Supporting Information

Cisplatin Resistance Reversal of Lung Cancers by Tumor Acidity-Activable Vesicular Nanoreactors via Tumor Oxidative Stress Amplification

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1. Materials

Cisplatin (≥ 99%) was purchased from Shandong Platinum Source Chemical Co., Ltd. Glucose oxidase (GOD) from *Aspergillus niger* (200 U/mg) was obtained from Sigma-Aldrich and used as received. Horseradish peroxidase (HRP, 250 U/mg) was procured from Klamar® (Shanghai, China). *β*-D-Glucose was obtained from TCI Development Co.,Ltd., fluorescein isothiocyanate (FITC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98%), *N*-hydroxysuccinimide (NHS), 4-dimethylaminopyridine (DMAP, 98%), and benzyl methacrylate (Bz, 98%) were supplied by Energy Chemical and passed through a silica column before use to remove the inhibitor. PEG-RAFT agent (M_n = 5000, M_n/M_w = 1.05), and 2-(piperidin-1-yl) ethyl methacrylate (PEMA) were synthesized according to the previous reported methods.[1] 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI, 94%), fluorescein diacetate (FDA), Genomic DNA Mini Preparation Kit with Spin Column, reactive oxygen species (ROS) Assay Kit (2',7'-dichlorofluorescin diacetate, DCFH-DA), Caspase 3 Kit, and Annexin V-FITC Apoptosis Detection Kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Fetal bovine serum (FBS), Roswell Park Memorial Institute (RPMI-1640) without glucose, and trypsin were purchased from GIBCO. The cisplatin-resistant human lung cancer cell line A549R and human lung cancer cell line A549 were obtained from Shanghai Fumengjiyin Biotechnology (FMGbio) Co. Ltd. Female BALB/c nude mice and female BALB/c mice with 4-Week-old were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal studies were carried out according to the regulations for the administration of affairs concerning experimental animals (Hefei, revised in June 2015).

2. Characterization

The chemical structures of all synthesized chemicals were characterized by ^{1}H NMR spectra on a Bruker AV300 NMR 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent. The molecular weight (M_w) and molecular weight distribution (M_w/M_n) of the prepared amphiphilic block copolymers were evaluated by gel permeation chromatography (GPC) equipped with a G1310B Iso. pump, a G1316A PL gel column, and a G1362A differential refractive index detector. The eluent was DMF with 1 g/L LiBr at a flow rate of 1.0 mL/min. A series of low-polydispersity PEG standards were employed for calibration. The morphology of self-assembled structures was examined by dropping 10 μL of sample on a copper grid placed on filter paper to remove excess solution, followed by drying process at room temperature before being examined by a JEOL-2100F Transmission Electron Microscope (TEM). SEM element analysis was performed on this equipment. The sample particle sizes, particle size distributions and zeta potentials were determined on a zeta-potential analyzer with dynamic laser light scattering (DLS) equipped a Malvern Zetasizer Nano ZS90, a He-Ne laser (633 nm), and 173^o collecting optics, For zeta potential evaluation, was applied and before polymersomes suspension were diluted and dispersed into ultrapure water at a final concentration of NaCl of 10 mM before being tested either at pH 7.4 or pH 6.8 by phase analysis light scattering (PALS) zeta potential mode, and all data were averaged over three measurements. UV-vis spectroscopy and fluorescence spectroscopy were recorded by UV-2401PC UV-VIS Spectrophotometer (Sahimadzu Corporation, Japan) and F-4600 Fluorescence Spectrophotometer (Hitachi, Japan), respectively. Reversed-phase high performance liquid chromatography (RP-HPLC) analysis was conducted on a Shimadzu HPLC system equipped with a LC-20AP binary pump, a C18 column, and a SPD-

20A UV-Vis detector. A Xenogen IVIS Spectrum optical imaging device (PerkinElmer) was used to study the sample bio-distribution *in vivo*.

