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Fabrication of aptamer decorated dextran coated nano-graphene oxide for targeted drug delivery

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ABSTRACT

In the current study, dextran (DEX) was covalently conjugated to the surface of nano-GO sheets, making stable biocompatible dextran coated GO (GO-DEX). The prepared GO-DEX was nontoxic to 4T1 mammary carcinoma cell line at concentrations up to 300 μg/mL.

AS1411 aptamer, a ssDNA aptamer which can improve the intracellular uptake by nucleolin recognition, also has been introduced to hydroxyl groups of DEX in GO-DEX to produce GO-DEX-Apt. Moreover, curcumin (CUR), a natural polyphenol, found in the rhizomes of *Curcuma longa* (turmeric) which shows antineoplastic effects, was loaded onto the GO-DEX and GO-DEX-Apt via π-π stacking interactions with a high loading capacity (~29 wt%). The GO-DEX-Apt-CUR could efficiently enter into 4T1 and MCF-7 nucleolin over-expressed cancer cells confirmed by fluorescence microscope and flowcytometry, and it also showed significantly higher cytotoxicity. These types of targeted nanoscale drug delivery vehicles on the basis of DEX coated GO may find potential application in cancer chemotherapy.

1. Introduction

Nanoparticle-based drug delivery systems for enhancing the therapeutic efficiency of chemotherapeutics are the hot spot of research in the field of nanobiotechnology(Zhang, Xia, Zhao, Liu, & Zhang, 2010). To date different drug delivery vehicles have been introduced including dendrimers (Hu, Qiu et al., 2016), liposomes (Espelin, Leonard, Geretti, Wickham, & Hendriks, 2016), polymerosomes (Liu, Yaszemski, & Lu, 2016) and inorganic nanomaterials such as gold nanoparticles (Tsai, Hsieh, Lu, Wang, & Mi, 2016) mesoporous silica (Hakeem et al., 2016) or carbonaceous materials (Panczyk, Wolski, & Lajtar, 2016). Although there are many advantages associated with these nanoparticles such as increasing the solubility of hydrophobic drugs or higher bioavailability (Lu, Wu, Yin, Zhang, & Cai, 2014), there are still a number of drawbacks such as burst release, limited stability of formulations leading to drug leakage and nonspecific cellular uptake resulting in undesired adverse effects(Hu & Zhang, 2009). Recently, graphene-based materials have received tremendous attention as drug delivery vehicles (Zhang, Yang, Feng, & Liu, 2011). Graphene is a novel carbon material with one atom thick two dimensional layers. However, due to strong graphene-graphene interactions, graphene is poorly soluble in aqueous solutions (Maity, Chakraborty, Mondal, & Jana, 2014).

Graphene oxide (GO) is the oxidized derivative of graphene which has been explored extensively in biological applications such as controlled drug delivery, nanocomposites and gene delivery (Zhang, Yang et al., 2011). Recent studies demonstrated that GO and derivatives are not only used as drug delivery platforms but also act as hyperthermia agent in photothermal therapy to elevate the cytotoxicity of antineoplastic agents in lower doses or to regulate the drug release profile (Li et al., 2015; Mauro, Scialabba, Cavallaro, Licciardi, & Giammona, 2015). Besides, due to its unique electronic, mechanical and thermal properties and good near-infrared (NIR) absorbance, GO has attracted considerable attention in developing biosensors and imaging contrast agents (Yang et al., 2016; Zhang, Yuan, Zhang, Wang, & Liu, 2011). High surface area and the presence of functional moieties such as hydroxyl, carboxyl, epoxy

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groups which allow the attachment of targeting agents and drug molecules, made GO an attractive material in biomedical studies. It was claimed that aromatic ring-containing anticancer drugs such as curcumin (CUR) and camptothecin (CPT) could be loaded on graphene-based materials with high efficiency (Liu, Robinson, Sun, & Dai, 2008). Although GO is dispersed more easily in water than graphene, it would aggregate in salt-rich solutions and cell medium or serum. Furthermore, studies claimed that GO and graphene exhibited certain cellular toxicity to cell cultures *in vitro* and animal cells *in vivo* (Zhang, Yang et al., 2011). However, GO which is coated by biocompatible polymers such as polyethylene glycol (PEG) or DEX does not seem to be toxic in the tested doses (Zhang, Yang et al., 2011), it is highly aqueous dispersible and stable in biological fluids compared to GO (Liu et al., 2008).

Dextran is a natural, hydrophilic homopolysacharide of glucose with unique favourable properties such as biodegradability, hydrophilicity and superior colloidal stability (Zhang et al., 2013). Studies demonstrated that DEX coated nanoparticles are more colloidal stable than those coated with PEG chains (Goodwin et al., 2008). Meanwhile, DEX has more reactive sites compared to PEG. The hydroxyl groups of DEX can be modified to react with various functional groups. Also, DEX-coated nanoparticles are less prone to non-specific protein adsorption(Xie et al., 2016; Zhang, Yang et al., 2011).

