Electronic Supplementary Information

Engineering graphene oxide with ultrasmall SPIONs and smart released drug for cancer theranostics

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1 Experimental

1.1 Chemicals

Diethylene glycol (DEG) was obtained from Sigma-Aldrich (St Louis, MO). 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC.HCl), and N-hydroxy- succinimide (NHS) were from GL Biochem (Shanghai, China). Generation 2 poly (amidoamine) dendrimers (G2.NH_2, molecular weight = 3256 g/mol) with a polydispersity index less than 1.08 was purchased from Dendritech (Midland, MI). Dulbecco's Modified Eagle's nutrient mixture (DMEM), fetal bovine serum (FBS), and penicillin were purchased from Gibco (New York, USA). DOX.HCl was purchased from Shanghai Aicheng biological technology Co., LTD. Iron (III) chloride hexahydrate, cis-aconitic anhydride, pyridine, triethylamine, dioxane, trisodium citrate dihydrate, and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 4T1 cells (a mouse breast cancer cell line) and normal L929 cells (a mouse fibroblasts cell line) were purchased from the Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). A Mi1li-Q Plus185 water purification system (Millipore, Bedford, MA) was employed to prepare ultrapure water, the resistivity is higher than 18.2 MΩ.cm.

1.2 Synthesis of the SPIONs@GO

2 g GO was prepared according to the references report.[1] Briefly, GO (10.2 mg) was dissolved into 10 mL deionized water and ultrasonic for 120 min to form a homogeneous solution. Then, anhydrous ferric chloride (3.2 mmol) was dissolved into 40 mL DEG under stirring to get a clear solution. The citric acid (1.6 mmol) was added to the above solution and the mixture was heated to 80 °C in a water bath until a clear solution formed. Then, the GO solution and $FeCl_3$/DEG solution were mixed and stirred the last 30 min to get a uniformity solution. After a thorough stirring, the GO/$FeCl_3$/DEG mixture solution was transferred into an autoclaved in a sealed pressure vessel with a volume of 100 mL at 200 °C. After 4 h reaction, the products were cooled down to room temperature (RT). The black products were collected by

centrifugation at 10 000 rpm 15 min. After that, the black precipitate was re-dispersed into anhydrous ethanol with ultrasound, and collected by centrifugation at 8 000 rpm 10 min, repeat this step three times. Finally, the SPIONs@GO products were re-dispersed into water and lyophilized to obtain the powder for further use.

1.3 Synthesis of the pH-sensitive drug (Cis-adriamycin aconitic acid-DOX, CAD)

DOX.HCl (116.0 mg) was dissolved into 10 mL chloroform with vigorous stirring, then 83 μL triethylamine was dropwise added into the DOX.HCl/Chloroform mixture. The reaction mixture was stirred for at least 12 h. Cis-aconitic acid anhydride (90.0 mg, 3 mL in dioxane) was dropwise added into DOX-HCl/Chloroform mixture stirred for at least 12 h in dark at 4 ^{o}C. The products were purified by an extraction process (ethyl acetate 15 mL, 5% sodium bicarbonate 15 mL, each time). Shake the mixture and let stand for 3 to 5 minutes, take the sublayer aqueous phase, repeat this step three times. Then, adjust the pH to 2.5-3.0 with 0.1 M HCl solution until the precipitation separates out. Subsequently, the precipitation was washed and collected by centrifugation at 7 000 rpm 10 min, repeat three times. The precipitation was lyophilized to obtain the powder (Named as CAD) for further use.

1.4 Synthesis of the CAD-SPIONs@GO composite

The CAD (34.1 mg in 10 mL DMSO), a DMSO solution of EDC (48.0 mg, 2 mL), NHS (29.0 mg, 2 mL) was dropwise added into the CAD DMSO solution with vigorous stirring 3 h in dark to active the carboxyl group. The CAD/EDC/NHS solution was dropwise added to G2.NH_2 (32.6 mg in 10 mL DMSO) and the reaction last 3 days in the dark at RT. The purified and lyophilized process were according our previous report to remove the DMSO and the excess of reactants and byproduct.[2] The SPIONs@GO (72.0 mg in 15 mL H_2O), a water solution of EDC (36.0 mg, 1 mL H_2O), NHS (36.0 mg, 1 mL H_2O) was dropwise added into the SPIONs@GO solution with vigorous stirring 3 h in dark at RT to activate the carboxyl group. Then, SPIONs@GO/EDC/NHS solution was dropwise added into G2.NH_2-CAD (72.0 mg in 20 mL H_2O) under vigorous stirring 3 days in the dark at RT. The unreacted G2.NH_2-CAD and the excess of reactants were removed by centrifugation at 8500 rpm 15 min, repeat three times. The final products of CAD-SPIONs@GO were lyophilized to obtain the powder. For further investigation the drug release of CAD-SPIONs@GO and the location of the SPIONs@GO after cellular uptake by cells, the reference was prepared simple physical mixture of the CAD and SPIONs@GO, FI-SPIONs@GO composite was formed by the similar pathway, respectively.

