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A graphene quantum dot@Fe_3O_4@SiO_2 based nanoprobe for drug delivery sensing and dual modal fluorescence and MRI imaging in cancer cells

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Abstract

A novel graphene quantum dot (GQD)@Fe_3O_4@SiO_2 based nanoprobe was reported for targeted drug delivery sensing, dual modal imaging and therapy. Carboxyl GQD (C-GQD) was firstly conjugated with T_2-weighted magnetic resonance imaging (MRI) agent Fe_3O_4@SiO_2 and functionalized with cancer targeting molecule folic acid (FA). The synthesized Fe_3O_4@SiO_2@GQD-FA nanoprobe provided targeted fluorescence and MRI dual-modality imaging for cancer cells. The loading of DOX drug molecules on the layered GQD surface of Fe_3O_4@SiO_2@GQD-FA nanoprobe via pi-pi stacking, resulted in a Fe_3O_4@SiO_2@GQD-FA/DOX conjugate with a FRET assay with GQD as donor and DOX as acceptor. The adsorption of DOX molecule on GQD surface could be detected with a limit of detection around 8 ng/mL. Meanwhile, using this Fe_3O_4@SiO_2@GQD-FA/DOX nanoprobe, we successfully performed *in vitro* MRI and fluorescence imaging of living Hela cells with the intracellular DOX release process monitoring. Cell viability study demonstrated the biocompatibility of Fe_3O_4@SiO_2@GQD-FA nanocarrier and the enhanced therapeutic efficacy of Fe_3O_4@SiO_2@GQD-FA/DOX nanoprobe for cancer cells. This luminomagnetic nanoprobe, characterized by excellent biocompatibility, real time drug delivery monitoring, dual modality fluorescence and MRI imaging and enhanced targeted cancer therapy efficacy, would be a potential platform for cancer accurate diagnosis and therapy.

Keywords: graphene quantum dots (GQDs), fluorescence resonance energy transfer (FRET), magnetic resonance imaging (MRI), cell imaging, drug delivery sensing

1. Introduction

Recently, development of nanoprobe with multiple functionalities, e.g. multimodal imaging, medical targeting, diagnostics, and therapeutic action in a single system, has attracted considerable interests for biomedical applications (Kelkar and Reineke, 2011). The use of multifunctional nanoprobe for early diagnosis and therapy of diseases is expected to provide significant improvements on diagnostic accuracy, therapeutic efficiency, and minimize the side effects. Functional fluorescent nanoparticles such as semiconductor quantum dots (QDs), have been used for bioimaging, diagnostics and therapy due to the good optical properties including high quantum yield, low photobleaching and narrow photoluminescence band (Bagalkot et al., 2007). However, semiconductor QDs are toxic to cells due to the heavy metal components and the large size of GQs will also lead to the change of cell functions (Valizadeh et al., 2012).

Graphene-based material is an especially popular class of multifunctional material for biomedical applications because of their unique mechanical, thermal and optical properties, such as single-atom-layered structure amenable to modification (Lee et al. 2008), excellent thermal and optical adsorption capability (Balandin et al., 2008; Neto et al., 2009), and low toxicity (Zhang et al., 2012). In particular, graphene quantum dots (GQDs), a new class of zero dimensional graphene nanomaterial,

exhibit tunable photoluminescence (PL) property, excellent photostability and great biocompatibility (Chen et al., 2012; Qian et al., 2014). Motivated by these significant features, GQDs are increasingly used as imaging and labelling probes in biomedical applications (Zheng et al., 2013; Shi et al., 2015; Ge et al., 2014). Recently, GQDs have been used to carry therapeutic molecules for simultaneous cell imaging and therapy applications (Wang et al., 2013; Wang et al., 2014). Unlike conventional nanoscale drug carriers, GQDs exhibit an ultrahigh loading capacity for highly aromatic molecules via strong π-π stacking interactions, whose loading ratio is up to 4000% (w/w) (Some et al., 2014). It is highly desirable to develop multimodal nanoprobes with selective targeting, dual-modality imaging, and real-time sensing drug release process in cells. Multimodal imaging has become increasing interesting by improving diagnostic accuracy which could not be achieved via just a single modality (Rieffel et al., 2015). Magnetic resonance imaging (MRI) has advantages of deep penetration and high spatial resolution and fluorescence imaging has high sensitivity (Zeng et al., 2014; Ma et al., 2015). A dual-imaging modality system combining GQD based fluorescence imaging and MRI will largely increase diagnostics efficiency. However, there has been no report on GQD@Fe_3O_4 based nanoprobe with fluorescence/MRI dual modality imaging and intracellular drug delivery sensing function.

