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Polyphenol-based nanoplatform for MRI/PET dual-modality imaging guided effective combination chemotherapy

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1. Chemicals and Materials:

Doxorubicin hydrochloride (DOX HCl), Simvastatin (SV), N,N-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), triethylamine (TEA), anhydrous N,N-dimethylformamide (DMF), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), iron (III) chloride ($FeCl_3$), and Manganese (II) sulfate hydrate, Zirconium (IV) chloride, 5-hydroxydopamine hydrochloride (98%), poly(ethylene glycol) methyl ether 2-bromoisobutyrate, poly(ethylene glycol) methyl ether 2-(dodecylthiocarbonothioylthio)-2methylpropionate (PEG DDMAT, macroCTA), pentafluorophenyl methacrylate (PFMA), 2,2-Azobis(2-methylpropionitrile) (AIBN) and 4-(2-Aminoethyl)-1,2-benzenediol hydrochloride were purchased from Sigma-Aldrich. Dead Cell Apoptosis Kit with Annexin V Alexa Fluor® 488 & Propidium Iodide (PI) were purchased from Thermo Fisher Scientific. Chlorin e6 monolysine amide trisodium salt (Ce6) was purchased from Santa Cruz Biotechnology, Inc. VECTASHIELD Antifade Mounting Medium with DAPI was purchased from Vector Laboratories. All above materials were used as received. High-purity water (Milli-Q water) with a resistivity of 18.2 MΩ cm was gained from a Millipore Milli-Q purification system (Millipore Corporation, U.S.A.).

2. General Characterization:

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Bruker AV300 scanner by using DMSO-d_6 as the solvent. Transmission electron microscopy (TEM) images were acquired with a Tecnai TF30 transmission electron microscope (TEM, FEI, Hillsboro, OR). Confocal microscopy images were acquired on a Zeiss LSM 780 microscope. Flow cytometry analysis was conducted with BD Beckman Coulter flow cytometer (Brea, CA) and FlowJo Software (TreeStar, Ashland, OR).

3. Animal model

The animal experiment procedures were conducted following a protocol approved by the National Institutes of Health Clinical Center Animal Care and Use Committee (NIH CC/ACUC).

4. Tumor treatment

The U87MG xenograft tumor mice were conducted by inoculating U87MG cells

(3×10^6) on the right flank of each mice. When the U87MG tumor volume reached to ~80 mm^3, all the mice were randomly divided into five groups as follows: (I) control group, (II) free DOX (1 mg/kg), (III) free SV (1 mg/kg), (IV) free SV (DOX dose of 1 mg/kg; SV dose of 1.6 mg/kg) and PFDS NPs (DOX dose of 1 mg/kg; SV dose of 1.6 mg/kg). All the above mice were three times tail vein injection every other day. The length and width of each tumor, and the weight of mice were measured by a digital caliper every other day. The tumor volumes were calculated following the formula: volume= length×$width^2$/2. The growth curve was calculated by comparing the tumor volumes (V) at all days to the initial tumor volumes as day 0 (V_0). One week after the treatment, one mouse from each group were euthanized, and the tumor sections were used for H&E staining.

5. In vivo ^{89}Zr-labelled PFDS NPs for PET imaging

U87MG tumor bearing mice were injected with 100 μCi of ^{89}Zr- PFDS NPs. A Siemens Inveon micro PET scanner was employed for the PET imaging at different time point post-injection. For each PET scan, 3-dimensional region of interests (ROIs) has been drawn for quantification. The %ID/g was calculated by the readings. The major organs were harvested for γ counting at 72 h post-injection.

6. In vivo T_1 Magnetic resonance imaging (MRI) of PFDS NPs

The Mn^{2+} doped PFDS NPs has been employed for T_1 MRI. The PFDS NPs were injected by tail vein to the mice. A Bruker MRI scanner (7.0 T, B-C 70/16, Bruker, US) was used for relaxation times calculation and MR imaging. The Imaging was performed pre- and post-injections at various timepoints.

Table S1. Half maximal inhibitory concentration (IC_{50}) of free drugs, drug combination, and PFDS NPs against U87MG cancer cells.

Drug Formulation	IC_{50} (μM)
Free DOX	0.76
Free SV	7.15
Free drugs combination (DOX)	0.20
Free drugs combination (SV)	0.39
PFDS NPs (DOX)	0.31
PFDS NPs (SV)	0.55

Table S2. Combination Index (CI) of free drugs combination and PFDS NPs.