1.5 Characterization

Transmission electron micrographs (TEM) and high-resolution transmission electron micrographs on a JEMe2010 electron microscope with an accelerating voltage of 200 kV. Atomic force microscopy (AFM) imaging was performed with a Vecco Dimension 3100 atomic force microscope. X-ray diffraction (XRD) spectra were obtained by using a Bruker D8 ADVANCE diffractometer (Germany) with Cu Kα (λ = 1.5406 Å). XPS spectra were measured using a PHI Quantum-2000 electron spectrometer (Ulvac-Phi, Japan) with 150 W monochromatized Al Ka radiation (1486.6 eV). Raman spectra were carried out on a confocal laser micro Raman spectrometer (Thermo Nicolet, USA). A Bruker AV400 nuclear magnetic resonance spectrometer was employed to obtain the ^{1}H NMR spectra. A TG 209 F1 (NETZSCH Instruments Co., Ltd., Bavaria, Germany) thermogravimetric analyzer was used for thermogravimetric analysis (TGA). Zeta potential and dynamic light scattering (DLS) measurements were conducted using a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) equipped with a standard 633 nm laser. UV-vis spectroscopy was collected using a Lambda 25 UV-vis spectrometer (Perkin Elmer, Boston, MA). The Fe concentration of the NPs in aqueous solution was analyzed using a Leeman Prodigy inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Hudson, NH). T_2 and T_1 relaxometry measurement were operated in the 0.5 T NMI20 Analysing and Imaging System (Shanghai NIUMAG Corporation, China). The parameters were as follows: T_2 relaxometry, TR =2 000 ms, TE =80 ms, resolution = 156 mm × 156 mm, section thickness = 0.5 mm; T_1 relaxometry, TR =800 ms, TE =60 ms, resolution = 156 mm × 156 mm, section thickness = 0.5 mm. The T_2 relaxivity or T_1 relaxivity value was obtained through linear fitting the inverse T_2/T_1 relaxation time ($1/T_2$ or $1/T_1$) as a function of Fe concentration.

1.6 pH-sensitive release behavior

The *in vitro* drug release kinetics of the CAD-SPIONs@GO composite was investigated under different pH conditions (pH = 5.5, 6.5, and 7.4). Briefly, CAD-SPIONs@GO (6.3 mg) was dispersed into 1 mL of PBS (pH = 7.4), 1 mL acetic acid-sodium acetate buffer solution (pH = 6.5), and 1 mL acetic acid-sodium acetate buffer solution (pH = 5.5) were placed into dialysis bags with a molecular weight cut off (MWCO) of 3500. Then, the dialysis bags were placed into 9 mL of corresponding buffer solutions in sample vials. All the samples were incubated in vapor-bathing constant temperature vibrator at 37 °C. At each predetermined time point, 1 mL of

solution was taken out from the outer phase and an equal volume of the corresponding buffer was replenished into each sample vial. The DOX concentration of the samples was quantified by UV-vis spectrometry at 480 nm. For further investigation, the specificity pH stimuli-responsive of the prepared CAD-SPIONs@GO NPs, DOX physically mixed with SPIONs@GO NPs was prepared. Then, the sustained release behavior of the physically mixed drugs was performed under the same conditions with CAD-SPIONs@GO NPs.

1.7 Cytotoxicity assay and anti-tumor cells activity

The cytotoxicity of the SPIONs@GO composite and the anti-tumor cells activity of CAD-SPIONs@GO composite was evaluated by CCK-8 assay of 4T1 cells or L929 cells. Generally, 4T1 cells or L929 cells were seeded into a 96-well plate with a density of 8×10^3 per well and incubated in regular DMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin for 24 h. The medium was replaced with fresh medium containing SPIONs@GO at different Fe concentrations (10, 50, 100, 150, 200 μg/mL) or CAD-SPIONs@GO composite at different DOX concentrations (1.3, 2.5, 5, 10, 20 μM). After 24 h incubation at 37 ^{o}C and 5% CO_2, CCK-8 solution (20 μL, 5 mg/mL) was added to each well and the cells were incubated for another 4 h under regular culture conditions. The absorbance at 570 nm in each well was measured using a Thermo Scientific Multiskan MK3 ELISA reader (Thermo Scientific, Massachusetts, USA). Mean and standard deviation for the triplicate wells were reported.

1.8 Cellular uptake

The uptake of the CAD-SPIONs@GO composite by 4T1 cells was confirmed by confocal microscopy analysis of the cells treated with the composites for 1, 2, and 4 h, respectively. In brief, 4T1 cells were seeded in a laser confocal petri dish at a density of 1×10^5 cells in 3 mL DMEM and incubated at 37 ^{o}C and 5% CO_2. After 12 h incubation, the medium was replaced with 1 mL fresh medium containing PBS (control), CAD-SPIONs@GO ([DOX] = 10 μM) or FI-SPIONs@GO composites at a Fe concentrations (75 μg/mL), respectively. When arrived each time points, the cells were washed 3 times with PBS, fixed by adding 2.5% glutaraldehyde solution. The localizations of DOX and carrier of FI-SPIONs@GO were directly observed by the Olympus Confocal Microscope (FluoView™ FV1000).

1.9 *In vivo* MR imaging of xenografted 4T1 tumor model

Animal experiments were carried out according to protocols approved by the institutional committee for animal care, and also in accordance with the policy of the National Ministry of Health. 6 weeks old female BALB/c nude mice (body weight ≈ 20 g, n = 5, Shanghai SLAC Laboratory Animal Center, Shanghai, China) were subcutaneously injected with 3× 10^6 cells/mouse in the left back. When the tumor nodules reached a volume of about 200 mm^3 at approximately 10 days postinjection, the mice were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg), and then the CAD-SPIONs@GO composites ([Fe] 2 mg/mL, in 100 μL saline) were intravenously delivered into the tumor-bearing mice via the tail vein. The mice were scanned by a 3.0 T clinical MR imaging instrument (SOMERTON Definition Flash, Siemens, Erlangen, Germany). The parameters of MR imaging in vivo were acquired as follows: TR = 1400 ms, TE =18 ms, matrix = 256× 256, section thickness = 2 mm, and FOV =80 × 80. T_1-weighted MR images were obtained before and after intravenous injection of CAD-SPIONs@GO composite at time points of 0, 15, 30, 60, 90, and 120 min, respectively.

1.10 *In vivo* chemotherapy

In briefly, female BALB/c nude mice (6 weeks old, body weight ≈ 20 g) were s.c. implanted with 3× 10^6 4T1 cells into left leg on day 0. The breast tumor mice (tumor size approximately 100 mm^3) were randomly divided into three groups (Saline group, DOX.HCl group, and CAD-SPIONs@GO group. The mice were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg). Subsequently, mice were treated with CAD-SPIONs@GO composite (20 mg/kg DOX equivalents in 100 μL saline, n = 5), free DOX.HCl (10 mg/kg DOX in 100 μL saline, n = 5), and the same volume saline (100 μL, n = 5) were intravenously delivered into the tumor-bearing mice via the tail vein on days 7 and days 14. The body weight, the volume of the tumor was recorded three times a week during the experimental period. Tumor volume was determined according to the previous literature.[3] For comparison, the mice with injection saline were used as a blank.

