Multi-functionalized graphene oxide based anticancer drug-carrier with dual-targeting function and pH-sensitivity†‡

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A dual-targeting drug delivery and pH-sensitive controlled release system based on multi-functionalized graphene oxide (GO) was established in order to enhance the effect of targeted drug delivery and realize intelligently controlled release. A superparamagnetic GO–Fe_3O_4 nanohybrid was firstly prepared *via* a simple and effective chemical precipitation method. Then folic acid, a targeting agent toward some tumor cells, was conjugated onto Fe_3O_4 nanoparticles *via* the chemical linkage with amino groups of the 3-aminopropyl triethoxysilane (APS) modified superparamagnetic GO–Fe_3O_4 nanohybrid, to give the multi-functionalized GO. Doxorubicin hydrochloride (Dox) as an anti-tumor drug model was loaded onto the surface of this multi-functionalized GO *via* π–π stacking. The drug loading capacity of this multi-functionalized GO is as high as 0.387 mg mg^{-1} and the drug release depends strongly on pH values. Cell uptake studies were carried out using fluorescein isothiocyanate labeled or Dox loaded multi-functionalized GO to evaluate their targeted delivery property and toxicity to tumor cells. The results show that this multi-functionalized GO has potential applications for targeted delivery and the controlled release of anticancer drugs.

1. Introduction

Since the discovery of the novel nanomaterial graphene in 2004,[1] graphene has attracted much attention for various biological deliveries, including gene and drug delivery and intracellular tracking, *etc*, due to its capability for traversing the plasma membrane and promoting the cellular uptake of small molecules[2,3] and macro-molecules.[4,5] One of the advantages of this nanomaterial is that graphene oxide (GO) can be well-dispersed in water and physiological environments due to its abundant hydrophilic groups, such as hydroxyl, epoxide and carboxylic groups on its large surfaces. In addition, its good biocompatibility and lack of obvious toxicity make it a promising material for drug carrier substances.[2,3]

Although many existing drug carriers have shown numerous advantages such as drug solubilization and prolonged blood circulation, their efficacy is largely constrained by their lack of the ability to achieve high targeting efficiency at tumor sites, because of their limited loading capacity and low degree of functionalization capability. Moreover, insufficient cell uptake further decreases the therapeutic efficacy of the anti-tumor drug, and nonspecific accumulation in normal tissues leads to serious side effects and thus limits their clinical usage. Therefore, many studies have focused on the development of efficient delivery systems with the abilities to enhance special cellular uptake of anti-tumor drugs and to realize intelligent controlled release. A well-known strategy to achieve efficient tumor targeting is to conjugate drug carriers with specific ligands that can recognize molecular signatures on the cancer cell surface. Targeting ligands that can serve such a purpose include folic acid (FA),[6] peptides,[7] transferrin,[8] polysaccharides[9] and monoclonal antibodies.[10] However, the drug delivery systems need to be directed to tumor sites in the first place before recognizing cell surface receptors. Therefore, an external targeting strategy, such as a guided magnetic field, is expected to improve drug delivery efficiency by driving the drug carriers effectively into tumor tissues. Magnetic nanoparticles have been widely used for targeted drug delivery.[11–13] It is believed that drug nanocarriers can be taken up by cells *via* the endocytosis process.[14,15] While the endocytic pathway begins near the physiological pH of 7.4, it drops to a lower pH value (5.5–6.0) in endosomes and approaches pH 5.0 in lysosomes.[16,17] Therefore, the pH-sensitivity of drug release is very important to avoid undesired drug release during the drug transportation in blood circulation and to improve the effective release of the anti-tumor drug in the tumor tissue or within tumor cells.

Due to their high aspect ratio[18] and abundant surface chemistry,[19,20] functionalized GO has shown great promise as a novel drug delivery system with high efficiency loading, multi-targeted drug delivery and intelligent controlled release. The use of functionalized GO for targeted drug delivery of small molecules such as anticancer drugs is seldom explored. Dai *et al.* reported PEG-ylated nanographene oxide for delivery of water-insoluble