3. Synthesis of FITC or Cypate-labelled Glucose Oxidase (FITC-GOD and Cypate-GOD)

FITC or cypate-labelled glucose oxidase were synthesised as follows. For FITC-GOD preparation, FITC in DMSO (25 μL, 10 mg/mL) was dropped into the aqueous solution of GOD (2 mL, 10 mg/mL containing 100 mM sodium carbonate) at 4°C. After 12 h reaction, the reaction mixture was dialyzed against deionized water in the dark and lyophilized. FITC number conjugated to GOD was determined to be 2.5 by the extinction coefficients of 44,100 M^{-1} cm^{-1} at 280 nm (GOD) and 81,000 M^{-1} cm^{-1}at 495 nm (FITC).

For Cypate-GOD conjugation, Cypate (0.1 g, 0.14 mmol), EDC (30 mg, 0.15 mmol), and NHS (19 mg, 0.15 mmol) were dissolved in dimethyl sulfoxide (DMSO) (500 μL) and stirred for 1 h at room temperature. Subsequently, cypate solution (25 μL) was added into GOD aqueous solutions (1 mL, 10 mg/mL). After reaction at 4°C for 12 h, the mixture solution was dialyzed against deionized water in the dark and lyophilized. Cypate number conjugated to GOD was determined to be 2.1 by the extinction coefficients of 44,100 M^{-1} cm^{-1} at 280 nm (GOD) and 224,000 M^{-1} cm^{-1} at 778 nm (cypate).

4. Cellular Uptake of Cisplatin and DNA Platination Measurements

To study the cellular uptake of cisplatin, A549R or A549 cells (5×10^5 cells) were cultured into 12-well plates at 37 °C overnight. Then the cells were incubated with fresh medium containing cisplatin, Cis@Bz-V or Cis/GOD@Bz-V in the presence of glucose (1 mg/mL) at pH 6.8 for 6 h. The equivalent cisplatin concentration was fixed to 1.5 μM. Next, the cells were

washed twice with PBS and collected by trypsinization. The platinum contents in cells were measured by ICP-MS.

To study DNA platination, A549R or A549 cells were incubated with cisplatin, Cis@Bz-V or Cis/GOD@Bz-V in the presence of glucose (1 mg/mL) at pH 7.4 or 6.8 for 48 h. The equivalent platinum concentration was fixed to 1.5 μM. Next, DNA in cells was obtained by Genomic DNA Mini Preparation Kit and the platinum concentration in DNA solution was measured by ICP-MS.

Supporting Reference

[1] (a) J. Li, Y. Li, Y. Wang, W. Ke, W. Chen, W. Wang, Z. Ge, *Nano Letters* **2017**, *17*, 6983-6990; (b) J. Li, A. Dirisala, Z. Ge, Y. Wang, W. Yin, W. Ke, K. Toh, J. Xie, Y. Matsumoto, Y. Anraku, K. Osada, K. Kataoka, *Angewandte Chemie International Edition* **2017**, *56*, 14025-14030.

