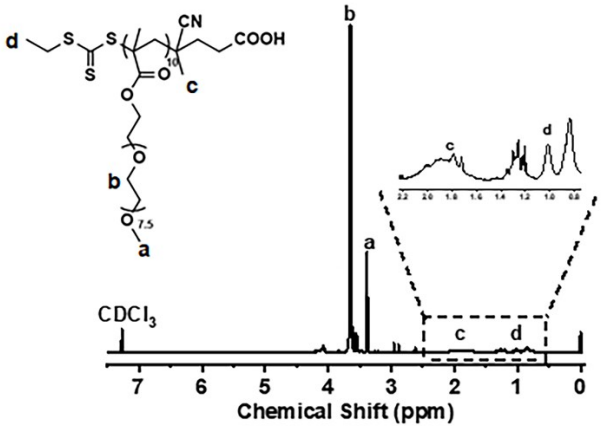


Figure S3. [1]H NMR spectrum of POEGMA.

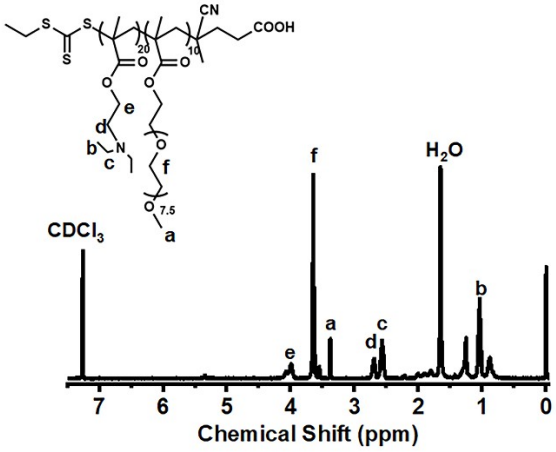


Figure S4. ^{1}H NMR spectrum of POEGMA-*b*-PDEAEMA.

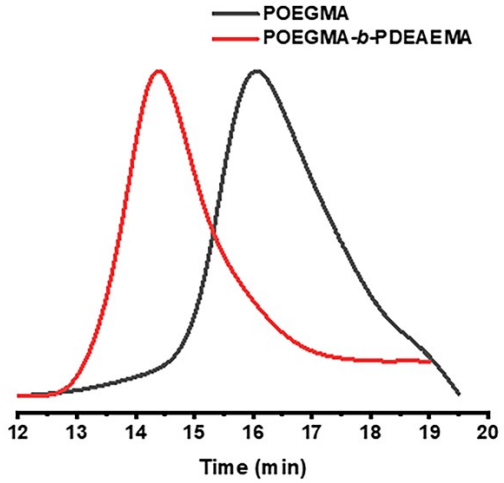


Figure S5. GPC traces of POEGMA and POEGMA-*b*-PDEAEMA.

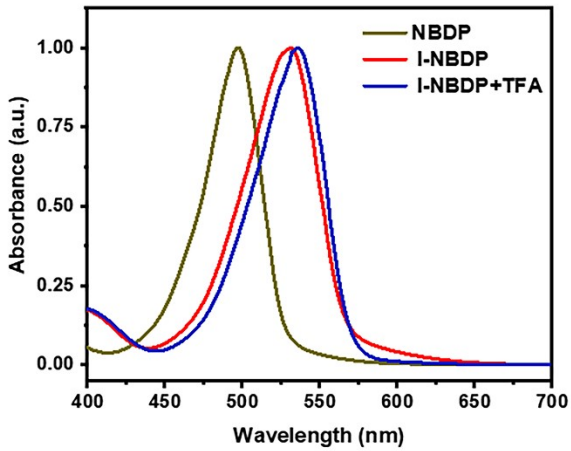


Figure S6. UV-*vis* absorption spectra of NBDP and I-NBDP in acetonitrile solution with and without additional TFA.