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cancer drugs and found that the functionalized nanographene sheets are biocompatible without obvious toxicity and can load an aromatic anticancer drug with high efficiency.[2] They also reported that the anti-cancer drug doxorubicin was loaded onto nanographene oxide functionalized with an antibody for selective killing of cancer cells.[3] Zhang *et al.* prepared FA conjugated sulfonic nanoscale GO and used them in loading two mixed anticancer drugs to realize the controlled loading and targeted delivery of mixed anticancer drugs.[21] Regarded as a promising candidate for drug delivery vehicles, GO based drug delivery systems combining dual magnetic and molecular targeting functions to tumor tissues and associated cells have not been reported. In this article, we describe a dual targeted delivery system based on multi-functionalized GO that contains a molecular targeting ligand and superparamagnetic iron oxide nanoparticles on the surface of GO for magnetic targeting. A superparamagnetic GO–Fe_3O_4 nanohybrid was firstly prepared *via* chemical precipitation method according to our previous work.[22] Then FA was conjugated onto Fe_3O_4 nanoparticles *via* imide linkage with amino groups of 3-aminopropyl triethoxysilane (APS) modified GO–Fe_3O_4 nanohybrid. Doxorubicin hydrochloride (Dox) as an anti-tumor drug model was then loaded onto the surface of this multi-functionalized GO *via* π–π stacking. Furthermore, the release of Dox exhibited pH dependence due to the carboxylic acid groups on GO. Cell culture experiments were conducted to evaluate the potential of multi-functionalized GO as a dual targeting delivery system with pH-sensitivity that can transport anticancer drugs to tumor cells effectively.

2. Experimental section

Materials

Graphite was purchased from Qingdao Tianhe Graphite Co. Ltd., with an average particle diameter of 4 μm (99.95% purity). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) and sodium hydroxide were purchased from Tianjin No. 3 Chemical Plant. 3-Aminopropyl trimethoxysilane (APS), N,N′-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS) and fluorescein isothiocyanate (FITC) were purchased from Aldrich and used without further purification. FA was bought from Nanjing Boquan Technology Co. and used as received. Doxorubicin hydrochloride (Dox) was purchased from Beijing Huafeng United Technology Co. Ltd. A dialysis chamber was purchased from Beijing Dingguo Biotechnology Co. (diameter = 36 mm), which had a molecular weight cutoff of 8000–15000 g mol^{-1}. RPMI 1640 culture medium was purchased from HyClone Co. and fetal bovine serum (FBS) was purchased from GIBCO Co. WST-1 was purchased from Biyuntian Biotechnology institute. All the other reagents were analytical grade and used without any further treatment.

Instrumentation

Transmission electron microscopy (TEM, FEI, TECNAI-20) was used to characterize the size and morphology of the samples. The magnetization curve of the GO–Fe_3O_4 nanohybrid was measured as a function of the applied magnetic field H with a 9600 VSM (LDJ Co.) superconducting quantum interference device (SQUID) magnetometer. The hysteresis of the magnetization was obtained by varying H between +6000 and −6000 Oe at 300 K. FTIR spectra were collected by using a Fourier transform infrared spectroscopy (FT-IR) (Tensor 27, BRUKER) and ultra-visible-near IR absorption spectrum (UV-vis-NIR) (JASCO, V-570) was used to characterize the functionalized GO. Confocal fluorescence microscopy (Olympus, FV1000) was used to detect the ability of the functionalized GO to be uptaken by the tumor cells. Absorbance in the WST assay was read by a Labsystems Dragon Wellscan MK2 microplate reader.

Preparation of GO-Fe_3O_4 nanohybrid *via* chemical deposition

Graphene oxide (GO) was prepared from purified natural graphite according to a modified Hummers method.[23] The GO-Fe_3O_4 nanohybrid was prepared according to the procedure in our previous work.[22] A typical procedure was as follows: GO (30 mg) was first sonicated in 50 mL dilute NaOH aqueous solution (pH 12) for several hours to transform the carboxylic acid groups to carboxylate anions, followed by thorough dialysis until the dialysate became neutral. The resulting product was condensed to 20 mL and placed in a 50 mL round-bottom flask. The flask was then purged with N_2 for 30 min. A solution of $FeCl_3 \cdot 6H_2O$ (36 mg) and $FeCl_2 \cdot 4H_2O$ (792 mg) in water (5 mL) was purged with N_2 for 30 min and then added to the flask. The mixture was stirred overnight under N_2 for ion exchange. After washing with water to remove excess iron salts, the solid product was re-dispersed in 25 mL water in a two-necked round bottom flask under a N_2 atmosphere. A NaOH aqueous solution (4 mL, 3 M) was added dropwise under N_2. The mixture was kept stirring at 65 °C for a further 2 h. Then the mixture was washed thoroughly with water to neutral pH and dried under vacuum at room temperature.