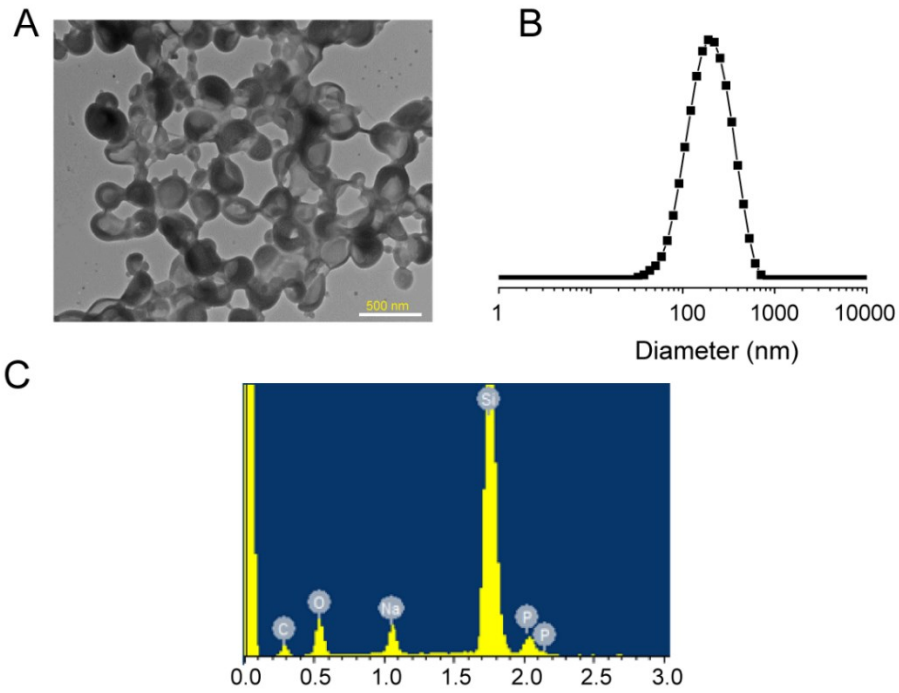

Figure S1. **(A)** TEM image, **(B)** size distribution by DLS and **(C)** SEM element analysis of PEG_{113}-*b*-P($BzMA_{120}$-*co*-$PEMA_{21}$) vesicle.

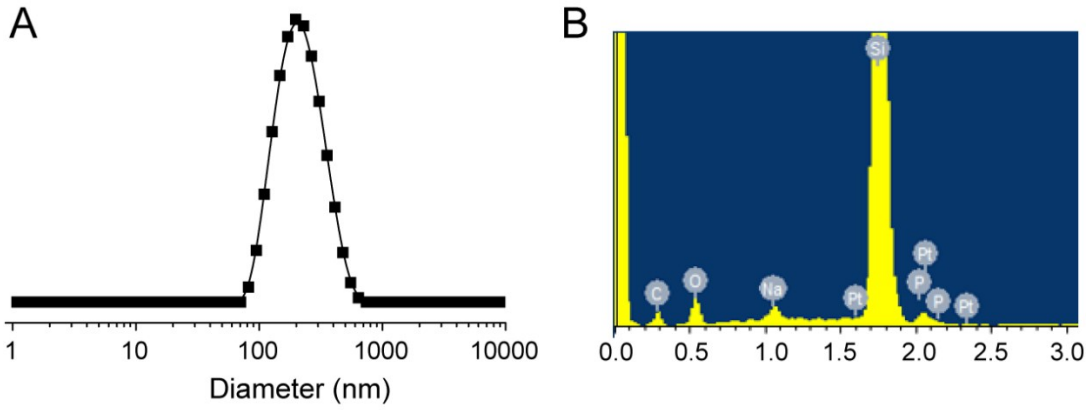

Figure S2. **(A)** Size distribution test by DLS. **(B)** SEM element analysis of cisplatin and GOD-coloaded PEG_{113}-*b*-P($BzMA_{120}$-*co*-$PEMA_{21}$) polymersomes (Cis/GOD@Bz-V).

