Supporting information

MWCNT-mediated Combinatorial Photothermal and chemo-immunotherapy of Melanoma

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Materials and methods

Materials

MWCNT-COOH (purity of 98% with -COOH content of 3.86%, 0.5-2 mm in length with a diameter of less than 8 nm) was purchased from Chengdu Organic Chemicals Co., Ltd. (Sichuan, China). CpG 7909 (5'-TCG TCG TTT TGT CGT TTT GTC GTT-3') was purchased from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Doxorubicin hydrochloride was purchased from Dakub Meilun Biology Technology Co., Ltd. (Dalian, China). Fetal bovine serum (FBS), RPMI-1640 and DMEM medium were provided by Gibco (Thermo Fisher Scientific, USA). Fluorescein isothiocyanate (FITC) was purchased from Invitrogen (Thermo Fisher Scientific, USA). Carboxy fluorescein diacetate succinimidyl ester (CFSE) was obtained from Sigma-Aldrich (St Louis, USA). IL-4 and recombinant mouse GM-CSF were purchased from PeproTech (Rocky Hill, USA). IFN-γ, TNF-α and IL-6 ELISA kits, and fluorescence-labeled antibodies against CD3e, CD4, CD8a, CD25, FoxP3, CD11b, F4/80, CD206, CCR7, MHC-I, MHC-II, CD40, CD80, CD86, CCR7, and CD11c were supplied by eBioscience (San Diego, USA).

Cells and animals

B16 cells, a mouse melanoma cells of C57BL/6 origin, were purchased from the Cell Bank of Peking Union Medical College. (Beijing, China). C57BL/6 mice (6 weeks, female), utilized for antitumor investigation, were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China). All animal procedures were conducted following the protocol approved by the Institutional Laboratory Animal Ethics Committee, and in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College.

Methods

1. Preparation of MWCNT-CpG and MWCNT-DOX complexes

One milligram MWCNT and 2 mg CpG were mixed in 1 mL Milli-Q water and sonicated for 30 min in an ice water bath to prepare MWCNT-CpG complex. Similarly, MWCNT-DOX complex was prepared by mixing 1 mg MWCNT and 3 mg DOX in 1 mL Milli-Q water with sonicating for 30 min. The complexes were subsequently stirred at 450 rpm for 12 h at room temperature under dark condition, followed by placing in a dialysis tubing with MWCO of 25,000 or 3,500 Da.(Solarbio Science&Technology Co., Ltd. (Beijing, China)) and dialyzing against Milli-Q water overnight to remove the unloaded CpG or DOX.

2. Characterization of MWCNT-CpG and MWCNT-DOX complexes

The surface morphology of the complexes was observed by transmission electron microscopy (TEM) (JEM-1010; JEOL, Tokyo, Japan). The size and zeta potential were measured with the Zetasizer Nano-ZS instrument (Malvern Instruments, Malvern, UK). The absorbance spectrum was analyzed using a UV spectrophotometer (Lambda 35 UV-VIS, PerkinElmer, USA). The NIR absorption of MWCNT-DOX and MWCNT-CpG was measured with Fourier transform infrared spectrometer (FTIR) (Nicolet 2000, Thermo Fisher Scientific, Waltham, MA) from 4000 to 400 cm^{-1} on KBr plates. The Raman spectrum was probed with the inVia Raman Microscope (Renishaw, UK).

3. Determination of the drug encapsulation and loading efficiency

The amount of CpG or DOX loaded onto MWCNT was quantified by measuring OD at 260 nm (CpG) or 480 nm (DOX) of the overnight dialyzate of MWCNT-CpG or MWCNT-DOX, using CpG or DOX dilution with predetermined concentrations as standards. The encapsulation efficiency and drug-loading efficiency were calculated by the following formulas:

encapsulation efficiency (%)=(weight of loaded drug)/(weight of feeding drug)×100%

drug-loading efficiency (%)=(weight of loaded drug)/(weight of loaded drug+weight of MWCNT)×100%