Conjugation of GO-Fe_3O_4 nanohybrid with FA (GO-Fe_3O_4-FA)

The folic acid active ester was prepared first. 1 g FA was dissolved in 30 mL DMSO in the presence of 0.5 mL triethyl amine thoroughly by sonication, then 1 g DCC and 0.56 g NHS were added into the reaction flask, followed by stirring for 24 h. The product was centrifuged to remove the sediment and the supernatant was added into the mixed reagent of ether and ethanol to produce sediment. Then the sediment was obtained by centrifugation and washing with ether. The straw-yellow folic acid active ester was obtained after drying.

The above GO-Fe_3O_4 nanohybrid (8 mL, 1.22 mg mL^{-1}) was added into 25 mL of ethanol in the presence of 0.3 mL APS and stirred at room temperature for 2 days. Then the suspension was ultracentrifuged and the precipitates were washed with ethanol several times. The APS-modified magnetic GO-Fe_3O_4 nanohybrid was transferred into 10 mL DMSO with 0.2 g folic acid active ester. The resulting mixture was brought to pH 8–9 by drop wise addition of triethylamine and stirred at 30–40 °C for 24 h. After the conjugating reaction, the suspension was ultracentrifuged and the precipitates were washed with DMSO three times and then dispersed into distilled water. The resulting GO-Fe_3O_4-FA was further purified with several ultracentrifugation and redispersion cycles. The absorbance of the supernatant was

recorded with UV absorption spectrum to ensure that excess free FA was removed from the solution.

Drug loading and release behaviors of GO-Fe_3O_4-FA

GO-Fe_3O_4-FA with the final concentration of 0.148 mg mL^{-1} was first sonicated with Dox with an initial concentration of 0.238 mg mL^{-1} for 0.5 h and then stirred overnight at room temperature in the dark. The samples were ultracentrifuged at 14000 rpm for 1 h. The Dox concentration in the upper layer was measured using a standard Dox concentration curve generated with an UV-vis-NIR spectrophotometer at the wavelength of 480 nm from a series of Dox solutions with different concentrations. The Dox loading capacity of GO-Fe_3O_4-FA was calculated according to the following formula:

$$\text{Drug loading capacity} = (W_{\text{administered dose}} - W_{\text{residual dose in solution}})/W_{\text{GO-Fe}_3\text{O}_4\text{-FA}}$$

Where $W_{\text{administered dose}}$ is the weight of initial drug for loading, $W_{\text{residual dose in solution}}$ is the weight of residual drug in solution after being loaded onto GO-Fe_3O_4-FA, and $W_{\text{GO-Fe}_3\text{O}_4\text{-FA}}$ is the weight of GO-Fe_3O_4-FA for loading, respectively.

The release behavior of Dox on GO-Fe_3O_4-FA was investigated by dialysis. The drug-loaded GO-Fe_3O_4-FA used for the release determination were placed into the dialysis chambers, which were dialyzed in 80 mL of aqueous solution under pH = 5, 7, 9, respectively. The drug release was assumed to start as soon as the dialysis chambers were placed into the reservoir. The release reservoir was kept under constant stirring, and one of the dialysis chambers was taken out for characterization at various time points. The concentration of Dox released from GO-Fe_3O_4-FA into aqueous solution was quantitatively analyzed by UV-vis-NIR spectrophotometer at the wavelength number of 480 nm.

Uptake of the multi-functionalized GO by human breast cancer cells (SK3)

Cell uptake studies were performed using SK3 cells, a human breast cancer cell. To investigate the targeted uptake of GO-Fe_3O_4-FA by SK3 cells, cellular uptake of the GO-Fe_3O_4-FA was observed by confocal fluorescence microscopy. FITC was loaded on GO-Fe_3O_4-FA by sonicating FITC solution (0.05 mg mL^{-1}, 2 mL) with an aqueous suspension of GO-Fe_3O_4-FA (1.22 mg mL^{-1}, 1 mL) for 30 min to mix them together, followed by stirring in the dark overnight. Unbound FITC was removed by ultracentrifugation at 14000 rpm for 1 h. As a control, GO-Fe_3O_4 was treated with FITC by the same steps. The generated GO-Fe_3O_4-FA-FITC and GO-Fe_3O_4-FITC were stored at 4 °C before using.

The SK3 cells were first cultured in 24-well plates in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and a fully humidified atmosphere at 37 °C containing 5% CO_2, followed by exposure to GO-Fe_3O_4-FA-FITC and GO-Fe_3O_4-FITC with the final concentration of 0.01 mg mL^{-1} at 37 °C for 1 h respectively. Finally the incubated SK3 cells were washed with PBS buffer.