1.11 *In vivo* biodistribution and toxicity assessment

The tumor-bearing nude mice after were euthanized at 4 h, 12 h and 24 h postinjection CAD-SPIONs@GO (n = 5 mice in each group, [Fe] 2 mg/mL, in 100 μL saline). And each time points, the heart, liver, spleen, lung, kidney, and tumor were extracted and weighed. The Fe content in different organ pieces was quantified by ICP-

AES according to our previous work.[4] For comparison, the mice with injection saline were used as a blank.

The biological safety assessment was performed by the routine blood-biochemical blood testing and HE stain assay. In briefly, female SCID/Slac mice (4 weeks old, body weight ≈ 15 g) were randomly assigned to four groups (n = 5 mice in each group). After postinjection CAD-SPIONs@GO (40 mg/kg, in 100 μL saline), the blood samples and major organs were collected on 0 days, 15 days, 30 days, and 45 days. The liver function indicators including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AKP), as well as kidney function indicators involving blood urea nitrogen (BUN) and creatinine (CREA), were examined.

1.12 Statistical analysis

One-way ANOVA statistical analysis was performed to evaluate the significance of the experimental data. A p-value of 0.05 was selected as the level of significance, and the data were indicated with (*) for $p< 0.05$, (**) for $p< 0.01$, and (***) for $p< 0.001$, respectively.

2. Results and discussion

Table S1: Variation in D, G, 2D and I_D/I_G band position of GO after *in-situ* growth of SPIONs.

Sample	Position of D band (cm^{-1})	Position of G band (cm^{-1})	Position of 2D band (cm^{-1})	I_D/I_G
GO	1347	1592	-	1.24
SPIONs@GO	1337	1589	2682	1.97

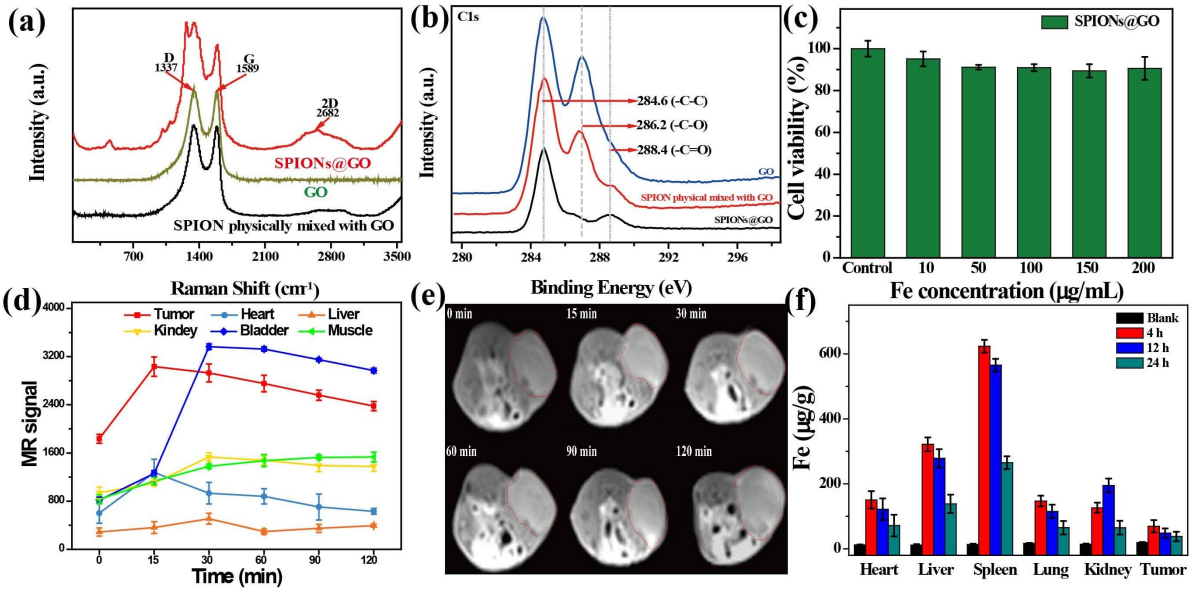

Figure S1. Raman spectrum of SPIONs@GO, GO, SPIONs physically mixed with GO (a); C1s XPS spectrum of GO, SPIONs physical mixed with GO and SPIONs@GO (b); The viability of 4T1 cells after treated with SPIONs@GO at the different Fe concentrations of 10, 50, 100, 150 and 200 μg/mL for 48 h at 37°C by the CCK-8 assay (c); *In vivo* MR signal of other organs after intravenous injection of the CAD-SPIONs@GO ([Fe] 1 mg/mL, in 200 μL saline) at different time points post *i.v.*-injection; and tumors(d); *In vivo* transverse T_1-weighted MR images of tumors (red circle) after intravenous injection of the CAD-SPIONs@GO ([Fe] 1 mg/mL, in 200 μL saline (e); *In vivo* biodistribution of the major organs of the mice including the heart, liver, spleen, lung, kidney, and tumor at 4, 12, and 24 h post intravenous injection of the CAD-SPIONs@GO ([Fe] 1 mg/mL, in 200 μL saline, f)

