Advanced Drug Delivery Reviews 65 (2013) 2111–2119

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Pharmacology of carbon nanotubes: Toxicokinetics, excretion and tissue accumulation ☆

Hanene Ali-Boucetta [a], Kostas Kostarelos [b,*]

[a] Pharmacy, Pharmacology and Therapeutics, School of Clinical and Experimental Medicine, Medical School, University of Birmingham, Birmingham B15 2TT, UK
[b] Nanomedicine Lab, Faculty of Medical & Human Sciences and National Graphene Institute, University of Manchester, AV Hill Building, Manchester M13 9PT, UK

ARTICLE INFO

Article history:
Accepted 22 October 2013
Available online 31 October 2013

Keywords:
Carbon nanotubes
CNT
Biodistribution
Toxicokinetics
Functionalisation
Nanotoxicology

ABSTRACT

Carbon nanotubes (CNT) are increasingly being investigated for their use in biomedical applications and nanomedicine. An emergent need for the understanding of their in vivo biodistribution and pharmacokinetics is therefore needed to establish the essential properties and criteria for their further development as targeted CNT delivery systems to specific tissues for diagnostics and therapeutic purposes. Until their biodistribution and toxicoketic profiles are fully understood, their translation into the clinic will be hindered. This review will highlight the important factors affecting the biodistribution and pharmacokinetic profile of CNT and address their toxicokinetics following systemic, pulmonary and dermal exposure.

Contents

1. Introduction

The unique properties of carbon nanotubes (CNT) make them promising candidates for a range of biomedical applications ranging from imaging to therapy [1–6]. There is an emergent necessity for a fundamental understanding of their pharmacological and toxicological aspects, a critical step in the development of any pharmaceutical product especially in the early stages of development. Knowledge of the in vivo biodistribution and pharmacokinetics of CNT would therefore provide the basis and a foundation for further development of targeted CNT delivery systems to specific tissues for diagnostics and therapeutic uses.

Several research groups have investigated the biodistribution profile of CNT using different tracking modalities to qualitatively and quantitatively determine the preferential site of accumulation of CNT and the

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Carbon nanotubes in medicine and biology — Safety and toxicology".

* Corresponding author.
E-mail address: kostas.kostarelos@manchester.ac.uk (K. Kostarelos).

0169-409X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.addr.2013.10.004

associated toxicity. The most commonly used method is the radiolabelling of CNT using radioactive isotopes which allows quantitative data collection using gamma scintigraphy as well as qualitative dynamic imaging of the in vivo fate of CNT using SPECT/CT and PET imaging modalities [7–10]. Other techniques have also been used, such as Raman spectroscopy in an attempt to identify nanotube signatures within tissues [11]. More recently, optical (IVIS) imaging was used to visualise the biodistribution of thermostable, luciferase conjugated CNT [12].

Several physicochemical properties of CNT such as functionalisation, size, aggregation, and dispersibility have been reported to play an important role in determining the in vivo fate of CNT. The need for different laboratories to systematically investigate such structure–function relationships is required to allow possibilities for clinical translation. Interestingly, some of these properties can be engineered to achieve the best possible kinetic profile as will be discussed in this review. Herein, we are attempting to provide an overview of what has been learnt today regarding the biodistribution and toxicokinetics of carbon nanotubes.

2. Factors affecting CNT biodistribution

2.1. CNT surface modification

The modification of CNT surfaces has rendered nanotubes dispersible in physiologically-relevant, aqueous environments, revealed their interaction with the biological milieu and allowed for their investigation in biomedical applications [1,2,5,13–15]. The biodistribution profiles determined for different CNT types have already shown a strong dependence on the nature of surface modification. Below, biodistribution studies are classified in two categories based on the type of surface modification of the material.

2.1.1. Non-covalently functionalised (or coated) CNT

The first study reporting non-covalently (coated) CNT was that of Cherukuri et al. who used intrinsic near infrared fluorescence as a tracking method for the intracellular accumulation of the Pluronic F108 coated single-walled carbon nanotubes (SWNT) in rabbits [16]. It was found that SWNT mainly accumulated in the liver and exhibited a very rapid blood clearance ($t_{1/2} < 1$ h) which was attributed to the formation of SWNT–protein complexes or SWNT aggregates following protein competition with the Pluronic coating. In another study, the biodistribution of ^{13}C-enriched backbone SWNT coated with Tween 80 was investigated taking advantage of the isotopic abundance $^{13}C/^{12}C$ to quantify CNT in organs via mass spectroscopy [17]. It was found that the coated SWNT exhibited high affinity for the liver, lungs and spleen and remained in these organs for 28 days [17]. The possible elimination route of the coated SWNT material was thought to be from the lungs (from 15 to 9.4%) towards the spleen, however, neither of the above studies performed systematic in vivo toxicity or physiology investigations.

