Supplementary Information

Functionalized carbon nanomaterials as nanocarriers for loading and delivery of poorly water soluble anticancer drug: A comparative study

Nanda Gopal Sahoo[a], Hongqian Bao[a], Yongzheng Pan[a], Mintu Pal[b], Mitali Kakran[a], Henry Kuo Feng Cheng[a], Lin Li[a*], Lay Poh Tan[b]

[a]School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

[b]School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

E-mail: mlli@ntu.edu.sg (L. Li)

Materials and methods

1) Materials

The MWCNTs were purchased from Iljin Nano Tech, Korea. Their diameter and length were about 10–20 nm and 20 μm, respectively. These nanotubes (purity of 95%) were produced by chemical vapor deposition. Graphite with an average particle size of 100 μm was obtained from Sigma-Aldrich. CPT was purchased from Guanyu Bio Inc from China. Poly (vinyl alcohol) (PVA) (M_w ~61,000) was obtained from Fluka. (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) and other mentioned reagents were purchased from Aldrich and used as received.

2) Preparation of MWCNT-PVA

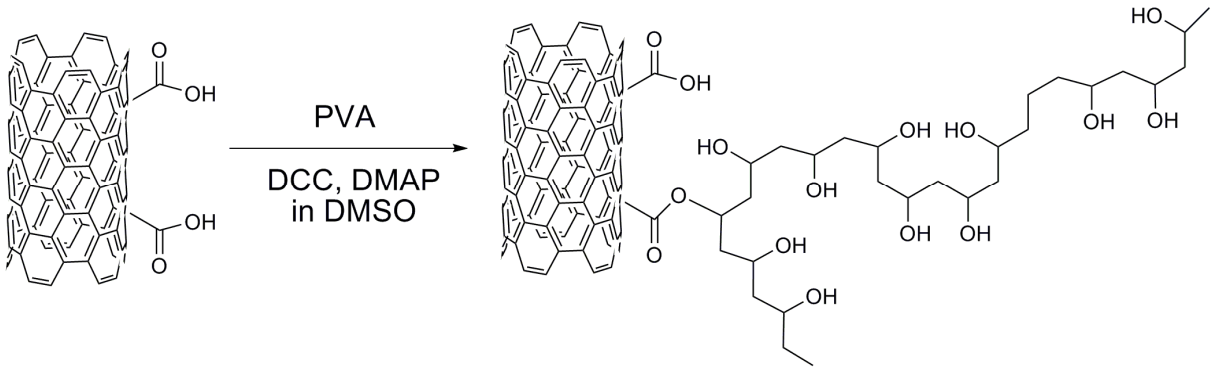
The MWCNTs were functionalized with PVA in a carbodiimide-activated esterification reaction. In a typical procedure, 55 mg purified CNT-COOH was dissolved in 10 mL dimethyl sulfoxide (DMSO) and sonicated for 30 min to obtain a homogeneous black-colored solution. The catalysts, N,N'-dicyclohexylcarbodiimide (DCC) (600mg, 2.91 mmol) and 4-dimethylaminopyridine (DMAP) (99 mg, 0.81 mmol) were gradually charged into the flask and stirred for 10 min. Then, a solution of PVA in DMSO (100 mg/mL, 5 mL) was added, and the mixture was sonicated to react at 50 °C for 24 h. After it was terminated, the dark suspension was filtered over a 0.2 μm PTFE microporous membrane, and the obtained solid was washed

thoroughly with acetone. Excess PVA was removed by dissolving the solid in water and heating at 60 ^{o}C, and the aqueous solution was filtered using a 0.2 μm Nylon membrane. After washing the filtered residues with a large amount of hot water, the PVA functionalized CNTs were obtained and dried in a vacuum oven. The FTIR spectra for the raw and functionalized MWCNTs are shown in Figure S 1.a. The very low intensity characteristic peaks of the raw MWCNTs were evident in the FTIR spectra at 3440 cm^{-1} (OH) and 1640^{-1} (C=O). After the H_2SO_4/HNO_3 treatment, the peaks of the MWCNT-COOH appeared at a higher intensity. These results suggested the increased number of carboxylic acid groups generated at the surface of the MWCNTs after the treatment with H_2SO_4/HNO_3. The MWCNT-COOH was further used for the preparation of MWCNT-PVA. The successful synthesis of MWCNT-PVA can be clearly verified from the FTIR spectra. The new intensity peak at 1720 cm^{-1} could be assigned to the C=O stretching of ester linkage of the MWCNT-PVA while the other peak at 1155 cm^{-1} was due to the C-O stretching. In addition, the peak at 2910 cm^{-1} was attributed to the C-H stretching of the PVA.