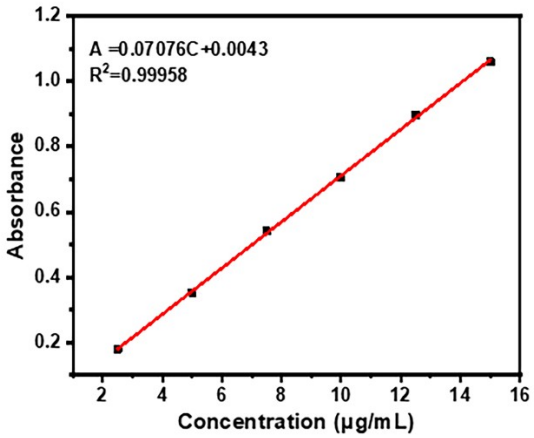


Figure S7. The relationship between the concentration and absorbance of I-NBDP in DMF at 525 nm.

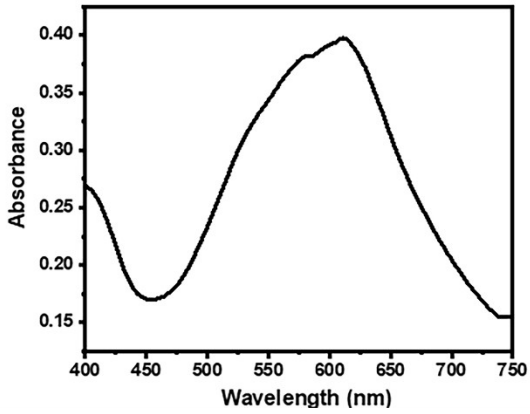


Figure S8. UV-*vis* absorption spectrum of the **IBPAAs** assemblies in PBS solution.

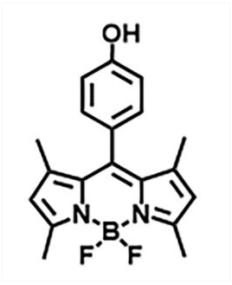


Figure S9. The molecular formula of OBDP.

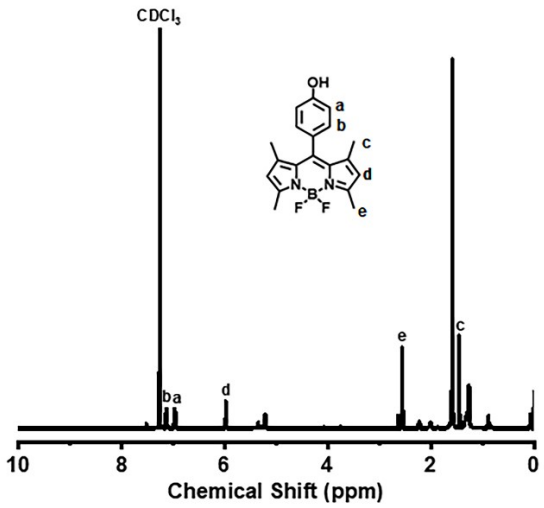


Figure S10. ^{1}H NMR spectrum of OBDP.

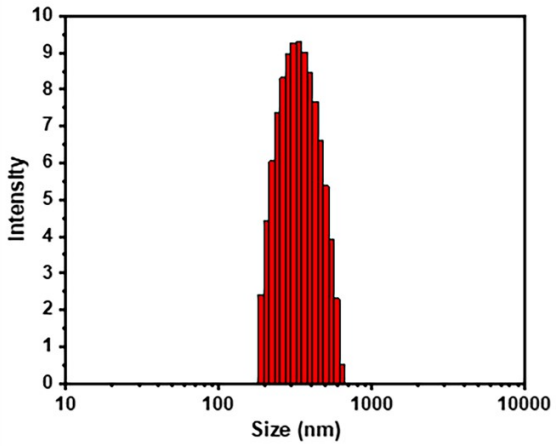


Figure S11. Particle size distribution of OBPAAs assemblies in PBS solution at pH = 7.4.

