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Controlled release of doxorubicin from graphene oxide based charge-reversal nanocarrier

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ABSTRACT

A number of anticancer drugs, such as doxorubicin (DOX), operate only after being transported into the nucleus of cancer cells. Thus it is essential for the drug carriers to effectively release the anticancer drugs into the cytoplasm of cancer cells and make them move to nucleus freely. Herein, a pH-responsive charge-reversal polyelectrolyte and integrin $\alpha_V\beta_3$ mono-antibody functionalized graphene oxide (GO) complex is constituted as a nanocarrier for targeted delivery and controlled release of DOX into cancer cells. The DOX loading and releasing *in vitro* demonstrates that this nanocarrier cannot only load DOX with high efficiency, but also effectively release it under mild acidic pH stimulation. Cellular toxicity assay, confocal laser scanning microscopy and flow cytometer analysis results together confirm that with the targeting nanocarrier, DOX can be selectively transported into the targeted cancer cells. Then they will be effectively released from the nanocarriers in cytoplasm and moved into the nucleus subsequently, stimulating by charge-reverse of the polyelectrolyte in acidic intracellular compartments. The effective delivery and release of the anticancer drugs into nucleus of the targeted cancer cells will lead to a high therapeutic efficiency. Hence, such a targeting nanocarrier prepared from GO and charge-reversal polyelectrolytes is likely to be an available candidate for targeted drug delivery in tumor therapy.

1. Introduction

The ideal effect for controlled drug delivery is for the drug to get to a desired therapeutic concentration at targeting sites while at other tissues is kept at safe levels [1–3]. And one of the major challenges to achieve this effect is to find a suitable delivery carrier. An ideal controlled carrier for anti-cancer drugs should have long circulation duration, the ability to target cancer cells, and eventually the ability to efficiently deliver and release drugs into cytoplasm. Currently, various polymeric particles [4–6], liposomes [7–9], microspheres [10,11] and nanoparticles [12–14] have been used as potential antitumor drug carriers. Among these carriers, nanomaterials demonstrate advantages in anti-cancer drug delivery including a tunable circulation life time, enhanced permeability and retention (EPR) effect that up-regulates intratumoral delivery due to the high permeability of tumor vasculatures, and multivalent effect that increases receptor targeting specificity by labeling multiple ligands on a single nanoparticle [15,16].

Recently, graphene oxide (GO) as a promising nanomaterial for drug delivery has attracted significant attentions for its good biocompatibility, endocytosis, and large specific surface area for loading drugs and other functional biomolecules [17–20]. Compared with other drug carriers, GO has much higher loading capacity, which makes it can effectively enhance the drug delivery into cells [17,19,21]. Furthermore, with a lot of active groups such as hydroxyl and carboxyl on the surface of GO, targeting molecules such as folic acid, antibodies can be conveniently immobilized on, and then enable GO to be specifically internalized into the targeted cells [22–24]. Accordingly, with the targeting molecule functional GO as the drug carrier, drugs can be efficiently and selectively transported into the targeted cells.

Doxorubicin (DOX), as a commonly used anticancer drug in chemotherapy, kills cells by intercalating with DNA, thereby preventing the DNA replication and then the cell division process [25,26]. Consequently, it operates only when being sufficiently transported into the nucleus of cancer cells. However, most of the drug carriers so far such as liposomes and nanoparticles, including GO, can neither migrate into the cellar nucleus due to their large size nor effectively release their cargoes in the cytoplasm [27,28]. Then only a small percentage of drugs delivered into the cytoplasm can reach the nucleus eventually. Thus, it is urgent to construct a drug carrier capable of effectively releasing anticancer drugs in the cytoplasm of cancer cells to make the drugs move into the nucleus, leading to a high therapeutic efficiency.

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Smart structures that are responsive to external stimuli, such as heat, light, pH or magnetic fields are ideal candidates for controlled release of anticancer drug [29–36]. By formulating these stimuli-responsive materials with drug carriers, DOX can be released from the delivery systems in cytoplasm under some specific stimuli, then move into the nucleus to kill cells. Different to the physiological pH (7.4) of normal cells, the extracellular environment of solid tumors is acidic (pH ~ 6.8), and the pH values in endosomes are even a lot lower (5.0–6.5) [32,37,38]. According to this, pH-responsive nanocarriers can be developed to deliver and efficiently release anticancer drugs in cancer cells, because it is reasonable to insert acid-cleavable linkers in a nanocarrier to induce destabilization of the carrier in endosome, resulting in the release of drugs from the nanocarrier into cytoplasm. The charge-reversal polyelectrolyte is a kind of polymer that could pH-dependently shift its charge nature between positive and negative. This property make charge-reversal polyelectrolyte an ideal material to constitute the pH-responsive nanocarriers for releasing the anticancer drugs, siRNA, genes and proteins in cytoplasm [39–45]. As an anionic carboxylate-functional polyelectrolyte, citraconic anhydride-functionalized poly(allylamine) (PAH-Cit) is a common charge-reversal polyelectrolyte, which can be readily converted back to cationic poly(allylamine) by amide hydrolysis upon exposure to mild acidic environments (Scheme 1A), such as those found within late endosomes and lysosomes [39,40,46].

Herein, we developed a GO based charge-reversal nanocarrier (GO-Abs/PEI/PAH-Cit/DOX) for enhanced delivery and controlled release of DOX into specific cancer cells. PAH-Cit was conjugated with a cationic polyelectrolyte (polyethyleneimine, PEI) coated GO by electrostatics. And DOX was loaded on GO by covalently linkage with PAH-Cit. As shown in Scheme 1A and C, after the drug delivery system is endocytosed into cells, the PAH-Cit/DOX will convert to cationic poly(allylamine) upon exposure to acidic environments of endosome or lysosome. Hence it is released from the positive charge surface of GO/PEI. It will be expected that this nanocarrier can specifically transport anti-cancer drugs into the targeted cancer cells with high efficiency, and then release them to move into nucleus to achieve the medical effect.

