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Targeted delivery and controlled release of doxorubicin into cancer cells using a multifunctional graphene oxide

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ABSTRACT

We have synthesized a new multifunctional graphene oxide as a drug carrier targeting to hepatocarcinoma cells. Surface modified graphene oxide with polyethyleneimine (PEI) sequentially derivatised with fluorescein isothiocyanate (FI) and polyethylene glycol (PEG)-linked lactobionic acid (LA), and acetylation of remaining terminal amines of the PEI produced a new multifunctional graphene oxide drug carrier (GO/PEI.Ac-FI-PEG-LA). Doxorubicin (DOX), an anticancer drug, was encapsulated in GO/PEI.Ac-FI-PEG-LA to give GO/PEI.Ac-FI-PEG-LA/DOX, with a drug loading percentage of 85%. We showed that both GO/PEI.Ac-FI-PEG-LA and GO/PEI.Ac-FI-PEG-LA/DOX were water soluble and stable between pH 5.0 and 9.0. In vitro release studies indicated that the release rate of DOX from GO/PEI.Ac-FI-PEG-LA/DOX complexes were significantly higher at pH 5.8 than that of the physiological pH. Another important feature of this carrier is its good cell viability in the tested concentration range (0-4 μM), and the GO/PEI.Ac-FI-PEG-LA/DOX can specifically target cancer cells overexpressing asialoglycoprotein (ASGPR) receptors and exert growth inhibition effect to the cancer cells. The enhanced target specificity and the substantial improvement in pH responsive controlled release have made this new carrier a potential choice for non-covalent encapsulation of drugs in GO, and a delivery system for cancer therapy.

1. Introduction

Chemotherapy is commonly used for cancer treatments. Anticancer drugs, such as doxorubicin (DOX), are typically non-specific to cancerous tissues, and also lead to adverse side-effect on normal tissues [1,2]. Moreover poor solubility of some anticancer drugs can significantly limit their bioavailability. In order to overcome these problems a drug delivery carrier with targeting specificity [3–5], good biocompatibility [6–8], sufficient stability [9–11], and prolonged circulation of half-lives [12,13] is needed to be developed. Among the currently available drug delivery carriers such as liposomes [14,15], nanoparticles [16,17], polymer micelles [18], and dendrimers [19], graphene oxide (GO) [20,21] is emerging as one of the most promising drug carrier for cancer diagnosis and chemotherapy due to their significant advantage features, including ultrahigh surface area, high drug-loading capability, effective transportation capability, low toxicity, good biocompatibility and enhanced cellular uptake [22–27].

It has been reported that asialoglycoprotein receptors (ASGPRs) are present on the surface of hepatocytes and several human carcinoma cell lines with a high density and show strong binding efficiency with galactose [28,29]. Nanoparticles modified with lactobionic acid (LA) [19,30], N-acetylgalactosamine [31,32], and galactose [33,34] have been shown to bind specifically to hepatocarcinoma cells (e.g., HepG2 and SMMC-7721 cells). In a recent study, Fu et al. developed a LA-modified multifunctional dendrimer-based carrier system and encapsulated DOX in the dendrimer for liver cancer cell targeting and DOX uptake in these cells [35]. In another study from the same research group, G5 dendrimers modified with polyethylene glycol-linked LA (PEG-LA) was used to entrap gold nanoparticles for targeted computed tomography imaging of hepatocellular carcinoma in vitro and in vivo [36].

In this work, we aim to synthesize and characterize a multifunctional GO drug carrier with targeting specificity to cancer cells and enhanced drug release capability. The design of the new GO based-carrier in the current study is to incorporate LA-PEG groups on the polyethyleneimine modified GO surface GO/PEI for target specific cancer cells (SMMC-7721) to eliminate side effects of drug toxicity. In addition the built in fluorescein label in the final product enables the confirmation of uptake of the newly designed carrier, GO/PEI.Ac-FI-PEG-LA/DOX, by the cancer cells.

2. Materials and methods

2.1. Materials

Graphene oxide was synthesized from natural graphite powder (1000 mesh) using a modified Hummers' method [37]. LA was

