Supplementary Information

Graphene oxide/Fe_3O_4 nanocomposite for combination of dual-drug chemotherapy with photothermal therapy

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SI 1: Hemolysis assay in vitro

Method: Nine aliquots of 100 μL of the diluted human red blood cells (HRBCs) suspension were added in a series of centrifuge tubes containing: (1) 1.9 mL of physiological saline solution as a negative control; (2) 1.9 mL of deionized water as a positive control; (3-9) 1.9 mL of CPT@uGO-COOH@MNP@OA@MTX composite at concentrations of 25, 50, 100, 200, 400, 800 and 1600 μg/mL. After shaken gently and placed at a standstill for 3 h at room temperature, the nine aliquots of the mixtures were centrifuged at 1500 rpm for 10 min, and the absorbance of the supernatants was measured at 541 nm. The hemolysis percentages of the samples were calculated according to the equation below:

$$\left(\text{Sample O.D.}_{541} - \text{Negative control O.D.}_{541}\right) / \left(\text{Positive control O.D.}_{541} - \text{Negative control O.D.}_{541}\right) \times 100\%$$

Results: The hemolysis percentages of nanocomposites slightly changed with an increased concentration in the range of 25～1600 μg/mL. Even at a high concentration of 1600 μg/mL, as low as 1.25% hemolysis percentages were detected and still fell within the negligible scope (＜ 5%).[3]

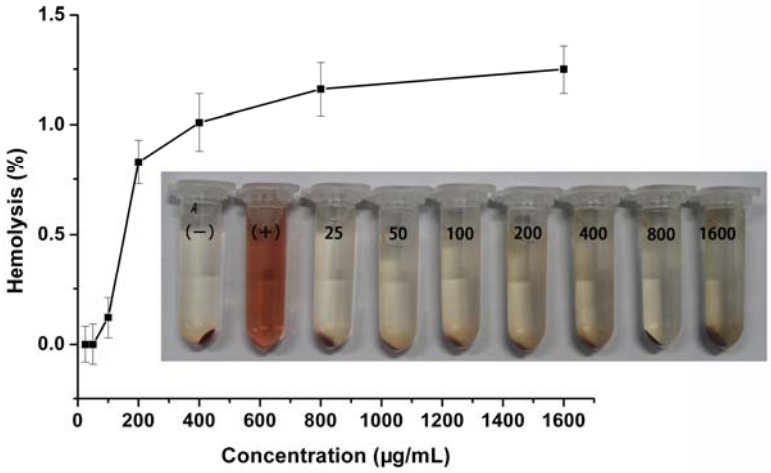

Fig. SI 1. Hemolysis percentages of CPT@uGO-COOH@MNP@OA@MTX composite at concentrations of 25, 50, 100, 200, 400, 800 and 1600 μg/mL. Inset on the right is a photograph of the hemolysis assay to detect the presence of hemoglobin in the supernatant.

SI 2: The labeling of MTX@uGO-COOH@MNP@OA@CPT composite with RhB

MTX@uGO-COOH@MNP@OA@CPT composite was labeled with rhodamine B (Rh B) to trace its bio-transportation by fluorescent probe. Labeling method is as follows: 100 μL of RhB solution (50 mg/mL, in PBS) was added dropwise to 6 mL of MTX@uGO-COOH@MNP@OA@CPT suspension (25 mg/mL, in PBS). After sonicated for 5 min at 35 kHz (Elma TI-H-5 MF2, Germany) followed by vibrating for 30 min at 25℃ in the dark, the mixture was centrifuged at 6000 rpm for 5 min and washed further three times with PBS under centrifugation. The products were denoted as Rh B-labeled MTX@uGO-COOH@MNP@OA@CPT composite.

SI 3: Zeta potential and dispersibility assay

In order to simulate physiological media for zeta-potential analysis, a human serum albumin (HSA, 0.6 mM)-containing PBS solution was used because albumin is the most abundant protein that occupies generally a concentration of 0.6 mM in serum.[1] The result is found that in the absence of HSA at pH 7.4, the zeta-potential charge of the uGO-COOH@MNP@OA nanocomposite was −53.4±4.5 mV. This absolute value slightly lower than that in the presence of HSA (−55.2±4.6 mV) (Table SM2), reflecting the insignificant effect of protein on the zeta potential of the nanocomposite. At pH 5.3, however, the nanocomposite possessed positive charges in two kind of media, with the zeta potential of 13.6±2.9 mV in PBS and 8.3±2.8 mV in HSA-containing PBS, respectively (Table SM2). In fact, the protein adsorption is a main effect factor on the zeta potential of the composites in serum.[2] At pH 7.4, the partial deprotonation of the carboxyl groups on the surface of the nanocomposite make them carry negative charges, while HSA molecules have also negative charges because the pH value is above its isoelectric point (pI = 4.7). The mutual repulsion between them became dominant over hydrophobic interactions. So, there was little fluctuation in the zeta potential values. When at pH 5.3, the carboxyl groups on the nanocomposite surface scarcely carry negative charges, while the solubility of HSA is relatively low because the pH value is close to its isoelectric point; so, the aggregating HSA tends to attract nanocomposite, which produces lower net positive charges of free nanoparticles in HSA-containing PBS than in PBS.

Table SI 3. Zeta potential of the uGO-COOH@MNP@OA nanocomposite in PBS and in HSA-containing PBS media at pH 5.3 and pH 7.4

Media conditions		Zeta potential (mV)
PBS	pH 7.4	-53.4±4.5
	pH 5.3	13.6±2.9
HSA- containing PBS	pH 7.4	-55.2±4.6
	pH 5.3	8.3±2.8

SI 4: Antitumor efficacy of free drugs

Antitumor efficacies revealed that free CPT, free MTX and their combination could inhibit the tumor growth to some extent, with the tumor inhibitory rate of 18.3%, 26.1% and 35.2%, respectively (Table SI4). Apparently these free drugs have much lower the tumor inhibitory rates than corresponding drug-loaded nanocomposites. Although the mice treated with free drugs were also exposed to near infrared radiation, there was no potential in hyperthermia due to the absence of the thermal seeds (MNP). Furthermore, the small molecule drugs (CPT and MTX) tend to diffuse distribution to different tissues in vivo, while nanocomposites primarily deliver drugs to tumor site under the external magnetic field guidance and act as the selective carrier of cytotoxic agents. With regard to the mice treated with the free dual-drug combination, their mean inhibition rate of tumor growth was slightly higher than that treated with free single drug, reflecting the potential efficacy of synergistic effect of dual drug.

Table SI 4 The anticancer properties of free CPT, free MTX and free CPT@ MTX in S-180 sarcoma-bearing Balb/c mice. The data are expressed as mean±S.D (n=5).

No.	Groups	NIR radiation	Tumor mass (g)	Tumor inhibitory rate (%)[a]
1	Control (PBS)	No	1.42 ± 0.31	—
2	free CPT	Yes	1.16 ± 0.34	18.3 ± 3.5
3	free MTX	Yes	1.05±0.25	26.1±3.9
4	free CPT@ MTX	Yes	0.92 ± 0.23	35.2 ± 4.4

References

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