In this paper, we report the development of a dual-imaging guided nanoprobe comprising GQDs attached on Fe_3O_4@SiO_2 core/shell structure with tumor target molecule folic acid (FA) and anti-cancer drug doxorubicin (DOX)

(Fe_3O_4@SiO_2@GQD-FA/DOX), which could provide fluorescence and MRI dual imaging modality, and sensing delivery process of drug molecules in the targeted tumor cells via fluorescence resonance energy transfer (FRET) process. The cell viability experiments demonstrated the low cytotoxicity of Fe_3O_4@SiO_2@GQD-FA nanocarrier. By loading DOX molecules, Fe_3O_4@SiO_2@GQD-FA/DOX nanoprobes showed enhanced therapeutic efficacy compared with free DOX molecules.

2. Materials and Methods

2.1 Materials

Iron (III) chloride hexahydrate was purchased from Alfa Aesar. 1-Octadence (tech. 90%), Oleic acid (tech. 90%), tetraethyl orthosolicate (TEOS, 99%), 3-Aminopropyl triethoxysiliane (APTES, 98%), ethanol (99.5%), hexane (99%), citric acid (99.5%), Igepal CO-520, folic acid (97%), thiazolyl blue tetrazolium bromide (MTT) assay kit and DOX (98%-102%) were purchased from Sigma Aldrich. Cyclohexane was acquired from Inthernational Laboratory. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuxxinimide (NHS, 98%) were purchased from Tokyo Chemical Industry and J&K Scientific respectively. Dulbecco's modified Eagle's medium (DMEM), fluorobrite DMEM, fetal bovine serum (FBS), penicillin streptomycin, and trypsin-EDTA were obtained from Life Technologies. All solutions were prepared in DI water or phosphate buffer saline (PBS).

2.2 Preparation of amino functionalized Fe_3O_4@SiO_2 nanoparticles.

Fe_3O_4 NPs were synthesized via thermal decomposition method (Park et al., 2004). In a typical experiment, 1 g of $FeCl_3 \cdot 6H_2O$ was dissolved in a mixture of 6 mL of DI water, 8 mL of ethanol and 14 mL of hexane. Then 1.9 mL of oleic acid and 0.24g NaOH were added and the mixture was kept at 70 °C for 4 h under vigorous stirring. After cooling down, the resultant iron–oleate complex was collected by separating funnel and washed tree times with DI water. Finally, hexane was evaporated off after drying at 80 °C overnight, resulting in iron–oleate complex in a waxy solid form. To obtain monodispersed Fe_3O_4 NPs, 3.6 g of iron–oleate precursor and 0.64 mL of oleic acid were dissolved in 25 mL of 1-octadecene and then degassed with argon for 30 min at room temperature. The mixture solution was heated to 320 °C and then kept at that temperature for 30 min under argon flow. The resultant solution was then cooled to room temperature, and access ethanol was added to the solution.

Core-shell Fe_3O_4@SiO_2 NPs were fabricated by a reverse microemulsion method (Ding et al., 2012). Typically, 5 mg of as-prepared Fe_3O_4 NPs were dispersed in a mixture of 2 mL of Igapal CO-520 and 50 mL of cyclohexane with continuous stirring, followed by adding 1 mL of ammonia solution. Next, 0.06 mL of TEOS was added dropwise and kept stirring for 12 h. The resultant Fe_3O_4 NPs were collected after magnetic separating and washing, and then redispersed in ethanol. After that,

0.06 mL of APTES silane was introduced to obtain amino functionalized Fe_3O_4@SiO_2 NPs. The final product was collected via magnetic separation.

