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Carbon nanotube lipid drug approach for targeted delivery of a chemotherapy drug in a human breast cancer xenograft animal model

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ABSTRACT

Carbon nanotube (CNT) possesses excellent properties as a drug carrier. To overcome the challenge of drug functionalization with CNT, we have developed a lipid-drug approach for efficient drug loading onto CNT, in which a long chain lipid molecule is conjugated to the drug molecule so that the lipid-drug can be loaded directly onto CNT through binding of the lipid 'tail' in the drug molecule to CNT surfaces *via* hydrophobic interactions. In a proof-of-concept study, drug paclitaxel (PTX) was conjugated with a non-toxic lipid molecule docosanol for functionalization with CNT. Folic acid was also conjugated to CNT for targeted drug delivery. High level of drug loading onto SWNT could be achieved by lipid-drug approach. Conjugation of FA to SWNT-lipid-PTX led to an increase in cell penetration capacity, and the targeted SWNT-lipid-PTX showed much improved drug efficacy *in vitro* in comparison to free drug Taxol and non-targeted SWNT-lipid-PTX at 48 h (78.5% *vs.* 31.6% and 59.1% in cytotoxicity respectively, $p < 0.01$). *In vivo* analysis using a human breast cancer xenograft mice model also confirmed the improved drug efficacy. The targeted SWNT-lipid-PTX was found non-toxic as evaluated by biochemical analysis using blood samples, and by histological analysis of major organs.

1. Introduction

Conventional chemotherapeutic drugs distribute throughout the body and often cause severe side effects. The advances in nanotechnology and nanomedicine enable the revolutionary solutions in the field of drug delivery. One of the major reasons for using nanoparticle in chemotherapy drug delivery is that the nanoparticles preferentially accumulate in tumors through the "enhanced permeability and retention" (EPR) effect. Indeed, nanoparticle drugs have demonstrated higher intratumoral drug concentration and lower normal-tissue concentrations than their parental small-molecule counterparts [1]. In addition, nanoparticles, containing high surface area-to-volume ratios, can be potentially engineered to be multifunctional nanoparticles that carry tumor-targeting molecules, tissue permeation enhancers, two or more types of therapeutics for more efficient cancer therapy [2]. Carbon nanotube (CNT), a new type of synthetic nanomaterial, offers opportunities for chemotherapy drug delivery. Structurally, CNT can be viewed as a tube rolled from layers of graphene sheets. Depending on the number of graphene layers, CNT is classified as single-walled carbon nanotube (SWNT) or multi-walled carbon nanotube (MWNT). Due to its well-organized structure, CNT presents remarkable physical properties, including ultra-high surface area, high aspect ratio, high tensile strength [3–5], excellent optical [6], electrical and thermal properties [7–9]. Besides, CNT are found easily penetrate all sorts of cells, including hard-to-transfect types of cells [10]. CNT is widely explored for potential biological applications because of its size, unique shape, and structure, as well as its attractive physical properties.

As a new type of nanomaterial, the potential toxicity of CNT has been intensively investigated *in vitro* and *in vivo*. It has been shown that appropriately functionalized CNT, *e.g.* polyethylene glycol (PEG) functionalized CNT does not cause noticeable toxicity to the treated animals [11]. Biodistribution of the lipid-polymer, phospholipid-PEG (PL-PEG), functionalized SWNT showed that CNT is safe because it can be excreted *via* the biliary and renal pathways after intravenous injection [12,13]. More importantly, a high tumor accumulation of PL-PEG functionalized SWNT could be achieved by conjugation of targeting ligands to SWNT [14]. These results have paved the way for applications of CNT in cancer therapy. In recent years, SWNT have been applied in a variety of biomedical applications ranging from cancer drug delivery, tumor imaging, detection and others [9]. We have tried to encapsulate drug-loaded CNT into artificial cells for

