Supporting information for

Multifunctional polyphosphazene-coated multi-walled carbon nanotubes for the synergistic treatment of redox-responsive chemotherapy and effective photothermal therapy

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Experiment section

Chemicals.

Carboxylated MWNTs (>98 wt% purity, length of 1–2 μm, diameter of 8–15 nm) were purchased from Chengdu Organic Chemistry Co., Ltd., China. Cut MWNTs were prepared by cutting and purifying the carboxylated MWNTs according to previously published methods (D. Wang, C. Hou and L. Meng, *J. Mater. Chem. B*, 2017, **5**, 1380-1387). Hexachlorocyclotriphosphazene (HCCP), reduced glutathione (GSH), Tween-80, bis(4-hydroxyphenyl)disulfide (HPS), doxorubicin hydrochloride (DOX), and other reagents were purchased from Aladdin Chemical Reagent Co., Ltd. LO2 and HeLa cells were obtained from American type culture collection (ATCC). The WST-1 cell viability assay kit was purchased from Beyotime Biotechnology Co., Ltd. Porous polyvinylidene chloride membrane (diameter of 50 mm and pore size of 0.22 μm) was purchased from Xi'an Kequan Instrument Co., Ltd. Analytical-grade chemicals were used as received. Ultrapure water (18.2 $M\Omega \cdot cm^{-1}$) obtained from a Millipore Milli-Q purification system was used throughout the experiments.

Preparation of MPDH.

First, cut MWNTs were dispersed in acetonitrile (0.25 $mg \cdot mL^{-1}$, 80 mL) and ultrasonicated for 10 min. Second, a mixture of DOX and HPS at different molar ratios (185.6 + 0, 139.2 + 20, 92.8 + 40, 23.2 + 60, or 0 + 80 mg) and TEA (10 mL) was added into the MWNT dispersion. After ultrasonicating the mixture for another 15 min, an HCCP acetonitrile

solution (5.5 mg·mL^{-1}, 10 mL) was slowly added. The resulting mixture was subjected to ultrasonication for another 10 h, followed by the collection of the sediment and filtrate. The sediments were washed by acetonitrile, ethanol, and ultrapure water (3 × 30 mL) to remove the unreacted HCCP, DOX, and HPS, as well as the generated TEA·HCl.

Characterization.

High-resolution transmission electron microscopy (HR-TEM) images were recorded on a JEOL-2100 electron microscope at 200 kV. TEM samples were prepared by the deposition of 5 μL of the clarifying suspensions on the carbon film with a 230-mesh copper micro-grid and air at ambient temperature prior to analysis. Fourier transform infrared (FTIR) spectra were recorded on a Paragon 1000 (Perkin Elmer) spectrometer. Samples were dried overnight at 45 °C under vacuum and thoroughly mixed and crushed with KBr for fabricating KBr pellets. Ultraviolet–visible (UV–vis) absorption spectra were recorded on a Lamba 20 spectrometer (Perkin Elmer, Inc.). Raman spectra were recorded using an argon ion laser operating at 514 nm. The photothermal properties of the modified MWNTs were examined using a NIR laser diode (808 nm, 1.18 W, spot size of 0.754 cm^2) and a digital thermometer (accuracy 0.1 °C, OMEGA Engineering Inc.). The degradation products were analyzed by LC-MS (WATERS I-Class VION IMS QTof). The viability of cells cultured with samples was examined using a microplate reader (Thermo Fisher).

GSH-responsive Drug Release

First, MWNT@PPZ-DOX-HPS was redispersed in different solvents (pH 7.4 PBS, pH 7.4 PBS + 20 μM GSH, pH 5.5 PBS, pH 5.5 PBS + 10 mM GSH) to form dispersions with the same concentration. The four aqueous suspensions of MPDH (1 mg·mL^{-1}, 30 mL) were added into 100-mL cone bottles and arranged in a thermostatic oscillator. The temperature was maintained at 37°C and at an oscillator frequency of 200 rpm. Next, 10 mL of the suspension was removed and subjected to centrifugation. Then, 9 mL of the supernatant was removed, and the sediment was dispersed with another 9 mL of the corresponding solvent and poured back into the cone bottle every 24 h.

Cytotoxicity studies by the WST-1 assay

The cell culture was carried out according to a previously published method[2b]. HeLa and LO2 cells were seeded in 96-well plates at a density of 5 × 10^4 per well for 24 h. Cells were treated with MPDH (0, 10, 25, 50, and 100 μg·mL^{-1}) for 2 h. Then, MPDH was discarded, and the cell was cultured in fresh medium. After 24 h, the cell viability was determined by the addition of 10 μL of the WST-1 agent. Cells cultured without modified MWNTs under the same conditions were used as controls.