4. In vitro drug release study

The in vitro release profile of CpG or DOX from MWCNT-CpG or MWCNT-DOX complex in response to pH variation and NIR irradiation was investigated using a dialysis method. Briefly, MWCNT-CpG or MWCNT-DOX suspension was placed into a dialysis tubing and dialyzed against PBS (pH of 7.4 or 5.5) in a 37℃ incubator with shaking at 300 rpm, with or without NIR irradiation (1 W/cm^2 for 5 min). 3 mL of the dialyzate was collected for analysis at pre-determined time intervals, and equal amount of fresh PBS was re-added to replace the removed

volume. The concentration of CpG or DOX was calculated as described above. All the samples were tested in triplicate. The cumulative release was calculated by the following formula:

$$\textit{cumulative release}\ (\%) = \frac{CnVn + \sum_{i=0}^{n-1} CiVi}{W \times LE\ \%}$$

（Cn: the concentration of samples taken at time point n; Vn: the total volume of the release medium; Ci: the concentration of samples taken at time point i; Vi: the total volume of samples taken at time point i; W: the weight of MWCNT-CpG or MWCNT-DOX; LE: the drug-loading efficiency of MWCNT-CpG or MWCNT-DOX.）

5. Evaluation of light-thermal conversion efficiency

MWCNT, MWCNT-CpG or MWCNT-DOX suspension with 10 μg /mL MWCNT was irradiated with 808 nm NIR laser (1W/cm^2, 1-5 min), and the temperature was recorded using a thermal imaging camera (E5, FLIR System Inc, Oregon, USA).

Results

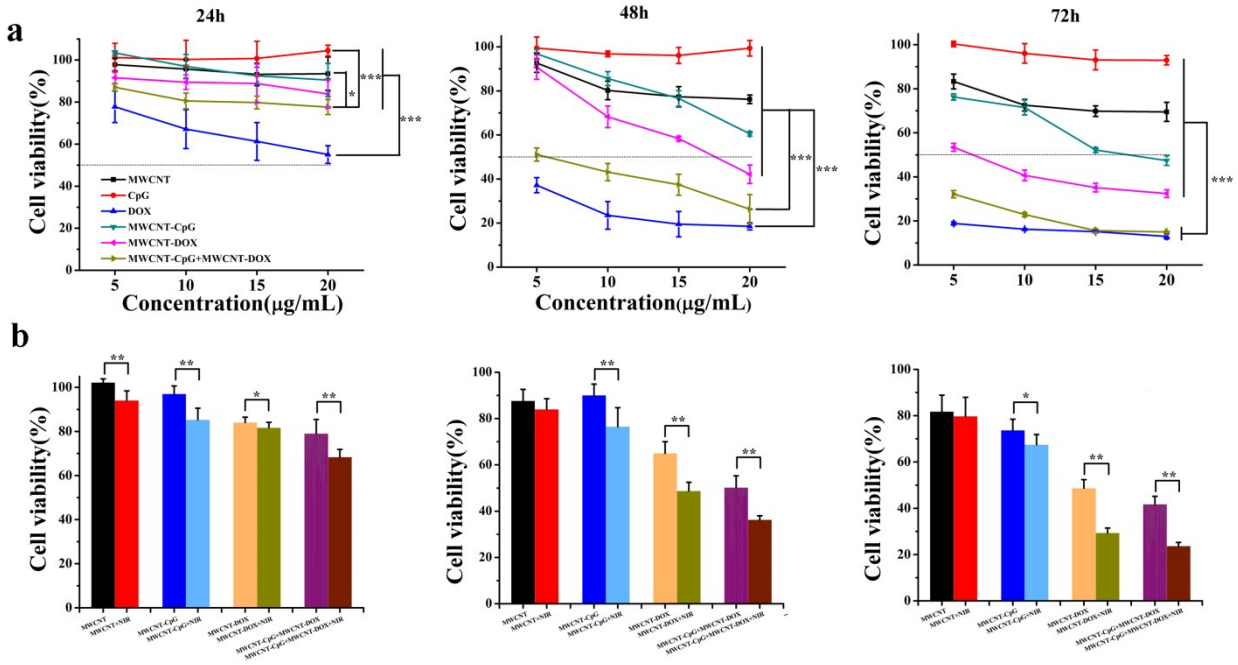

Fig. S1. Viability of L02 cells. (a) Viability of L02 cells treated with different concentrations of MWCNT, CpG, DOX, MWCNT-CpG,

MWCNT-DOX or MWCNT-CpG+MWCNT-DOX; (b) Viability of L02 cells treated with MWCNT, MWCNT-CpG, MWCNT-DOX and MWCNT-CpG+MWCNT-DOX with or without NIR irradiation (808 nm). Data were presented as mean ± SD (n=5). The statistical significance in the difference was analyzed using one-way ANOVA with Tukey's post-test. *P<0.05, **P<0.01, ***P<0.001.