Liu et al. have investigated the biodistribution of SWNT coated with PEGylated phospholipid by positron emission tomography (PET) and Raman spectroscopy [18]. The effect of polyethylene glycol (PEG) chain length (2000 or 5400) in targeting cancer using the RGD peptide was investigated. Significant accumulation of all types of lipid-coated SWNT was observed in the liver and spleen. While these lipid-coated SWNT showed no tumour uptake in xenograft-bearing animals, the more integrin targeted constructs (SWNT-PEG_{5400}-RGD) demonstrated higher tumour accumulation of 10–15% of ID per gram tissue. In a more recent study, Liu et al. further investigated the effect of length and branching of PEG on the biodistribution of PEG-lipid coated SWNT [11]. Although, the liver and spleen were still the main organs of accumulation, there was an increase in blood circulation (up to 15 h) and a reduction in the liver uptake with the branched (7 kDa) PEG coated-SWNT. In addition to the biliary excretion being the main elimination route for these non-covalently PEGylated SWNT over a 2-month period, the possibility of SWNT of smaller dimensions (<50 nm in length, 1–2 nm in diameter) excreted via the urine at early time points was also suggested. Prencipe et al. [19] later synthesised different surfactant polymers based on poly(γ-glutamic acid; γPGA) and poly(maleic anhydride-alt-1-octadecene; $PMHC_{18}$) and increased PEGylation. It was found that SWNT coated with the polymers exhibited a very long circulation half-life of up to 22 h following i.v. administration which is considered to be due to the highly dense PEG coating resulting from the multiple anchoring domains and PEG chains compared to single anchoring points with lipid PEG coating. Similarly, Liu et al. [20] used $PMHC_{18}$ polymer anchored with different PEG lengths (2 kDa–5 kDa) at several densities (5%–100%). Following intravenous injection of $PMHC_{18}$-PEG coated SWNT in 4T1 murine cancer bearing Balb/C mice and covalently that tumour and skin uptake of SWNT increased in correlation with the increase in degree of PEGylation (length and density of PEG chains). Although the longest circulation half-life was observed with 100% 5 kDa (PEG-$PMHC_{18}$)-SWNT, RES organ uptake was still observed. The same study also reported blood half-lives of 12–13 h as optimum to achieve a balanced tumour-to-normal organ uptake ratio.

It is important to note that these studies used the same type of nanotubes (SWNT) and the same strategy of non-covalent functionalisation using different types of coating. Moreover, most of the techniques employed to study the biodistribution of these macromolecule-coated CNT require preservation of the pristine carbon backbone, since any structural defects may damage their electronic properties and would not allow the use of near infrared or Raman spectroscopy. Overall, it has been found that pristine, non-covalently functionalised SWNT accumulate in RES organs (mainly the liver).

2.1.2. Covalently-functionalised CNT

Several studies have been published describing the biodistribution of covalently functionalised CNT in vivo, most of which used SWNT [10,21–25]. The first such study was that of Wang et al. using iodine-125 labelled hydroxylated SWNT (^{125}I-SWNT-OH) injected intraperitoneally (i.p.) [23]. A rapid distribution of ^{125}I-SWNT-OH was reported in most organs with the highest affinity to the stomach, kidney and bone. More significantly, ^{125}I-SWNT-OH were found to be excreted mainly via the urine (94%). No significant differences in tissue distribution were reported for the different administration routes (subcutaneous, stomach gavage and intravenous) assessed. In a more recent study, Wang et al. looked at the in vivo fate of ^{131}I-SWNT-OH shortly after i.v. and i.p. administrations and reported their fast distribution into organs as observed within 2–60 min post-injection [25]. Interestingly, the kidneys showed the highest uptake with 24% ID after 2 min post-injection with a slight reduction in the signal within 60 min of the experiment.

Guo et al. reported a very similar biodistribution study to that of Wang et al. in which MWNT were functionalised with glucosamine, subsequently labelled with Technetium-99 (^{99m}Tc-MWNT-glu) and injected i.p. into mice [22]. ^{99m}Tc-MWNT-glu exhibited a rapid organ biodistribution and a blood circulation half-life ($t_{1/2}$) of 5.5 h. In addition, significant amounts of MWNT were retained in the kidneys over 24 h with more than 75% of radioactivity found in the urine and faeces. However, in a later study using taurine-functionalised MWNT (^{14}C-taurine-MWNT) the same group observed high affinity to the liver (80% of ID) after i.v. administration and residual retention for 28 days with gradual elimination within 3 months [24]. Deng et al. also investigated the effect of alternative administration routes other than i.p., by stomach gavage and intratracheal instillation (i.t.) to compare the in vivo fate of *f*-MWNT [24]. High accumulation in the small and large intestines for the stomach gavage and higher affinity to the lungs after i.t. administration were reported respectively. Similar findings of predominant liver accumulation were reported by the same group [26] in which a novel radiotracing technique was adopted yielding ^{125}I-taurine-MWNT. In another study, Yang et al. used covalently PEGylated SWNT to observe 30% ID retained in the blood compartment one day post-injection, quantified by isotope-MS [27].

Other studies performed in our laboratories, looked at the tissue distribution of *f*-CNT using the surface functionalisation chemistry based on the 1,3-dipolar cycloaddition reaction yielding ammonium functionalised CNT. Singh et al. prepared SWNT-NH_3^+ further functionalised with the chelating molecule diethylenetriaminepentaacetic (DTPA) and subsequently radiolabelled with indium-111 ([^{111}In] DTPA-SWNT) which was then intravenously injected into mice [21]. The study demonstrated tissue distribution of *f*-SWNT in the kidney, muscle, skin, bone and blood after 30 min post-injection. Interestingly, *f*-SWNT were observed to be excreted from the body via the bladder–urine route with a maximum blood circulation half-life of 3.5 h.

In a follow-up study, radiolabelled multi-walled nanotubes [^{111}In] DTPA-MWWNT were administered i.v. in rats and found to be mainly localised in the kidneys within 30 min post-injection and subsequently excreted via the urine as studied by dynamic whole body single photon emission computed tomography (SPECT/CT) [28]. We have highlighted the importance of the degree of chemical surface functionalisation as a key determinant of the extent of nanotube individualisation that appears to lead to urinary excretion [7,8,29]. All these observations were broadly in agreement with the data reported by others on hydroxylated (^{125}I-SWNT-OH) and glucosamine functionalised (^{99m}Tc-MWNT-glu) despite the very different chemical processing, chemical treatment and starting material.