Drug Formulation	Combination Index (based on IC_{50})
free drugs combination	0.32
PFDS NPs	0.49

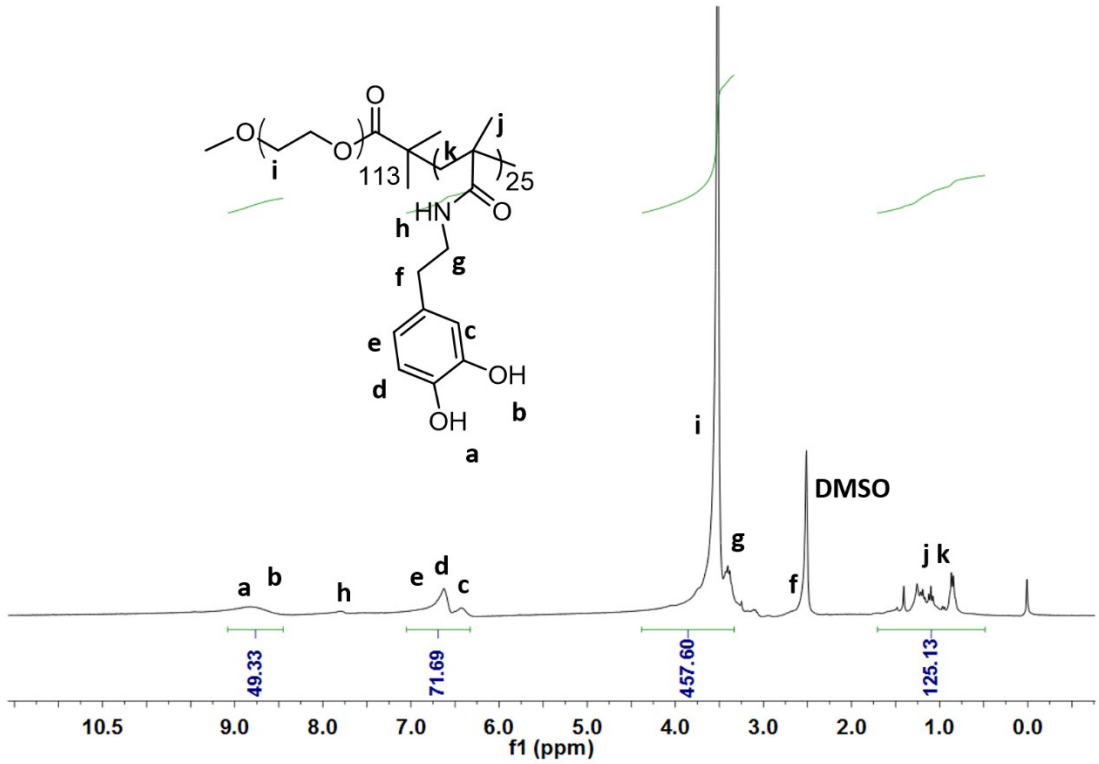

Fig. S1. ^{1}H NMR spectrum of the PEG_{5000}-PDA_{25} copolymers.