Cytotoxicity of the Dox loaded multi-functionalized GO to Hela cells

To investigate the cytotoxicity of the GO-Fe_3O_4-FA loaded with the anti-tumor drug Dox towards tumor cells, WST assays were performed and Hela cells were employed. Dox was loaded on GO-Fe_3O_4-FA by sonicating Dox solution (0.60 mg mL^{-1}, 1 mL) with an aqueous suspension of GO-Fe_3O_4-FA (0.21 mg mL^{-1}, 3 mL) for 30 min to mix them together, followed by stirring in the dark overnight. Unbound Dox was removed by ultracentrifugation at 14000 rpm for 1 h. GO-Fe_3O_4 was treated with Dox by the same steps as the control. The generated GO-Fe_3O_4-FA-Dox and GO-Fe_3O_4-Dox were stored at 4 °C before using.

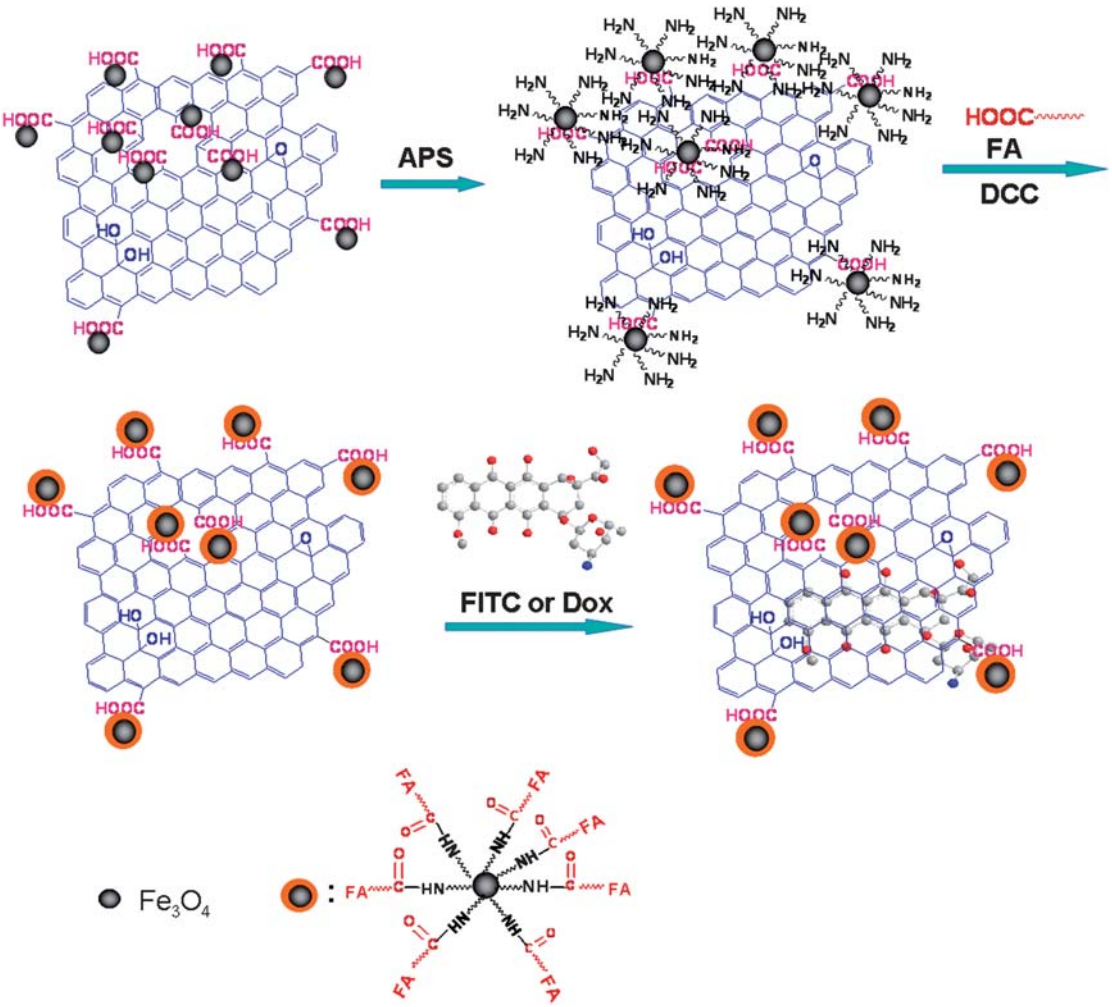
For WST assays, the Hela cells were seeded in 96-well plates at a density of 1×10^4 cells per well in normal RPMI-1640 medium or FA-free RPMI-1640 medium supplemented with 10% FBS and maintained for 24 h. Then, the cells were incubated with GO (with the final concentration of 0.050 mg mL^{-1}), GO-Fe_3O_4 (with the final concentration of 0.020 mg mL^{-1}, equal to the concentration of GO-Fe_3O_4 in GO-Fe_3O_4-FA-Dox), free Dox (with the final concentration of 0.0088 mg mL^{-1}), GO-Fe_3O_4-Dox (with the final concentration of the loaded Dox of 0.0088 mg mL^{-1}), or GO-Fe_3O_4-FA-Dox (with the final concentration of the loaded Dox of 0.0088 mg mL^{-1}) for 24 h. The cells incubated with GO-Fe_3O_4-FA-Dox were seeded in FA-free RPMI-1640 medium before use to ensure overexpression of folate receptor (FR) on the surface of the cells due to the FA-starved cells overexpressing FRs on the cell surfaces.[24] The tumor cells of other groups were cultured in normal RPMI-1640 medium to give few available free FRs on the cell surfaces. The relative cell viability was checked by the WST assay.