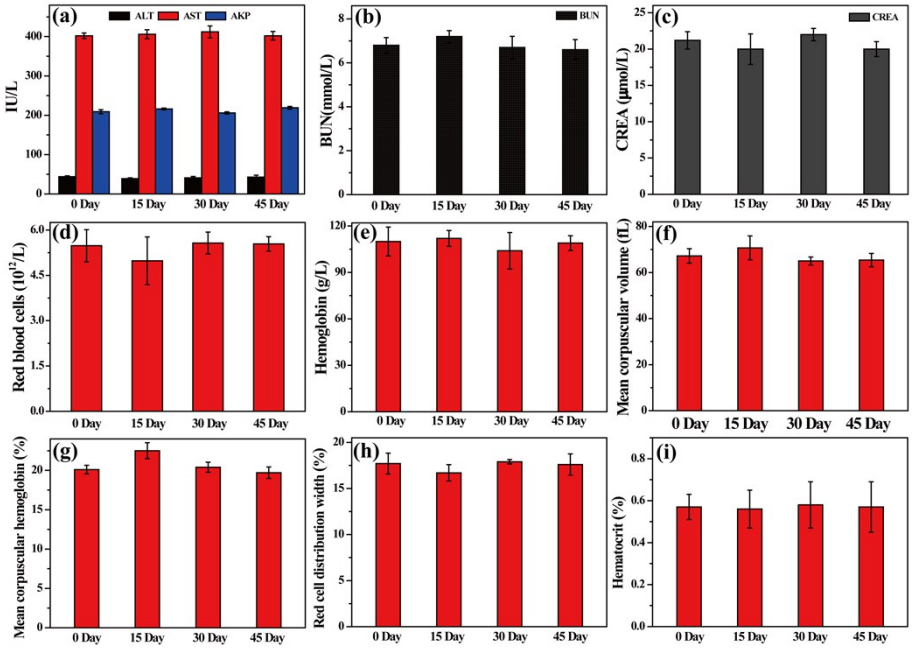

Figure S2. *In vivo* toxicity studies of CAD-SPIONs@GO. Healthy Balb/c mice were intravenously injected with CAD-SPIONs@GO saline solution at dosages (40 mg/kg) and sacrificed at 15, 30, and 45 days (n = 5). Age- and weight-matched healthy mice were used as a control. (a) The blood levels of ALT, AST, and AKP from control and treated mice as liver function markers. (b) BUN levels and (c) CREA levels in the blood representing kidney functions. (d-i) The complete blood panel data: (d) red blood cells; (e) hemoglobin; (f) mean

corpuscular volume; (g) mean corpuscular hemoglobin; (h) red cell distribution width; (i) hematocrit.

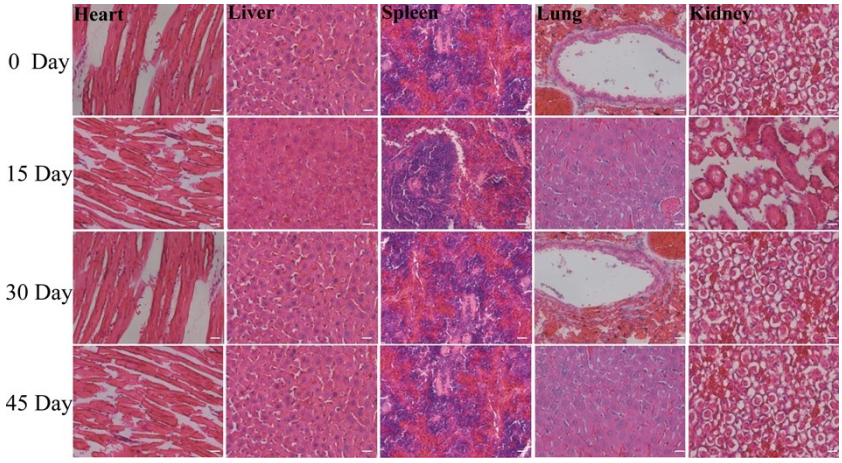

Figure S3. Histopathological examinations of major organs (*e.g.* heart, liver, spleen, lung, and kidney,) from mice of intravenous injection groups at 40 mg/kg dose of CAD-SPIONs@GO for 0, 15, 30, and 45 days. Scale bar = 20 μm.

Atomic force microscopy (AFM, Figure S4a) and TEM images show the prepared GO nanosheets have the thickness of 0.5 to 1.1 nm (inset of Figure S4b), with around size of length 90 × width 70 nm. Figure S4c indicates that the ultrasmall SPIONs are homogeneously distributed onto the GO nanosheet, and the corresponding selected area electron diffraction (SAED) pattern (inset) shows the weak polycrystalline nature of SPIONs@GO. The obvious narrow peak at 2θ of 10° (Figure S4d) confirms the 2D layer structure of GO, while after loading with SPIONs, the peak of GO at around 10° disappears. XRD pattern of SPIONs@GO composite in Figure S4e presents weak crystalline peaks and corresponds well to the magnetite phase (PDF#19-0629), indicating that the *in-situ* growth of SPIONs onto GO nanosheets could induce the destruction of the long-range ordered layer structure. The XPS spectrum (Figure S4f,) shows two obvious peaks at around the 710.8 eV and 724.6 eV, respectively corresponding to Fe^{2+} and Fe^{3+}, indicating the synthesized NPs should be magnetic Fe_3O_4.[5]

The colloid stability of CAD-SPIONs@GO is one of the core premises for the further biologic application. It is found that after dispersed in PBS, water and cell culture medium for 7 days, the hydrodynamic sizes of CAD-SPIONs@GO have only a slight change, but without visible aggregations or precipitations (the inset of Figure 1G, Figure S4g, Figure S4h, and Figure S4i, ESI), indicative of colloidal stability. Additionally, ICP-AES was used to monitor the Fe content change of CAD-SPIONs@GO for 7 days, as shown in Figure S5 (ESI), a very small amount (1.5% ±

0.3) of Fe was released into PBS after 7 days, revealing that the SPIONs were stably located on the GO sheet *via* such *in-situ* growth strategy.

The zeta potentials and hydrodynamic sizes of the GO, SPIONs@GO, and CAD-SPIONs@GO composite are shown in Table S2. It is found that the zeta potential of GO is -52.5 mV due to the abundant carboxyl groups, and the zeta potentials of the SPIONs@GO and CAD-SPIONs@GO composites increase to -40.3 and +20.7 mV, respectively, indicative of successful surface modifications.