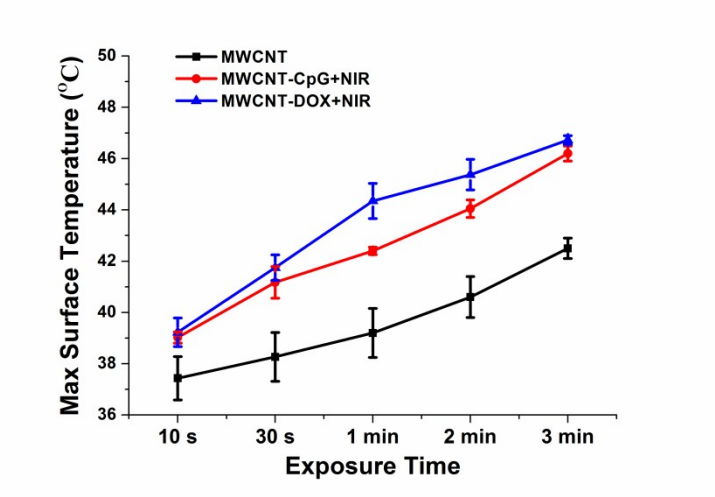

Fig. S2. Surface temperature increase of tumor bearing mice after treatment with MWCNT, MWCNT-CpG or MWCNT-DOX, and subsequent NIR irradiation. Data were presented as mean ± SD (n=6).

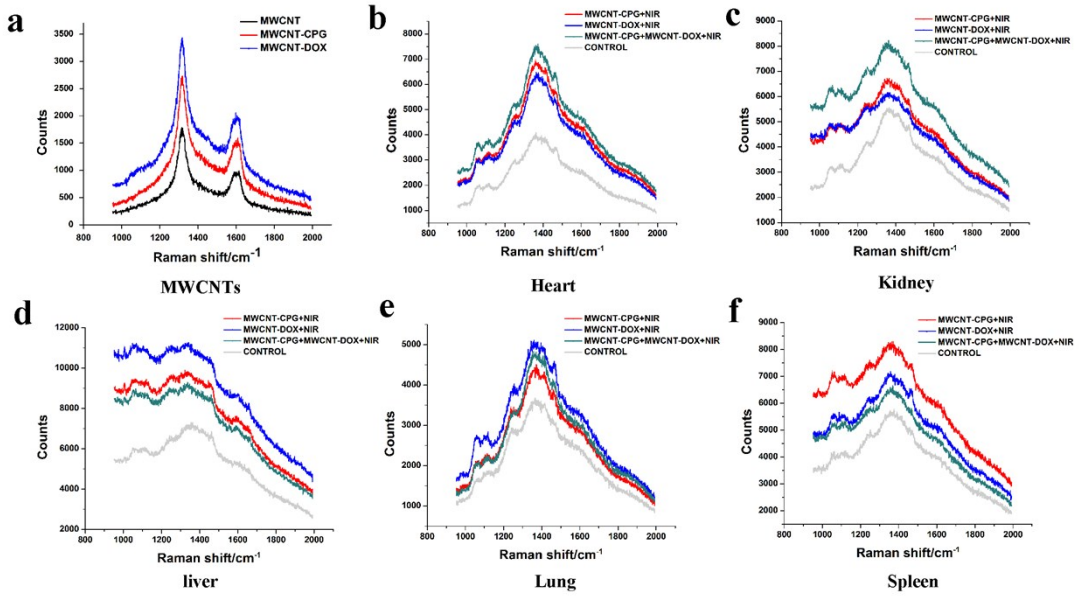

Fig. S3. Biodistribution of MWCNT in mice organs probed by in vitro Raman spectroscopy. (a) MWCNT, MWCNT-CpG and MWCNT-DOX; (b) Heart; (c) Kidney; (d) Liver; (e) Lung; (f) Spleen.