3. Results and discussion

The preparation of the multi-functionalized GO based anti-cancer drug-carrier with dual-targeting function and pH-sensitivity was shown in Scheme 1. Firstly, the superparamagnetic GO-Fe_3O_4 nanohybrids were prepared by chemical deposition of iron ions using soluble GO as carriers. Then APS were used to modify GO-Fe_3O_4 nanohybrids *via* the hydrolysis of APS, with the aid of surface hydroxyl groups on the surface of the Fe_3O_4 nanoparticles on the GO to generate the reactive amino groups. FA was then conjugated onto Fe_3O_4 nanoparticles *via* the amide linkage between the amino groups of APS modified GO-Fe_3O_4 nanohybrids and the carboxylic groups on FA. Thus, the multi-functionalized GO with magnetite and tumor dual-targeting properties was obtained. Cell uptake studies were carried out using labelled fluorescein isothiocyanate or Dox loaded multi-functionalized GO to characterize the targeted delivery and toxicity of the delivery system to tumor cells.

The morphology of the GO-Fe_3O_4 nanohybrid was characterized with TEM (see ESI‡, Fig. S1). Many Fe_3O_4 nanoparticles on GO with the size of several nanometres can be seen from the TEM images, and the size of most GO-Fe_3O_4 nanohybrid particles was below 200 nm. This suggests that a large amount of Fe_3O_4 nanoparticles are immobilized onto GO sheets.

The specific saturation magnetization of GO-Fe_3O_4 nanohybrid was measured with a superconducting quantum interference device magnetometer at room temperature. The saturate



Scheme 1 The preparation of the multi-functionalized GO based anticancer drug-carrier with dual-targeting function and pH-sensitivity.

magnetization *Ms* of the GO-Fe_3O_4 nanohybrid is 8.57 emu g^{-1}. The magnetization curves are S-like curves with near zero magnetic hysteresis loops (see ESI‡, Fig. S2). This indicates that the GO-Fe_3O_4 nanohybrid exhibits a superparamagnetic behavior.

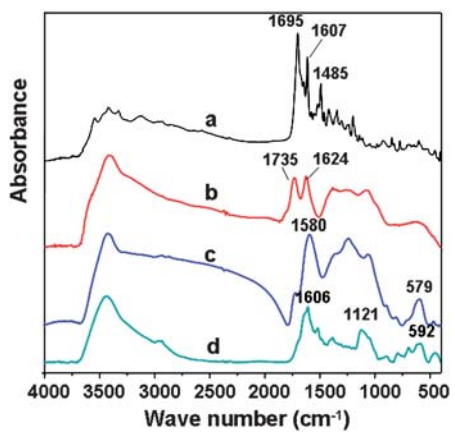


Fig. 1 FTIR spectra of FA (a), GO (b), GO-Fe_3O_4 (c) and GO-Fe_3O_4-FA (d).