A reference SPIONs without GO was similarly synthesized for comparison and shows irregular spherical morphology with the larger size of *ca.* 5~6 nm, and obvious aggregations (Figure S6, ESI), indicating that the GO plays an important role on the homogeneous nucleation and growth. Interestingly, compared with the reference SPIONs, the r_1 values of composite SPIONs@GO and CAD-SPIONs@GO present obvious increase, while r_2 values show a significant decrease. As confirmed in the Figure S7, the T_1-weighted MR images of CAD-SPIONs@GO show increasing signals with the Fe concentrations (Figure S7b). Compared with those of the current clinical used Gd-based T_1 MR imaging agents (r_1 relaxivity$\approx$3.8 $mM^{-1}s^{-1}$), the CAD-SPIONs@GO nanocomposite in our work display similar MR imaging performance. The resulting r_2/r_1 value of SPIONs@GO (r_2/r_1 = 2.37), CAD-SPIONs@GO (r_2/r_1 = 2.64), and *in vitro* MR imaging performance indicate that the prepared CAD-SPIONs@GO could be used as potential T_1-weighted positive contrast agents for MR imaging. For comparison, the physical mixture of SPIONs and GO was also synthesized, and the r_1 and r_2 of the mixture sample are calculated to be 0.43 $mM^{-1}s^{-1}$ (Figure S8, ESI) and 15.13 $mM^{-1}s^{-1}$ (Figure S9, ESI), respectively, similarly to those of reference SPIONs, indicating that simple physical mixture of SPIONs and GO could not change the relaxation rate of SPIONs. Completely different from the composites SPIONs@GO, such low r_1 and large r_2/r_1 value of this mixture sample cannot be used as T_1-weighted positive contrast agent for MR imaging, suggesting that the GO in the composite could play important roles on the change of relaxation rates.

Furthermore, to obtain the pH-response DOX, cis-aconitic anhydride was reacted with DOX *via* amidation reaction, and the formed CAD drug was characterized by ^1H NMR (Figure S10a, and Figure S10b, ESI). The peak at around 7.9 ppm belongs to the protons of the anthracene moiety of DOX, and additional signals at 6.1 and 6.4 ppm appear in the CAD, attributing to the protons (CH–COO–) of the cis-aconitic anhydride linkages. Additionally, the FTIR spectrum in Figure S11c shows the signal

at around 1548 cm^{-1} (amide II) in CAD, which is assigned to the characteristic signal of the formed amide bond. Both the ^1H NMR and FTIR results indicate that the CAD has been successfully synthesized. The percentage of organic compound in the CAD-SPIONs@GO was quantitatively analyzed by using TGA (Figure S11, ESI). The weight of citrate in the pristine SPIONs@GO was measured to be 11.9%, and the weight of G2.NH_2-CAD was measured to be 27.2%, indicative of a successful surface modification of G2.NH_2-CAD. Combining with the ^1H NMR results, the amount of CAD on the composites was calculated to be 12.8%, and the drug conjugate efficiency was achieved as high as up to 93.7%.

The location of the drug carriers, FITC (green fluorescence dye) was marked onto the SPIONs@GO. As shown in Figure S12A (FITC and merged column), it is found that the green signal greatly enhances at 4 h, indicating that the drug carriers are concentrated in the cytoplasm. The endocytosis process and the distribution of DOX or SPIONs@GO could be observed by laser confocal microscopy. 4T1 cells were incubated with CAD-SPIONs@GO for 0, 1, 2, 4 h, and cell nuclei were labeled with DAPI (blue fluorescence dye), while red fluorescence comes from DOX. As shown in Figure S12B (the merged column), the pink signal (the overlap of blue and red) enhances with the co-culture time, and the strongest signal was observed at 4 hours. It is worth noting that the red fluorescence (the DOX column) mainly concentrates in the nucleolus, further confirming that DOX can release from the drug carriers of CAD-SPIONs@GO in the acidic environment of cytoplasm, and quickly entered into the nucleolus. To study the side effects of CAD-SPIONs@GO on normal cells, L929 cells (a normal mouse fibroblasts cell line) were chose to measure the toxicity of the CAD-SPIONs@GO after coculture with 24 h by the CCK-8 assay. The results indicate that compared to free DOX, L929 cells have above 80% cell viability at DOX concentration range of 0-20 μM, suggesting little cytotoxicity in the given concentration range (Figure S13).

Table S2: Zeta-potential and hydrodynamic size of the GO, SPIONs@GO, and CAD-SPIONs@GO.

Sample	Zeta potential (mV)	Hydrodynamic size (nm)	PDI
GO	-52.5	127.2 ± 3.3	0.217
SPIONs@GO	-43.4	132.4 ± 2.8	0.304
CAD-SPIONs@GO	+20.7	175.3± 2.7	0.338