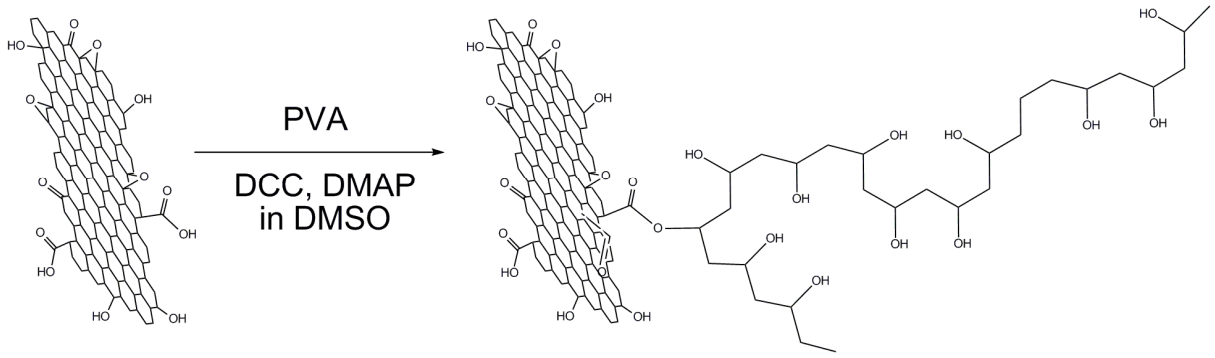
3) Preparation of GO-PVA

Preparation of GO. GO was synthesized using a modified Hummer's method from natural graphite powder.[1] Graphite powder (3 g) was put into an 80 °C solution of concentrated H_2SO_4 (97 %, 12 mL), $K_2S_2O_8$ (2.5 g), and P_2O_5 (2.5 g). The mixture was kept at 80 °C for 6 h using a hotplate. Successively, the mixture was cooled to room temperature and diluted with 0.5 L of de-ionized water and left for overnight. Then the mixture was filtered and washed with de-ionized water using a 0.2 μm Nylon filter to remove the residual acid. The product was dried under ambient condition for overnight. This pre-oxidized graphite was then oxidized by the Hummers' method.[2] Briefly, 1 g of the pretreated graphite and 0.5 g of $NaNO_3$ were placed into a flask. Then, 25 mL of H_2SO_4 was added with stirring in an ice-water bath, and 3 g of $KMnO_4$ was slowly added over about 1 h. Stirring was continued for 2 h in the ice-water bath. After the mixture was stirred vigorously at room temperature for 2 days, 100 mL of 5 wt% H_2SO_4 aqueous solution was added over about 1 h with stirring, and the temperature was kept at 98 °C. The resultant mixture was further stirred at 98 °C for 2 h. The temperature was reduced to 60 °C, and 3 mL of H_2O_2 (30 wt% aqueous solution) was added, and the mixture was stirred at room temperature for 2h. The oxidation product was purified by rinsing with a 10% HCl solution,

repeatedly washing with copious amounts of de-ionized water, and filtering through a 0.2 μm Nylon membrane.



a.