There are many studies exploring the efficiency of electrostatic loading of aromatic, water insoluble anticancer drugs on nanographene oxide (Zhang, Yang et al., 2011)

In this regard, CUR is a naturally occurring polyphenol which is the main component of *Curcuma longa* and it is found to have wide range of medicinal properties(Aggarwal & Sung, 2009). Many studies proved its antimicrobial(Barua et al., 2014), anti-inflammatory (Hatamie et al., 2015) and anticancer effects (Some et al., 2014). The anticancer properties of CUR are due to the down-regulation of different transcription factors such as NF-kB, AP-1 and beta-catenin (Pillai et al., 2015). However, the therapeutic use of CUR is limited due to the low solubility, high clearance rate and low bioavailability leading to suboptimal blood concentrations (Naksuriya, Okonogi, Schiffelers, & Hennink, 2014). To enhance the poor biopharmaceutical properties of CUR and improve the aqueous solubility, we loaded CUR via noncovalent physisorption on to the surface of graphene oxide-dextran nano-hybrid (GO-DEX).

Recently, it was demonstrated that targeted delivery of nanomedicine could be a promising approach to specifically deliver the drug to the target cell population and reduce the undesired effects associated with anticancer agents (Gao et al., 2012). Nucleolin is 76 kDa transmembrane glycoprotein highly expressed in the plasma membrane of tumor cells comprising DU145 (prostate cancer cell line), MDA-MB-231, MCF-7 (breast cancer cell lines) and 4T1 (murine metastatic breast cancer cell) (Li, Tong et al., 2014), A549 (lung cancer cell line), and HeLa (cervical cancer cell line)(Alibolandi, Ramezani, Abnous, & Hadizadeh, 2016). Accumulating evidences validated that cell surface nucleolin acted together with protein complexes associated with tumorgenesis and angiogenesis suggesting that nanoparticle-conjugated aptamer could enhance the cellular uptake and the efficiency of the drug delivery while reducing the side effects associated with the cytotoxic drugs (Koutsioumpa & Papadimitriou, 2014; Malik et al., 2015).

In order to increase the cellular uptake and improve the drug delivery efficiency, AS1411 aptamer was covalently attached to our newly designed nanoparticle composed of DEX coated nano-GO. Until now, the targeted delivery of CUR using GO-DEX-AS1411 aptamer nanocomplex to tumor cells is not reported in the literature. Thus, the main goal of the current study is to enhance the delivery of CUR loaded GO-DEX-Apt to the nucleolin overexpressed 4T1 and MCF-7 cells.

2. Materials and methods

2.1. Materials

Graphite powder was obtained from Loba Chemie (Delhi, India). All reagents and solvents were of analytical grade and obtained from commercially available suppliers (Merck AG and Sigma Aldrich) and used without further purification. CUR was purchased from Euroasian Chemicals Pvt. Ltd. (Delhi, India). Pharmaceutical grade DEX (MW 5000 Da) was procured from Pharmacosmos (Holbaek, Denmark).

N-hydroxysulfosuccinimide (NHS), 1-ethyl3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 1,2-ethylenediamine (EDA), sodium cyanoborohydride ($NaCNBH_3$) and carbonyl diimidazole (CDI) were purchased from Sigma-Aldrich (Schnelldorf, Germany).

An ssDNA-aptamer for nucleolin reported previously was employed as the targeting ligand. The oligonucleotides were synthesized by Microsynth Co. Ltd. (Basel, Switzerland). The sequence of the modified aptamer was 5′-NH_2-C_6-GGTGGTGGTGGTTGTGGTGGTGGTGG-3′.

2.2. Graphene oxide (GO) synthesis

Graphene oxide was synthesized from graphite flake according to improved Hummers' method with some modifications (Marcano et al., 2010).

Briefly, a 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (120:13.3 mL) was added to a mixture of graphite flakes (1 g) and $KMnO_4$ (6 g).

The reaction was then heated to 50 °C and stirred for 18 h. The reaction was cooled to room temperature and poured onto ice (135 mL) with 30% H_2O_2 (10 mL). The mixture was then sifted through a metal sieve (400 μm).

The filtrate was centrifuged (8000 rpm for 30 min), and the supernatant was poured out. The remaining solid material was then washed with HCl 0.1 N and in succession with double distilled water. The synthesized GO was freeze-dried overnight to obtain ready to use final product.

2.3. Synthesis of 1,2-ethylenediamine-terminated dextran (EDA-DEX)

We aimed to prepare amine-terminated dextran in order to conjugate the terminal amine groups to the carboxylic (COOH) groups on the surface of GO through classical amide coupling. Then the 1,2-ethylenediamine (EDA) was introduced to the end of the dextran.

The amine terminated dextran (EDA-DEX) was prepared according to our previous study implementing DEX-terminal reductive amination reaction with sodium cyanoborohydride ($NaCNBH_3$) as reducing agent (Alibolandi, Alabdollah et al., 2016). Briefly, 1 g DEX (5000 Da) and 1,2-ethylenediamine (10-fold molar excess over DEX) was dissolved in 10 mL DMSO and stirred (600 rpm) at 60 °C for 7 days. Then $NaCNBH_3$ (20 mg) was added to the reaction each day.

Finally the reaction was cooled to room temperature, and the aminated-DEX was precipitated by addition of 20 mL methanol.

The EDA-DEX was accurately purified by vacuum filtration and successive washing with methanol. The purified EDA-DEX was lyophilized and stored at −20 °C until use.

^{1}H NMR spectra were recorded in DMSO-*d*6 at room temperature using a *Bruker* Avance 300 MHz *NMR spectrometer* (Rheinstetten, Germany) to verify DEX amination (Fig. S1).