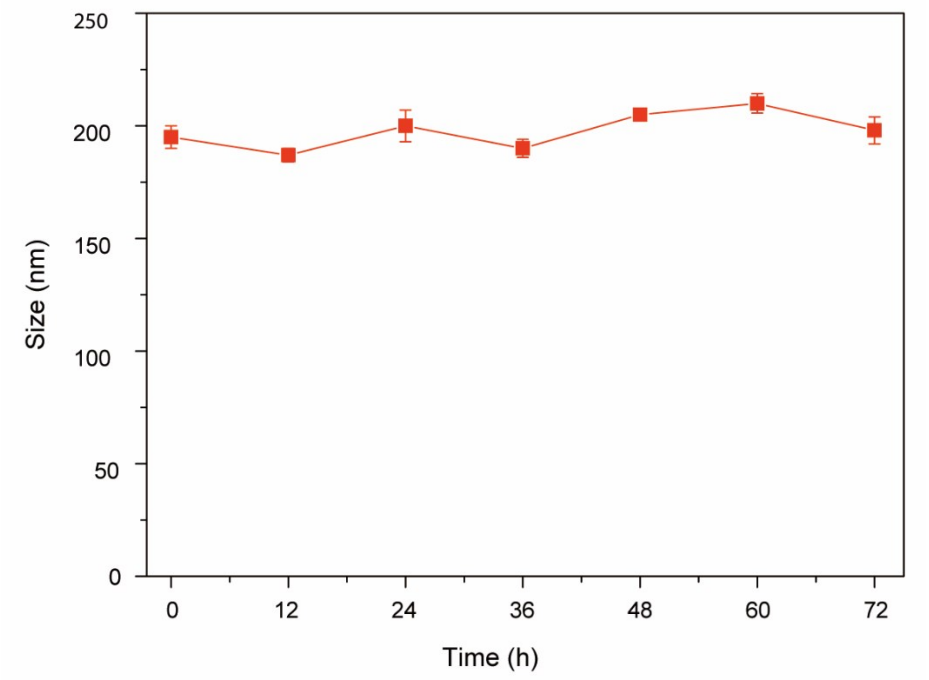

Figure S3. The Cis/GOD@Bz-V nanoreactor size stability analysis by DLS at different time points at pH 6.8 and 37 °C. Mean ± SD, n = 3.

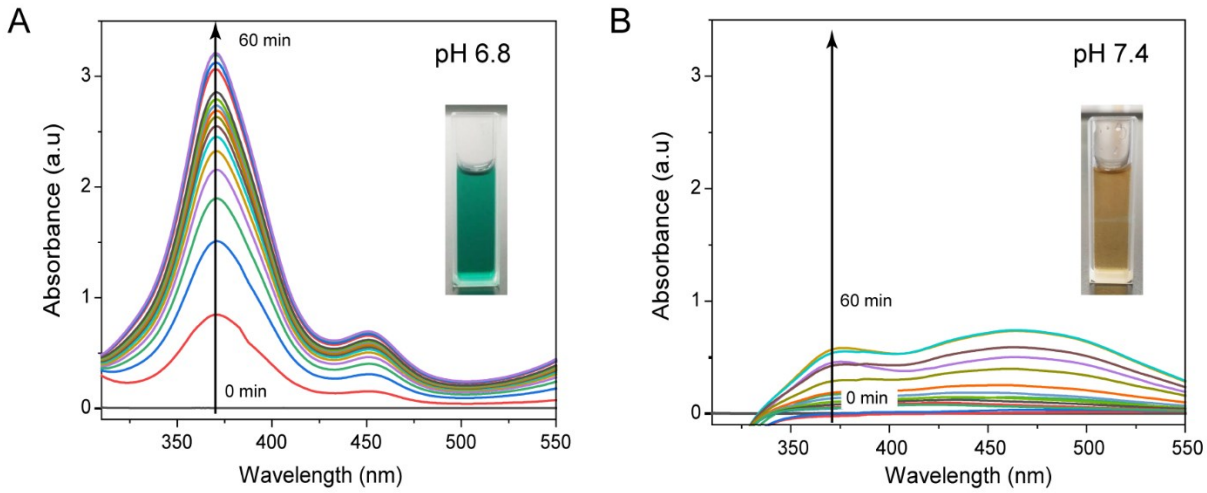

Figure S4. Analysis of pH-responsive membrane selective permeability based on oxidized TMB color absorbance intensity evaluation at 370 nm as the product of cascade reactions of Cis/GOD@Bz-V (GOD: 50 mU/mL) after being incubated in PBS solution in the presence of glucose (1 mg/mL), HRP (150 mU/mL), and TMB (100 μM) at pH 7.4 or pH 6.8. The inset digital images show the color of the solution at pH 6.8 7.4 after 1 h of incubation.