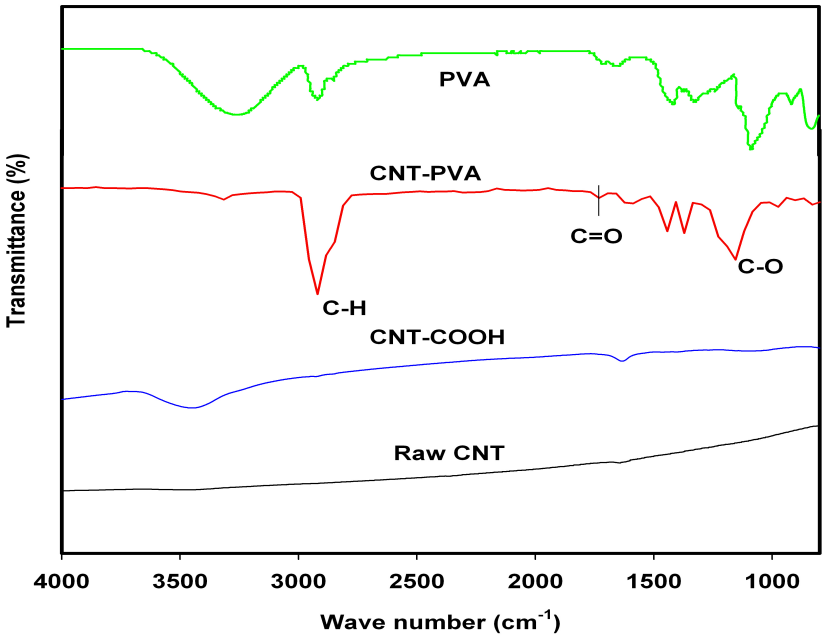


b.

Scheme S 1. Synthesis of (a) MWCNT-PVA and (b) GO-PVA

Preparation GO-PVA. The GO was functionalized with PVA in a carbodiimide-activated esterification reaction that was the same as that used for the preparation of MWCNT-PVA. In this case, 50 mg purified GO was dissolved in 15 mL DMSO and sonicated for 30 min to obtain a homogeneous brown-colored solution. The catalysts, DCC (2.3 g, 11 mmol) and DMAP (0.17 g, 1.4 mmol) were gradually added into the flask and stirred for 10 min. Then, a solution of PVA in DMSO (100 mg/mL, 5 mL) was added, and the resulting mixture was stirred at 50 °C for 3 days. After the reaction was terminated, the suspension was filtered over a 0.2 μm PTFE microporous membrane, and the obtained solid was washed thoroughly with DMF and acetone. In order to eliminate the unreacted PVA, the solid was dissolved in hot water, and the suspension was filtered using a 0.2 μm Nylon membrane. After washing the filtered residues

with a large amount of hot water, the PVA functionalized GO was obtained and dried in a vacuum oven.



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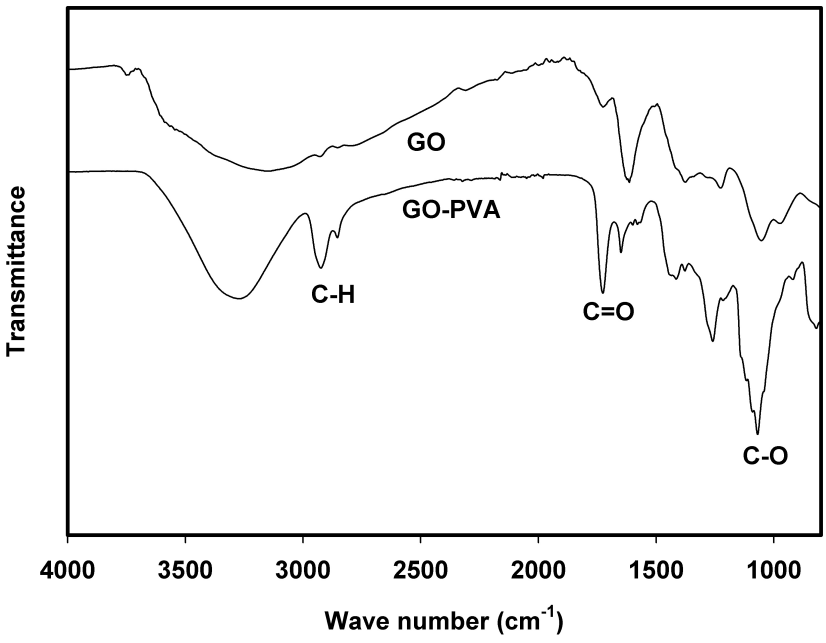


b.

Figure S1. FTIR spectra for (a) raw MWCNTs, MWCNT-COOH, MWCNT-PVA, and PVA; (b) GO and GO-PVA.

