PROCEEDINGS OF THE INTERNATIONAL CONFERENCE NANOMATERIALS: APPLICATIONS AND PROPERTIES
Vol. **1** No 2, 02NNBM12 (3pp) (2012)

PEG-grafted Hyperbranched Polyethyleneimine-Oxidized Single Walled Carbon Nanotube Complex (PEG-PEI-SWNT) for Sustained Delivery of Doxorubicin

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(Received 06 June 2012; published online 19 August 2012)

To take advantages of single-walled carbon nanotubes (SWNTs) for cellular delivery of chemotherapeutic agents (e.g. doxorubicin) in order to decrease doxorubicin toxicity and increase its efficacy, we aimed to develop a novel approach to aqueous disperse and stabilize SWNTs through consequent steps of oxidation (oxSWNT) and PEG-PEI complexation (PEG-PEI-SWNT). Doxorubicin was loaded onto the modified SWNTs in alkalione pH with more considerable capacity (~°900 %) than those previously reported, due to complex formation with PEI proved by UV-visible spectroscopy. The loaded carrier was stable in physiologic simulated medium. Drug release was prolonged and dilution independent, but exhibited pH-dependent burst release that makes SWNTs as suitable *in vivo* drug carriers in acidic tumor milieu.

Keywords: Carbon nanotube, PEGylation, Polyethyleneimine, Drug delivary, Cancer therapy.

PACS number: 87.55.ne

1. INTRODUCTION

Among the numerous drug delivery systems currently under investigations, carbon nanotubes (CNTs) have shown great promise as novel delivery systems [1]. Single-walled carbon nanotubes (SWNTs), a kind of these novel polyaromatic molecules, offer potential advantages over the more widely studied nanoparticle systems, including ultrahigh surface areas (that give them the ability to carry a high cargo loading of multiple molecules along the length of the nanotube sidewall) [2], high mechanical strength but ultra-light weight and structural flexibility (which could prolong the circulation time and hence the bioavailability of the carried drug molecules), rich electronic properties (which facilitates their surface modification and ligand attachment) , and excellent chemical and thermal stability [3, 4]. They are considered to be comparatively safe and capable to penetrate cell membranes, providing a route for delivery of cargoes into the cytoplasm. Also, the intrinsic spectroscopic properties of nanotubes, including Raman and photoluminescence, provide additional advantages for tracking, detecting, and imaging to understand drug delivery efficacy in vivo [5].

CNTs have highly hydrophobic surfaces, and are not soluble in aqueous solutions. For biomedical applications, surface chemistry or functionalization is required for water dispersion of CNTs, and to render biocompatibility and low toxicity. Modifying CNTs by attaching hydrophilic polymers such as poly ethylene glycol (PEG), yield CNT-polymer conjugates stable in biological environments [6]. In addition to solubilization, PEGylation is one of the most effective methods to prolong the blood-circulation time due to the known "stealth" properties of PEG to overcome the phagocytic activity of the reticulo-endothelial system. Moreover PEGylation provide a platform for further modifications such as conjugating specific ligands toward biologic targets. PEG functionalized SWNTs were observed to persist within liver and spleen macrophages for 4 months without apparent toxicity. The intracellular PEGylated SWNTs were highly dynamic and the cell penetration of PEGylated SWNTs appeared as bidirectional [5].

In present study, we aimed to establish a new approach to disperse and stabilize SWNTs for drug delivery application by means of PEG grafted hyperbranched polyethylenimine (PEI) as a hydrophilic biocompatible block copolymer.

2. MATERIALS AND METHODES

We started with powder of pristine SWNTs (purity >90%, mean diameter ~ 1 – 2 nm, length ~ 1 – 3 μm, SSA 380 m^2/g, Neutrino Co., Germany), Polyethylene glycol (PEG) 2 KD (methoxy, amine, NHS terminated) (JenKem, USA), Hyperbranvhed polyethyleneimine 25 KD (Sigma Aldrich, Germany), Doxorubicin hydrochloride hydrochloride (Adriblastina®, Pharmacia, Italy), N-(3-Dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDC) (Sigma Aldrich, Germany), N-Hydroxysuccinimide (NHS) (Sigma Aldrich, Germany).