Following these initial studies, McDevitt et al. repeated independently the same functionalisation chemistry as Singh et al. [21] and Lacerda et al. [28,29] to produce ammonium functionalised SWNT labelled with ^{111}In, using 2-(4-isothiocyanatobenzyl)-1,4,7,10-tetrraazacyclodecane (DOTA) as the chelating molecule [10]. In that work monoclonal antibodies (Rituximab) were also conjugated onto the SWNT to offer tissue targeting. The differences in tissue biodistribution of [^{111}In] DOTA-SWNT-NH_3^+ and the antibody-targeted construct ([^{111}In] DOTA-SWNT-Rituximab) were assessed. The [^{111}In] DOTA-SWNT accumulated mainly in the kidney, spleen, liver and bone showing rapid blood clearance. Antibody conjugation affected the tissue biodistribution as evidenced by a decrease in kidney residence time and a 2-fold increase in liver affinity with no change in spleen accumulation. This study and a subsequent similar investigation by the same group [9] are generally in good agreement with the original observations of Singh et al. and confirmed that rapid clearance and urinary excretion of CNT occur, provided appropriate chemical functionalisation has been performed.

Although pharmacokinetic studies are quantitative in nature, scarce data have been in currently available biodistribution studies with CNT. The half-life of CNT in blood circulation is the only parameter identified in most CNT pharmacokinetic studies, even though not accurately, nor reproducibly among different techniques (Table 1). In addition, the type of CNT functionalisation, the route of administration and the methods of CNT tracking in vivo are also highlighted in Table 1.

2.2. Surface density of functionalisation (mainly covalent)

We have recently reported that by tuning the degree of surface functionalisation on multi-walled nanotubes, a greater control over their organ biodistribution and clearance could be achieved [8]. It was found that the higher the functionalisation density on the surface of CNT, the less RES accumulation and the higher the urinary excretion of CNT observed as highlighted in Fig. 1. This systematic study correlated with previous studies from our laboratory [7,29] as well as Ruggiero et al. [30] who showed that high ammonium functionalised SWNT (via the 1,3-dipolar cycloaddition) were excreted intact via the glomerular filtration system as shown in Fig. 2.

Jain et al. [31] also highlighted the importance that density of functionalisation on carboxylated MWNT played on the tissue distribution and toxicokinetic profile. It was found that well-individualised MWNT with shorter lengths (<500 nm) and a high degree of oxidation (surface carboxyl density > 3 μmol/mg) were rapidly cleared from the body through renal excretion without causing nephrotoxicity. In contrast, pristine MWNT and those with lower degrees of carboxylation showed higher retention in RES organs such as the liver, spleen and lungs. The effect of increasing PEG grafting density on the biodistribution of covalently PEGylated CNT is further discussed in the following section.

2.2.1. PEGylation of CNT

In order to enhance the circulation half-life of nanoparticles a polymeric coat consisting of polyethylene glycol (PEG) is very commonly introduced on their surface using a variety of methodologies. This PEG coat can offer a shield against protein adsorption, recognition by circulating macrophages and RES organs, therefore enhance their blood circulation [32]. Carbon nanotube PEGylation has been described by several laboratories for the development of CNT in biomedical applications [15,18,33,34]. PEGylation can be performed either by physisorption and coating of the polymer around the CNT [11,18] or by covalent attachment

Table 1
Pharmacokinetic parameters of carbon nanotubes in vivo.

CNT type	Functionalisation	Route of administration	Half-life (h)	Volume of distribution (ml)	Method of CNT tracking	References
SWNT	1,3 dipolar cycloaddition (covalent)	Intravenous	3–3.5	/	Gamma scintigraphy (Indium^{111})	[21]
SWNT	Copolymer (Pluronic F108) (non-covalent)	Intravenous	1	/	Near infrared fluorescence	[16]
MWNT	Carboxylation (covalent)	Intraperitoneal	5.5	/	Gamma scintigraphy (Technetium^{99m})	[22]
SWNT	PEGylated-lipid (non-covalent)	Intravenous	5 (SWNT-linear-5K PEG) 15 (SWNT-branched-7K PEG)	/	Raman spectroscopy	[11]
SWNT	PEGylated-lipid (non-covalent)	Intravenous	3.3	/	Raman spectroscopy	[33]
SWNT	Polymer m-PEG-$PMHC_{18}$[a] (non-covalent)	Intravenous	20.8 (100% 5K PEG)	/	Raman spectroscopy	[20]
SWNT	Polymer m-PEG-$PMHC_{18}$[a] (non-covalent)	Intravenous	30 (5K PEG)	/	Near-infrared fluorescence	[60]
MWNT	1,3 dipolar cycloaddition carboxylation + amidation (low functionalisation) Carboxylation + amindation (high functionalisation) (covalent)	Intravenous	0.019 0.053 0.013	3.995 5.219 1.904	Gamma scintigraphy (Indium^{111})	[8]

[a] Poly(maleic anhydride-alt-1-octadecene), methoxy poly (ethylene glycol).