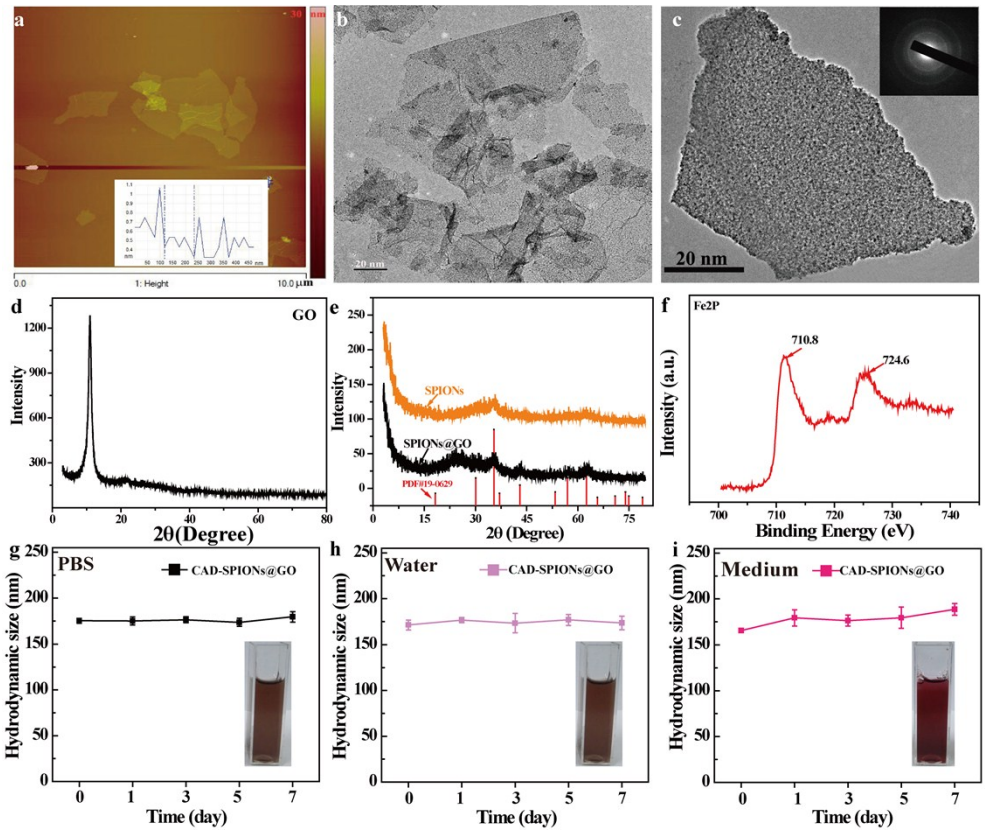

Figure S4. AFM image of GO, the inset is the height profile of GO (a); TEM image of GO (b) and SPIONs@GO (c), the inset is the selected area electron diffraction pattern of SPIONs@GO; XRD pattern of GO (d) and SPIONs@GO (e); Hydrodynamic size of the CAD-SPIONs@GO, 1 mg/mL, dispersed in PBS (g), water (h), and cell culture medium (with 10% FBS, i) at different time periods, respectively, the inset are the photographs of the CAD-SPIONs@GO composite (1 mg/mL) dispersed in PBS, water, and cell culture medium after 7 days.

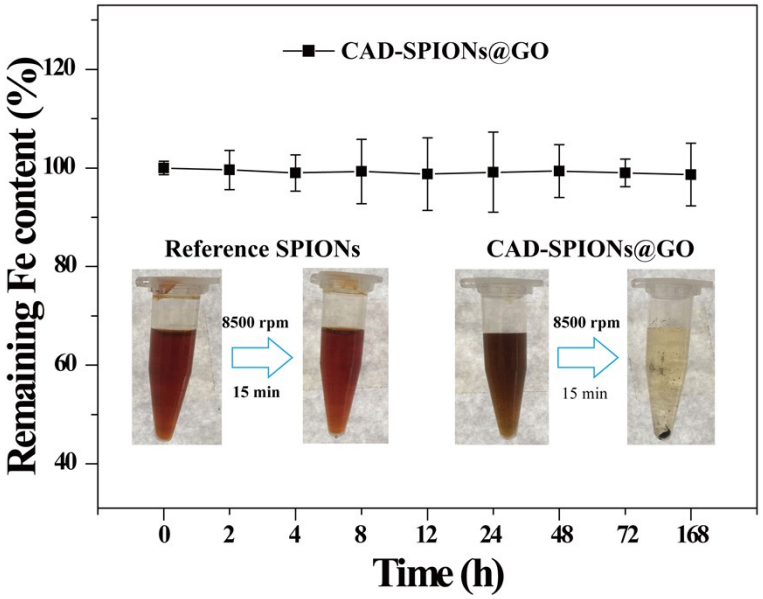

Figure S5. The stability of CAD-SPIONs@GO dispersed in PBS within 7 days. In briefly, CAD-SPIONs@GO were dispersed in PBS and collected by centrifugation at 8500 rpm for 15 min when each time point arrived. The remaining Fe content of CAD-SPIONs@GO was calculated by subtracting the Fe content of in supernatant *via* the ICP-AES assay. (The insets are the digital photos of the reference SPIONs and CAD-SPIONs@GO dispersed in PBS for 7 days before and after centrifugation at 8500 rpm for 15 min, respectively.)

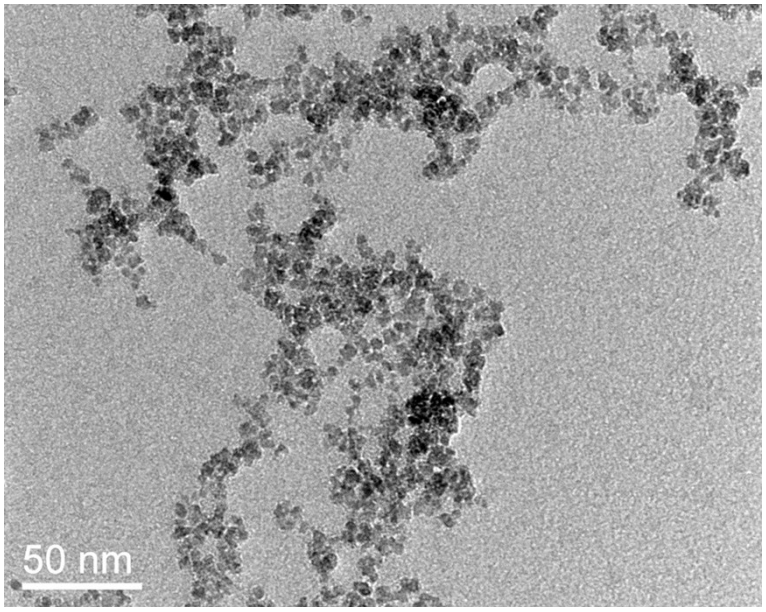

Figure S6. TEM imaging of the reference SPIONs.

