*Critical Reviews*™ *in Oncogenesis*, 19(3-4):261–268 (2014)

# Fibrillous Carbon Nanotube: An Unexpected Journey

**Michael R. McDevitt[1,2,*] & David A. Scheinberg[1,3]**

[1]Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; [2]Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; [3]Department of Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center, New York, NY 10065

***Address all correspondence to**: Michael R. McDevitt, Departments of Medicine and Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; Tel.: 646-888-2192; Fax: 646-422-0640; m-mcdevitt@ski.mskcc.org.

**ABSTRACT:** The emergence of nanomedicine, a discipline at the nexus of materials engineering, chemistry, biology, and pharmacology, has generated much excitement in the field of translational medical research and provided some unexpected results. Nanomedicine seeks to introduce nanoscale technology to the practice of medicine via the design and development of nanomaterials possessing therapeutic or diagnostic functions. However, as expected, any modification of the base nanomaterial platform to decorate it with solublizing, targeting, therapeutic, or diagnostic modalities yields a material with a very different pharmacological profile than the original platform. Clearly, the goal of nanotechnology is to put into practice a novel synthetic substance in which the function of the complex is greater than the sum of its components. These new compositions must be thoroughly evaluated *in vivo*. Therefore, reliance on pharmacokinetic predictions based solely on the baseline profile of the original platform can confuse the field and delay progress. Carbon nanotube pharmacokinetic profiles provide an interesting example of this situation. Covalently functionalized nanotubes exhibit fibrillar pharmacology while those nanotubes that are not covalently functionalized transiently behave as fibers and then tend toward an overall colloidal profile *in vivo*.

**KEY WORDS:** carbon nanotube, fibrillar, pharmacokinetics, pharmacology, nanomaterial

**ABBREVIATIONS: PK:** pharmacokinetic; **ADME:** absorption, distribution, metabolism, and excretion; **SWCNT:** single-walled carbon nanotubes; **MWCNT:** multiwalled carbon nanotubes; **CNT:** carbon nanotube; **PEG:** polyethylene-glycol; **PET:** positron emission tomographic; **NIR:** near-infrared; **f-CNT:** functionalized carbon nanotube; **DOTA:** 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; **DFO:** desferrioxamine B; **SPECT:** single photon emission computed tomography.

## I. INTRODUCTION

Pharmacokinetic (PK) profiling informs how the host processes a molecule by examining the absorption, distribution, metabolism, and excretion (ADME) of that molecule *in vivo*. These ADME factors and the administered dosage determine the concentration of a drug at its sites of action, and thus the intensity of its effects as a function of time.[1] The PK behavior can also be used to evaluate biocompatibility and address issues of untoward toxicity. Therefore, in the course of developing a nanomaterial for use *in vivo*, it is imperative to examine and describe the PK profile of that molecule in a relevant animal model. The use of classic tracer methodology is a powerful tool to interrogate these ADME parameters.[2] Radionuclide tracer studies are most valuable if the molecular construct is pure and well characterized; furthermore, the appended tracer should be stable and not alter the overall PK profile of the nanomaterial.[3] In addition, it is important to include appropriate control experiments, have a sufficiently large numbers of test subjects,[4] and obtain data over the timescale of several biological half-lives. A thorough physicochemical characterization of the nanomaterial will supply pertinent supporting data and assist with interpretation of subtle PK effects. It is important to note that this methodology reports only the PK fate of the tracer and it should be demonstrated that the reporter and the macromolecule under investigation remain intact over the course of the experiment.

## II. CARBON NANOTUBES

Single-walled and multiwalled carbon nanotubes (SWCNT and MWCNT, respectively) are graphene cylinders with extremely large aspect ratios

11040-8401/14/$35.00 

(length ≫ diameter) and surface areas (~$10^3$ $m^2/g$). These synthetic nanoscale materials display an array of unique intrinsic chemical, physical, electronic, thermal, and optical properties that could be exploited in drug design and sensing applications, and both CNTs are actively being investigated for potential biomedical applications. These materials must be solubilized and dispersed for systemic administration. Appending various modalities [e.g., targeting ligands (e.g., proteins, peptides, oligonucleotides, small molecules), radionuclides, fluorophores, therapeutic drug molecules, and amphiphiles] to the sidewall of the nanotube is an established strategy to disperse, solubilize, and augment utilitarian biochemical features.

