Supporting Information

Hydrophilic Multi-Walled Carbon Nanotubes Decorated with Magnetite Nanoparticles as Lymphatic Targeted Drug Delivery Vehicles

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Experimental

Materials: MWNTs, produced by chemical vapor deposition method, were purchased from Shenzhen Nanotech Port Co., Ltd. Activated carbon was purchased from Chongqing Lummy Pharmaceutical Co., Ltd. Polyvinylpyrrolidone (PVP, K30 grade) was purchased from Shanghai Lite Chemical Technology Co., Ltd. $FeCl_2 \cdot 4H_2O$ (purity ≥ 99.0%) and $FeCl_3 \cdot 6H_2O$ (purity > 97%) were purchased from Fluka. Ammonia was purchased from Shanghai Chem. Reagent Co. All the reagents were used as received.

The distribution of MN-MWNTs in vivo: Male SD rats (Shanghai SLAC Laboratory Animal Co., Ltd.), 4 to 5 weeks of age, were housed in sterilized cages and fed autoclaved food and water *ad libitum*. All animal procedures were approved by the institutional animal care committee. All guidelines met the ethical standards required by law and also complied with the guidelines for the use of experimental animals in China. Subcutaneous injection of MN-MWNTs physiological saline solution (0.1 mL) into the left footpad was carried out. The rats were euthanized after subcutaneous injection as early as 3 h to examine the distribution of MN-MWNTs. The draining lymph nodes and major internal organs were examined visually and photographed. At necropsy the lymph nodes and selected internal organs were carefully extirpated and fixed with 10% formalin. The samples were treated serially by paraffin embedding, sectioning and *H&E* staining. The lymphatic distribution of MN-MWNTs was examined by light microscopy and Transmission electron microscopy (TEM, Hitachi H-600).

*Lymphatic targeted drug delivery studies***:** SD rats were randomly allocated to five groups. Each group contained 32 rats, with each rat administered a dose of gemcitabine 15 mg kg^{-1}. Four rats were chosen from each group after final administration for 6, 12, 24, 48, 72, 120, 192, and 240 h, respectively. 1 mL blood was collected into a heparinized vacutainer tube from the tail vein. The blood was centrifuged at 3000 rpm for 10 min to separate cells from plasma, and then the samples were frozen at –80 ^{o}C. Then, the rat was euthanized, and the popliteal lymph nodes were harvested. High performance liquid chromatographic (HPLC) was utilized to determine the concentration of GEM in plasma and popliteal lymph nodes.

Statistical analysis: The data were expressed as mean ±SD. Statistical analysis was performed with ANOVA using SPSS 15.0 software ($P < 0.05$ as significant difference).

Results:

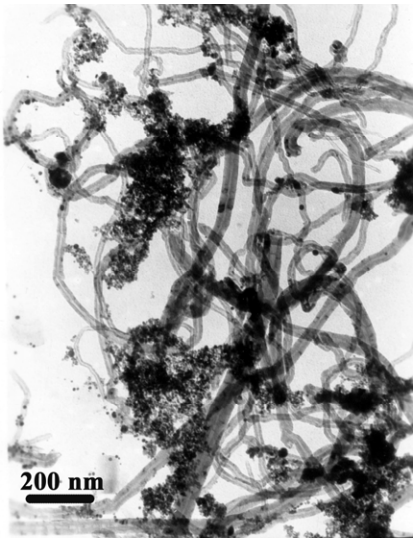

Fig. S1 TEM image of magnetite nanoparticles decorated pristine multi-walled carbon nanotubes.

