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Leading opinion

Single walled carbon nanotubes as drug delivery vehicles: Targeting doxorubicin to tumors☆

Lingjie Meng [a], Xiaoke Zhang [a], Qinghua Lu [a,*], Zhaofu Fei [b], Paul J. Dyson [b]

[a] Department of Polymer Science and Engineering, School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland

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ABSTRACT

Single walled carbon nanotubes (SWNTs) are emerging as promising delivery vehicles for cancer diagnostics and chemotherapies due to their unique properties, including, remarkable cell membrane penetrability, high drug-carrying capacities, pH-dependent therapeutic unloading, prolonged circulating times and intrinsic fluorescent, photothermal, photoacoustic and Raman properties. In this leading opinion paper, we systemically discuss and evaluate the relationship of the biological safety of SWNTs with their physicochemical properties such as their length, purity, agglomeration state, concentration and surface functionalization. Other relevant issues, including the cellular uptake mechanism, biodistribution and metabolism of SWNTs are also reviewed. The design and preparation of SWNT-based drug delivery systems (DDSs) and their pharmacokinetic, cancer targeting and therapeutic properties both *in vitro* and *in vivo* are highlighted. Future opportunities and challenges of SWNT-based DDSs are also discussed.

1. Introduction

The aim of chemotherapy, the method of choice in cancer treatment, is to destroy cancer cells whilst minimizing side effects to healthy tissue [1]. However, many current chemotherapeutic agents are far from ideal. Many drugs target systemic biomolecules, such as DNA, and consequently have a high general toxicity. Take, for example, the anthracycline, doxorubicin (DOX), a drug widely used in chemotherapy due to its efficacy in fighting a wide range of cancers [2], with particularly important applications in the treatment of breast cancer. Nevertheless, like most anticancer drugs, DOX has some drawbacks, including limited solubility, a poor non-selective biodistribution and accordingly, treatment with DOX results in severe damage to healthy tissue.

In 1986, Matsumura and Maeda described a new concept using relatively large polymers to target drugs specifically to cancer cells *via* what became known as the enhanced permeability and retention (EPR) effect [3]. The EPR effect results from physical differences between cancer tissue and healthy tissue, since the rapid growth of tumor tissues leads to ineffective lymphatic drainage and abnormalities in the architecture of the blood vessels that feed them. These abnormalities include poorly-aligned endothelial cells with wide fenestrations, the lack of a smooth muscle layer, and innervations with a wider lumen. Such structural defects lead to hyper-vasculature, enhancing the permeability of tumors to macromolecules, and once the macromolecules have entered, they are retained as there is little recovery through either blood vessels or lymphatic vessels. Since this first report, this property has been shown to allow tumor targeting and accumulation of natural and synthetic macromolecular drug carriers.

The possibility to accumulate large species in cancerous tissues opens the opportunity to use macromolecular delivery vehicles to specifically target small molecule drugs to tumor sites. There are several important properties of such carriers, and perhaps the most important, is that the macromolecule should have a low intrinsic toxicity, thereby simply enhancing the selectivity of current chemotherapeutics. Various nanosized delivery vehicles, including liposomes, polymeric nanoparticles, and inorganic nanoparticles, have been employed to improve the pharmacological and therapeutic properties of drugs [4–6]. Among these delivery vehicles carbon nanotubes (CNTs) would appear to be increasingly promising candidates [7–9].

☆ *Editor's Note*: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

* Corresponding author. Tel.: +86 21 54747535; fax: +86 21 54747535.
E-mail address: qhlu@sjtu.edu.cn (Q. Lu).

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The CNTs are well-ordered tubular allotropes of carbon with a diameter in the nanometer range and their length can reach several centimeters. They can be envisaged as a single graphene sheet rolled into a seamless tube (single walled carbon nanotubes; SWNTs) [10,11] or multiple layers of graphene rolled simultaneously to form seamless concentric tubes (multi-walled carbon nanotubes; MWNTs) [12]. The specific structure of SWNTs is usually described by a (n, m) vector that defines its diameter and chirality [13]. Fig. 1 gives the schematic of three typical SWNTs, and compared to MWNTs, SWNTs tend to exhibit richer electrical and optical properties [14].

There are several important properties of SWNTs that may be exploited in biomedical applications. In addition to targeting *via* the EPR effect, they are relatively easily internalized by cells opening the possibility for their use in the delivery a variety of payloads for therapy and diagnosis [15]. With special sp^2-hybridized carbon surfaces and a large surface area (theoretically 1300 m^2/g), SWNTs have a high capacity for drug loading – including loading inside the tube.

Not only drugs, but also specific targeting molecules can be integrated onto the surface of SWNTs by either covalent or non-covalent interactions [8]. In some cases, the loading is dependent on the medium. For instance, anticancer drugs with condensed aromatics, such as DOX, can be loaded by π-π stacking interactions. Such loading is pH-dependent, providing another handle for tumor cell targeting. Loading is significantly higher at healthy blood pH than in the acidic pH environment of, for example, endosomes, lysosomes, and the micro-environments of rapidly growing tissues of tumor origin as well as parasitically or virally infected cells [16–18]. Furthermore, functionalization of the SWNTs can optimize the properties for drug delivery, including solubility, blood circulation time, selectivity and retention in the tumor [19–24]. Combined, these properties suggest a potential application of SWNTs in targeting drug delivery for treating cancer and other diseases [8,15,25].