Shortening, purification and water dispersion of SWNTs was carried out via oxidation, using a mixture of 96 % H_2SO_4/65 % HNO_3 (3:1 V/V). UV-vis-NIR spectroscopy method at $\lambda = 808$ nm was established to calculate SWNT concentration. The density of carboxylic acid moieties of the oxSWNTs was quantified by acid-base back-titration potentiometric method.

To prepare PEG-PEI-SWNT polyionic complex, NHS-functionalized PEG (2 KD) was attached to amine group of hyperbranched PEI (25 KD) in PEG to PEI ratio of 2:1. Free PEG was removed by precipitating PEG-PEI copolymers in the presence of acetone. Aliquots of diluted oxSWNT were drop wise added to PEG-PEI solution at weight ratios of 1:1, 2, 5, 10, 20, 50 and 100 of SWNT to PEI. Free copolymer was removed by consecutive centrifugation and washing steps. To choose the optimal ratio, dispersibility of the

products was examined by addition of isotonic NaCl solution. Free copolymer was determined using ammonium ferrothiocyanate and ninhydrin assay.

PEG-PEI-SWNTs were introduced into phosphate buffer (pH 8) by ultrasonication. Various concentrations of doxorubicin hydrochloride (DOX) were mixed in range of 50 – 500 μg/ml with PEG-PEI-SWNTs at 4 °C overnight. Unbound excess DOX was removed by centrifugation. The amount of DOX loaded onto SWNTs was measured from supernatant by the absorbance peak at 490 nm.

DOX-SWNT suspensions were allowed to stand in simulated biologic media containing PBS pH 7.4 or 5.4 with moderate shaking at 37 °C. After different time intervals, free SWNTs were separated by centrifugation, and the concentration of released DOX in the supernatant was estimated by spectrofluorometry.

3. RESULTS AND DISCUSSION

The SWNT recovery during oxidative acid treatment was > 80 %. OxSWNTs exhibited water dispersible characteristic, but in contrast to PEG-PEI stabilized SWNTs, could not stand the presence of salt and agglomerated in such medium or similarly under physiologic conditions. COOH content of oxSWNTs were determined to be about 2.5 % W/W (5 mmole COOH/g SWNT).

The PEG-PEI-SWNTs were stable in water and physiological buffers for at least several weeks at room temperature without agglomeration and precipitating out of the solution indicating of single or small bundled, short SWNTs rather than large aggregates. These results suggest strong and stable noncovalent absorption of the PEG-PEI molecules on SWNT sidewalls. PEGylation of PEI minimizes aggregation of the nanoparticulates.

Formation of DOX-SWNT complexes was evidenced by the reddish color of DOX-SWNT samples due to adsorbed DOX and its characteristic UV–vis absorbance peak on top of the characteristic SWNT absorption spectrum (Fig. 1).

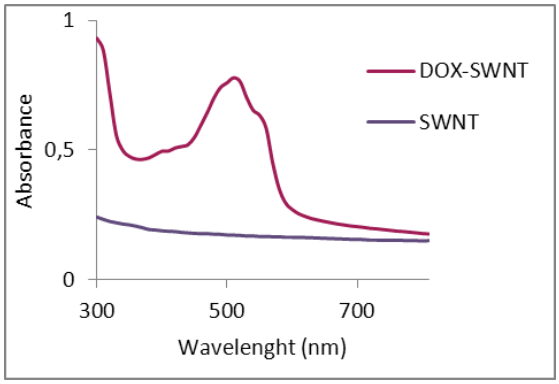

Fig. 1 – UV-vis-NIR spectrum of DOX-SWNT

Loading efficiency was observed to be pH dependent, so that loading factor (defined as loading efficiency/maximum efficiency in pH 8.5) decreased from 100 to ~ 19.5 to ~ 11 percent as pH was reduced from 8.5 to 7.4 and 5.4 respectively (Fig. 2).

