Electron Supplementary Information (ESI)

of

Graphene Quantum Dots-Capped Mesoporous Silica Nanoparticles through an Acid-Cleavable Acetal Bond for Intracellular Drug Delivery and Imaging

Tao Chen,[a] Hao Yu,[b] Nianwang Yang,[a] Mingdong Wang,[a] Chendi Ding[a] and Jiajun Fu*[a]

[a] School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, China.
Fax: +8625 84315609; Tel: +8625 84315206;
E-mail: fujiajun668@gmail.com
b Center for Molecular Metabolism, School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, China.

Materials and Methods

Tetraethylorthosilicate (TEOS, 99.98 %) and *N*-Cetyltrimethylammonium (CTAB) were provided by J&K Scientific, sodium hydroxide, 3-aminopropyltriethoxysilane (APTES), acetone, succinic anhydride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl (EDC), N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. 3,9-bis(3-aminopropyl)-2,4,8,10-tetraoxaspiro[5.5]undecane was obtained from TCI. Graphite powder (spectral grade) was provided by Sinopharm Group Chemical Regent Co., Ltd. (Shanghai, China). All the solutions were prepared using high-purity Milli-Q water (18.2 MΩ cm) throughout the experiment.

Characterization

The lateral size and thickness of GQDs were measured with AFM using a multi-mode Nanoscope V scanning probe microscopy system (Bruker, USA). AFM sample were prepared by drop-casting the dispersive GQDs onto freshly cleaved mica substrates. Transmission electron microscopy (TEM) images of samples were obtained on a JEOL JEM-2100 with an accelerating voltage of 200 Kv. The morphologies of samples were observed on S-4800 (Hitachi, Japan). The real-time release behavior of DOX was monitored by a RF5301PC fluorospectrophotometer using a 1 cm quartz cell. UV/vis measurements were performed on a Shimadzu UV-1800 spectrophotometer at the wavelength of 480 nm. Small-angle powder X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advanced diffractometer using Cu Kα radiation (λ = 1.5406 Å). X-ray photoelectron spectra (XPS) were recorded on a RBD upgraded PHI-5300 system (Perkin Elmer) with Al Kα radation (hν = 1486.7eV). Fluorescence images were carried on an Olympus optical system microscopy. The N_2 adsorption–desorption isotherms were obtained at 77 K on a Quanta chrome Nova 1000 Micrometric apparatus. The solid state ^{13}C and ^{29}Si CPMAS NMR spectra were collected on a Bruker AVANCE 400 NMR spectrometer (Switzerland) at 400 MHz with 7 mm sample rotors in a triple-resonance probe head. Zeta potentials were measured by a Zeta potential analyzer (ZetaPALS, Brookhaven Instruments Corp.). Elemental analysis was performed by Elemental Vario EL Ⅲ. The carbon, hydrogen and nitrogen contents of each sample were determined using the thermal conductively detector.

Experimental details

Synthesis of fluorescent grapheme quantum dots (GQDs): Graphene oxide (GO) was prepared from natural graphite powder by a modified Hummers method.[1] Graphene sheets (GSs) was obtained by deoxidizing GO in a tube furnace at 300 ℃ for 2 h in nitrogen atmosphere. GSs (0.05 g) were oxidized in a mixture of concentrated H_2SO_4 (10 mL) and HNO_3 (30 mL) for 48 h under ultrasonication (500 W, 40 kHz). The mixture was then diluted with deionized (DI) water and filtered through a 0.22 μm microporous membrance to remove the acids. Oxidized GSs (0.2 g) were redispersed in DI water (40 mL) and the pH was adjusted to 8 with NaOH. The black solution was transferred to an autoclave (50 mL) and heated at 200 ℃ for 24 h. After cooling to the room temperature, the colloidal solution was dialyzed in a dialysis bag (retained molecular weight: 3500 Da) for 48 h to yield GQDs.