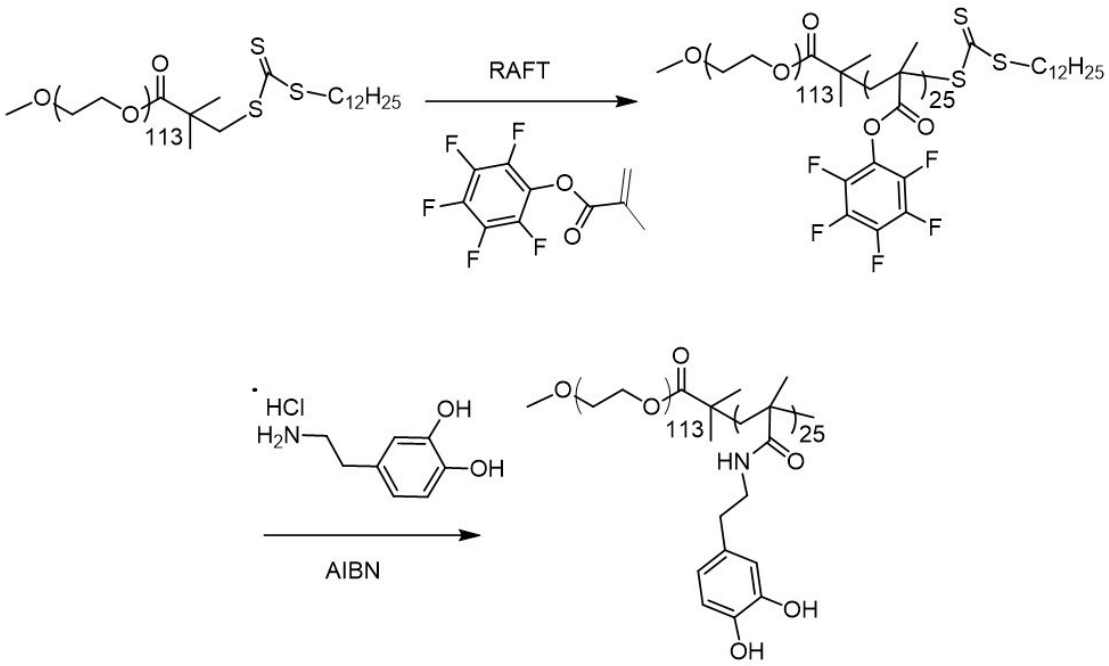

Fig.S2. The synthesized procedure of the PEG_{5000}-PDA_{25} copolymer.

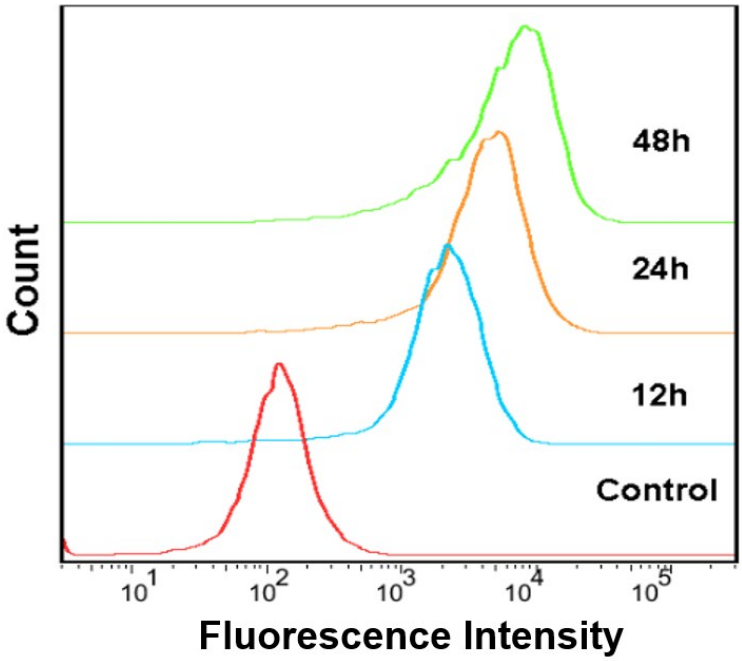

Fig. S3. Flow analysis of cellular uptake after incubating AF488-labelled PFDS NPs under different time.

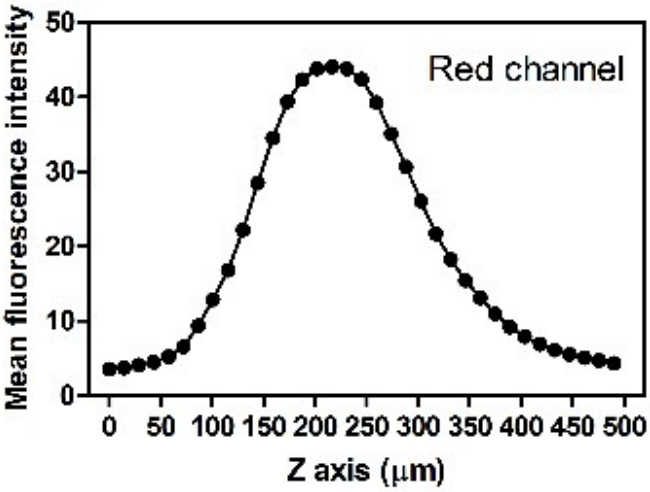

Fig. S4. The mean fluorescence intensity of confocal microscope images (U87MG cell spheroid, red channel).

Fig. S5. In vitro T_1-weighted MR images of PFDS NPs at different Mn concentration.