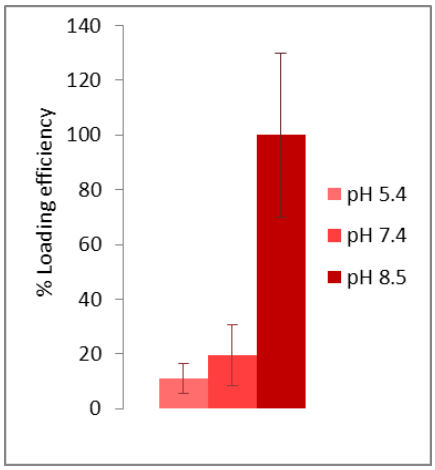

Fig. 2 – The effect of loading medium pH on loading efficiency

The loading extent increases as the DOX concentration increases (Fig. 3). It was estimated loading efficiency and capacity of ~ 97 % and 605 % for DOX/SWNT = 5, and ~ 75 % and 933 % for DOX/SWNT = 10 respectively. The optimum DOX/SWNT weight ratio for maximum loading efficiency and capacity to be obtained, estimated to be 8.

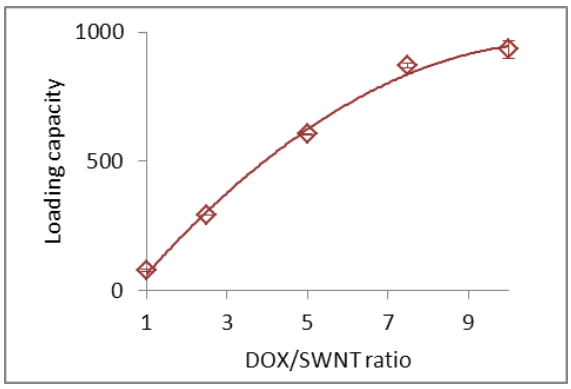

Fig. 3 – Effect of DOX/SWNT weight ratio on loading

Dox release profile exhibit pH-dependency, triggered at acidic conditions, due to protonation of doxorubicin at acidic pH (Fig. 4). This made SWNTs ideal as *in vivo* drug carriers; since the micro-environments in the extracellular tissues of tumors and intracellular lysosomes and endosomes are acidic.

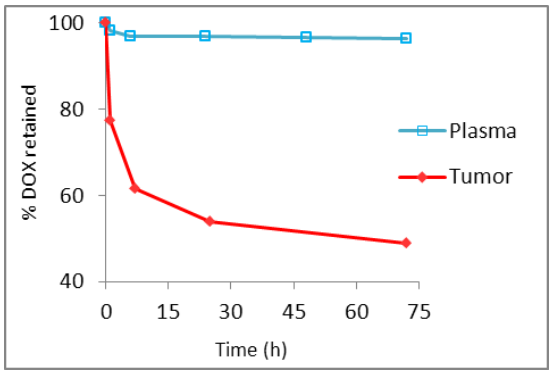

Fig. 4 – pH-dependent release of DOX in two different simulated environments; plasma (PBS pH 7.4) and tumor (PBS pH 5.5)

Herin we tried to establish a new approach to stabilize SWNTs and form superfacial matrix for drug delivery application with exclusive properties such high loading capacity and pronounced sustained and pH-dependent drug release.

REFERENCES

1. G. Pastorin, *Pharm. Res.* **26**, 746 (2009).
2. Z. Liu, S. Tabakman, K. Welsher, H. Dai, *Nano Res.* **2**, 85 (2009).
3. Y. Lin, S. Taylor, H. Li, et al. *J. Mater. Chem.* **14**, 527 (2004).
4. C.J. Gannon, P. Cherukuri, B.I. Yakobson BI, L. Cognet, J.S. Kanzius, C. Kittrell, et al. *Cancer* **15**, 2654 (2007).
5. F. Liang, B. Chen, *Curr. Med. Chem.* **17**, 10 (2010).
6. Z. Liu, S. Tabakman, K. Welsher, H. Dai, *Nano Res.* **2**, 85 (2009).