Synthesis and functionalization of mesoporous silica nanoparticles (*MSNs*): Mesoporous silica nanoparticles were synthesized as previously reported in literature.[2] In brief, CTAB (1.0 g), NaOH (3.5 mL, 2M), and H_2O (240 mL) were mixed and the temperature of the mixture was adjusted to 80 ℃. After stirring for 30 min, TEOS (5.0 mL) was added dropwise over 10 min. The mixture was stirring vigorously for another 2 h to form a white precipitation. The resulting nanoparticles were filtered and washed with method and DI water. To remove CTAB surfactants, the nanoparticles were reacted in a mixture of methanol (80 mL) and concentrated HCl (5 mL) at 50 ℃ for 24 h. The resulting MSNs were obtained by centrifugation, washing repeatedly with methanol, and dried under vacuum.

The functionalization procedures of MSNs were divided into three steps. First, MSNs were functionalized with amino group. MSNs (0.5 g) were refluxed in anhydrous toluene (40 mL) containing APTES (0.375 mL) under nitrogen for 20 h. After centrifugation, the products were extensively washed with toluene and methanol, and then dried. The sample was obtained as MSNs-NH_2. Second, MSNs were grafted with succinic anhydride molecules. Prepared MSNs-NH_2 (200 mg) was suspended into acetone at room temperature under stirring for 4 h. Then, succinic anhydride (80 mg) was dissolved acetone solution (10 mL), and the mixture solution was dropwise added and stirred at room temperature for 24 h. The resulting product was filtered and rinsed with ethanol and distilled water three times. The product was collected as MSNs-COOH. Third, MSNs

were modified with acetal linker. Synthesized MSNs-COOH (80 mg) were dispersed in H_2O (3 mL) containing EDC (50mg) and NHS (20mg). Then, 3,9-bis(3-aminopropyl)-2,4,8,10-tetraoxaspiro[5.5]undecane was added to the above solution and the mixture was stirred at room temperature for 8 h. After centrifugation, the product was washed with extensive distilled water. The obtained product was donated as MSNs-acetal. Fourth, MSNs were gated by fluorescent GQDs. MSNs-acetal (10 mg) were suspended in H_2O (2 mL), then EDC (10 mg), NHS (5 mg) and GQDs (1 mg) was added. The mixture was stirred at room temperature for 8 h to complete the capping process. The product was filtered, thoroughly washed with distilled water, and dried under vacuum. The sample is referred to as GQDs@MSNs.

Loading, capping and release experiments: For DOX loading, MSNs-acetal nanoparticles (10 mg) were suspended into PBS solution of DOX (5 mL, 1 mg mL^{-1}) and stirred for 24 h. The particles were then collected by centrifugation. In order to seal the DOX loaded MSNs-acetal, GQDs aqueous solution (5 mL, 1 mg mL^{-1}) was added into the suspension, followed by addition of EDC (10 mg) and NHS (5 mg). The mixture was stirred for another 6 h. The solid was centrifuged, washed extensively with PBS buffer solution (pH 7.0) and dried under vacuum oven overnight. The real-time drug release behavior of GQDs@MSNs-DOX was investigated by a fluorescence spectrometer. The GQDs@MSNs-DOX (2 mg) was placed into dialysis bag, which was immersed into the cuvette filled with 3 mL three different media: (a) phosphate buffer pH 7.0; (b) acetate buffer pH 5.0; (c) acetate buffer pH 4.0. The long-term released DOX from GQDs@MSNs-DOX was measured at predetermined time intervals and investigated by UV/vis spectroscopy with 480 nm wavelength.

Cell Culture: Human lung adenocarcinoma (A549) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Gibco) under 5% CO_2 atmosphere at 37 ℃. The cell culture medium was changed every 48 h, and the cells were seeded at a density of 10^4 /well in 96-well plates.

Cytotoxicity Assay by MTT: A549 cells were seeded in a 96-well plate at a density 10^4 cells/well and cultured in 5% CO_2 at 37 ℃ for 24 h. Then the cells were treated with free DOX, MSNs-DOX

and GQDs@MSNs-DOX at desired concentration. After incubation for 12, 24 and 48h, MTT solution (0.5 mg mL^{-1}) was added per well and incubated for another 4 h. The medium containing MTT was removed and dimethyl sulfoxide (150 μL) was added. The absorbance of solution was monitored using the enzyme-linked immunosorbent assay (PowerWave XS) at the wavelength of 550 nm.