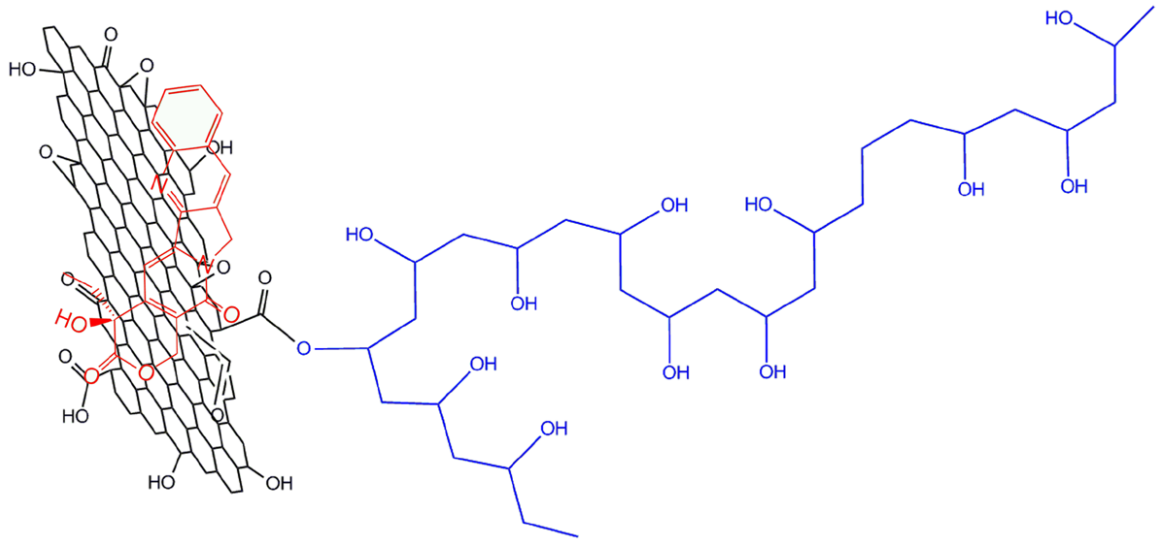
From the FTIR spectra shown in Figure S1.b, the absorbance peaks at 1057 cm^{-1}, 1379 cm^{-1}, 1614 cm^{-1}, 1726 cm^{-1}, 3147 cm^{-1} can be attributed to C-O stretching (epoxy or alkoxy), O-H stretching (carboxyl), C=C assigning to skeletal vibrations of unoxidized graphite domains, C=O in carboxylic acid and carbonyl moieties, and O-H broad coupling stretching (hydroxyl), respectively. After functionalization of GO with PVA, all the peaks of GO appeared at a higher intensity. Moreover, methylene ($-CH_2-$) stretching vibration at 2914 cm^{-1} and 2850 cm^{-1} were also prominent due to the presence of PVA in the GO-PVA.

4) Loading of CPT on MWCNT-PVA and GO-PVA

5 mL of 0.5 mg/mL MWCNT-PVA or GO-PVA in DI water were separately mixed with 0.5 mL of 8.6 mM CPT DMSO solution and stirred at room temperature for overnight. Excess CPT precipitated as solid was removed by centrifugation. The supernatant was filtered through a 0.8 μm filter to fully remove any solid. The solution was then dialyzed (molecular weight cut-off (MWCO) = 3 kDa) against distilled water for 2 days to remove the small amount of solubilized free CPT and DMSO. The produced MWCNT-CPT and GO-CPT were preserved in darkness at 4°C.

5) In vitro drug release experiments

To determine the stability and release rate of CPT loaded on MWCNT-PVA and GO-PVA, 2 mL of prepared MWCNT-PVA-CPT or GO-PVA-CPT solution was loaded into an inner dialysis tube (MWCO = 10 kDa) and dialyzed against 15 mL of PBS buffer in outside vial. The sealed vial was incubated in a shaker at 37 °C for a period of 3 days. At selected time intervals, 2 mL dialyzate was taken out from the vial and equal volume of fresh PBS buffer was replenished to it. The released CPT was evaluated by measuring the UV-vis absorbance at 369 nm.