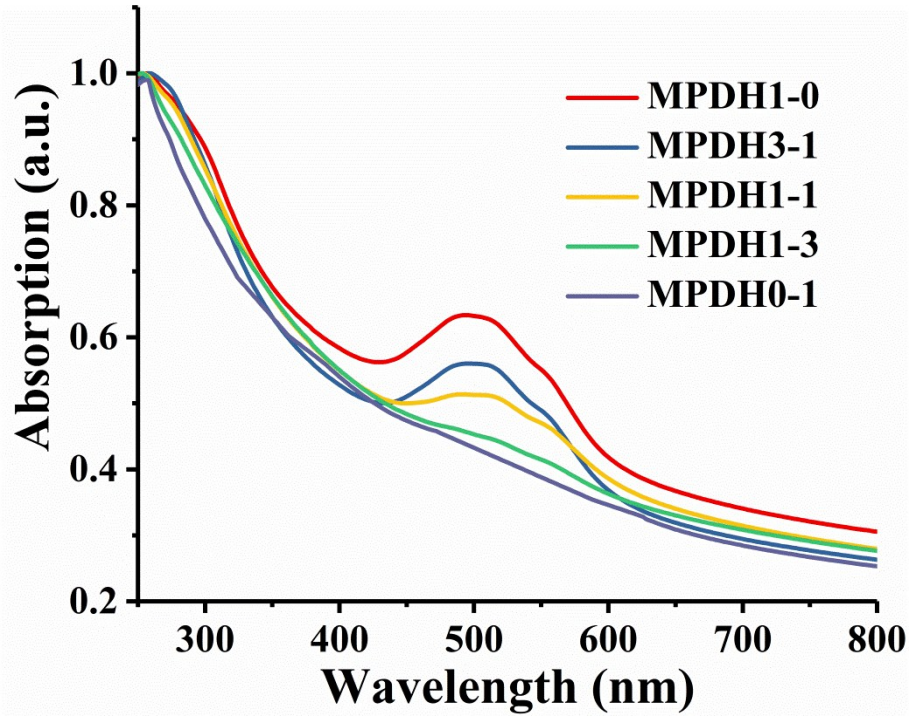

Fig. S1 UV-Vis spectra of the prepared MPDH.

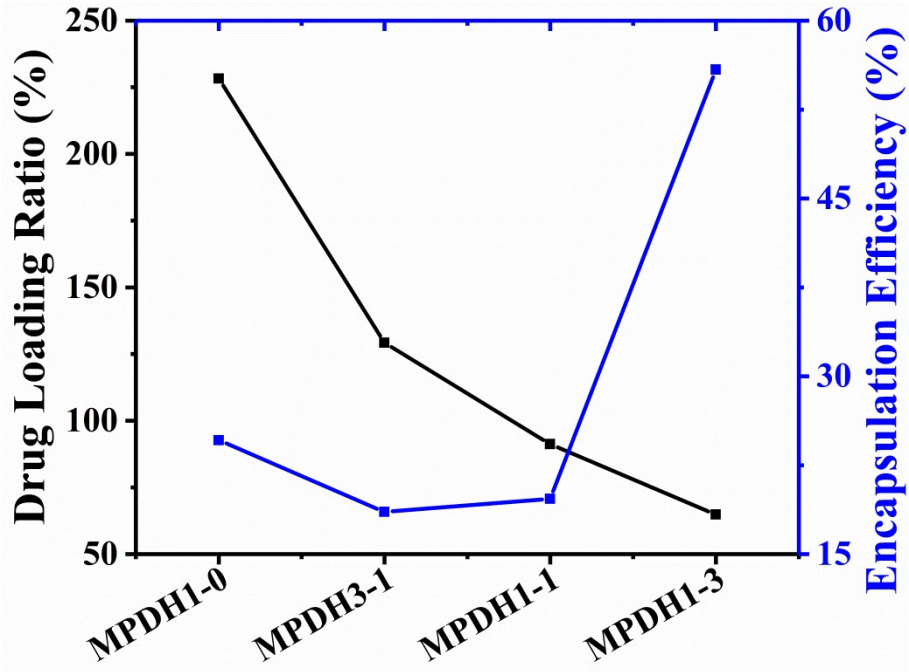

Fig. S2 The drug loading ratio and encapsulation efficiency of MPDH.