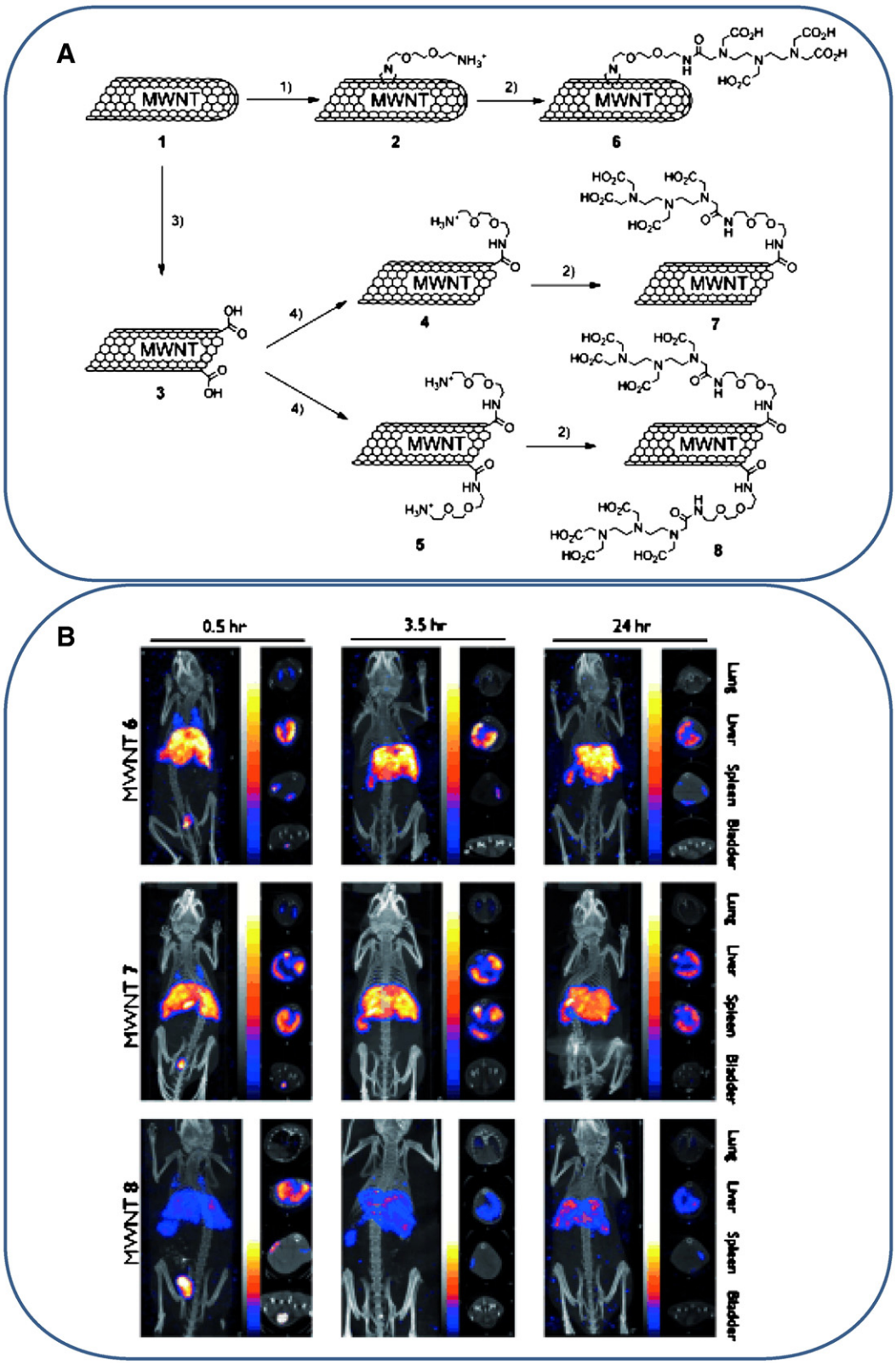


Fig. 1. (A) Synthesis of the different DTPA-MWNTs. 1) 1. Boc-NH-$(CH_2CH_2O)_2$-CH_2CH_2-NH-CH_2-COOH/$(H_2CO)n$ in dimethylformamide (DMF); 2. 4 M HCl in dioxane. 2) *N*,*N*-diisopropylethylamine/DTPA in dry DMSO/DMF. 3) H_2SO_4/HNO_3 (3:1), sonication. 4) 1. Neat $(COCl)_2$; 2. Boc-NH-$(CH_2CH_2O)_2$-CH_2CH_2-NH_2, dry tetrahydrofuran; 3. 4 M HCl in dioxane. Boc = *tert*-butyloxycarbonyl (B) Whole-body imaging. Nano-SPECT/CT fused images of the whole mouse. Images were taken at 0.5, 3.5 and 24 h post-injection for MWNTs 6, 7, and 8 after injection of 50 μg of ^{111}In-DTPA-MWNTs with a scanning time up to 0.5 h. Adapted from reference [8].

of the polymer on the CNT backbone. The stability of the PEG coat in the bloodstream is generally believed to be stable, especially following numerous studies [11,19,20] reporting that non-covalent PEGylation provides longer blood circulation half-life when PEGylation density is increased. While most of these studies were performed using SWNT, it is still to be demonstrated that the same effect is observed for MWNT.