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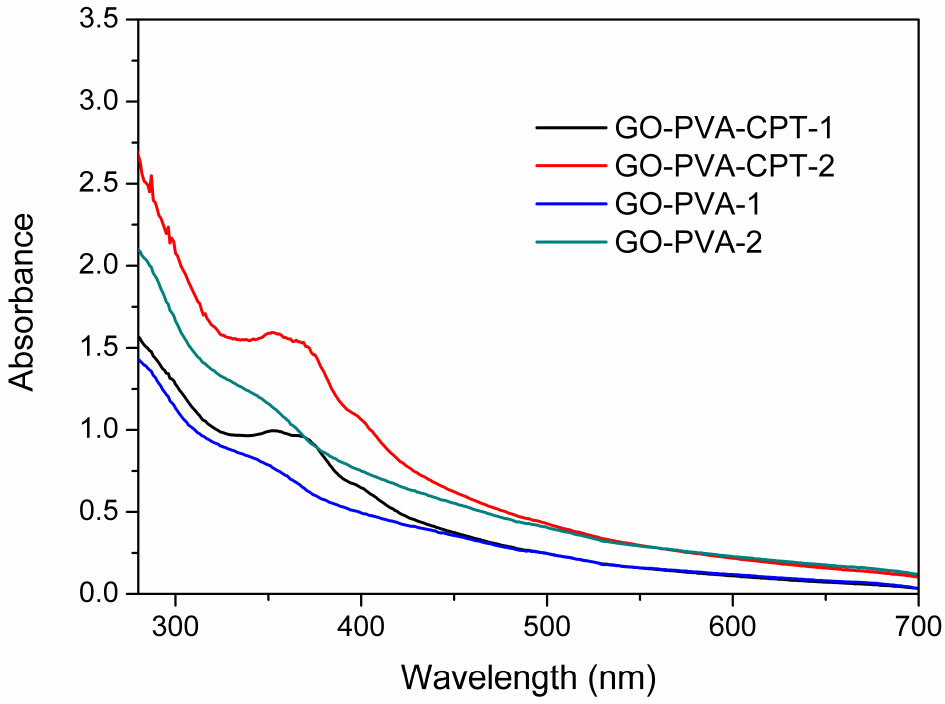


b.

Figure S2. Loading of CPT on GO-PVA: (a) schematic depiction of CPT loaded GO-PVA, and (b) UV spectra of GO-PVA and GO-PVA-CPT with different concentrations.

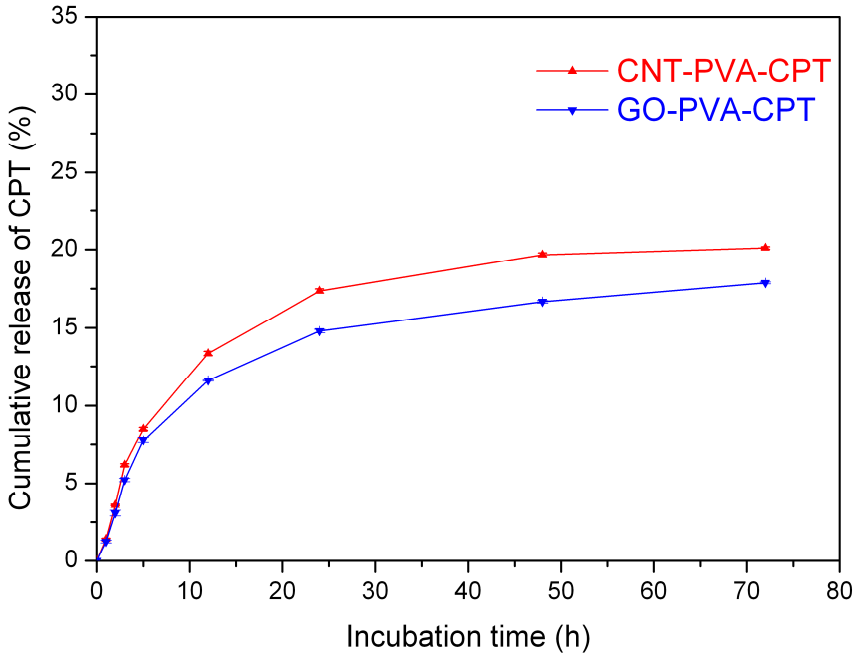
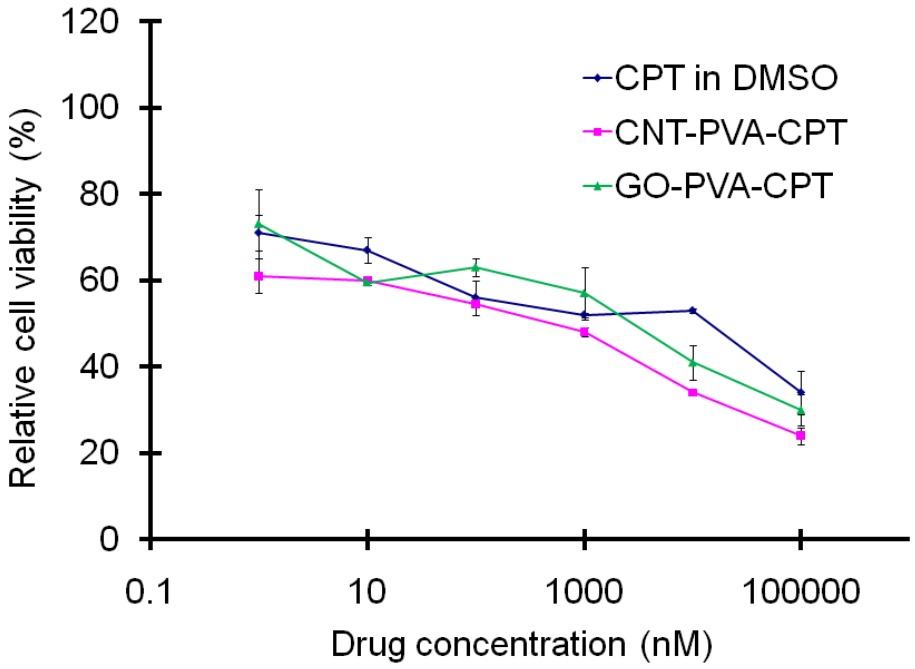