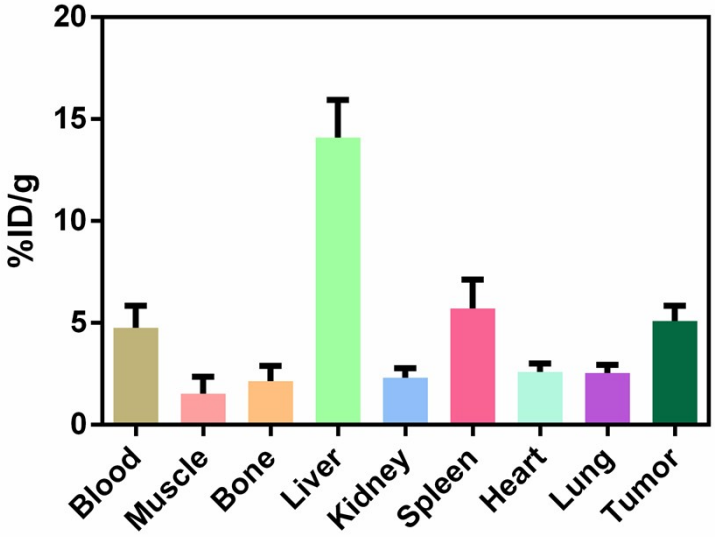

Fig. S6. The biodistribution of PFDS NPs in most organs at 72 h post-injection by gamma counter.

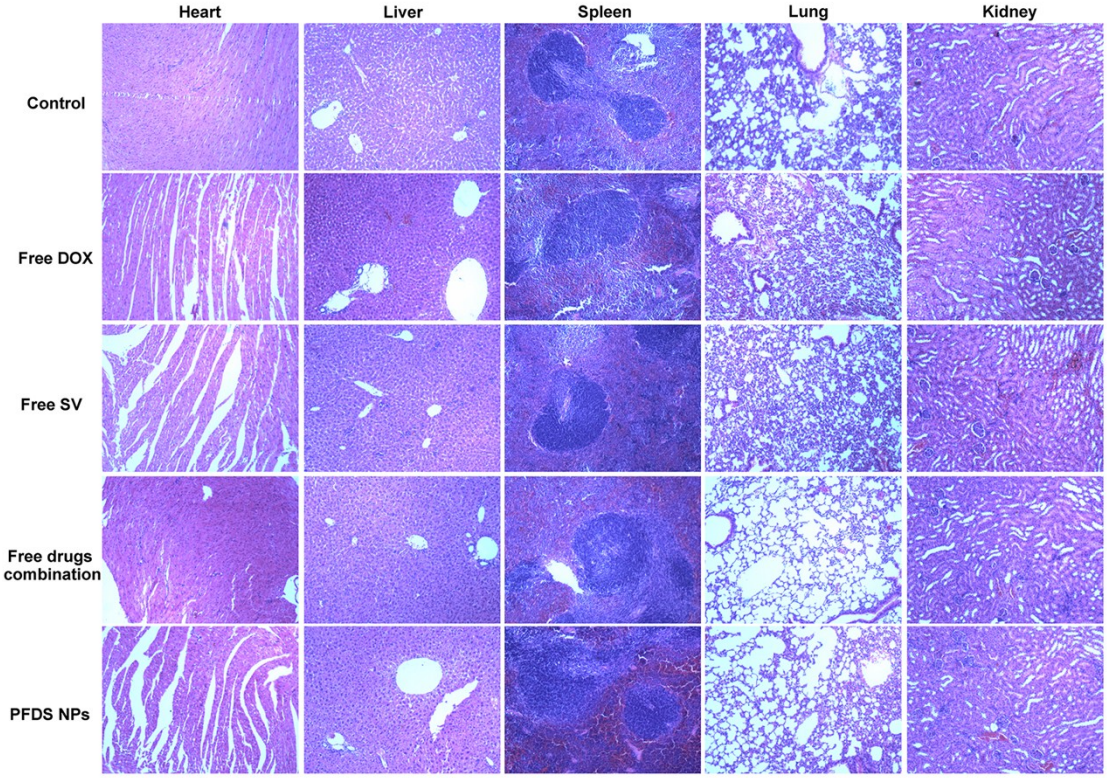

Fig. S7. The H&E staining of major organs under various treatments.