Fig. 1 displays the FTIR spectra of FA (a), GO (b), GO-Fe_3O_4 (c) and GO-Fe_3O_4-FA (d). In the GO-Fe_3O_4 nanohybrid spectra, the peak at 1735 cm^{-1} corresponding to γ(C═O) of –COOH on the GO shifts to 1580 cm^{-1} due to the formation of $–COO^-$ after coating with Fe_3O_4. The peak at 579 cm^{-1} is the characteristic peak corresponding to the stretching vibration of Fe–O bond in the Fe_3O_4. After the GO-Fe_3O_4 nanohybrid was conjugated with FA, the peak at 579 cm^{-1} shifts to 592 cm^{-1} in the GO-Fe_3O_4-FA spectrum due to the modification of the GO-Fe_3O_4 nanohybrid with APS. At the same time, the peak at 1121 cm^{-1} corresponding to the Si–O–Si anti-symmetric stretching vibration absorption emerged. The characteristic peak of FA at 1606 cm^{-1} is clearly observed in Fig. 1d, which is slightly shifted from 1607 cm^{-1} in the FTIR spectrum of FA. Also, the clear peak at 1648 cm^{-1} corresponding to the characteristic peak of N–O in the FTIR spectrum of the folic acid active ester was observed (see ESI‡, Fig. S3). This suggested that FA was successfully conjugated onto the GO–Fe_3O_4 nanohybrid.

The multi-functionalized GO before and after loading with Dox was further confirmed by UV-vis absorption spectra as shown in Fig. 2. The peak at 283 nm in the UV-vis spectrum of GO-Fe_3O_4-FA in Fig. 2c is attributed to the characteristic absorption of FA, which has a strong peak at 282 nm, as shown

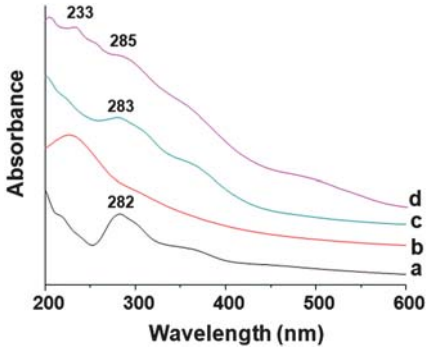


Fig. 2 UV spectra of FA (a), GO (b), GO-Fe_3O_4-FA (c) and GO-Fe_3O_4-FA-Dox (d).

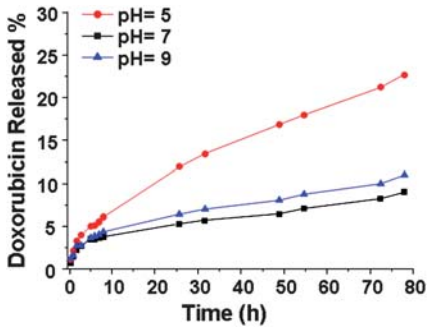


Fig. 3 The release of Dox on GO-Fe_3O_4-FA at different pH value.

in Fig. 2a. The slight shift from 282 to 283 nm for FA species before and after the FA conjugating with GO-Fe_3O_4 may be due to the formation of an amide linkage between the carboxylic acid group of FA and the amino group of the APS-modified GO-Fe_3O_4 nanohybrid. After the GO-Fe_3O_4-FA was loaded with Dox, the UV-vis peaks at around 233 and 497 nm attributed to the loaded Dox molecules were observed in the UV-vis spectrum of Dox loaded GO-Fe_3O_4-FA, as shown in Fig. 2d. The slight shifts of the UV-vis spectra for the conjugated FA components from 283 nm in Fig. 2c to 285 nm in Fig. 2d may originate from the interaction of the loaded Dox drugs and the conjugated FA components. All these results demonstrated that Dox molecules were successfully loaded onto GO-Fe_3O_4-FA.

Dox as an anti-tumor drug model was loaded onto the surface of this multi-functionalized GO *via* a simple mixture and sonication method by π–π stacking and hydrophobic interactions between multi-functionalized GO and Dox, which has been proved in our previous work.[25] The unbound drug was removed by centrifugation and the loading efficiency of Dox on multi-functionalized GO were calculated by measuring the concentration of unbound drug using UV-vis spectra. The Dox loading capacities of this multi-functionalized GO is as high as 0.387 mg mg^{-1} (or 38.7% in percentage) when the solution of Dox with an initial concentration at 0.238 mg mL^{-1}. Although some surface areas on multi-functionalized GO have obviously been occupied by Fe_3O_4 nanoparticles or/and even FA molecules and this results in the decline of drug loading capacities comparing with the original GO, such a loading value is still higher than that of some common drug carrier materials, such as liposomes,[26] where the loading capacity is always below 10%.