2.3 Preparation of Fe_3O_4@SiO_2@GQD-FA nanocarriers.

The GQDs were synthesized via directly pyrolyzing citric acid (Dong et al., 2012). Typically, 0.25 g of citric acid was heated to 200 °C for 20 minutes using an oven. Then the generated yellow liquid was added dropwise in 10 mL of 0.25 M NaOH solution. The blue luminescent GQD aqueous solution was obtained after NaOH neutralization. The conjugation of GQDs with amino functionalized Fe_3O_4@SiO_2 nanoparticles was achieved by the classic carbodiimide crosslinking chemistry between carboxyl groups of GQDs and amine groups on the surfaces of Fe_3O_4@SiO_2. Briefly, 80 mg of EDC and 10 mg of NHS was added to 5 mL of GQDs solution (10 mg/mL in PBS buffer) and incubated for 20 min in dark environment. Subsequently, 1 mg of amino functionalized Fe_3O_4@SiO_2 nanoparticles were added and the mixture was stirred gently overnight. The final product was collected via magnetic separation. Fe_3O_4@SiO_2@GQDs-FA was then assembled by incubating Fe_3O_4@SiO_2@GQD solution (5.0 mL, 10 mg/mL) with 80 mg NHS in 10 mg MES buffer and 50 mg FA for 30 min at room temperature. The mixture was then stirred in the dark for 24 h, and centrifuged and filtered to remove the unreacted FA and NHS. The final product was then redispersed in PBS solution for further usage.

2.4 Characterization

The shape, size and morphology of the synthesized nanoassembly was investigated with a JEOL-2100F transmission electron microscopy (TEM) equipped with an Oxford Instrument energy-dispersive X-ray (EDX) spectrometry system. Fourier transform infrared spectrum (FTIR) was collected using a PerkinElmer Spectrum 100 FT-IR spectrometer (PerkinElmer Inc., USA). UV-Vis spectra were performed on a UV/Visible spectrophotometer (Biochrom., England). Zeta potential measurements were performed on a Zetasizer Zeta Potential Analyzer (Malvern Instruments Ltd., England). Excitation and emission spectra were measured by a FLS920P Edinburgh Analytical Instrument. Confocal optical micrographs were obtained from a confocal laser scanning microscope (Leica SP8).

2.5 In vitro cellular MRI

All MRI images were collected in a clinical MR scanner (Siemens MAGNETO Trio, 3T, Germany) at room temperature. Fe_3O_4@SiO_2@GQDs-FA at various Fe concentrations: 0.00, 0.04, 0.08, 0.12, and 0.16 mM were suspended in 96 well plates for T_2-weighted MRI imaging. For cell experiments, Hela cells were incubated with Fe_3O_4@SiO_2@GQDs-FA at Fe concentration of 0.1 mM in cell culture medium for 24 hours. After careful washing, Hela cells were then detached by tripsinization and centrifuged in a tube before imaging.

2.6 In vitro cellular fluorescence imaging

Hela cells with a concentration of 1×10^5 cells/mL were firstly incubated on coverglass bottom dish for 24 hours. Solution with Fe_3O_4@SiO_2@GQDs-FA/DOX

(40 μg/mL) was then added and co-cultured with Hela cells. After washing with PBS buffer twice, the Hela cells were monitored at various time points with the same optical conditions using confocal microscopy for the following channels: GQDs channel (Ex: 405 nm; Em: 475 nm-505 nm), DOX channel (Ex: 485 nm; Em: 585 nm-615 nm), DOX FRET channel (Ex: 405 nm; Em: 585 nm-615 nm).

2.7 Cell viability assay

The viability of Hela cells exposed to Fe_3O_4@SiO_2@GQD-FA/DOX, Fe_3O_4@SiO_2@GQD-DOX, DOX, Fe_3O_4@SiO_2, Fe_3O_4@SiO_2@GQD, and Fe_3O_4@SiO_2@GQD-FA was assessed by MTT assay. Hela cells were seeded at a density of 1×10^4/cell into 96-well plate. After 24 h, the cells were exposed to various samples for another 24 h of incubation. Then 20 μL of MTT solution (5 mg/mL) was added to each well and incubated at 37 ^{0}C in 5% CO_2 atmosphere for 4 h. Next, the MTT solution was discarded and 200 μL of DMSO was added to each well to dissolve formazan crystals. Absorbance of each sample was measured at 570 nm using a microplate reader (Infinite F200, Tecan, Switzerland).