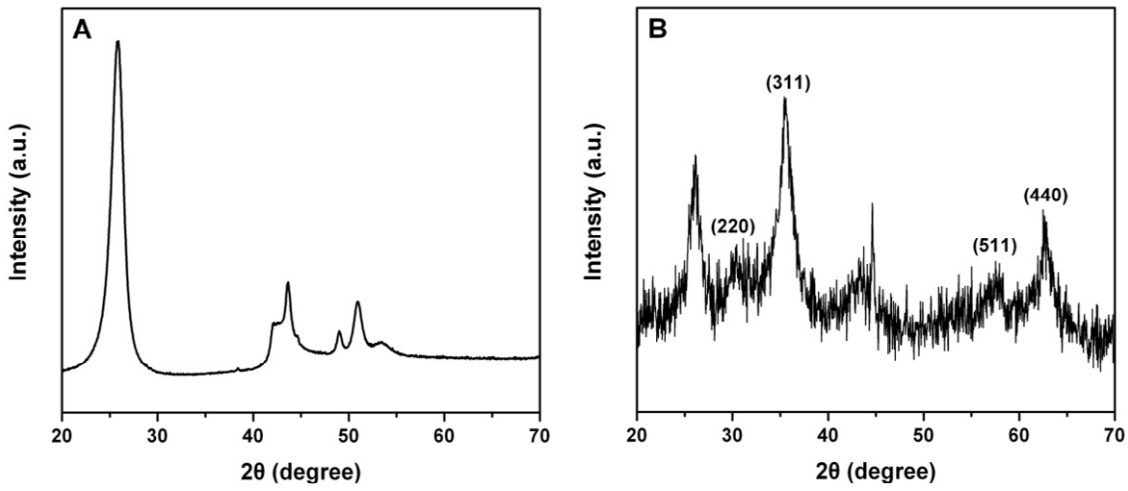

Fig. S2 XRD spectra of A: pristine MWNTs and B: MN-MWNTs.

In natural world, there exists different kinds of iron oxide, *e.g.* α-Fe_2O_3 (hematite), γ-Fe_2O_3 (maghemite),

Fe_3O_4 (magnetite), and β-FeOOH (ferric hydroxide), all of them possess unique X-ray diffraction (XRD) pattern [3]. To confirm the structure of the nanoparticles attached on the outer surface of MWNTs, we measured the XRD of pristine MWNTs and MN-MWNTs. As shown in Figure S2, in contrast to pristine MWNTs, which show three clear characteristic peaks of the graphite structure in MWNTs at 26.2°, 43.6° and 50.7°, XRD spectra of MN-MWNTs show the extra characteristic peaks at 30.1° (220), 35.5° (311), 57.0° (511) and 62.6° (440), which is consistent with the standard peaks of Fe_3O_4 nanoparticles. XRD result indicates the nanoparticles prepared by chemical coprecipitation of Fe^{2+} and Fe^{3+} was Fe_3O_4.