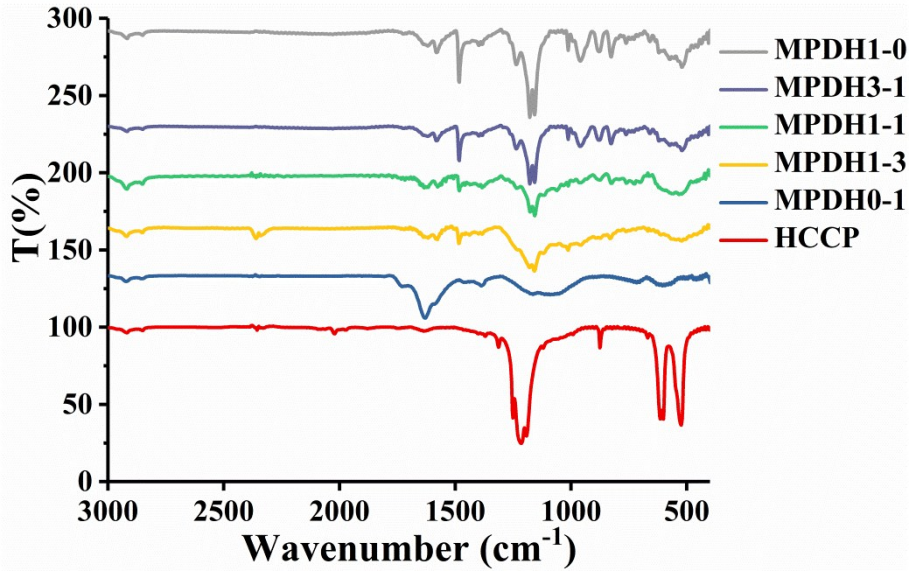

Fig. S3 FT-IR spectra of the prepared MPDH materials and HCCP.

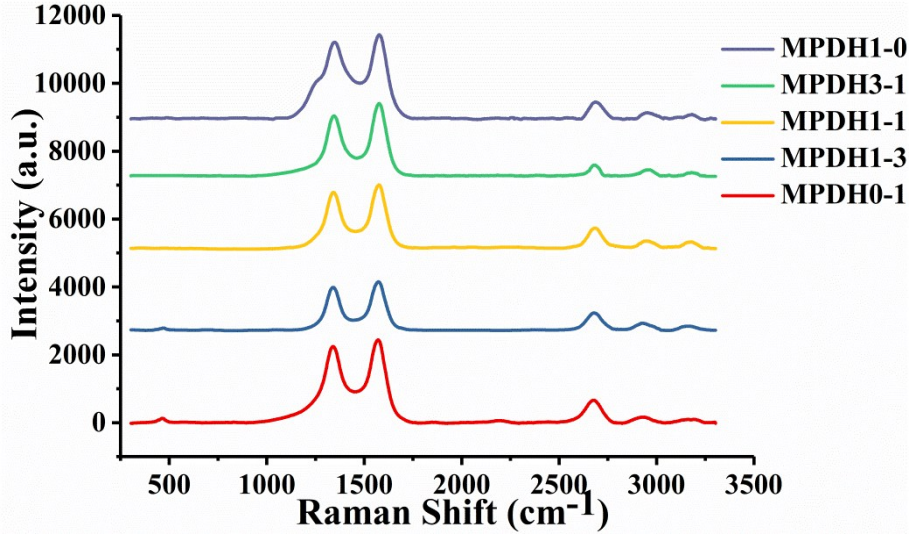

Fig. S4 Raman spectra of the MPDH materials.