3. Results

3.1 Mechanism of nanoprobe FRET sensing system.

As shown in Fig. 1a**,** this Fe_3O_4@SiO_2@GQD-FA/DOX nanoprobe is composed of four components: 1) Fe_3O_4@SiO_2 core-shell structure for MRI imaging; 2) GQDs, covalently conjugated with Fe_3O_4@SiO_2 core-shell via amine-carboxyl linking, which

serve a dual function of fluorescence probe and donor molecule in FRET; 3) Folic Acid (FA), conjugates with GQDs via amine-carboxyl linking, which serves as a target molecule for cancer cells; 4) Doxorubicin (DOX) with well know fluorescent properties, conjugates with GQDs via pi-pi stacking interaction, which serves a dual function of anti-cancer drug and acceptor molecule in FRET. The assembly of this system results in a FRET system with GQD as donor molecule and DOX as acceptor molecule, where the fluorescence emission of GQDs is quenched by the adsorption of DOX on GQD surface via pi-pi adsorption. At this stage, GQD is on fluorescence "OFF" stage and DOX is on fluorescence "ON" stage via energy transfer under excitation. After the nanoassembly is taken up by targeted cancer cells, DOX molecules are released from GQDs' surface in the presence of acidic intracellular environment, which induces the recovery of fluorescence of GQDs to "ON" stage (Fig. 1b). Therefore, the localization of nanoprobes and sensing the release of DOX in cancer cells could be monitored by observing FRET signal change.

Here Fig. 1

3.2 Characterizations of GQDs and the synthesis process of nanoprobe

Fe_3O_4 nanoparticles were synthesized with a thermal decomposition method, which showed good water dispersity (Fig. 2a). The selected area electron diffraction

(SAED) pattern confirmed the typical cubic spinel structure of Fe_3O_4 (Fig. S1a). Core-shell Fe_3O_4@SiO_2 nanoparticles were formed with a reverse microemulsion method. The prepared Fe_3O_4@SiO_2 contained a core-shell structure, in which a 5 nm of darker Fe_3O_4 core and 10 nm of SiO_2 shell could be observed (Fig. S1b). The silica shell avoided the possible fluorescence quenching effect by the Fe_3O_4 cores of the nanoparticles. The average size of synthesized Fe_3O_4@SiO_2 nanoparticles was around 25 nm (Fig. S1c). GQDs were synthesized using a hydrothermal method with citric acid as the starting material. From the high-resolution TEM (HRTEM) image, the synthesized GQD showed good water dispersity with the lattice fringe of around 0.2 nm (Fig. 2b). The average size of GQDs was around 4 nm (Fig. S1d). FA was then conjugated with GQD via carbodiimide crosslinking chemistry. The conjugation of GQDs-FA with Fe_3O_4@SiO_2 composite led to the attachment of multiple GQDs on Fe_3O_4@SiO_2 surface to form a satellite structure (Fig. 2c).

To further characterize the formation of Fe_3O_4@SiO_2@GQD-FA system, UV-Vis spectra of nanoassembly formation were recorded (Fig. 2d). GQDs show an absorption peak at 325 nm. The characteristic absorption peak of FA is around 280 nm. For Fe_3O_4 NPs and silica modified Fe_3O_4 NPs, no obvious absorption peaks are observed in the spectra. After conjugation with GQDs and FA, the absorption spectrum of Fe_3O_4@SiO_2@GQD-FA shows the characteristic peaks of GQDs and FA at 325 nm and 280 nm, respectively, indicating the successfully formation of Fe_3O_4@SiO_2@GQD-FA nanoassembly. Fig. 2e shows the Fourier transform infrared spectroscopy (FTIR) spectra of nanoassembly formation. Fe_3O_4 nanoparticles show

the characteristic Fe-O peak at 603 cm^{-1}. Wide bands at about 1645 and 3450 cm^{-1} appear in all IR spectra, indicating the bending and stretching vibration of -OH. After coating silica shell on Fe_3O_4 core, two bands associated with Si-O-Si are observed at 1105 $cm^{-1}$and 490 cm^{-1}, respectively, which presents SiO_2 shell on the surface of Fe_3O_4. The stretching vibration of -CH is observed at 2941 cm^{-1} and 2870 cm^{-1}, presenting the successful conjugation of (3-aminopropyl)triethoxysilane (APTES) on Fe_3O_4@SiO_2 surface. After covalent binding with GQDs, new peaks appear at 1305 cm^{-1}, which should be assigned to C–N stretching of EDC/NHS. Another characteristic band 1645 cm^{-1} attributes to vibration of –NH–CO–, which indicates the formation of amide groups in Fe_3O_4@SiO_2-GQD. Moreover, the zeta potential (ξ) of Fe_3O_4@SiO_2 after APTES saline modification is around +40 mV due to the presence of NH_2^+ groups from silane molecules (Fig. S2**)**. After conjugation with GQDs, the zeta potential is shifted to -25 mV due to the abundant negative groups on GQD surface. The Zeta potential is further shifted to -18 mV after FA conjugation.