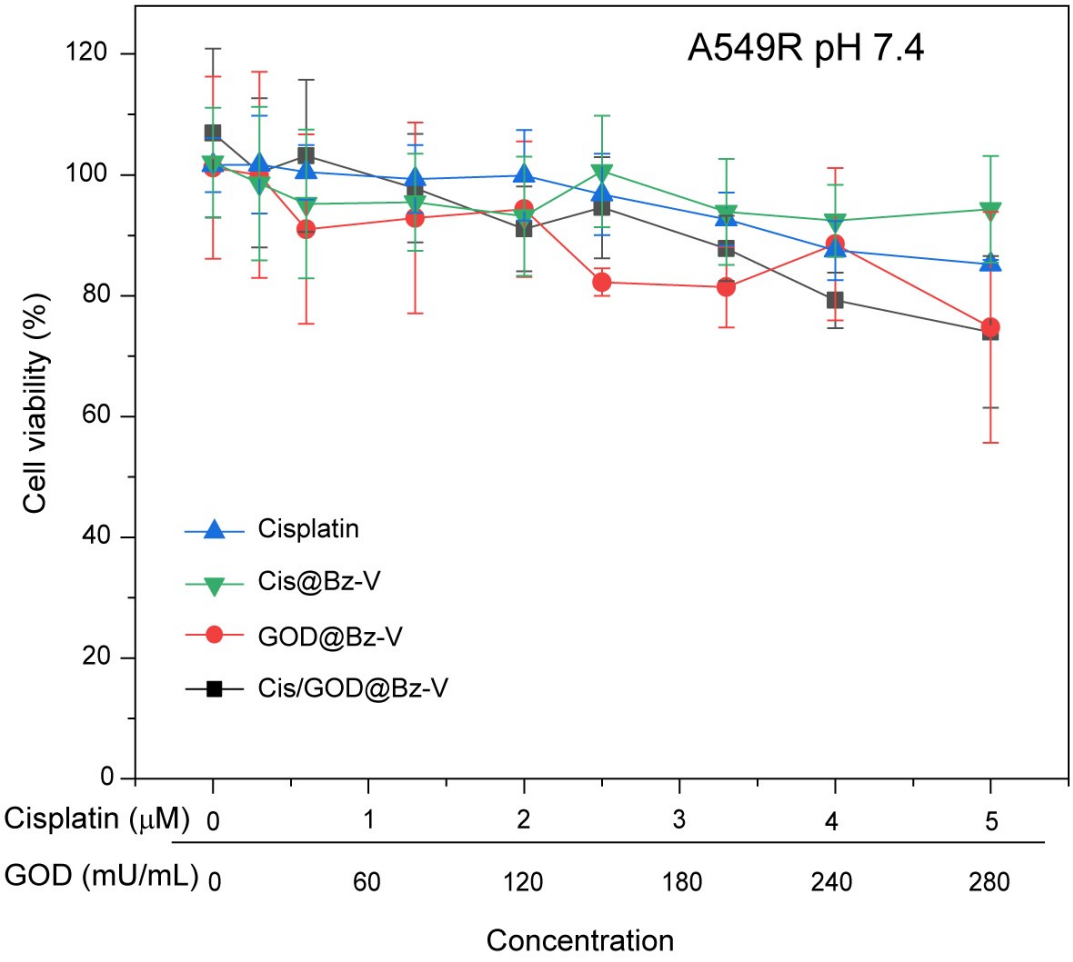

Figure S5. Cell cytotoxicity analysis by treating A549/R cells with various concentrations of free cisplatin, Cis@Bz-V, GOD@Bz-V or Cis/GOD@Bz-V for 48 h at pH 7.4. Mean ± SD, n = 4.