2. Experimental section

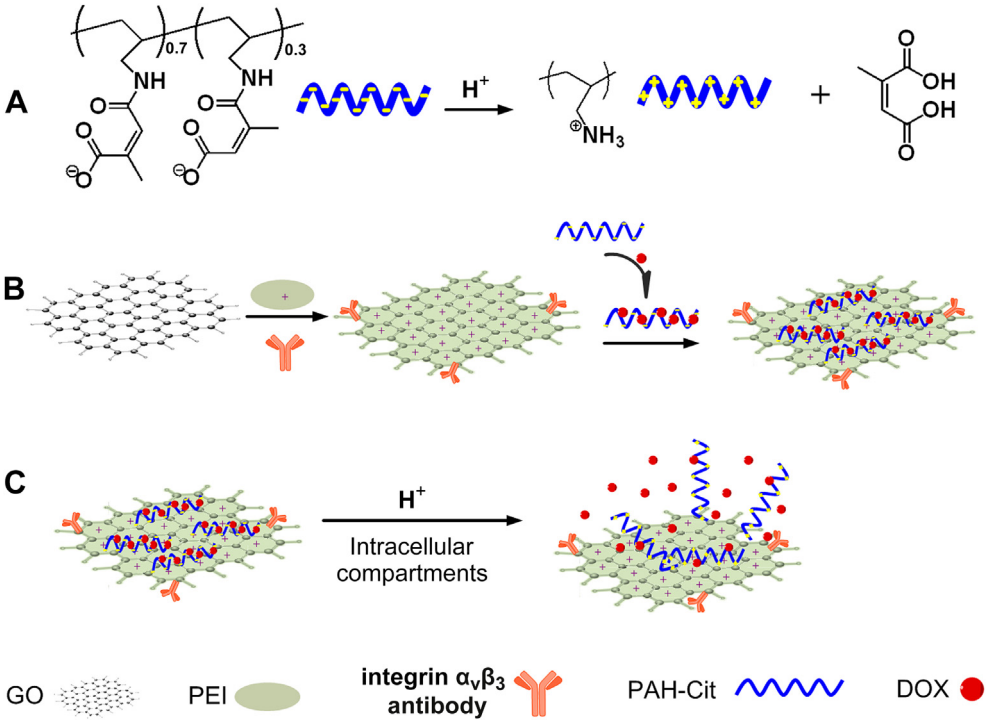
2.1. Materials and characterization

Citraconic anhydride and polyethyleneimine (PEI, M_w ~ 60 KDa) were purchased from TCI Tokyo Chemical Industry Co.,Ltd; Doxorubicin hydrochloride (DOX), poly (allyamine hydrochloride) (PAH, M_w ~ 15 kDa), N-hydroxy succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich Co. Integrin $\alpha_V\beta_3$ (23C6) mono-clonal antibody (integrin $\alpha_V\beta_3$ mAb) was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA); CCK-8 was purchased from Dojindo Laboratories (Kumamoto, Japan). The graphene oxide (GO) was the product of XF NANO, INC (Nanjing, China). Since all the chemicals were analytical grade and were used without further purification. The high-purity deionized water (resistance >18 MΩ cm) is used throughout.

The optical absorbance characteristics of various samples were investigated by visible absorption spectra (Lambda-35 UV–Vis spectrophotometer, Perkin–Elmer, USA) and fluorescence spectra (LS-55 fluorescence spectrophotometer, Perkin–Elmer, USA) with an excitation of 490 nm. Tapping mode atomic force microscopy (Agilent 5500AFM) purchased from Agilent Technologies, Inc. (Englewood, USA) was used for detecting the size of GO and GO/PEI/PAH-Cit/DOX. The zeta potential of various samples were measured by affordable molecular/particle size and zeta potential analyzer (Malvern instruments, Zetasizer Nano-ZS) at 25 °C, and all the samples were dispersed in phosphate buffer solution (PBS) of pH 7.4.

2.2. Synthesis of charge-reversal polyelectrolyte and the conjugation of DOX

We synthesized the charge-reversal polyelectrolyte according to the Lynn [46]. PAH (40 mg) was dissolved in NaOH (1 M) and stirred for several hours. Citraconic anhydride was then added dropwise into the PAH solution, with NaOH (6 M) added during the reaction to keep the pH above 8.0. After overnight reaction, the resultant mixture was filtrated through 3 kDa filters (Millipore) to remove excess reagents. Then 100 μL of 5 M DOX was put into the 1 mL as-prepared polyelectrolyte, followed by 100 μL of EDC and 100 μL of NHS added to the resultant solution in succession to catalyze the attachment of DOX to the polyelectrolyte. The mixture was incubated for 24 h, protected from light illumination. Unbound excess DOX was also removed by filtration through 3 kDa filters (Millipore) and repeated rinsing.



Scheme 1. (A) Hydrolysis of the citraconic amide side chains of anionic charge-reversal polyelectrolyte (PAH-Cit) under mild acidic conditions yields cationic PAH (poly(allylamine)). (B) Construction of the targeted charge-reversal nanocarrier (GO-Abs/PEI/PAH-Cit/DOX). (C) Controlled release of DOX at endosome or lysosome, stimulating by the pH-dependent charge-reverse of the charge-reversal polyelectrolytes on GO.