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PEGylated multi-walled carbon nanotubes as versatile vector for tumor-specific intracellular triggered release with enhanced anti-cancer efficiency: Optimization of length and PEGylation degree

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ABSTRACT

PEGylated multi-walled carbon nanotubes (PEG-MWCNTs) were optimized as versatile vector for tumor-specific intracellular triggered release of doxorubicin (DOX), based on the effect of their length and PEGylation degree on the cytotoxicity and DOX-loading capacity. The length and surface carboxyl groups of the carboxylated multi-walled carbon nanotubes (CMWCNTs) were easily tailored by adjusting the oxidation time. The longer CMWCNTs or those with high carboxyl group content showed obvious cytotoxicity, while the PEG-MWCNTs $\leq$ 300 nm showed better cytocompatibility. The PEG-MWCNTs-3 of about 300 nm was selected as drug delivery vector, possessing a high drug-loading capacity of 0.55 mg/mg. They released DOX rapidly under lower pH media mimicking the tumor microenvironment with cumulative release of 57% within 24 h, while the premature leakage under the simulated physiological condition was only 10%. The WST-1 assays demonstrated the DOX-loaded PEG-MWCNTs-3 exhibited the enhanced inhibitory efficiency against HepG2 cells, in comparison with free DOX.

1. Introduction

In the last decades, carbon nanotubes (CNTs) have attracted considerable attention in the variety of different fields and created a revolution in chemistry and condensed matter physics, owing to their amazing unique physical and chemical properties since first appearance in 1991 by Japanese physicist Sumio Iijima [1]. Due to its unique structure of the sp^2-hybrirdized carbon atom, CNTs possess excellent properties, which have opened remarkable pathways for their potential applications, energy storage, hydrogen storage, field-emitting devices, transistors, nanoprobes and sensors, composite material, templates, and so on [2].

The cytotoxicity of CNTs can be related to several factors: metal impurities, length and size distribution, surface area, dispersion and aggregation status, coating or functionalization, immobilization, cellular uptake or internalization, and cell types. It has been well-known that the highly water-soluble and serum stable CNTs are biocompatible and nontoxic or very low toxic as confirmed by several *in vitro* and *in vivo* toxicity studies [3]. So the functionalized CNTs have also demonstrated immense potentials, particularly in the areas of drug delivery [4], and tissue engineering [5] in biomedical field. The ability for CNTs, especially the single-walled carbon nanotubes (SWCNTs), to absorb and convert electromagnetic radiation, specifically near infrared (NIR), into heat or sound energy has been exploited for successful photoacoustic therapy [6] or photothermal therapy (PTT) [7] against cancer cells. Among numerous functional materials in the delivery of therapeutic agents, CNTs have also been attracted extensively as carriers for controlled and targeted drug delivery [8–10], in oder to improve the pharmacological activity of bioactive molecules and simultaneously diminish their undesirable systemic side effects [4,11], as well as cancer imaging applications [4,12].

In contrast to normal tissues, the physicochemical and microenvironmental features of tumor tissues include weak acidity [13], overexpressed proteins and enzymes [14] and abnormal temperature gradients [15]. Most importantly, the endosomes and lysosomes of tumor tissues have drastically different acidic condition [16]. Based on these apparently differences abovementioned, outstanding advances have been made in manipulating these physicochemical properties that can be administered directly multifunctional nanocargo to deliver anticancer drugs to the desired target sites. Meantime, due to the unique sp^2 carbon structure

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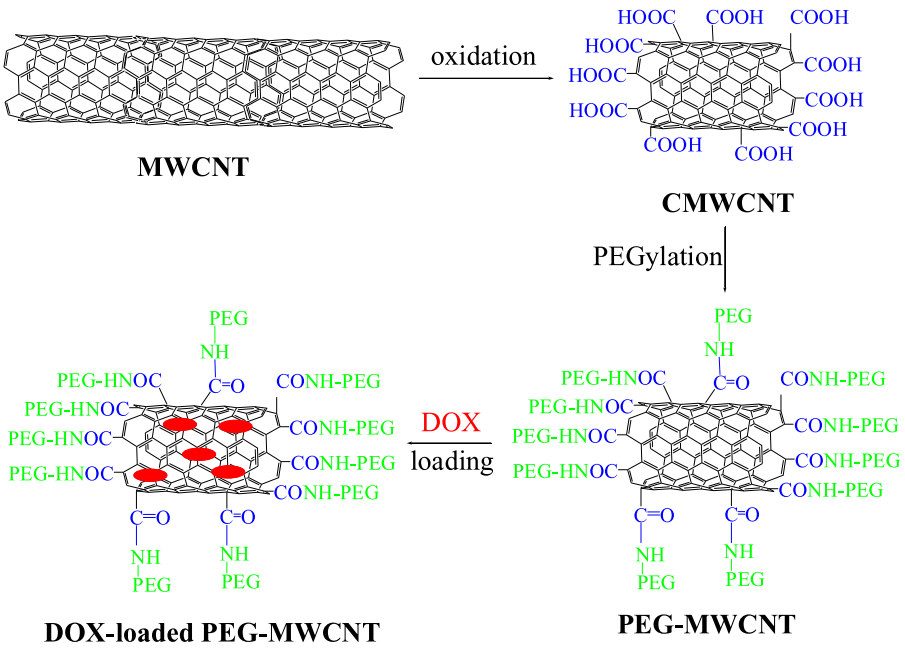