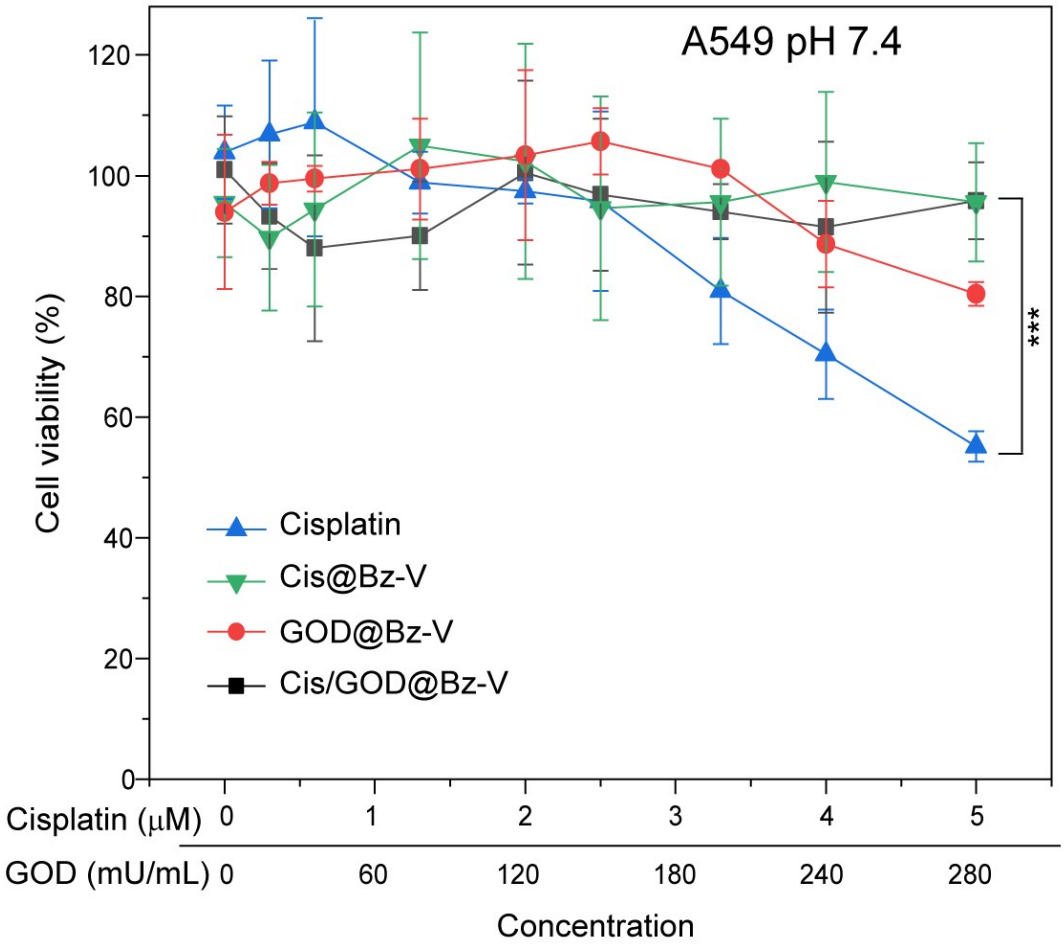

Figure S6. Cell cytotoxicity analysis by treating A549 cells with various concentrations of free cisplatin, Cis@Bz-V, GOD@Bz-V or Cis/GOD@Bz-V for 48 h at pH 7.4. Mean ± SD, $n = 4$.