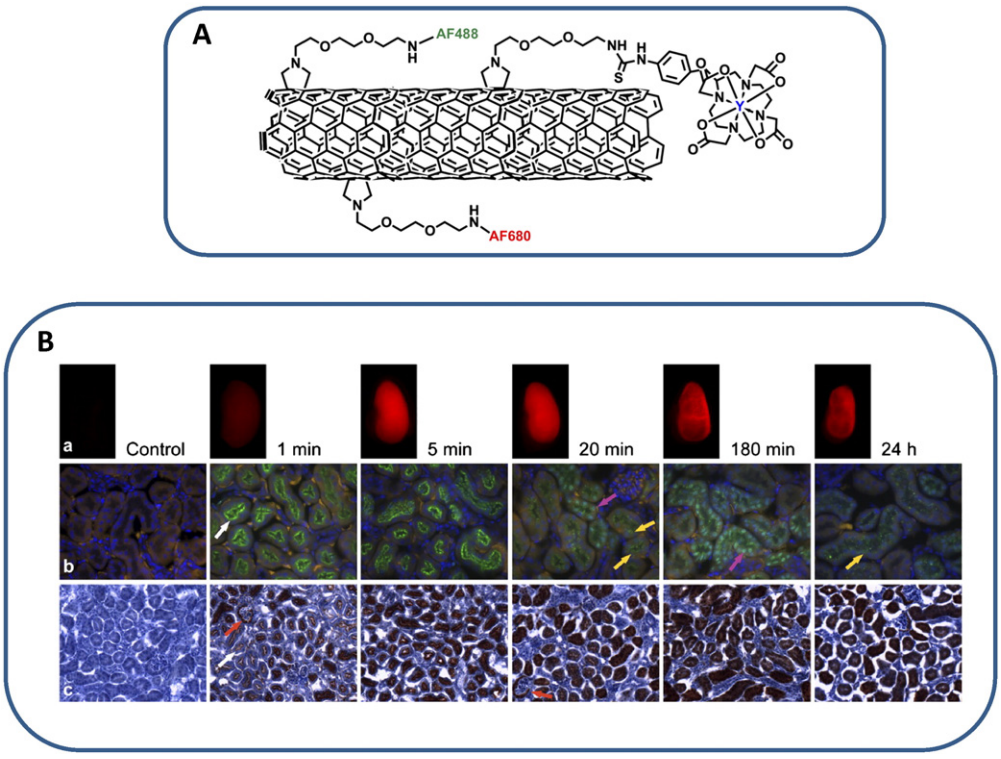


Fig. 2. (A) Water-soluble CNT covalently functionalised with DOTA, AF488, and AF680. Schematic representation of the key appended moieties of the SWCNT-[([^{86}Y] DOTA)(AF488)(AF680)] construct. (B) NIR images of harvested kidneys and corresponding IF and IHC microscopic sections of the kidneys of mice injected with SWCNT-[(DOTA)(AF488)(AF680)]. Time course imaging of kidneys of animals injected with SWCNT-[(DOTA)(AF488)(AF680)] using NIR imaging (a), IF (composite image: DAPI + AF488 + TRITC) (b), and IHC (c). Reported is construct accumulation in the proximal tubule brush border (white arrows) and glomerulus (red arrows) in the first minutes and progressively cytoplasmic (yellow arrows) and nuclear accumulation (magenta arrows) in tubular cells.
Adapted from reference [30].

Most covalently PEGylated CNT have been based on the conjugation of amine terminated PEG to oxidised or hydroxy-terminated CNT. The first study to describe covalently PEGylated CNT was performed by Yang et al. in 2008 [27] using ^{13}C-enriched SWNT with diamine-terminated PEG (PEG_{1500N}). High aqueous dispersibility was reported (at ~20 mg/ml) and an increased blood circulation half-life of 15.3 h. However, liver and spleen uptake was observed with this construct up to 7 days post-injection as confirmed by isotope ratio mass spectroscopy. Bhirde et al. [35] further investigated the potential of PEGylation on the in vivo fate of SWNT and their therapeutic potential in cancer therapy. Qualitative information based on the Raman signature of SWNT in excretion samples suggested that PEGylated SWNT circulated for up to 7 days in the body, while their pristine counterparts were not detected in the excreted materials after 2 days post-injection that could indicate their entrapment in vital organs. Histopathological examination showed that the lungs were the preferred site of accumulation for the oxidised SWNT with signs of inflammatory responses due to the presence of inflammatory mononuclear cells. PEGylated SWNT still showed lung accumulation, but to a lower degree and with a mild inflammatory reaction.

Few data on the toxicity from intravenously injected CNT has been generally been reported. Zhang et al. [36] assessed liver toxicity of PEGylated MWNT in comparison to pristine MWNT dispersed in 1% Tween20 after i.v. injection. PEGylated MWNT induced less hepatic injury compared to pristine MWNT as indicated by no significant changes in levels of liver enzymes. These results indicated that PEGylation of CNT could partially but not exclusively improve the biocompatibility of CNT.

In more recent work, Yang et al. [37] showed that MWNT-PEG_{4000}-OH had a short blood half-life of 5.2 min, thought to be due to the change in the type of CNT (MWNT versus previously used SWNT) and hence the difference in length/diameter ratio. Another reason could be attributed to the difference in the terminal group of the PEG. Previously, amine-terminated PEG [38] was used to confer a long blood circulation half-life, in contrast to this latter study, where hydroxyl-terminated PEG was used. Similar differences have been reported for amino-terminated PEG functionalised quantum dots (QDs) that showed longer circulation half-lives compared to hydroxyl and carboxyl terminated PEG-QDs [39]. Interestingly, increases in PEG chain length to 20 kDa also led to a significant reduction in hepatic accumulation (for MWNT-PEG_{20000}-OH).

2.3. Dispersibility, aggregation and individualisation of CNT

The second most important factor to determine CNT biodistribution is the degree of aqueous dispersibility of the administered CNT population. Highly dispersed CNT show better pharmacokinetic profiles than agglomerated CNT because it is harder for RES to recognize individual nanotubes compared to aggregated bundles. In order to disperse pristine CNT, they are commonly mildly sonicated or vortexed in serum, surfactants or block copolymers, therefore can be considered 'coated' CNT according to the classification described earlier. Lacerda et al. [29] showed that serum-coated pristine MWNT accumulated mainly in the liver, spleen and lungs compared to ammonium functionalised MWNT. MWNT aggregates were also observed in the lungs which was not the case for their functionalised counterpart. Cherukuri et al. [16] highlighted that serum proteins may compete in vivo with the non-covalent coating at the CNT surface leading to desorption and subsequent rapid aggregation in the bloodstream which will favour recognition by macrophages. Generally, a well-functionalised CNT population should also achieve effective individualisation of the nanotubes and stable dispersion in physiologically-relevant environments.