## III. FUNCTIONALIZATION OF CARBON NANOTUBES FOR USE *IN VIVO*

Pristine (i.e., raw or as-produced) CNT materials are hydrophobic, highly aggregated bundles of carbon fibers that are relatively inert in aqueous milieu. Two predominant strategies to debundle and disperse CNT for use *in vivo* depend on the following modifications: (i) covalent functionalization of a fraction (1–2%) of the aromatic sidewall $sp^2$-carbon scaffold with aliphatic amino acid appendages,[5–8] and (ii) non-covalent decoration with physisorbed surfactants, oligonucleotides, or polyethylene-glycol (PEG) amphiphiles. The aim in both these approaches is to debundle the CNT aggregates and individualize the fibers to employ the CNT as a delivery platform or an active constituent for executing some extrinsic or intrinsic imaging or therapeutic effect. Either modification can render the CNT well dispersed, soluble, and biocompatible so that it can be used systemically *in vivo*. However, the non-covalent strategy appears to be a transient dispersal effect that rapidly dissipates once the agent is administered systemically. These modifications strongly influence the nature of the CNT's molecular interaction with biological systems and thus yield different PK profiles. Once they are dispersed and individualized, these novel macromolecules possess an extremely large aspect ratio that confers unique reactivity *in vivo* and pharmacologically distinguishes them from globular-shaped molecules and particulate colloids.

## IV. LABELING OF CARBON NANOTUBES FOR TRACER STUDIES

CNTs are ideal scaffolds to append with one or several different tracer functionalities when intrinsic reporting is not an option. The large surface area and aspect ratio permit an increase in both signal diversity and signal amplification per macromolecule relative to conventional imaging agents.[9–11] CNT constructs have been designed to report information using a range of different tracer modalities (e.g., fluorophores, radionuclides, and MRI active metal ions) to take advantage of the strengths and overcome the weaknesses of each modality.[9–12] However, when used in combination, these different modalities can telescopically report the global (i.e., whole animal), local (i.e., tissue), cellular, and even subcellular organelle location of the CNT tracer *in vivo* and *ex vivo*. This telescopic imaging paradigm, using dynamic positron emission tomographic (PET) imaging, near-infrared (NIR) fluorescence imaging, and immunohistochemical and immunofluorescence microscopy, was utilized to describe the paradoxical PK profile of a covalently functionalized SWCNT in a murine model and propose the unexpected mechanism for renal clearance.[13]

## V. PHARMACOKINETICS OF CARBON NANOTUBES

### A. Distribution and Excretion

Distribution and excretion are profoundly affected by the modification method selected to individualize the CNT. The systemic delivery of functionalized CNT (f-CNT) to a tissue will depend on vascular or lymphatic system access to that tissue. In turn, retention at that tissue will depend on specific cellular accumulation that may occur by numerous biological or physical ways. What is apparent is that CNTs do not accumulate in every cell type; there are specific

cells that accumulate f-CNT.[13] Excretion of nanomaterials from the host is mediated by the kidneys, liver, and skin into the urine, feces, and perspiration, respectively, and from the blood into breast milk.[14] Renal excretion is an ideal route for elimination, especially if the nanomaterial has the appropriate dimensions to rapidly filter.[15,16]

Covalent modification of CNT with numerous sidewall ammonium functionalities yielded f-CNT with the composition SWCNT-$(NH_3^+)_n$; this f-CNT could subsequently be labeled with a mixture of tracer modalities (i.e., [$^{86}$Y]DOTA, [$^{111}$In]DOTA, [$^{89}$Zr]DFO, Alexa Fluor 488, Alexa Fluor 568, and Alexa Fluor 680) and used to image and examine the PK profile of f-CNT constructs in animal models.[13,17,18] These well-characterized carbon nanomaterials showed minimal accumulation off-target, quick clearance from the blood, and rapid excretion by renal filtration.[13,17] Further, a chromatographic analysis of the excreted f-CNT showed that this fibrillar macromolecule was filtered intact into the urine. Detailed mechanistic and imaging analyses confirmed that the f-CNTs were being filtered by the glomerulus and not actively transported from blood to urine.[13] This finding was surprising because these f-CNTs ranged from 350 to 500 kD in molecular weight and were well above the molecular weight threshold for macromolecular renal filtration. The PK data was modeled and a mechanism was proposed to explain the observed glomerular filtration as a consequence of the f-CNT alignment with blood flow. The high aspect ratio f-CNT was aligned with flow and not "tumbling" in the blood; the molecule encountered the filtration slit diaphragm "end-on" (the long axis of the molecule was perpendicular to the slit opening) and readily transited across that boundary. Because of fluid forces and flow dynamics, the small 1 nm diameter dimension of this very large macromolecule was presented to the slit diaphragm with the profile of a small molecule. The surprising f-CNT transport phenomenon was termed fibrillar pharmacology to distinguish it from the more traditional PK behavior of globular macromolecules and of the colloidal particulates.[19]