Fluorescence microscopy: To investigate the cellular uptake and DOX release, A549 cells were cultured in the incubation medium (DMEM) for 24 h. GQDs@MSNs-DOX was added into DMEM at a concentration of 50 μg mL^{-1} for 2 h incubation in 5 % CO_2 at 37 ℃. Subsequently, the cells were collected and washed twice with PBS (pH = 7.4). The cell images were taken using fluorescence microscopy (Olympus, Japan)

Data analysis

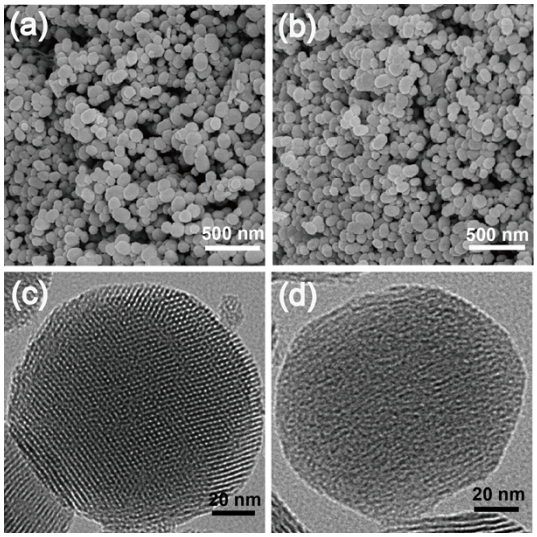

Fig. S1 Scanning electron microscopy (SEM) images of MSNs (a) and GQDs@MSNs (b); Transmission electron microscopy (TEM) images of MSNs (c) and GQDs@MSNs (d).

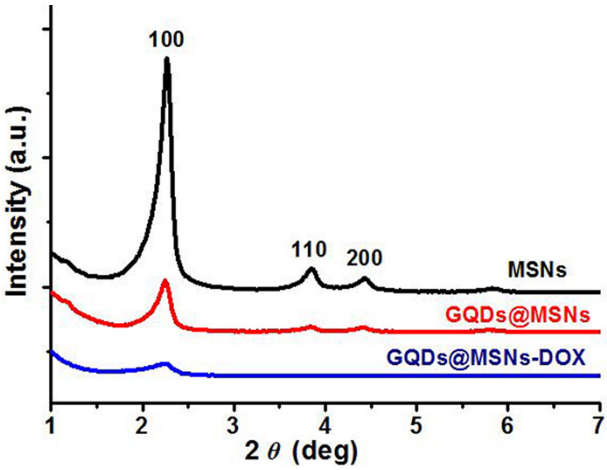

Fig. S2 Small-angle powder X-ray diffraction (XRD) patterns of MSNs, GQDs@MSNs and GQDs@MSNs-DOX.

The XRD pattern of MSNs exhibited three well-resolved reflections (100), (110), and (200), which is typical of a hexagonal mesoporous structure. GQDs@MSNs also showed similar peaks to MSNs, indicating that the acetal linkage had no influence on the mesoporous structure of silica matrix. However, the three peaks were hardly detectable after loading with DOX and capping with GQDs.

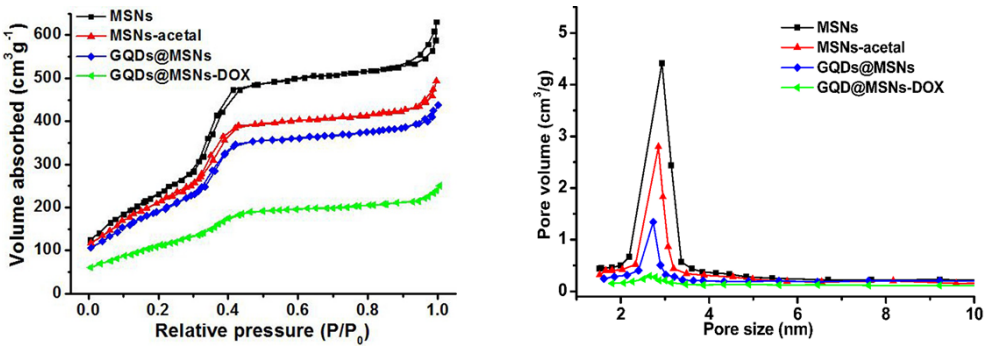

Fig. S3 Nitrogen adsorption-desorption isotherms (left) and pore size distribution (right) of MSNs, MSNs-acetal, GQDs@MSNs and GQDs@MSNs-DOX.