2.4. Other factors affecting the biodistribution of CNT

It is challenging to compare between different studies, mainly due to the different CNT preparation methodologies, functionalisation

strategies and dose regimes followed. More coordinated work is needed to determine possible differences between the biodistribution pattern of various CNT types, even between SWNT and MWNT. Based on our work, both SWNT and MWNT that have been ammonium-functionalised using the 1,3 dipolar cycloaddition have been shown to have a similar biodistribution profile that at high degrees of functionalisation can lead to predominant urinary excretion [7,21]. The length of CNT is also an important factor to consider. Murphy et al. [40] reported that shorter nanotubes, currently being investigated in nanomedicine applications, were cleared from the pulmonary pleural space and into the lymph nodes after intrapleural administration. The importance of length in CNT biodistribution was also discussed by Jain et al. [31]. Well-individualised and short MWNT (<500 nm) were rapidly excreted through the kidneys without causing nephrotoxicity. However, pristine MWNT and longer MWNT showed a stronger retention in RES organs such as the liver, spleen and lungs and induced significant hepatotoxicity in mice.

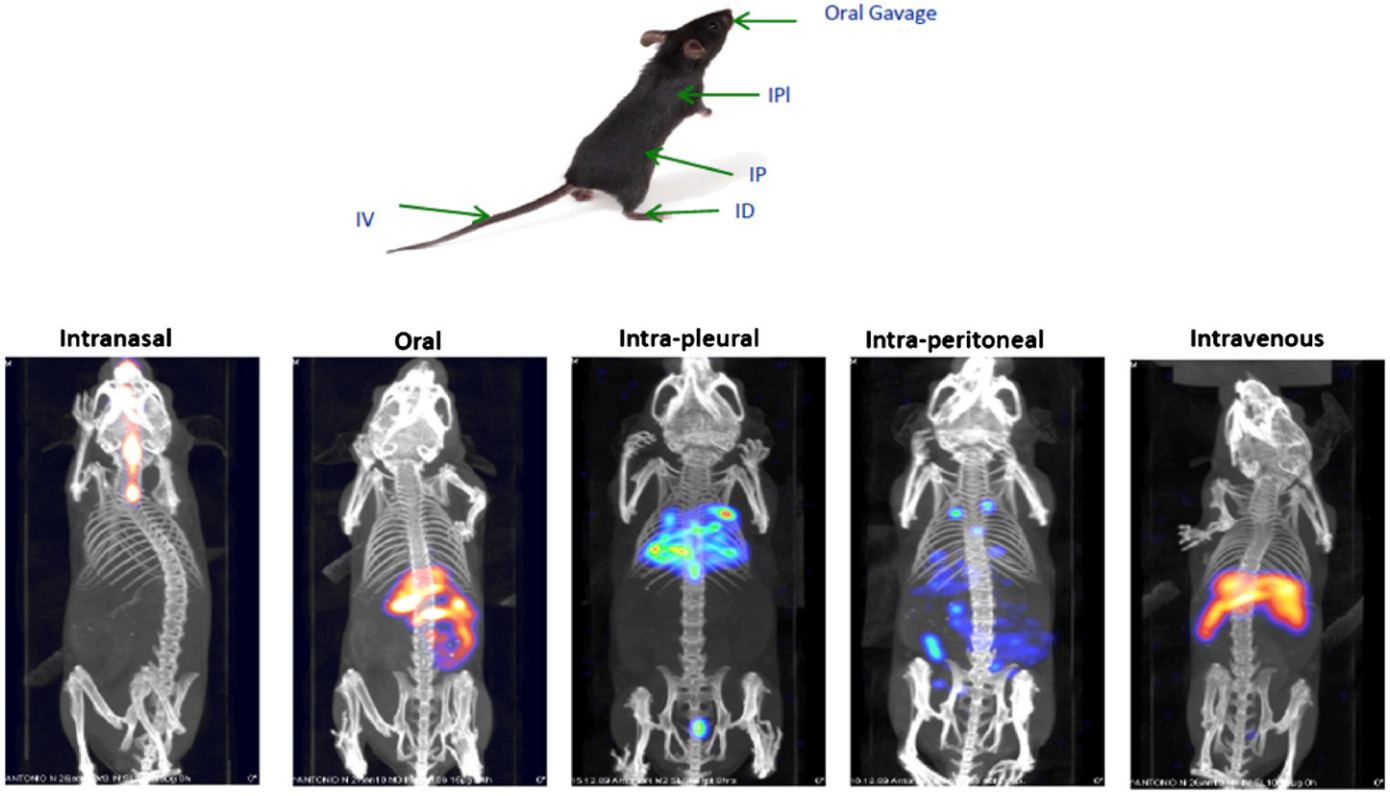
3. Carbon nanotube toxicokinetics

Several laboratories have investigated the toxicokinetics of CNT using different animal models and toxicity paradigms in an attempt to investigate whether CNT may be associated with possible adverse effects on human health. Most investigations have focused on pulmonary and dermal tissue exposure, with only a few studies examining the effects of nanotubes administered systemically for the development of therapeutics and diagnostics. The toxicokinetic profile of any nanomaterial, including CNT, will be highly dependent on the route of administration that will determine the tissues of maximum exposure; hence we reviewed different studies according to this categorisation. Scheme 1 highlights the importance of routes of administration on the biodistribution and toxicokinetic profile of CNT.

3.1. Systemic blood circulation

Most studies exploring the toxicological impact of CNT after systemic administration have been conducted in the context of developing CNT for biomedical applications. Following subcutaneous injection of oxidised MWNT, Sato et al. [41] found that longer MWNT (825 nm) mediated a stronger immune response compared to their shorter counterparts (220 nm in length). The results were explained in this case on the basis of macrophages inability to engulf the longer MWNT. Koyama et al. [42] studied the effect of metal impurities on the toxicological profile of subcutaneously injected non-purified and purified MWNT and observed the induction of immunological toxicity and localised alopecia with as-produced, non-purified MWNT. In another study, Carrero-Sanchez et al. [43] described no signs of toxicity or biochemical and histopathological alterations after oral and intraperitoneal administration of pristine and N-doped MWNT. However, when SWNT and MWNT dispersed in Tyrode solution were intravenously injected by Radomski and co-workers [44], platelets were easily stimulated by CNT causing an enhancement in the rate of carotid artery thrombosis formation.