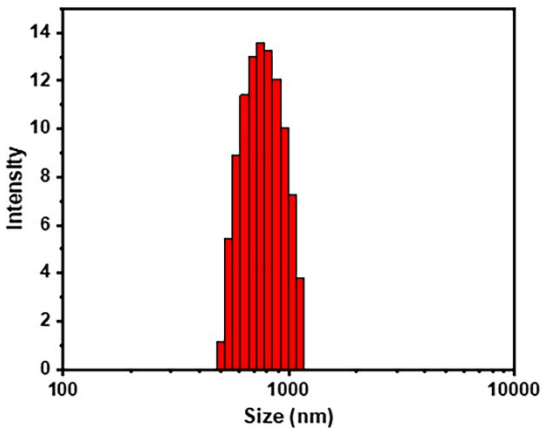


Figure S12. Particle size distribution of OBPAAs assemblies in PBS solution at pH = 5.5.

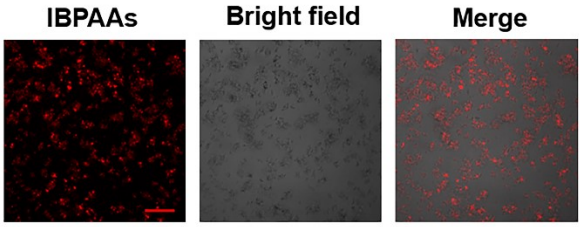


Figure S13. Fluorescence confocal microscopy images of *S. aureus* after incubation with the **IBPAAs** at pH = 5.5. Scale bar: 20 μm.

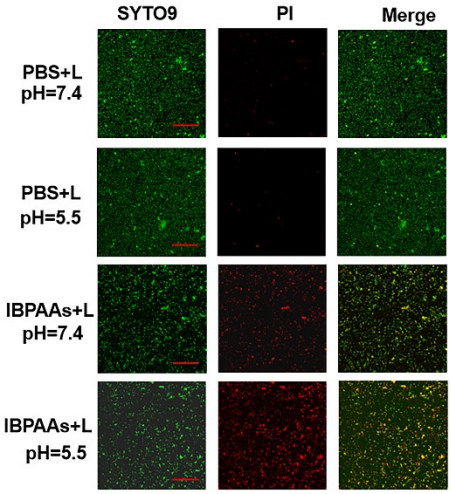


Figure S14. Confocal images of live/dead staining of *S. aureus* before and after treated with **IBPAAs** at pH = 5.5 and pH = 7.4 under light irradiation, respectively. Scale bar: 50 μm.

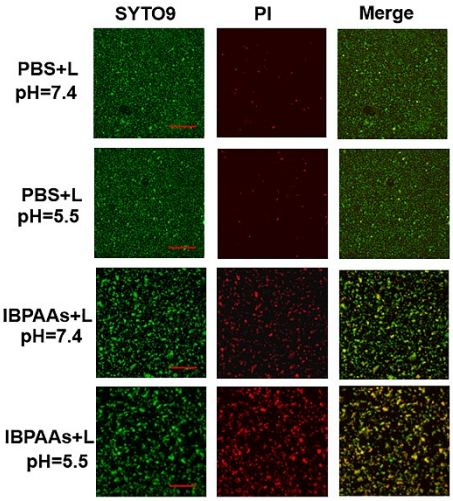


Figure S15. Confocal images of live/dead staining of *E. coli* before and after treated with **IBPAAs** at pH = 5.5 and pH = 7.4 under light irradiation, respectively. Scale bar: 20 μm.

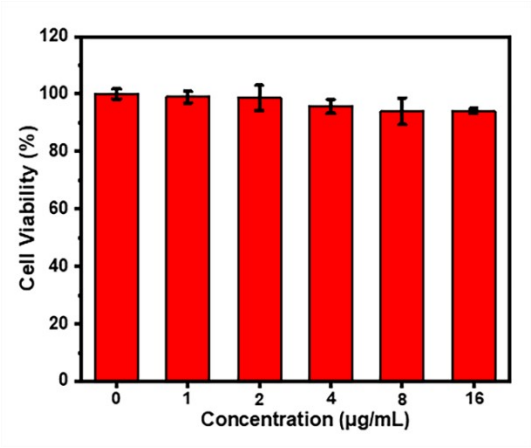


Figure S16. Cell viability of L929 treated with **IBPAAs**.