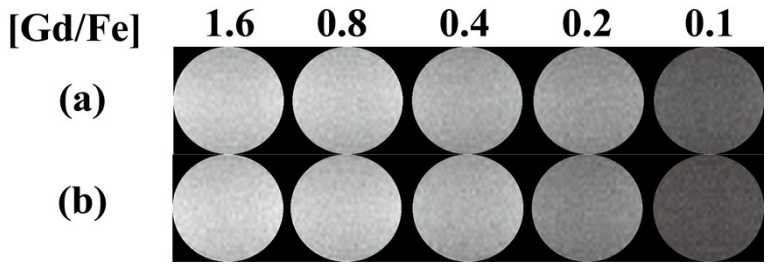

Figure S7. T_1-weighted MR imaging of the DOTA-Gd (a, at the Gd concentration of 0.1, 0.2, 0.4, 0.8, and 1.6 mM) and CAD-SPIONs@GO (b, at the Fe concentration of 0.1, 0.2, 0.4, 0.8, and 1.6 mM), respectively.

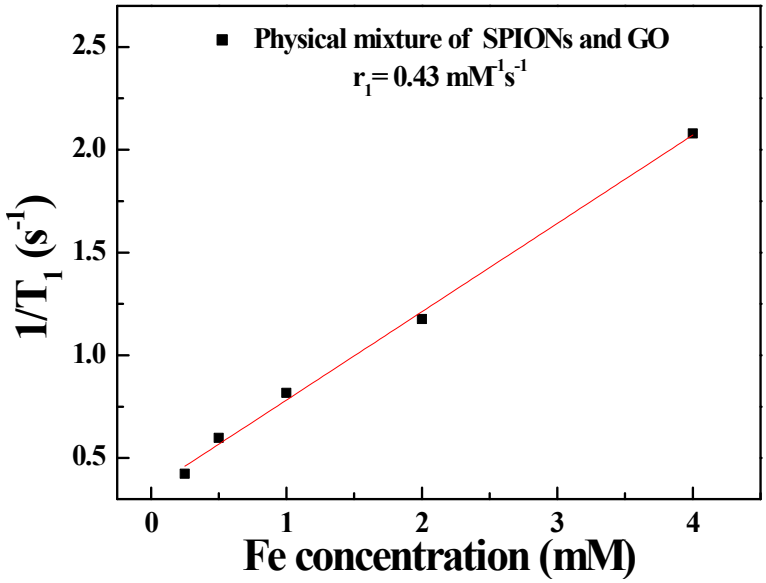

Figure S8. Linear fitting of $1/T_1$ of the physical mixture of SPIONs and GO at the Fe concentration of 0.25, 0.5, 1, 2, and 4 mM, respectively.

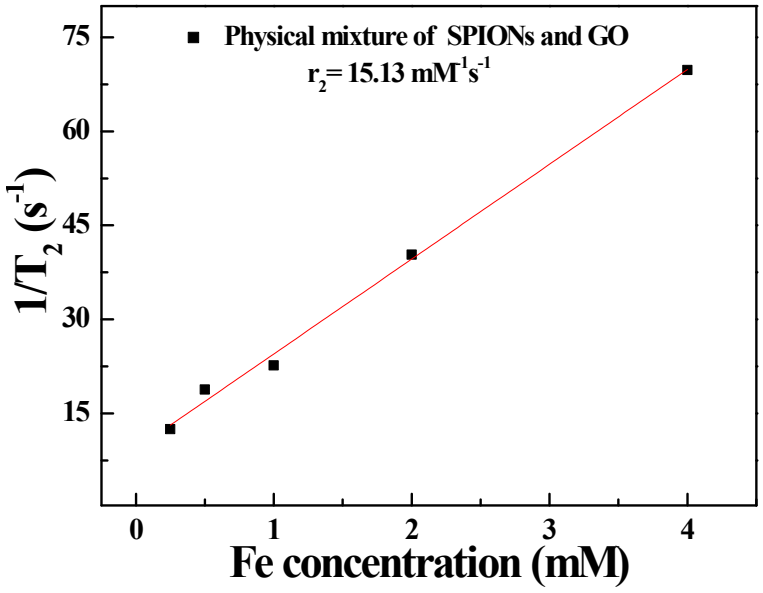

Figure S9. Linear fitting of $1/T_2$ of the physical mixture of SPIONs and GO at the Fe concentration of 0.25, 0.5, 1, 2, and 4 mM, respectively.

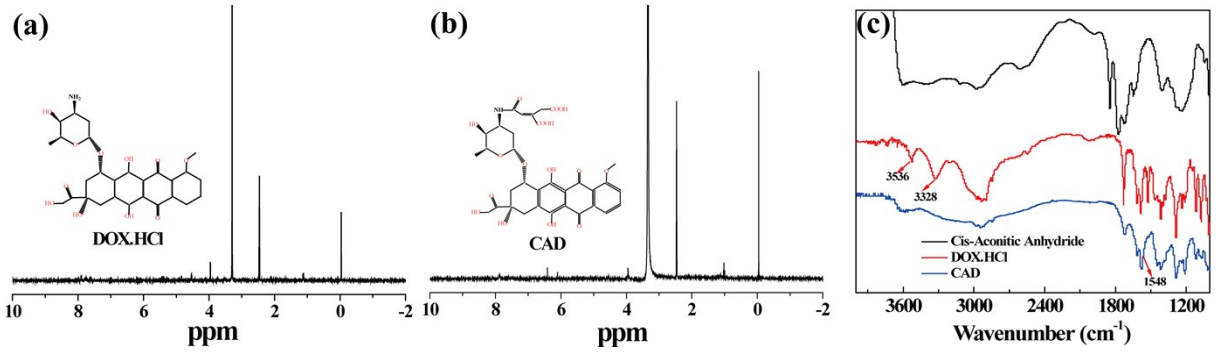

Figure S10. The ^{1}H NMR spectrum of DOX.HCl (a) and CAD dissolved in DMSO-d_6 (b), respectively. The FTIR spectrum of cis-aconitic anhydride, DOX.HCl, and CAD (c).