Here Fig. 2

3.3 Establishment of FRET system based on GQD and DOX

GQDs exhibit blue photoluminescence with emission peak at 460 nm under UV excitation of 365 nm (Fig. S3**)**. DOX is a well-known fluorescent drug molecule with excitation peak around 470 nm. The good matching between emission spectra of GQDs and excitation spectra of DOX makes it possible to establish a FRET system

between GQDs and DOX. Fig. 3a shows the representative emission spectra of GQDs, Fe_3O_4@SiO_2@GQD, Fe_3O_4@SiO_2@GQD-FA and Fe_3O_4@SiO_2@GQD-FA/DOX respectively. It shows that the emission peaks of GQD and Fe_3O_4@SiO_2@GQD are around 465 nm. The emission peak of Fe_3O_4@SiO_2@GQD-FA is blue-shifted by ~25 nm, suggesting the presence of FA on GQD surface. Upon DOX loading on GQD surface via pi-pi stacking interaction, the fluorescence signal of GQDs was quenched due to energy transfer between GQDs and DOX, which was attributed to spectral superimposition between the absorption spectrum of acceptor (DOX) and the emission spectrum of donor (GQDs). Instead, an emission peak at 600 nm by DOX was observed due to FRET effect. To further explore this FRET system, a fixed concentration of Fe_3O_4@SiO_2@GQD-FA (1μM) was incubated with DOX with a series of increasing molar ratios. Sequential decrease of the fluorescence signal of GQDs and the continuous increase of fluorescence signal of DOX were observed upon more and more DOX adsorption on GQD surface (Fig. 3b). The fluorescence decay curves of GOD before and after DOX adsorption were measured with 365 nm laser pulses (Fig. S4). The fluorescence lifetime of GQD was observed to be shortened after formation of GQD-DOX composites, which demonstrated that this process indeed induced non-radiative FRET effect. The adsorption of DOX on GQD surface can be detected by monitoring the DOX FRET signal with a limit of detection around 8 ng/mL based on control group plus 3 times of standard derivation (Fig. 3c).

To explore the feasibility of Fe_3O_4@SiO_2@GQD-FA as anti-tumor drug carriers, DOX was firstly loaded by mixing 50 mg of DOX with 50 mg of

Fe_3O_4@SiO_2@GQD-FA in 2 mL of PBS buffer (pH 7.4) for 24 h. The loading capacity of DOX on Fe_3O_4@SiO_2@GQD-FA is calculated as 67±2%. The cumulative DOX release profiles of Fe_3O_4@SiO_2@GQD-FA/DOX under physiological condition (pH=7.4) and acidic condition (pH=5.5) are then monitored and shown in Fig. 3d. Fe_3O_4@SiO_2@GQD-FA/DOX presented good stability under physiological conditions, with the release of only 7% of DOX at 6 h and 18% of DOX at 24 h. In contrast, 20% and 62% of DOX was speedily released at 6 h and 24 h in the acidic environment, respectively. After 3 days, up to 70% of DOX was released at pH=5.5. However, only 26% of DOX is released at pH=7.4. This pH-triggered drug release of DOX could be explained by higher solubility of DOX due to increased protonated carboxyl and amino groups on DOX at lower pH environment which weakens the binding force between DOX and GQDs (Niu et al., 2013; Liu et al., 2007; Liu et al., 2009). This pH dependent DOX release property of this drug delivery system is highly needed for targeted cancer therapy, because tumor cells often exhibit an acidic environment (Gao et al. 2012; Ganta et al., 2008). Thus, the pH-sensitive Fe_3O_4@SiO_2@GQD-FA/DOX drug release system is feasible for intracellular targeted cancer therapy.