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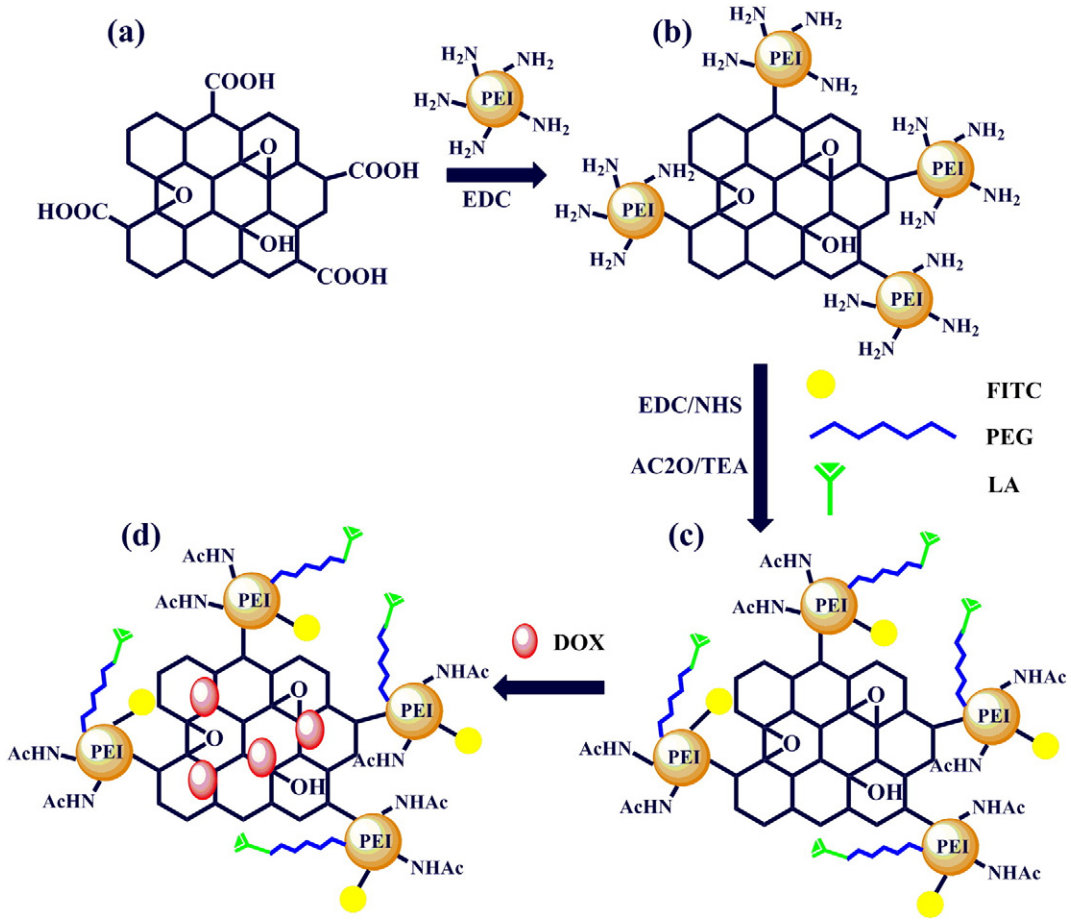

Fig. 1. Schematic illustration of the synthesis of GO/PEI.Ac-FI-PEG-LA.

purchased from Acros Organics (Beijing, China). NH_2-mPEG-COOH (Mw ≈ 2000) was obtained from Shanghai Yarebio Biotechnology Corporation (Shanghai, China). Hyperbranched PEI (Mw ≈ 25,000) and all other chemicals and solvents were purchased from Aldrich (St. Louis, Missouri) and used as received. LA-functionalized PEG (COOH-mPEG-LA) was synthesized and characterized according to a literature method [36]. SMMC-7721 cells (a human liver cancer cell line) and PIEC cells (a pig endothelial cell line) were obtained from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, China). RPMI 1640 medium, DMEM medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Shanghai Yuanxiang Biomedical Technology (Shanghai, China). Regenerated cellulose dialysis membranes with molecular weight cut-off (MWCO at 50,000, 14,000 and 1000) were acquired from Fisher (Pittsburgh, PA). Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ cm.

2.2. Synthesis of GO/PEI.Ac-FI-PEG-LA

The modification of GO with PEI to form GO/PEI was adapted from a reported method [38]. Briefly, GO with carboxyl residues (100.0 mg) dispersed in DMSO (50 mL) were activated with EDC (120.0 mg) co-dissolved into DMSO (5 mL) under vigorous magnetic stirring. The reaction was continued for 3 h to activate the carboxyl residues of GO, followed by the addition of PEI solution (150 mg in 10 mL DMSO). The reaction mixture was sonicated for 24 h to obtain GO/PEI conjugates. Finally, the DMSO and the excess of reactants and byproduct were removed from the reaction mixture by extensive dialysis against phosphate buffered saline (pH = 7.4) solution (3 times, 2 L) and water (6 times, 2 L) using a dialysis membrane with MWCO of 50,000 for 3 d. The GO/PEI conjugates were obtained following lyophilization (185 mg), Fig. 1(a,b,c).

Multifunctional GO (GO/PEI.Ac-FI-PEG-LA) were synthesized by sequential conjugating FI and COOH-PEG-LA onto the surface of GO/PEI. Firstly, GO/PEI (40 mg) was dispersed into water (10 mL). Then, FI (1.4 mg) dissolved into DMSO (0.5 mL) was added into the water solution of GO under vigorous magnetic stirring. The reaction was continued for 3 h to get the crude product of GO/PEI-FI. Then, COOH-PEG-LA (33 mg) in water (10 mL) was activated by EDC (28 mg) and NHS (17.2 mg) co-dissolved into water (5 mL) for 3 h and was added dropwise into the water solution of GO/PEI-FI under vigorous magnetic stirring. The reaction was continued for 24 h to obtain the crude product of GO/PEI-FI-PEG-LA. The GO/PEI-FI-PEG-LA composites were subjected to an acetylation reaction to the remaining amine groups of PEI using a literature procedure. In brief, the composites were thoroughly mixed with triethylamine (360 μL). Then, 240 μL acetic anhydride was added dropwise into the composites/triethylamine solution. The reaction mixture was vigorously stirred for 24 h. The GO/PEI.Ac-FI-PEG-LA product (53 mg) was purified and lyophilized according to the procedure used for preparation of GO/PEI conjugates.

2.3. Characterization techniques

^{1}H NMR spectra of functionalized GO were recorded using a Bruker DRX 400 nuclear magnetic resonance spectrometer (Bruker, Switzerland). Samples were dissolved in D_2O before measurements. UV–Vis spectra were collected on a Shanghai Jinhua UV-1800 UV–Vis spectrophotometer (Shanghai, China). Zeta potential measurements were carried out using a Zetasizer Nano ZS system (Malvern, UK) equipped with a standard 633 nm laser. The morphology of the GO/