Table S1. BET and BJH parameters of MSNs, MSNs-acetal, GQDs@MSNs and GQDs@MSNs-DOX.

Sample	**Special surface Area(m^2 g^{-1})**	**BJH average pore diameter (nm)**	**Pore volume(cm^3 g^{-1})**
MSNs	1098	2.9	0.89
MSNs-acetal	839	2.8	0.68
GQDs@MSNs	765	2.6	0.55
GQDs@MSNs-DOX	374	--	0.38

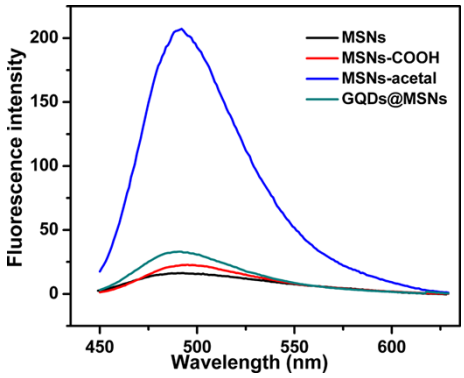

Fig. S4 Fluorescence spectra of fluorescamine solutions after reacting with MSNs, MSNs-COOH, MSNs-acetal and GQDs@MSNs (excitation wavelength of 420 nm).

Fluorescamine spectra provide direct evidence of the formation of the GQDs@MSNs based on the fact that the amount of primary amine groups ($-NH_2$) is proportional to the fluorescence intensity. Thus, the fluorescence intensity of GQDs@MSNs became much lower than that of MSNs-acetal, since the free amine from acetal linker were consumed after coupling with carboxyl groups of GQDs.

Table S2. Zeta potentials of MSNs, MSNs-NH_2, MSNs-COOH, MSNs-acetal and GQDs@MSNs.

Sample	Average zeta potential (mV)
MSNs	-32.1
MSNs-NH_2	21.5
MSNs-COOH	-38.9
MSNs-acetal	19.4
GQDs@MSNs	1.7

The average zeta potential of MSNs-NH_2, MSNs-COOH and MSNs-acetal were 21.5, -38.9 and 19.4 mV, implying the successive introduction of organosilane amide group, carboxyl group and acetal linker (Table S2). After capping with GQDs, zeta potential of GQDs@MSNs increased to 1.7 mV due to the success of EDC/NHS chemistry.

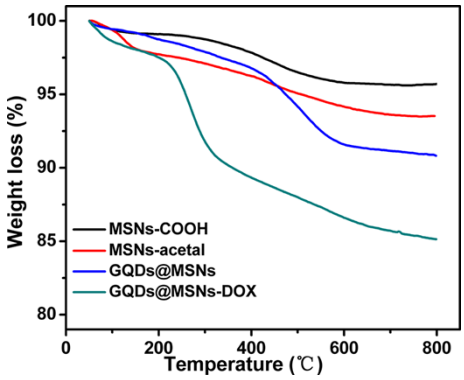

Fig. S5 Thermogravimetric analysis (TGA) of MSNs-COOH, MSNs-acetal, GQDs@MSNs and GQDs@MSNs-DOX.

TGA data show that the weight loss of MSNs-COOH, MSNs-acetal, GQDs@MSNs and GQDs@MSNs-DOX were 3.5 %, 5.7%, 9.2 and 14.9%, respectively. The degree of degradation of GQDs@MSNs increased by 3.5% compared with MSNs-acetal, owing to the weight loss of GQDs capping on the surface of MSNs. The loading amount of DOX was 57 mg g^{-1} SiO_2, calculated by the difference of weight loss b GQDs@MSNs and GQDs@MSNs-DOX.