Here Fig. 3

3.4 In vitro MRI imaging

Fe_3O_4 nanoparticles have been proved to be an excellent T_2 contrast agent for MRI (Zeng et al., 2014). Fig. 4a shows the M–H hysteresis loops of Fe_3O_4 nanoparticles before and after coating with silica and GQD, reflecting the characteristic superparamagnetic property of Fe_3O_4@SiO_2@GQD. To evaluate the potential of Fe_3O_4@SiO_2@GQD-FA as diagnostic MR contrast agents, the MR signal of Fe_3O_4@SiO_2@GQD-FA at different Fe concentrations was measured by a 3T MRI scanner (Fig. 4b). The results indicated that T_2-weighted signal intensity decreased significantly with the increase of Fe concentrations, which was attributed to the dipolar interaction between magnetic moments of Fe_3O_4 and protons in water. The spin-spin relaxation time was thus shortened, leading to the darker images (Ma et al., 2015). Fig. 4c shows the transverse ($1/T_2$) relaxation rates of Fe_3O_4@SiO_2@GQD-FA as a function of Fe concentrations in de-ionized water. The results show a linear relationship between relaxation rates and Fe concentrations with a high relaxation value of 62.8 $mM^{-1}S^{-1}$, indicating the as-prepared Fe_3O_4@SiO_2@GQD-FA is feasible for efficient T_2 MRI. The feasibility of Fe_3O_4@SiO_2@GQD-FA nanoassembly for *in-vitro* MRI in Hela cells was then investigated. Fig. 4d clearly shows the negative image contrast enhancement of Hela cells incubated with Fe_3O_4@SiO_2@GQD-FA with Fe concentration of 0.1 mM for 24 h compared with the bright image of untreated cells.

Here Fig. 4

3.5 Intracellular imaging and drug release monitoring

To explore the functionality of Fe_3O_4@SiO_2@GQD-FA/DOX nanoprobe for intracellular imaging and drug delivery sensing *in vitro*, the uptake of nanoprobes and change of FRET signals in Hela cells were investigated via fluorescence microscopy over extended time periods. The cell samples were washed carefully with PBS solution several times to remove unbound nano- assembly before fluorescence images were taken. As shown in Fig. 5, after 0.5 h incubation with Fe_3O_4@SiO_2@GQD-FA/DOX composite, red fluorescence signal (DOX FRET channel) with excitation at 405 nm was observed at the boundary region of Hela cells, indicating the start of entry of nanocarriers by Hela cells via receptor-mediated endocytosis. At this initial stage, the fluorescence of GQDs was largely in the "OFF" state (GQD channel) and the energy was transferred to DOX, indicating that DOX molecules were mostly stacked on the surface of GQDs. After 6 h of incubation, an obvious increase of blue fluorescence (GQD channel) was observed in cell cytoplasm with "ON" state due to the detachment of DOX molecules from the carriers. The FRET signals were mainly in the cell cytoplasm, whereas red fluorescence of DOX under direct excitation (DOX channel, Ex: 485 nm) was observed in both cytoplasm and nuclei. Since FRET signals mainly represented unleased DOX molecules on carriers, it was concluded that some released DOX molecules entered into nuclei and nano-carriers with unreleased DOX molecules were mainly in the cell cytoplasm. After 12 h incubation, strong red fluorescence could be observed in cell nuclei (DOX 485 ex channel) with a decrease of FRET signals (DOX FRET channel), which indicated most DOX molecules were released from carriers and accumulated inside

nuclei. Blue fluorescence was also observed inside cell nuclei (GQD channel), suggesting that some nanocarriers entered into cell nuclei at this stage. The results here demonstrated that this nanocarrier is sensitive to detect drug release process inside cancer cells on a single cell level in vitro.

Here Fig. 5

3.6 Cell viability and therapeutic efficacy study

To confirm the biocompatibility of the nano-carriers, the cytotoxicity of Fe_3O_4@SiO_2, Fe_3O_4@SiO_2@GQD, and Fe_3O_4@SiO_2@GQD-FA on Hela cells were studied with an MTT assay. All samples show excellent biocompatibility as cell viability was greater than 80% and Fe_3O_4@SiO_2@GQD-FA showed almost no effect on cell viability up to up to 100 μg/mL (Fig. 6a). Therapeutic efficacy was also compared with pure DOX, Fe_3O_4@SiO_2@GQD/DOX and Fe_3O_4@SiO_2@GQD-FA/DOX for a series of DOX concentrations. As shown in Fig. 6b**,** Hela cells showed lower viability with Fe_3O_4@SiO_2@GQD-FA/DOX and Fe_3O_4@SiO_2@GQD/DOX compared with that of pure DOX for all the concentrations, indicating the largely increased therapy efficiency with nanoassembely drug carriers. Moreover, for a DOX concentration of 10 μg/mL, the cell viability incubated with Fe_3O_4@SiO_2@GQD-FA/DOX (15.4%) was even lower than Fe_3O_4@SiO_2@GQD-DOX (20.4%), which could be due to enhanced targeting efficiency to Hela cells of FA modified Fe_3O_4@SiO_2@GQD compared to that of non-targeted Fe_3O_4@SiO_2@GQD carriers.