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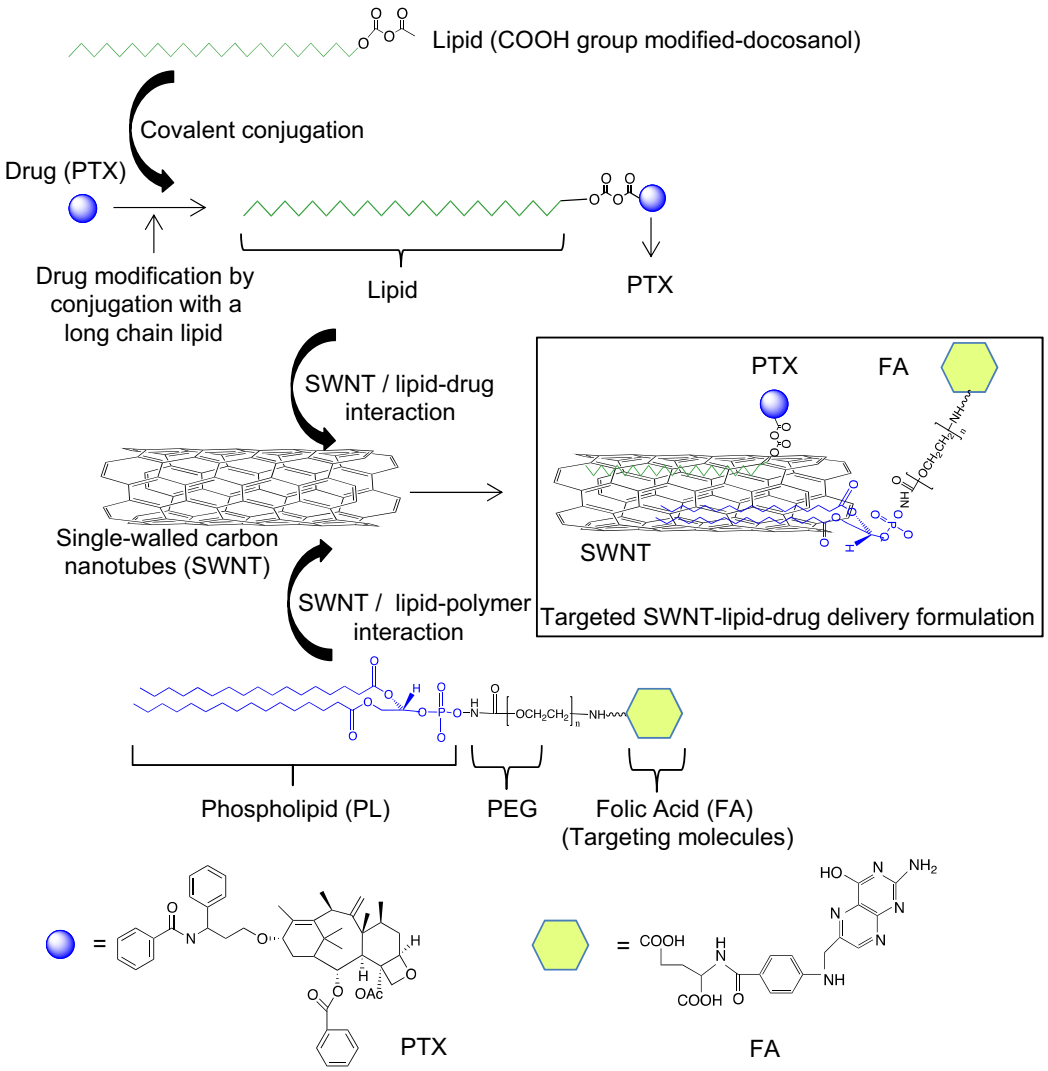
targeted delivery [15]. The polymeric membrane of artificial cells could prevent drug degradation from the harsh gastrointestinal environment in the route of oral delivery, and the surface of artificial cells could be engineered for the targeted delivery. We have also incorporated pro-angiogenic genes functionalized CNT into stents for efficient gene therapy [16]. Another group has successfully delivered functional siRNA into tumors tissues for cancer therapy [17]. Currently, the CNT-based siRNA formulation for cancer treatment is finalizing for moving into human studies [18].

Since CNT is pre-formed supramolecular nanotubes, the drug loading to this pre-formed structure is very challenging. SWNT can be filled with a large variety of compounds, including organic molecules [19,20] and inorganic materials [21,22]. Chemotherapeutic drugs are loaded into the interior of SWNT through a simple capillarity-induced filling. However, the loading amount is below 5% (w/w) [23]. Researchers have also investigated the loading of small molecule drugs directly onto the CNT surface. They found that pre-functionalized CNT exists large surface areas allowing for direct attachment to some hydrophobic drugs that contain flat benzene ring structures. One study has investigated the adsorption of doxorubicin on SWNT [24]. High loading amount could be reached (400% by weight). When the drugs are loaded directly to CNT, the CNT-coating polymers are freed for conjugation of other functionalities, *e.g.* targeting molecules, antibodies, fluorescence molecule or other drugs for multifunctional delivery [24]. However, for drugs with bulky structures, *e.g.* paclitaxel, the absorption of the drugs on nanotube is not good because the resulting formulations are not stable. These bulky drugs are usually conjugated to CNT-dispersing polymers for delivery [25]. When linking drugs to CNT-dispersing polymer for delivery, the polymer ends are occupied by drugs, and therefore the multifunctional drug delivery is not possible due to lack of spaces for other functionalities. It is imperative to develop a general approach for construction of multifunctional CNT-based drug delivery system that is suitable for various types of drugs, which is the main aim of this study.

2. Materials and methods

2.1. Reagents

All the reagents used in experiments were purchase from Sigma Aldrich, Canada except otherwise indicated specifically.



Scheme 1. Design strategy of a novel targeted SWNT-lipid-drug delivery system. The drug molecule, *e.g.* paclitaxel (PTX), was conjugated to a long chain lipid *via* a reversible ester bond. The SWNT-lipid-drug was made by exploiting the lipid 'tail' in the drug to form lipid-drug/SWNT complex through strong hydrophobic interactions. The formulation was made multifunctional delivery using a tumor targeting molecule folic acid (FA) that was conjugated to the end of the PL-PEG exploiting an amide bond formed between amine group of PL-PEG and carboxyl group of FA. This formulation could overcome the existing challenges of CNT in drug deliveries and broadened their use for multifunctional deliveries. The Use of lipid molecule is unique and advantageous as it allows delivery of a range of drugs in multifunctional delivery applications.

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