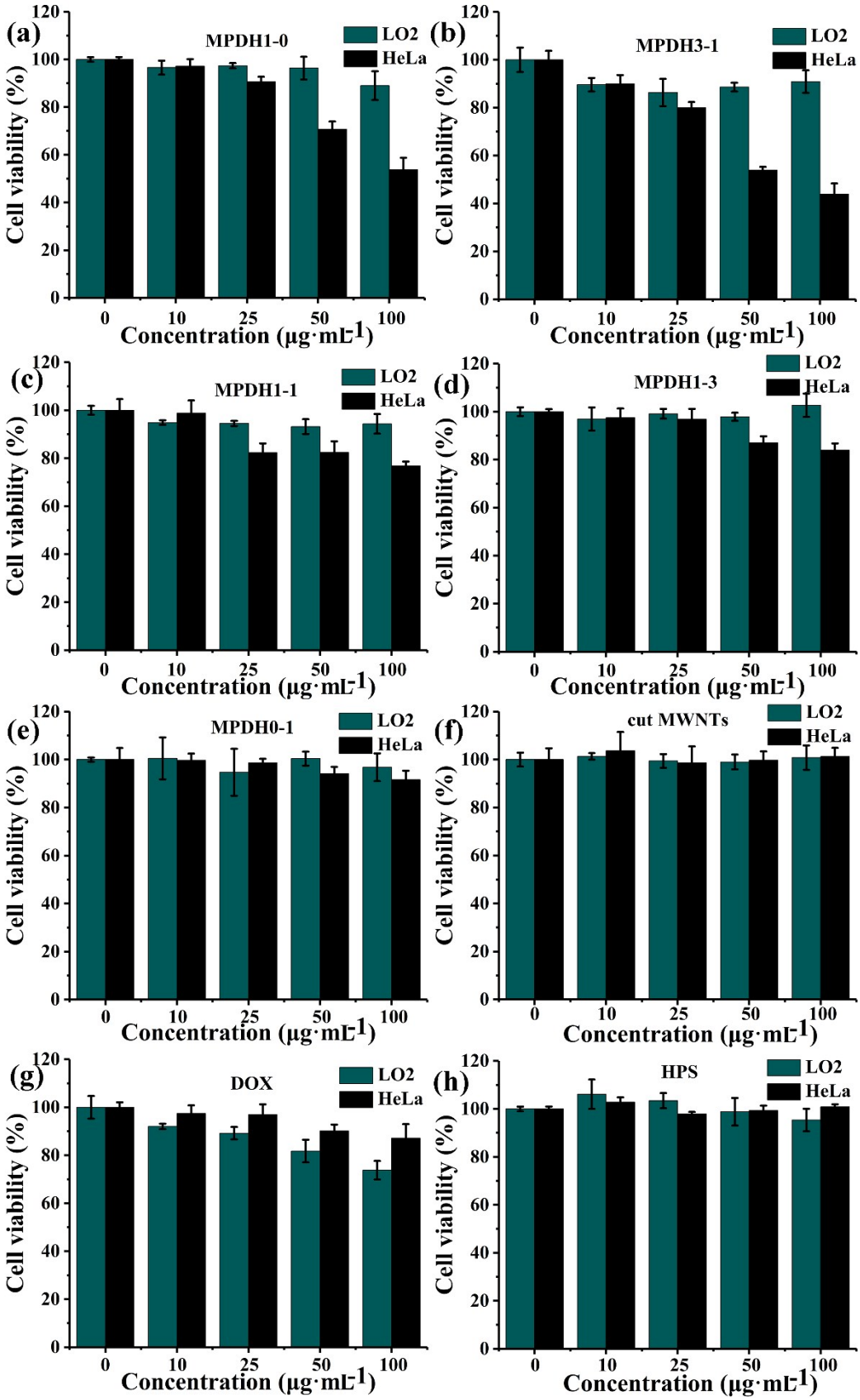

Fig. S5 Cell viability of LO2 and HeLa cells after incubation with the prepared MPDH materials, cut MWNTs, DOX and HPS as a function of concentration over 24 h.

Table. S1 Main degradation species containing DOX of MPDH1-0 in LC-MS result.

Number	Species	Proportion
A	DOX	1
B	HCCP-DOX-OH_2	0.85
C	HCCP-DOX-OH_3	4.31
D	HCCP-DOX-OH_4	0.67
E	HCCP-DOX_2	0.84
F	HCCP-DOX_2-OH	0.99
G	HCCP-DOX_2-OH_2	0.56
H	HCCP-DOX_2-OH_3	1.89
I	HCCP-DOX_2-OH_4	1.20

Table. S2 Main degradation species containing DOX of MPDH3-1 in LC-MS result.

Number	Species	Proportion
A	DOX	1
B	HCCP-DOX_2-HPS-OH	2.59
C	HCCP-DOX-OH_3	2.44
D	HCCP-DOX-HPS_2	1.50
E	HCCP-DOX_2-OH_4	1.44
F	HCCP-DOX_2-HPS-OH_2	1.13
G	HCCP-DOX_2-OH_3	0.97
H	HCCP-DOX_2-HT_2	0.54
I	HCCP-DOX-HPS-OH_2	0.51

HT: p-hydroxy thiophenol