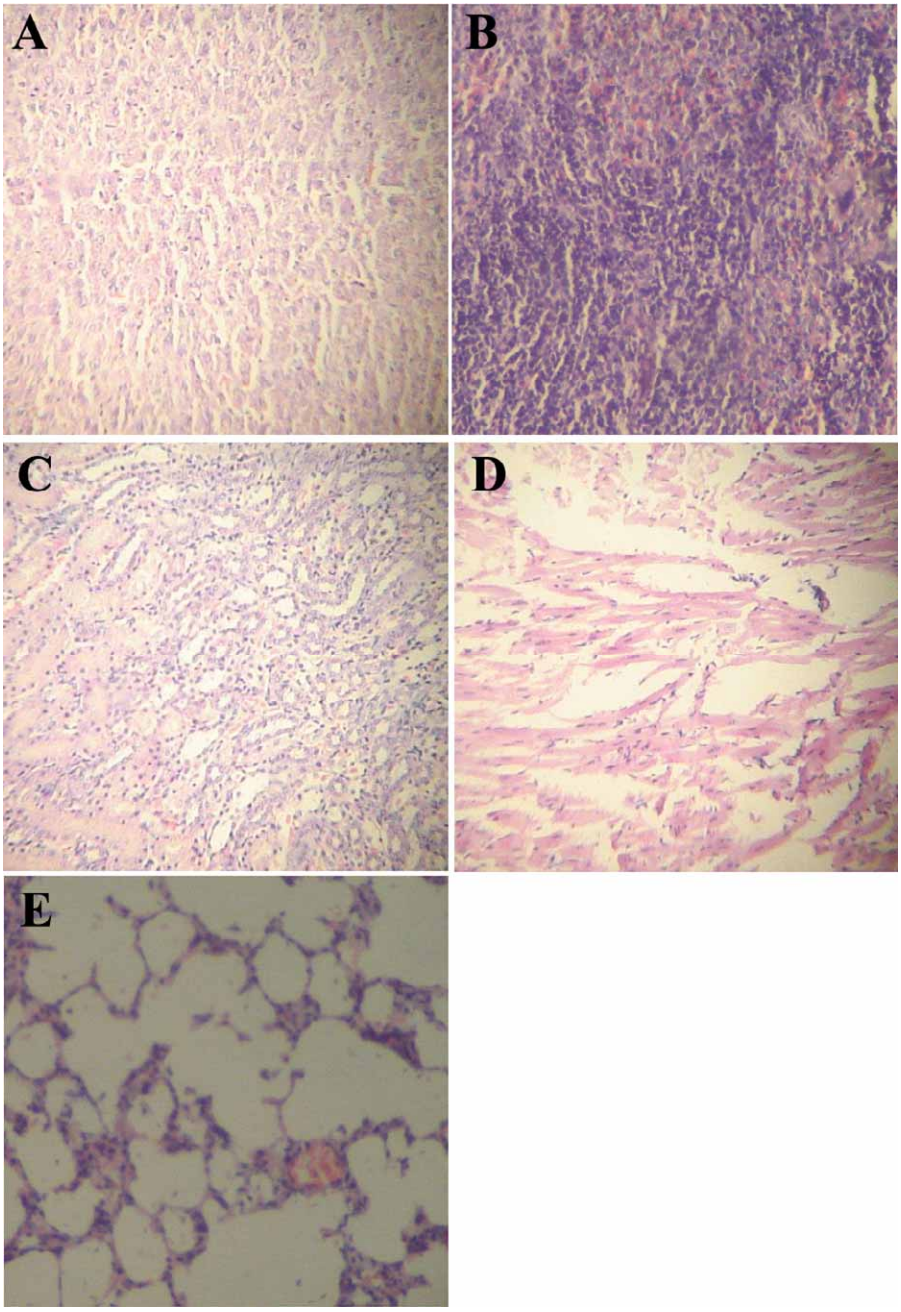

Fig. S3 Microscopy (×100) images of the internal organs, A: liver, B: spleen, C: kidney, D: heart and E: lung, after *H&E* staining.

The drug loading efficiency for the MN-MWNT-Gem was measured. 10 mg of MN-MWNTs were dispersed in 10 mL water by sonication for 10 min, and then 10 mg of gemcitabine were added into the solution. After stirred for 24 h, MW-MWNT-Gem was separated by applying an external magnetic field. The concentration of residual gemcitabine in the solution was measured by UV. The drug loading efficiency, calculated according to the following equation, was about 62%.

$$\text{Drug loading efficiency} = \frac{\text{initial weight of gemcitabine - residual weight of gemcitabine in solution}}{\text{weight of MN-MWNT}}$$

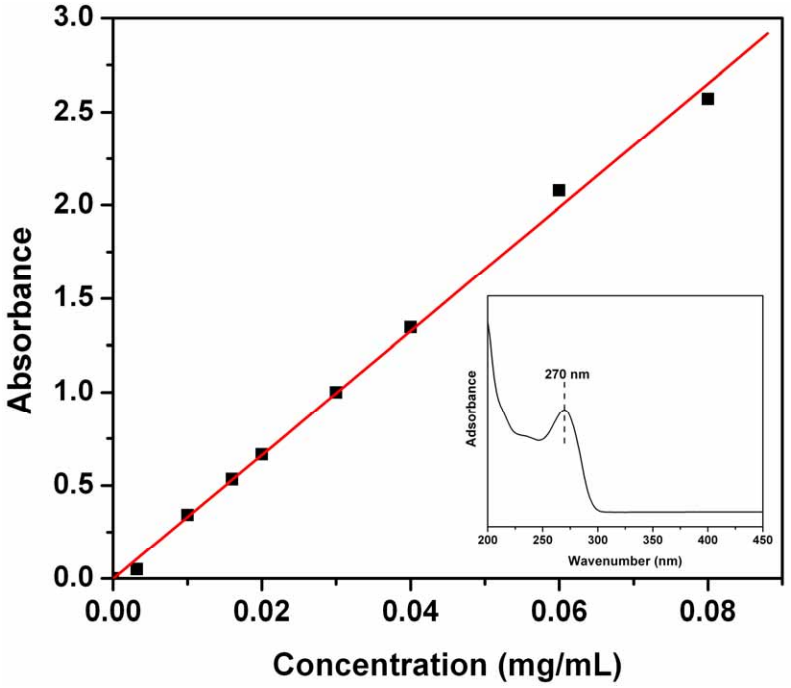

Fig. S4 The standard curve for concentration of gemcitabine *vs.* ultraviolet absorption at 270 nm. The insert is UV spectrum of gemcitabine.

References

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[3] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. V. Elst and R. N. Muller, *Chem. Rev.*, 2008, **108**, 2064.