Table S3. Element analysis of MSNs, MSNs-COOH, MSNs-acetal and GQDs@MSNs.

Type of silica	Nitrogen (%)	Carbon (%)	Hydrogen (%)
MSNs	0.12	0.45	0.32
MSNs-COOH	0.25	3.13	0.65
MSNs-acetal	0.71	4.06	0.60
GQDs@MSNs	0.54	7.80	0.45

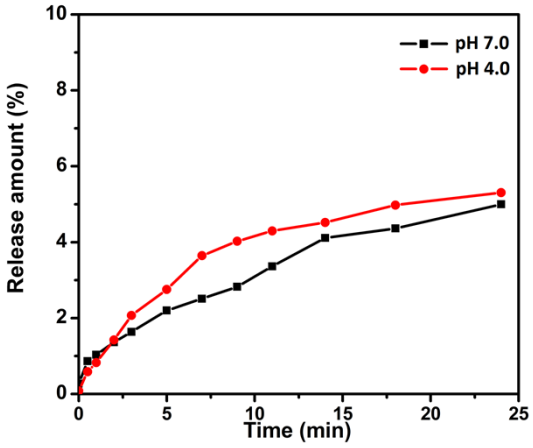

Fig. S6 Release profiles of DOX-loaded MSNs-acetal at pH 7.0 and 5.0 without GQDs capping.

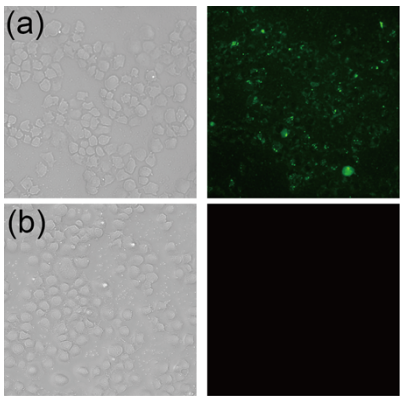

Fig. S7 Fluorescence microscopy images of A549 cells incubated with GQDs@MSNs (a) and without GQDs@MSNs (b).

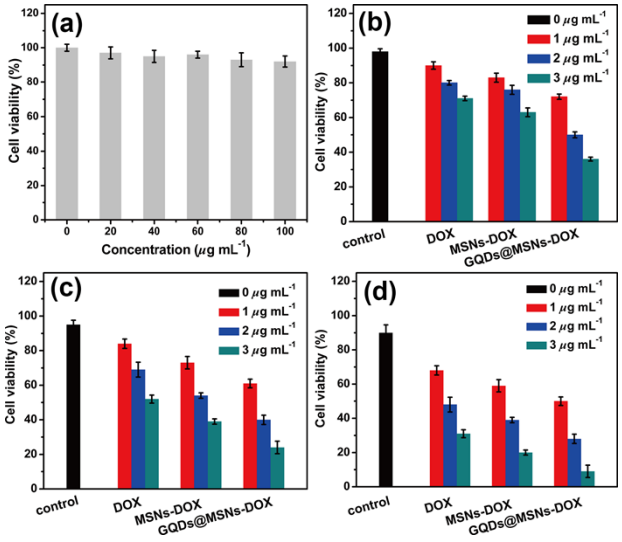

Fig. S8 Cell viability of A549 cells after incubating with GQDs@MSNs for 24 h (a). Cell viability of A549 cells after incubating with free DOX, MSNs-DOX and GQDs@MSNs-DOX for 12 h (b), 24 h (c) and 48 h (d).

Supporting References

1. (a) Y. Xu, H. Bai, G. Lu, C. Li and G. Shi, *J. Am. Chem. Soc*. 2008, **130**, 5856; (b) D. Pan, S. Wang, B. Zhao, M. Wu, H. Zhang, Y. Wang and Z. Jiao, *Chem. Mater*. 2009, **21**, 3137.
2. C. Y. Hong, X. Li and C. Y. Pan, J. Mater. Chem., 2009, **19**, 5155.