A biodistribution and excretion study of water-soluble, hydroxylated SWCNT described stomach, kidney, and bone accumulation in a mouse model with accompanying renal excretion of 94% of the injected dose into urine.[20] This curious tissue uptake profile could be a consequence of the pendant hydroxyl moieties or the degradation and release of the $^{125}$I tracer from the CNT following cellular internalization. Another PK study of f-SWCNT (using the sidewall ammonium functionalization methodology) reported no significant tissue accumulation in a mouse model; the f-SWCNT underwent rapid clearance from the blood ($t_{1/2}$ ~3 h) and elimination into the urine.[21] Transmission electron microscopy showed images of f-MWCNT molecules transiting directly from the blood compartment into the Bowman capsule.[22] A dynamic single photon emission computed tomography (SPECT) study imaged only the distribution of the injected dose to the kidney and renal clearance into the urine of radiolabeled f-MWCNT in a rat model following intravenous injection.[23] The SWCNTs have also been filled with the $^{125}$I tracer and then sealed and subsequently sidewall functionalized with biantennary carbohydrates.[24] This high-density radioemitting crystal was administered intravenously to mice, and SPECT imaging demonstrated that the tracer cleared the blood rapidly (within a few hours) and was accumulated almost exclusively in the lung; no description of renally eliminated activity was reported. The appended carbohydrate moieties may have directed this material to a target in the lung; however, the absence of any activity in the urine may now be hypothesized to relate to the particle's geometric shape *in vivo*. A pre-targeting approach to tumor therapy and imaging employing a radiolabeled f-SWCNT modified with sense oligonucleotide strands as the rapid clearance component of this system was examined in a mouse model; the radiolabeled f-SWCNT-sense showed minimal off-target tissue accumulation and rapid renal excretion while able to bind and combine with the tumor-targeting antibody-antisense oligonucleotide partner.[25] The goal was to have the rapidly clearing f-SWCNT-sense platform radiolabeled to high specific activity with the potent alpha particle emitting $^{225}$Ac radionuclide for therapy[26] and the $^{111}$In radionuclide for imaging.

Filamentous polymer micelles (22 < diameter < 60 nm; length ~0.008 mm) were reported to exhibit

very different PK profiles than spherical micelles with a log greater lifetime in the blood. The explanation provided for this persistence in the blood was that globular shapes were more readily accumulated by cells than the filamentous shapes, since the latter were extended under fluid flow conditions.[27] The filomicelle dimensions were very different compared with f-CNT, but highlight the impact of shape on PK.

Pristine SWCNTs were dispersed in Pluronic F108 and the biodistribution evaluated *ex vivo* in a rabbit model using the intrinsic NIR fluorescence of the SWCNT.[28] The stability of Pluronic-dispersed SWCNT was challenged by serum proteins that rapidly dislodged the synthetic surfactant from the CNT surface within seconds following intravenous administration. The SWCNT concentration in the blood serum decreased exponentially ($t_{1/2}$ = 1.0 h) and one day after administration the SWCNT was observed only in the liver. This study demonstrated that pristine SWCNTs exhibited high-contrast NIR fluorescence that could be sensitively tracked *ex vivo* using optical methods. The biodistribution of PEG-coated SWCNT was investigated in murine models using PET to image the biodistribution of the tracer *in vivo*.[29] The PEG phospholipids stabilized the SWCNT *in vivo* and the PEG chain length played a role in biodistribution and blood clearance times. The liver was the predominant target tissue (accumulated 20–40% of the injected dose per gram); lesser amounts of radioactivity were reported in the intestine, heart, lung, kidney, spleen, and stomach; the tracer cleared the blood compartment within 2–5 h and there was no activity reported to be renally cleared. The SWCNT has an intrinsic Raman spectral signature that was used to examine tissues *ex vivo* and confirmed the high liver uptake. Raman microscopy and NIR photoluminescence imaging modalities rely on intrinsic CNT properties; however, the challenges to these techniques continue to remain the attenuation of signal and the high background due to auto-fluorescence when attempting to measure tissue deeper than 2 mm.[30,31] Photoacoustic imaging techniques may avoid some of the shortcomings associated with optical bioimaging in whole animals.[32]

Studies utilizing PET and SPECT radionuclides as the imaging component of the CNT[13,17,18,23,24,29,33] have been very useful in evaluating PK *in vivo* and *ex vivo*. PET is an extremely sensitive tomographic imaging modality and a diverse assortment of radionuclides are available to optimize the radiochemical half-life and chemistry.[34,35] The most informative PK data were obtained using dynamic PET imaging. Furthermore, radioactive tracers permit one to measure the activity balance and account for the entire administered dose.