Figure S3. In vitro release profiles of CPT from CNT-PVA-CPT and GO-PVA-CPT in PBS buffer (pH 7.4) at 37 °C.

6) Cell culture and in vitro cell viability assay

Human breast cancer cell line (MDA-MB-231) and metastatic skin tumor cell line (A-5RT3) were grown into a confluent monolayer in the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 100 $U.L^{-1}$ of penicillin/streptomycin at 37 °C in a 5 % CO_2 humidified incubator. Both cells were routinely subcultured using 0.2 % trypsin and 1 mM ethylenediaminetetraacetic acid (EDTA). (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) was used as an indicator of cell viability which is determined by its mitochrondrial-dependent reduction to formazone. Cancer cells were plated at a density of 3×10^3 cells/well into 96-well plates for 24 h, followed by treatment with different concentrations of each compound for further 48 h. Cells were washed with phosphate-buffered saline (PBS) for three times, and MTT (5 mg/mL in PBS) was added to the medium followed by incubation at 37 °C for 2 h at dark. Then, the supernatant was removed, and the formazone crystals were dissolved using DMSO, followed by shaking at 150 rpm for 5

mins. Triplicates were maintained for each treatment. The absorbance was read at 560 nm using Bio Rad Model 3550 Microplate Reader. Data of cellular viability were expressed as the mean ± standard deviation in the present study.



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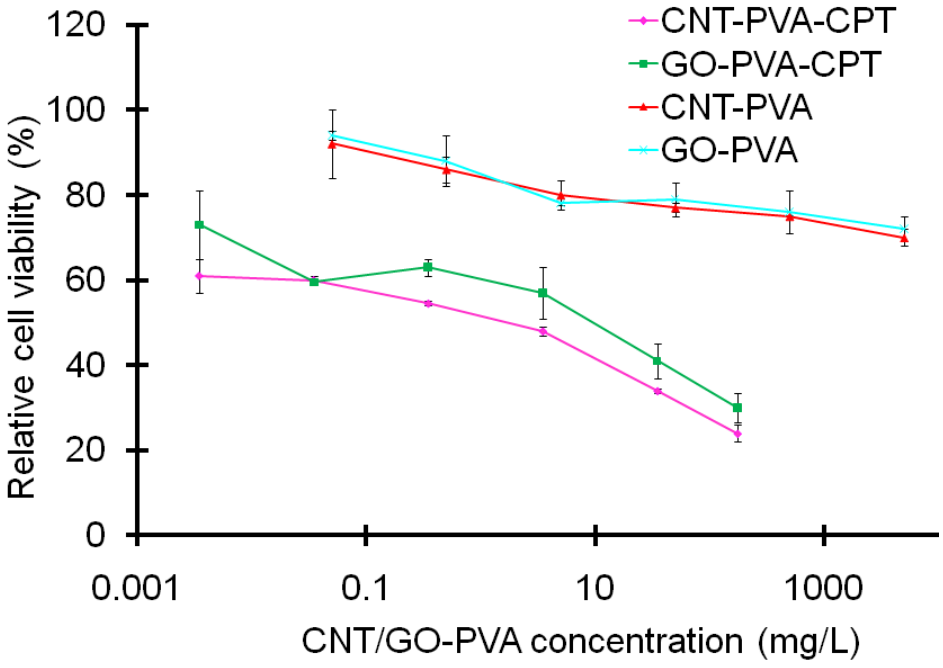