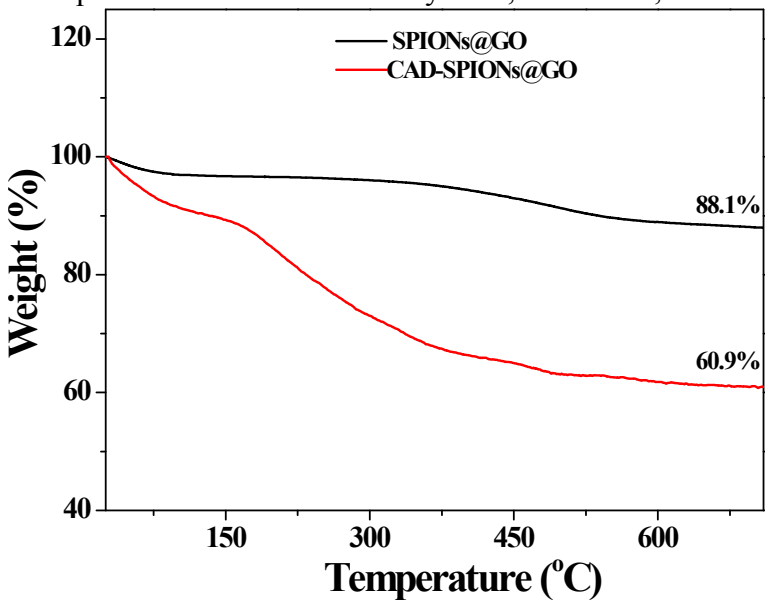

Figure S11. TG analysis curves of SPIONs@GO, CAD-SPIONs@GO.

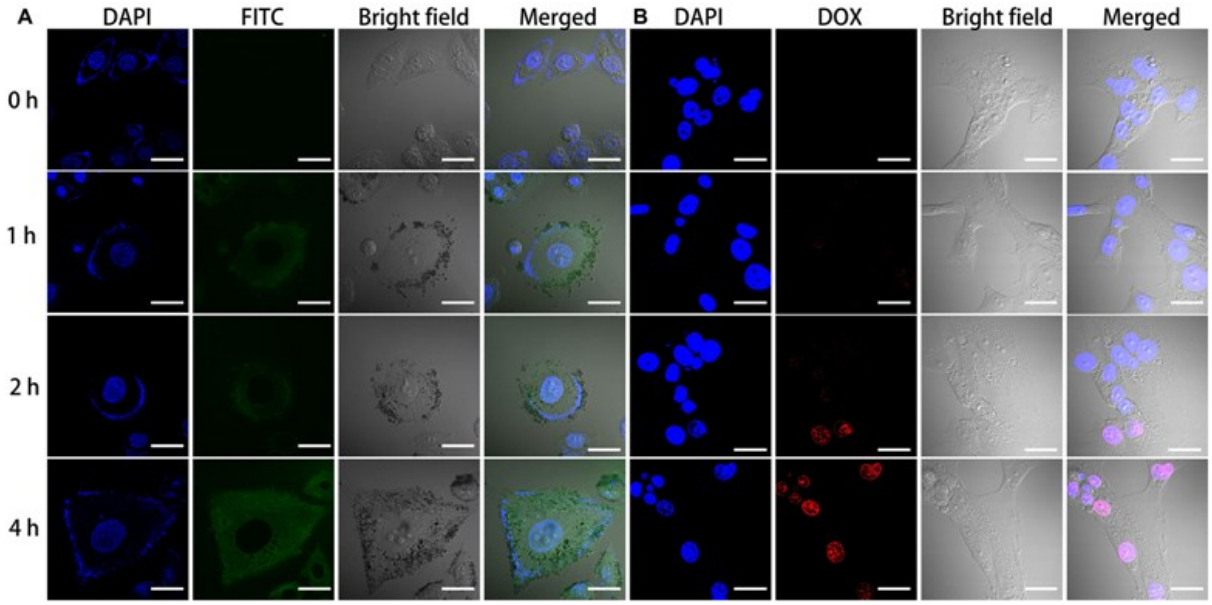

Figure S12. The cellular uptake of 4T1 cells after the cells was treated with the FI-SPIONs@GO ([Fe] 200 μg/mL) at a different period (A), 4T1 cells treated with PBS were used as the 0 h control, Scale bar = 10 μm. Confocal microscopy images of 4T1 cells treated with CAD-SPIONs@GO for 0, 1, 2, and 4 h at the DOX concentration of 10 μM, respectively, Scale bar = 10 μm (B).

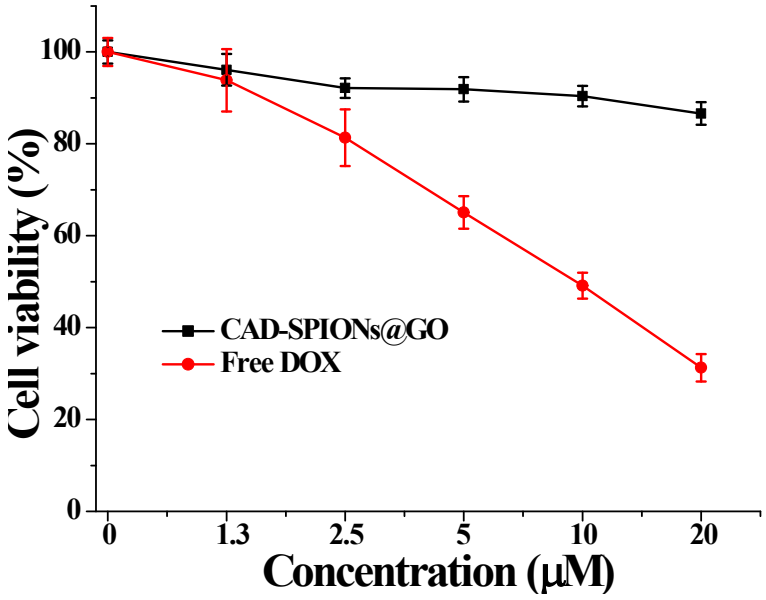

Figure S13. The viability of L929 cells after treated with free DOX and CAD-SPIONs@GO at the different DOX concentrations of 1.3, 2.5, 5, 10 and 20 μM for 24 h at 37°C by the CCK-8 assay.

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