4. Conclusion

In summary, Fe_3O_4@SiO_2@GQD-FA/DOX multifunctional nanoprobes have been developed with functions of fluorescence/MRI imaging and FRET based drug release sensing. The adsorption of DOX molecule on GQD surface could be detected with a limit of detection around 8 ng/mL. By monitoring the FRET signals change in the cancer cells, the cellular uptake of nanoprobes and intracellular DOX drug molecule release process were successfully monitored. Moreover, the therapeutic efficacy of DOX could be enhanced by the nanocarriers. Overall, this synthesized GQD@Fe_3O_4 based multifunctional luminomagnetic nanoprobe could be a promising platform for targeted cancer accurate diagnosis and therapy with drug molecule tracking function.

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Figure Captions

Fig. 1 (a) Schematic illustration of the synthesis process of Fe_3O_4@SiO_2@GQD-FA multifunctional nanoassembly. (b) Schematic illustration of DOX loading and release process on Fe_3O_4@SiO_2@GQD-FA and the FRET system with GQD as donor molecule and DOX as acceptor molecule.

Fig. 2 TEM images of (a) Fe_3O_4 nanoparticles, (b) GQDs, (c) Fe_3O_4@SiO_2@GQD-FA; (d) UV–Vis spectra of GQD, FA, Fe_3O_4, Fe_3O_4@SiO_2-NH_2, Fe_3O_4@SiO_2@GQD and Fe_3O_4@SiO_2@GQD-FA; (e) FTIR spectra of Fe_3O_4, Fe_3O_4@SiO_2-NH_2, Fe_3O_4@SiO_2@GQD and Fe_3O_4@SiO_2@GQD-FA.

Fig. 3 (a) PL spectra of GQDs, Fe_3O_4@SiO_2@GQD, Fe_3O_4@SiO_2@GQD-FA and Fe_3O_4@SiO_2@GQD-FA/DOX. Inset: Photograph of the solution of GQDs taken under UV light. (b) PL spectra of Fe_3O_4@SiO_2@GQD-FA with increasing molar ratio of DOX (from 0 to 20); (c) Relative DOX FRET signal change with concentration of DOX molecules; (d) Cumulative DOX release (%) from Fe_3O_4@SiO_2@GQD-FA/DOX in PBS buffer with various pH values at different time intervals. Quantitative data are presented as mean ± SD with n=3.

Fig. 4 (a) M–H hysteresis loops of Fe_3O_4@SiO_2@GQD-FA nanoparticles; (b) T_2-weighted MRI of Fe_3O_4@SiO_2@GQDs-FA at various Fe concentrations: 0.00, 0.04, 0.08, 0.12, and 0.16 mM; (c) T_2 relaxation rates of Fe_3O_4@SiO_2@GQDs-FA at different Fe concentrations; (d) T_2-weighted MRI of Hela cells incubated without and with Fe_3O_4@SiO_2@GQDs-FA at Fe concentrations of 0.1 mM for 24 h.

Fig. 5 Fluorescence images of HeLa cells treated with Fe_3O_4@SiO_2@GQDs-FA/DOX at 0.5 h, 6 h, and 12 h. GQDs channel (Ex: 405 nm; Em: 475 nm-505 nm), DOX channel (Ex: 485 nm; Em: 585 nm-615 nm), DOX FRET channel (Ex: 405 nm; Em: 585 nm-615 nm).

Fig. 6 (a) In-vitro cytotoxicity of Fe_3O_4@SiO_2, Fe_3O_4@SiO_2@GQD and Fe_3O_4@SiO_2@GQD-FA and GQDs. (b) Viability profiles of DOX, Fe_3O_4@SiO_2@GQD-DOX and Fe_3O_4@SiO_2@GQD-FA/DOX after 24 h incubation. Data were displayed as means ± standard deviation with n = 5.