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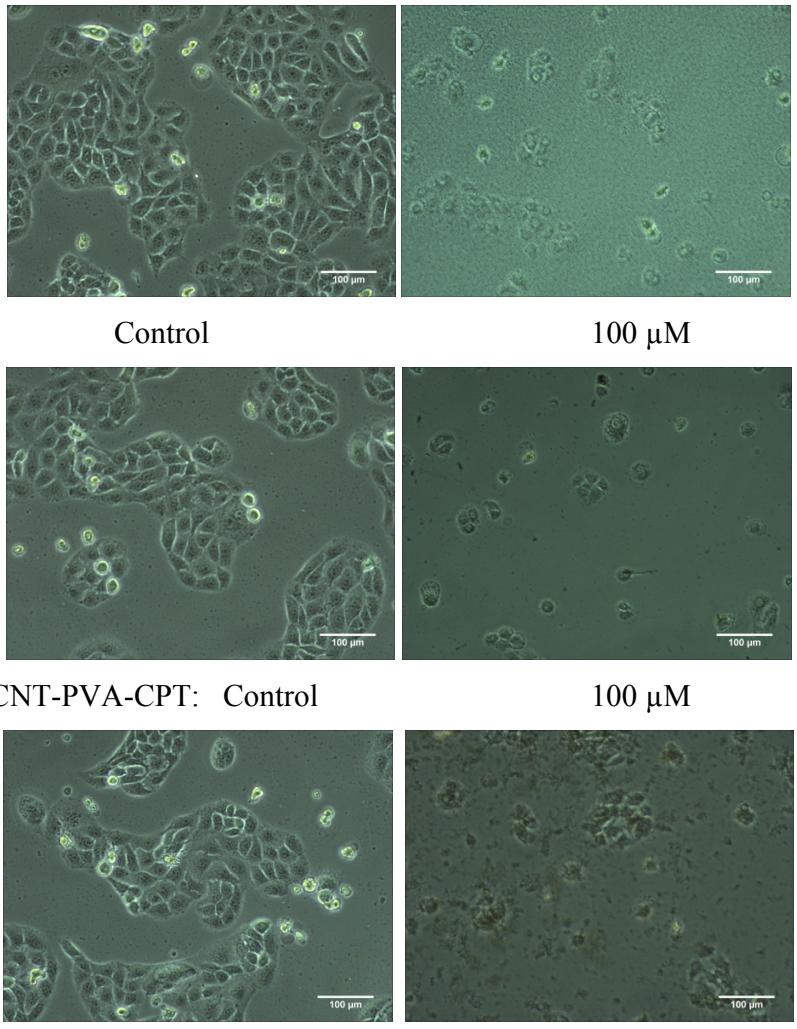

c.

Figure S4. (a) Relative cell viability of A-5RT3 cells cultured with free CPT, MWCNT-PVA-CPT and GO-PVA-CPT at different concentrations of CPT respectively; (b) Relative cell viability of A-5RT3 cells cultured with MWCNT-PVA and GO-PVA in the presence of or the absence of CPT loading respectively, (c) Optical images of A-5RT3 cells after cultured with CPT, MWCNT-PVA-CPT and GO-PVA-CPT, respectively.

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2 W.S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.* **1958**, *80 (6)*, 1339.