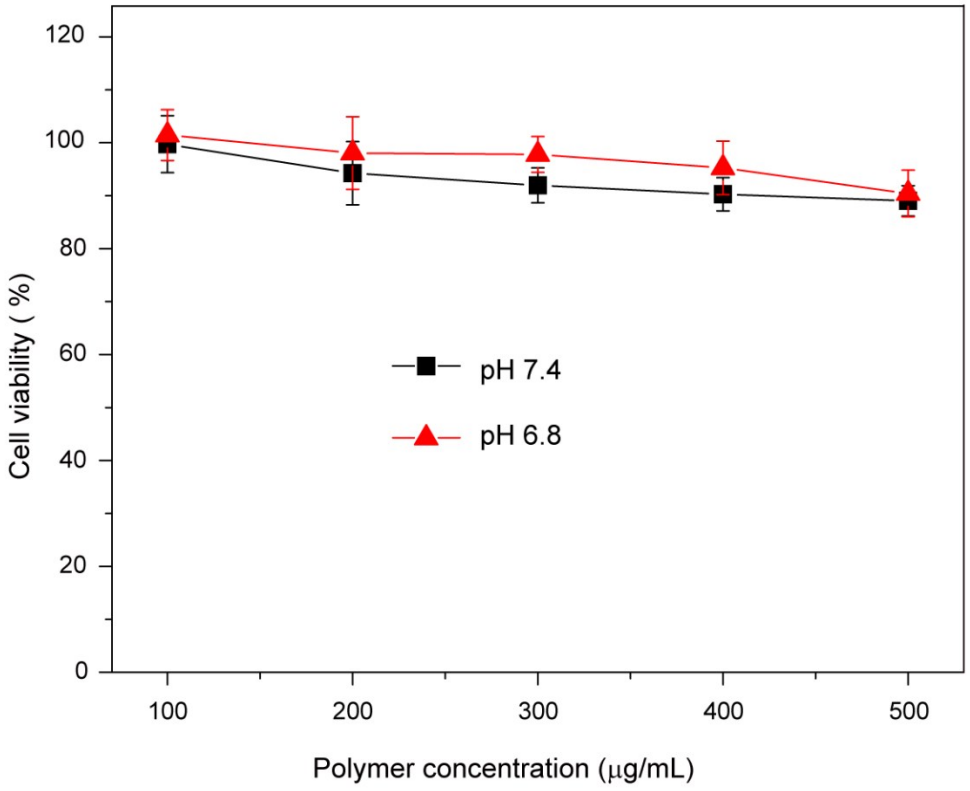

Figure S7. Cell cytotoxicity analysis by treating A549R cell line with various concentrations of PEG_{113}-*b*-P($BzMA_{120}$-*co*-$PEMA_{21}$) polymer at pH 6.8 and 7.4 for 48 h. Mean ± SD, $n = 4$.

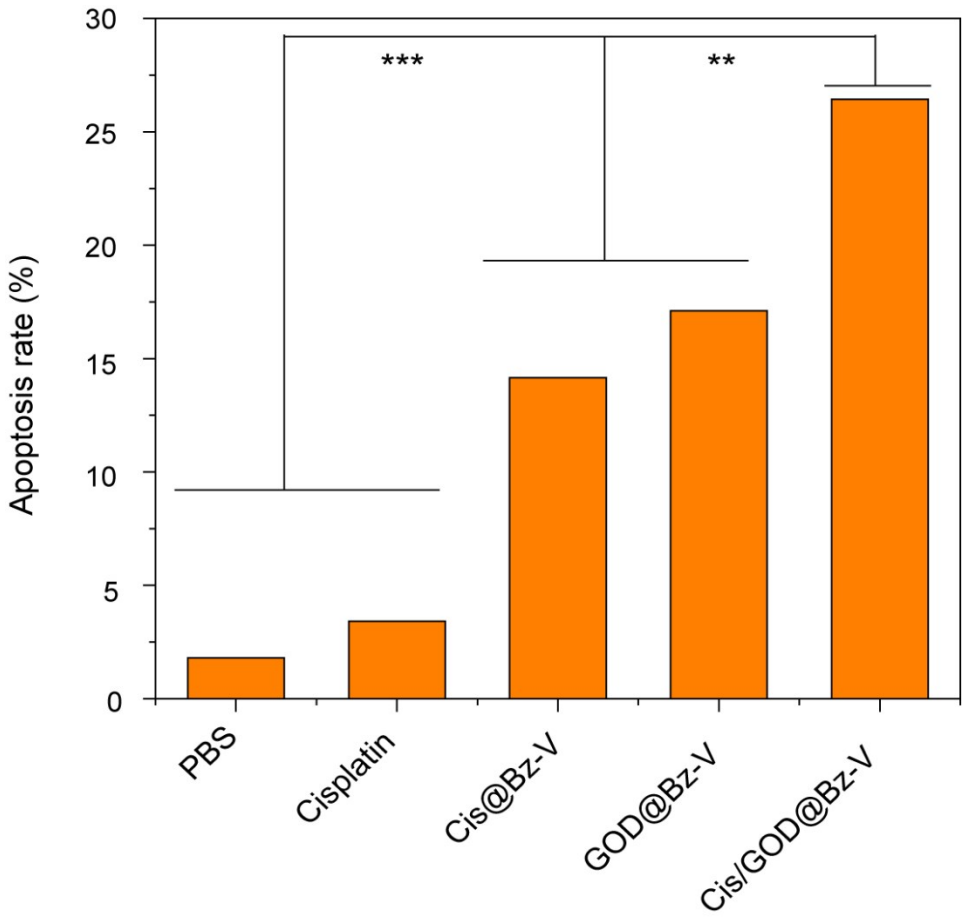

Figure S8. Apoptosis rate quantification in A549R cells after treatments with PBS, free cisplatin, Cis@Bz-V, GOD@Bz-V, or Cis/GOD@Bz-V at the cisplatin-equivalent concentration of 1.5 μM or GOD of 91.4 mU/mL for 24 h in the presence of glucose (1 mg/mL) at pH 6.8. $^{**}p < 0.05$, $^{***}p < 0.01$ (*t*-test).