In addition to offering potential drug delivery, SWNTs have intrinsic properties that facilitate tracing and therapy. For example, they have unique optical properties, such as near-infrared region (NIR) fluorescence and absorption, Raman scattering, and photoacoustic properties. These optical properties are potentially useful for tracing, bio-imaging, disease diagnosis and additional drug activation (see below). It could be envisaged that, combined with a load of drugs and targeting molecules, CNT based drug delivery systems could allow *in-situ* imaging of the response of the tumor to the loaded drug that could help guide the treatment regimen.

Both SWNTs and MWNTs exhibit strong optical absorptions in the NIR. In contrast, biological tissues and blood are maximally transmissive in this range [26,27]. These properties might allow CNTs targeting tumor cells to adsorb irradiation of a NIR laser and emit heat, leading to localized hyperthermia and the death of tumor cells with minimal damage to surrounding tissues. Combined with a chemotherapeutic drug delivered by the CNTs, NIR photothermal therapy may enhance the treatment efficacy [28].

Semiconducting SWNTs can emit NIR fluorescence in the range of 800–2000 nm, depending on their structures. The fluorescence range spans the entire biological tissue transparent window and is, therefore, promising for drug detection and biological imaging in superficial tissues and small animals [29]. Moreover, all SWNTs exhibit strong resonance Raman scattering in a tangential mode (G-band, around 1580 cm^{-1}) associated with the perfect graphene nature of their side walls. The sharp and strong inherent peak can be easily distinguished from fluorescence backgrounds and can be used in optical imaging [30] including multi-color Raman imaging [31].

As mentioned above, CNTs can effectively absorb NIR laser light and convert it into heat. Their nanoscale dimension and high thermal conductivity allow fast heat transition to the surrounding medium efficiently generating high frequency ultrasound [32]. Photoacoustic imaging has the potential to overcome the resolution and depth limitations of optical imaging whilst maintaining a relatively high contrast [33].

While SWNTs can improve the efficacy of DOX (and other drugs), their inherent physical properties provide options to construct a unique platform for potential multimodality disease diagnosis and therapy. These unparalleled advantages have fueled research exploring the biological applications of these structures (Fig. 2). Nevertheless, there are genuine concerns regarding the safety of CNTs. Reports have described asbestos-like toxicological behavior of MWNTs with lengths > 20 μm [34] and the formation of cancerous lesions following treatment with MWNTs [35]. Thus, key questions remain to be fully answered and below a critical analysis of the literature, and some new data are provided.

2. Are SWNTs safe for drug delivery?

A drug carrier is expected to have an intrinsically low systemic toxicity, and thus, the safety of CNTs is of utmost importance when considering their biomedical applications. The apparent toxicity of CNTs has caused concern in the past decade [36,37], however, data are often inconsistent and conflicting. The toxicity of CNTs appears to depend on various parameters, such as structure (SWNTs vs. MWNTs), length, purity, agglomeration and surface functionalization [13,21,28,36–41]. Therefore, caution must be applied when generalizing the toxicity data obtained on one particular type of CNT evaluated under specific conditions. Notably, the most commonly used cell viability test, the MTT assay, is unsuited to cytotoxicity determinations of SWNTs because the MTT-formazan crystals tend to be associated with the SWNTs, reducing their solubility in DMSO and isopropanol, leading to errors in the assay [42].

2.1. In vitro studies

Raw, freshly prepared SWNTs are usually tens of micrometers to several centimeters in length and tend to cluster into large bundles contaminated with metal residues. Purified SWNTs and cut SWNTs (<500 nm) are derived from these raw SWNTs [17,43]. SWNTs can

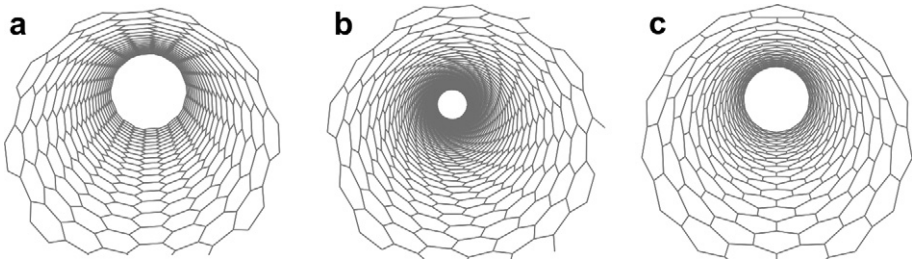

Fig. 1. Schematic of three typical SWNTs, (a) Armchair (10, 10), (b) Chiral (13, 6), and (c) Zigzag (14, 0).