Focusing more on studies that used chemically-functionalised CNT for biomedical applications, Yang et al. [17] found that neither the pristine, nor the PEGylated SWNT showed signs of acute toxicity even at higher doses of 80 mg pristine and 24 mg PEGylated SWNT per kg body mouse weight. Interestingly, Lacerda et al. [29] found that intravenously injected ammonium functionalised MWNT did not cause any physiological or pathological abnormalities 24 h post-injection even at high injected doses (20 mg/kg) in Balb/C mice. That work again emphasized the importance of adequate chemical functionalisation leading to individually dispersed CNT as a critical factor for the prevention of toxicity and organ accumulation. In addition, Schipper et al. [45] showed no haematological or histopathological alterations when SWNT coated with PEGylated lipids were injected i.v. and assessed up to 4 months



Scheme 1. SPECT/CT imaging of CNT injected through different routes of administration. The toxicokinetic profile of CNT is highly dependent on the route of administration as it determines the main organs of high exposure to CNT (e.g. intravenous injection of low-functionalised CNT accumulate in the liver and spleen, while if orally administered they accumulate in the stomach and intestines).

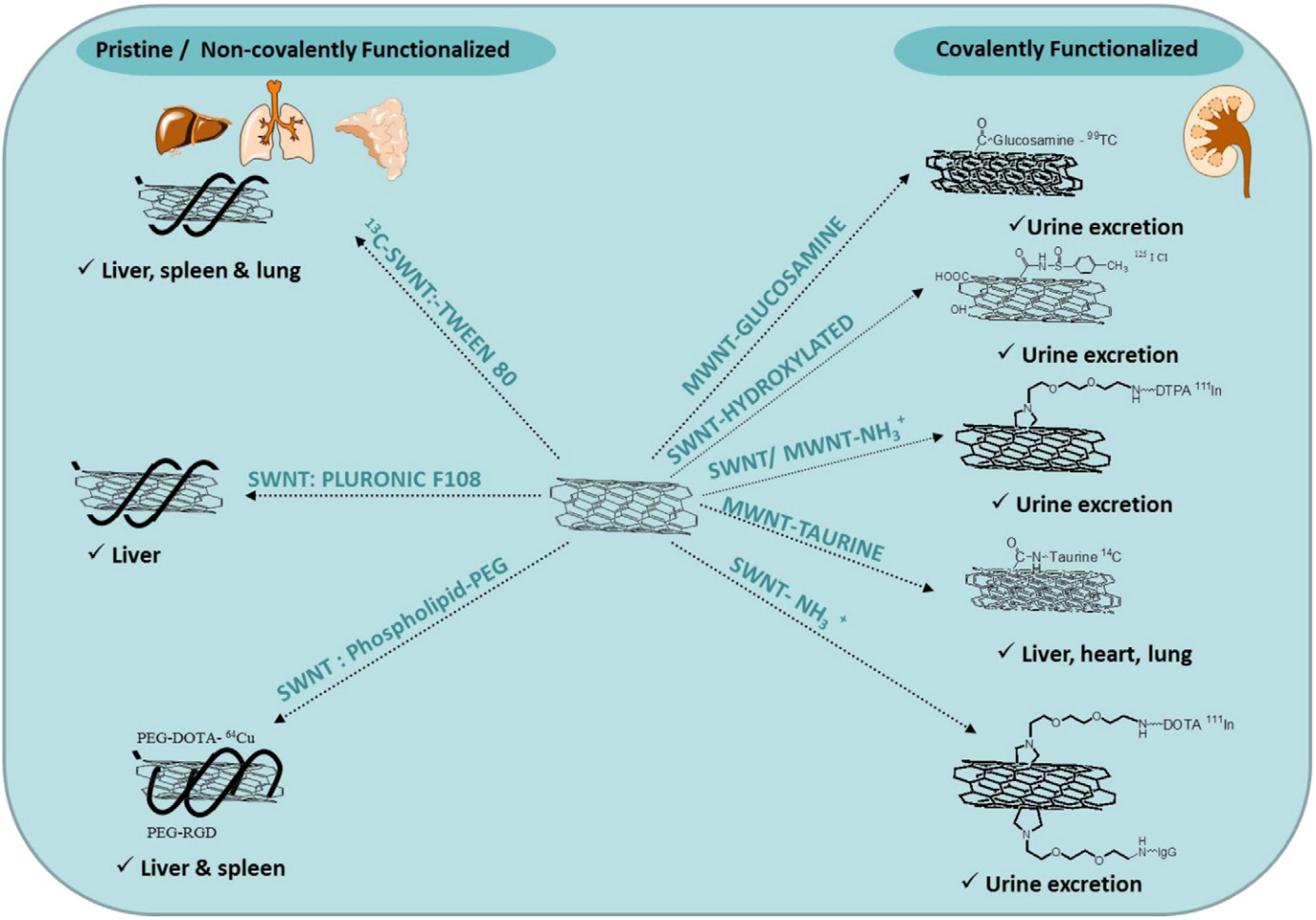
post-injection. However, when Yang et al. [46] injected purified SWNT (coated with 1% Tween 80) i.v. in (male CD-ICR) mice, serum biochemical changes and pulmonary inflammation were observed in the absence of induction of apoptosis or changes in immunological markers 3 months post-injection. In order to study whether splenic toxicity (the spleen being one of the major organs of CNT accumulation) was occurring, Deng et al. [47] found that glucosamine-functionalised MWNT (i.v. injected) did not cause any change in the phagocytic activity nor a reduction in glutathione, superoxide dismutase of the spleen over 2 months.