Fig. 1. Schematic illustration of the synthetic routine to DOX-loaded PEG-MWCNTs.

and inherent hydrophobic nature of CNTs, multiple copies of chemotherapeutic drugs or DNA/RNA molecules can easily be absorbed onto their surface through hydrophobic interactions or π–π stacking to imporve enhance the tumor-targeting drug transportation and minimize the severe side effects along with the chemotherapy [17], as well as theranostic applications [18].

Although CNTs have been widely used in DDS for tumor treatment in which PEGylation was the most used method to improve their biocompatibility [19–23], and the proven success in other fields may not translate to the use of CNTs in medicine for reasons including inconsistent data on cytotoxicity and limited control over functionalized-CNT behavior [24]. By now, there is no comprehensive report on the effects of length and PEGylation degree. The two factors are the most decisive on the anti-cancer efficiency of the CNTs-based DDSs, affecting the blood circulation duration, cellular uptake, drug-loading and controlled release performance.

Inspired by excellent properties of CNTs and difference between physicochemical characteristic of normal tissues and tumor tissues, in the present work, multi-walled carbon nanotubes (MWCNTs) with different lengths were obtained by regulating oxidation time [25]. To enhance the biocompatibility and make the drug delivery platforms stealthy during blood circulation, poly(ethylene glycol) (PEG) was applied to improve the surface chemistry of the MWCNTs. It is well known that PEG is one of the most commonly used hydrophilic polymers, which possesses high biocompatibility and can be rapidly and spontaneously cleared from human bodies as excellent candidates of biomedical materials. It is also believed to impart low fouling properties to surfaces due to the screening of interfacial charges, repulsion (entropic and osmotic), and excluded volume effects [26]. Furthermore, the facilitated drug release by the protein competition could be avoided during the blood circulation [27]. Finally, the PEGylated carboxyl functionalized multi-walled carbon nanotubes (PEG-MWCNTs) were optimized for favorable biocompatibility and pH-triggered controlled release characteristics in delivering chemotherapeutic anticancer-drug to reduce server side effects (Fig. 1). Therefore, promising CNTs-based DDS was established for application in delivering chemotherapeutic drugs in cancer treatment in the future.

2. Experimental

2.1. Materials and reagents

MWCNTs (purity of 95%, diameter of 10–20 nm and length of 5–15 μm) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Amino-terminated PEG (PEG–NH_2, M_n = 2000) was provided by Beijing Kaizheng Biological Engineering Development Co., Ltd. (Beijing, China). 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDCl) was purchased from Fluorochem. N-Hydroxylsuccinimide (NHS) was purchased from Aladdin Chemistry Co. Ltd. Doxorubicin hydrochloride (DOX) was purchased from Beijing Huafeng United Technology Co. Ltd. All other reagents were analytical reagent grade purchased from Tianjin Chemical Company, Tianjin, China. Double distilled water was used throughout.

2.2. Carboxyl functionalized MWCNTs (CMWCNTs) with tailored length

CMWCNTs with different lengths, different oxidation time (2 h, 4 h, 6 h, and 8 h) was chosen as a control condition to handle the pristine MWCNTs [25]. In a typical procedure of oxidation time of 8 h, pristine MWCNTs (2.0 g) were charged into 200 mL of a mixture of concentrated H_2SO_4/HNO_3 (3:1) equipping with ultrasound for 8 h. Subsequently, the above mixture was vigorously stirred at 80 °C for 8 h. The resulting product was neutralized with NaOH solution, and subsequently the most of the water were removed at reduced pressure by a rotary evaporator. After that, the mixture was transferred into dialysis tube (MWCO: 3500) against abundant deionized water for 48 h to remove inorganic salt originated from the neutralization reaction. Finally, the obtained carboxyl functionalized multi-walled carbon nanotubes (CMWCNTs) were lyophilized and stored at 4 °C.

2.3. PEG-MWCNTs

The CMWCNTs were PEGylated with PEG–NH_2 via amidation reaction between carboxyl groups of the CMWCNTs and the amino

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