The drug release at different pH values were investigated at pH 5, 7, 9, respectively, as shown in Fig. 3. The Dox were released very slowly from multi-functionalized GO at neutral and basic conditions, and only about 7.5% and 11% of the total bound Dox was released for 80 h under neutral conditions (pH 7) and basic conditions (pH 9) respectively. However, in acidic conditions, Dox was released very quickly in the early stage but the release rate gradually declined after 5 h and about 24% of the total bound Dox was released from the nanohybrid in the first 80 h. As discussed in our previous work,[25] the hydrogen-bonding interaction between –OH and $–NH_2$ groups in Dox and the –OH and –COOH groups on GO is the strongest at the neutral condition, resulting in an inefficient release. The stronger hydrogen-bonding interaction under basic conditions than that under acid conditions results in a slower release rate under basic conditions. It is well known that there are acidic lysosomes inside tumor cells. As expected for an ideal delivery carrier for an anticancer drug, the multi-functionalized GO first specifically transported the drugs to the cancer cells, then the drug loaded carriers are taken up to the tumor cell interior through endocytosis. So, at lysosomal acidic pH (<5.5), protonation of amine groups on Dox can break the part of the hydrogen bond between Dox and the multi-functionalized GO carriers, leading to a larger desired release of Dox. In view of the different releasing behaviors of Dox on multi-functionalized GO under different pH environment, this multi-functionalized GO can be used as a good candidate material for intelligent drug release.

The targeting effect of the multi-functionalized GO to tumor cells was evaluated by selective uptake of the multi-functionalized GO by tumor cells *in vitro*. The FITC labelled multi-functionalized GO was then incubated with human breast cancer cells (SK3) (FA receptor positive) at 37 °C for 1 h, and the cells were observed by confocal fluorescence microscopy. Fig. 4 shows the confocal fluorescence images of SK3 after being incubated with GO-Fe_3O_4-FA-FITC and GO-Fe_3O_4-FITC, respectively. Much stronger fluorescence can be seen in the SK3 cells after incubation with GO-Fe_3O_4-FA-FITC than with GO-Fe_3O_4-FITC, which suggests specific targeting of multi-functionalized GO under the leading of FA molecules. This indicates that the multi-functionalized GO can be quickly and effectively delivered into the targeted tumor cells which over-express FRs.

We then investigated the cytotoxicity of the Dox loaded multi-functionalized GO to tumor cells. WST assays were performed and Hela cells were employed. GO, GO-Fe_3O_4, Dox, GO-Fe_3O_4-Dox, GO-Fe_3O_4-FA-Dox were incubated with Hela for 24 h, respectively. As shown in Fig. 5, no obvious toxicity was observed for GO without drug loading under the obvious higher concentration (0.050 mg mL^{-1}) than other experimental groups (with GO-Fe_3O_4 concentration of 0.020 mg mL^{-1}). The cytotoxicity increases after GO loaded with magnetic Fe_3O_4 nanoparticles. The cytotoxicity to Hela of Dox loaded GO-Fe_3O_4 nanohybrid is much higher than before loading with Dox, but lower than that of GO-Fe_3O_4-FA-Dox under the same drug concentration. It indicates that GO-Fe_3O_4-FA-Dox has the potential for selectively killing cancer cells *in vitro*. However, Dox shows the highest toxicity to Hela cells under the same condition due to the partial inefficient release of Dox on multi-functionalized GO.

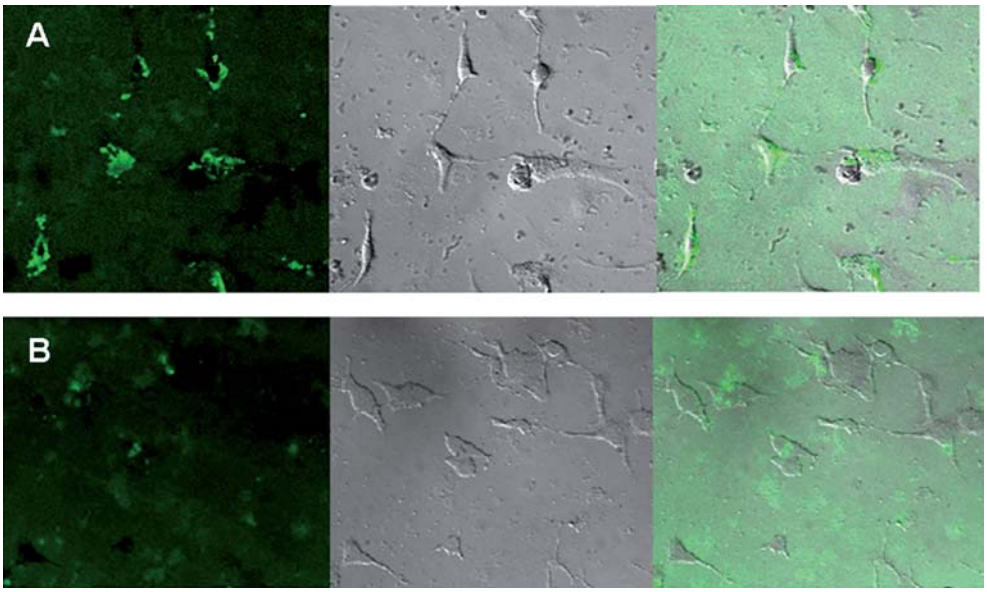


Fig. 4 Confocal fluorescence images of GO-Fe_3O_4-FA-FITC (A) and GO-Fe_3O_4-FITC (B) after incubation with SK3 at 37 °C for 1 h.