In summary, a tendency has been noted for the amphiphile-coated pristine CNT materials to favor liver accumulation and subsequent hepatobiliary excretion, and eschew the kidneys and renal clearance. These materials clear the blood within hours and the report of amphiphile displacement by serum proteins[28] could be describing a loss of fibrillar shape as a consequence of (i) CNT aggregating onto serum proteins forming high-molecular-weight complexes or (ii) a reaggregation of fibers into colloidal bundles that were unable to renally filter and accumulated in the liver. In contrast, there was a trend for the covalently modified CNT to undergo rapid renal excretion with only a minimum of hepatobiliary clearance. The more robust covalent sheath maintained the integrity of the individual fibers and prevented aggregation *in vivo*. A mechanism describing renal excretion of covalently modified SWCNT has explained glomerular filtration that was accompanied by a small amount of transient reabsorption in the proximal tubule cells.[13] The pharmacokinetic profile of these novel materials can be altered by understanding the biophysical clearance parameters and the judicious improvement in design specifications.

## B. Absorption

Absorption issues are important when considering the safe and responsible use of pristine CNTs in order to avoid accidental exposure via dermal absorption, inhalation, or ingestion. Among the potential scenarios for accidental exposure are included mishandling or uncontrolled release into the environment. Preventing exposure and absorption by the above-mentioned routes can be managed by appropriately engineered barriers, ventilation, and protective respiratory bar-

riers and attire. In addition, training and safety protocols designed to minimize exposure should be implemented. Environmentally responsible regulatory mandates could be enabled and enforced to manage disposal and containment of bulk quantities used in devices or in construction materials. The amounts anticipated for medical application should be much less in scale, but absorption prevention measures must still be considered.

### C. Metabolism

Metabolic biotransformation of CNTs *in vivo* will be relevant when using unstable covalent linkages, non-covalently assembled amphiphilic moieties, and other potentially biodegradable biologic components (e.g., protein, peptide, or nucleic acid). Interestingly, there have been reports that described the enzymatic degradation of functionalized CNT *in vitro* and *in vivo*.[36–40] The role of enzymatic biodegradation of functionalized CNTs is an intriguing characteristic to consider in the development of CNTs.

## VI. CONCLUSIONS

Nanotechnology concerns materials and systems whose structures and components exhibit novel and significantly improved physical, chemical, and biological properties, phenomena, and processes due to their nanoscale dimensions. The efficient development of CNT-based constructs will depend a great deal on performance *in vivo* as described by the distribution and excretion profiles of these agents. The development of a nanomedicine will depend on the incremental biodistribution profile of each synthetic modification step. The translational path to clinical use for any molecule begins with a PK analysis in an animal model and that data extrapolated to predict biodistribution and excretion in humans. CNT drug constructs built with targeting, reporting, and therapeutic capabilities should possess an acceptable PK and biocompatibility profile. As an example, in most bioimaging applications, rapid accumulation at the target site and a brief blood half-life is desired so that the signal-to-background ratio is optimized. Therefore, it is practical if the intended target can be easily accessed and marked by the CNT while the untargeted agent is removed from circulation and excreted.[13,17,25] Ideally, the f-CNT tracer will be administered, quickly locate the target, and bind while any untargeted CNT rapidly clears the host. In comparison, monoclonal antibody-based imaging agents have blood half-lives on the order of days, thus extending the time to optimally image. An appropriately designed CNT could achieve sufficient signal-to-background ratio to enable imaging within hours of administration. Targeting a vascular epitope is another potential strategy given the PK profiles described for either the covalent or non-covalently modified CNT. To date, CNTs have been covalently appended with a variety of radionuclide and fluorescent imaging modalities[13,17,18,21,23,24,29,33] and have shown to have an ability to target tumor[18,29,33] and tumor vascular endothelium[41] in animal models. Improving the PK performance of CNT with non-covalently appended moieties could be realized by knowing the binding affinity of the amphiphile to the CNT. For example, if one considers that the $K_d$ of an oligonucleotide/ammonium-SWCNT complex has nM affinity[42] and oligonucleotide/pristine SWCNT has μM affinity,[43] then the administered onboard dose can be tailored to maintain an assembled construct. In serum, once the concentration of the assembled construct drops below the $K_d$ value, the construct and amphiphile will dissociate and the SWCNT will cease to exhibit fibrillar pharmacology.

## ACKNOWLEDGMENTS

The paper was initiated on the basis of discussions by the authors with collaborators and colleagues at various meetings over the course of our research investigations in this area. This work was supported by the Office of Science (BER), U.S. Department of Energy (Award No. DE-SC0002456); Grants No. NIH R21 CA128406, No. R01 CA166078, No. R01 CA55399, and No. R25T CA046945; the Memorial Sloan-Kettering Center for Molecular Imaging and Nanotechnology (CMINT); and the Memorial Sloan-Kettering Experimental Therapeutics Center.

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