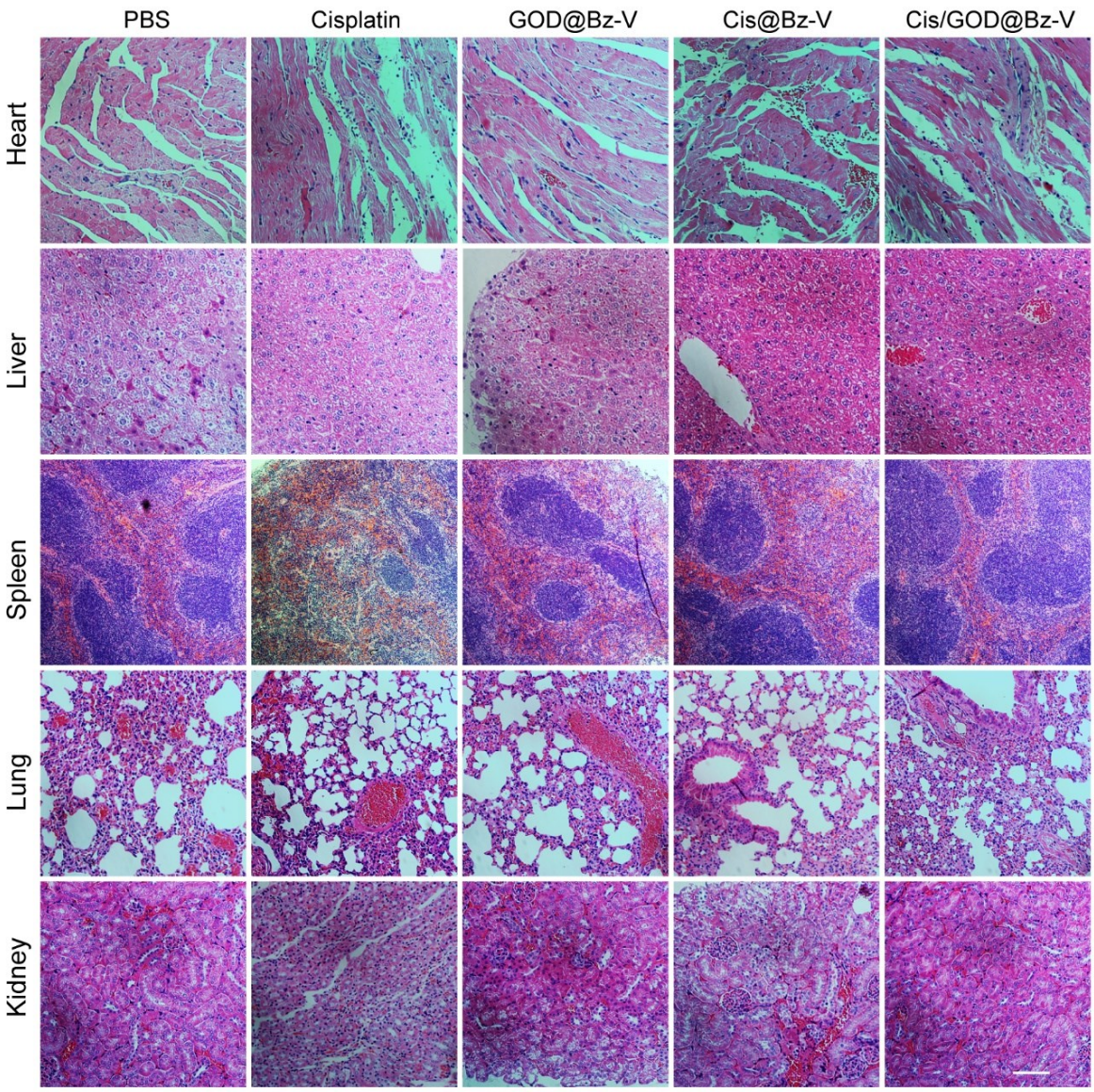

Figure S9. Post-treatment H&E staining of heart, liver, spleen, lung, and kidney organs for the groups treated with PBS, free cisplatin, Cis@Bz-V, GOD@Bz-V, or Cis/GOD@Bz-V formulation. Scale bar represents 100 μm.