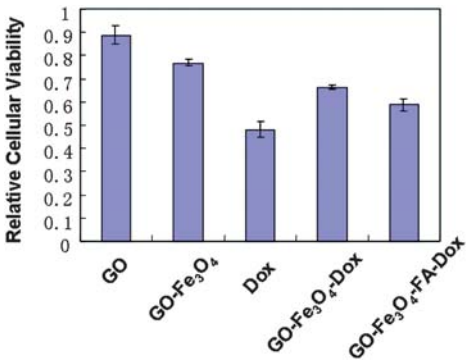


Fig. 5 Relative cellular viability of Hela after treatment with GO, GO-Fe_3O_4, Dox, GO-Fe_3O_4-Dox and GO-Fe_3O_4-FA-Dox.

4. Conclusions

Multi-functionalized GO, which can realize dual-targeted delivery based on the force of a magnetic field and the specific interaction between the FA on the drug carriers and the over-expressed folate receptor on the surface of some tumor cells, were prepared by conjugating GO-Fe_3O_4 nanohybrid with FA with the aid of APS. The multi-functionalized GO was confirmed by the results from TEM, FTIR spectra, UV-vis spectra and magnetization curves. The size of most multi-functionalized GO was below 200 nm and they show superparamagnetic property with the saturation magnetization of 8.57 emu g^{-1}. The Dox loading capacity is as high as 0.387 mg mg^{-1} in the case of the initial concentration of Dox at 0.238 mg mL^{-1}. Also, the release of drug from this multi-functionalized GO can be controlled by pH conditions in the environment. Cell uptake studies indicate that the multi-functionalized GO can specifically transport the drugs to SK3 cells and show toxicity to Hela cells after loading Dox. All these results make it possible to use GO as an ideal multi-functionalized drug-carrier for tumor combination therapy.

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Supplementary Material (ESI) for Journal of Materials Chemistry

Multi-functionalized graphene oxide based anticancer drug-carrier with dual-targeting function and pH-sensitivity

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Supporting Information

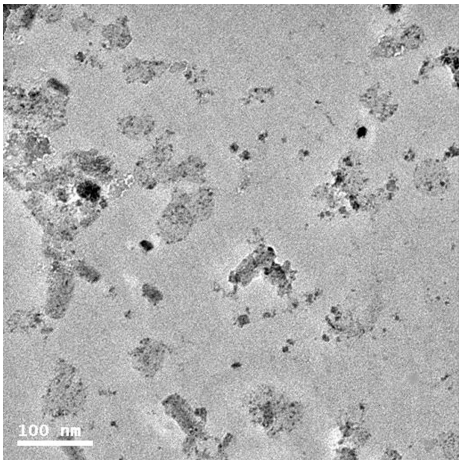

Figure S1. TEM of GO–Fe_3O_4 nanohybrid

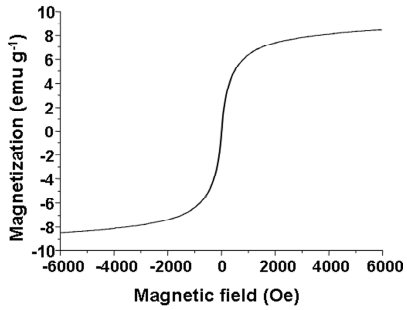

Figure S2. Magnetization curve of GO–Fe_3O_4 hybrid

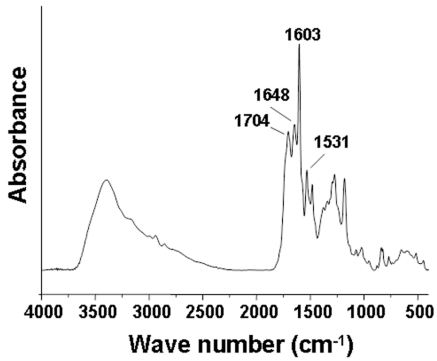

Figure S3. FTIR spectrum of folic acid active ester