Although comparisons between the currently published data on CNT toxicokinetics are difficult because of differences in several important material parameters such as size, dispersibility, functionalisation, and metal catalyst contaminants, one can observe that agglomeration and low dispersibility of as-produced, pristine CNT are major contributing factors for toxicity. Chemical functionalisation that improves CNT dispersibility leads to dramatically reduced cytotoxicity both in vitro and in vivo. Another major contributing factor is the presence of metal catalysts which complicates the cytotoxicity profile as the intrinsic nanotube contribution to toxicity is difficult to identify. Detailed chemical and structural knowledge and characterisation of the nanomaterial should always be provided in toxicokinetics assessments of CNT to allow a clearer determination of contributing factors. More systematic studies should also be carried out with chemically functionalised CNT developed for biomedical applications, to determine the effect of material characteristics (such as shortening) that make CNT biocompatible with the biological milieu.

3.2. Pulmonary

Most in vivo toxicokinetic studies have focused on the pulmonary route of exposure to CNT because of its importance to public and occupational health. From the toxicokinetic point of view, the lungs and residence time of the nanomaterial in the pulmonary tissue are of primary concern. Maynard and co-workers [48] have investigated the release of particles from unrefined SWNT into the air and its effect on workers at a small scale production facility. They reported that very low airborne particle concentrations were generated as a consequence of handling unrefined materials with no effects. That is the only published work to date studying direct human exposure with CNT. Li et al. [49] reported that MWNT caused proliferation and thickening of lung alveolar walls in mice as a result of the size and aggregation of MWNT. Mitchell and co-workers [50] observed no lung damage after 14-day exposure of mice to MWNT, but instead detected suppression of systemic immunity. More recently, the same group has also offered a mechanistic explanation of this observation suggesting that protein signalling from the lung (where the CNT reside) activates pathways in the spleen leading to suppression of immunity [51].

Intra-tracheal instillation (i.t.) is one of the main methodologies used to study the pulmonary toxicokinetics of CNT. Carrero-Sanchez et al. [43] stated that N-doped MWNT were safer than non-doped pristine nanotubes after i.t. injection which might be due to their improved dispersibility. In addition, Li et al. [49] observed that aggregated MWNT can reach the lung directly by by-passing the mucocilliary system, leading to persistent pathological lesions in bronchi and alveoli. Other



Scheme 2. The current status of CNT biodistribution based on the nature of functionalisation. Pristine and non-covalently functionalised CNT accumulate predominantly in liver, spleen and lungs, while covalently functionalised CNT are reported to be excreted in the urine.

groups have also reported that the agglomeration of SWNT in the airways is the primary cause of morbidity [52] and granuloma formation [53]. Using guinea pigs, Huczko et al. [54,55] examined the effect of CNT characteristics and the duration of exposure on the degree of respiratory distress observed together with any induced lung pathology. Elgrabli et al. [506] used bovine serum albumin (BSA) to improve the dispersibility of MWNT and observed no inflammatory, physiological or histological pathologies, however BSA-coated MWNT were found inside alveolar macrophages which became apoptotic in an attempt to eliminate the CNT. Lastly, metal catalyst impurities seem to also play a role on the in vivo toxicity as studied by Lam et al. [53] using SWNT that contained residual catalyst.

Other studies have also reported that CNT can translocate the pulmonary tissue and reach the systemic circulation [50]. It may therefore be the case that pulmonary toxicity as well as risk of exposure from systemic side effects can occur as a result of low level chronic inhalation exposure to CNT [57]. In the same context, Erdely et al. [58] have emphasized the possible inter-connections between pulmonary residence and systemic circulation, reporting that exposure to SWNT and MWNT could induce acute pulmonary and systemic effects as characterised by an increase in lung and blood gene and protein expression markers [58]. More studies to further explore possible complex mechanisms triggered and correlation with the kinetic profile of the material from the lungs to the lymphatic system and consequently into blood circulation are needed.

3.3. Dermal

Maynard et al. [48] investigated the effect of handling unrefined CNT in a laboratory production facility and found about 0.2–6 mg per hand of CNT deposits on gloves, indicating that dermal exposure can occur on unprotected body regions. The use of protective clothing in order to minimise dermal exposure to CNT was therefore suggested. Interestingly, Huczko et al. [59] found no association between working with soot containing CNT and the risk from possible skin allergies as assessed by various dermatological tests on human volunteers and rabbits. More work is prerequisite in this field to further explore the toxicokinetic profile of CNT after dermal exposure; however we speculate that their distribution profile will be highly dependent on the physicochemical characteristics of the nanotubes, the dispersing agents, coating materials or functional group and their length.

4. Conclusions

Even though CNT have been explored as novel nanomaterials at the cutting edge of biomedical applications, we are still learning about their biokinetic behavior in vivo. More mechanistic understanding of the interactions between well-characterised CNT needs to be obtained to allow their translation in a clinical setting. A comparison between the different pharmacological studies using nanotubes published today, offers a broad and not conclusive, albeit very informative overall picture. The indication is that chemical functionalisation of CNT can lead to significant and rapid urinary excretion. On the other hand, pristine or non-covalently coated CNT show preferential and predominant accumulation in RES organs (liver and spleen). This overall picture can be summarised schematically in Scheme 2. It has to be noted however that such observations are not absolute, due to the complexity of the processes taking place. Accurate determination of fractions of the injected nanotube material and the duration of residency to different tissues will depend on the sensitivity of the techniques used. The degree of chemical functionalisation as well as the quality of the dispersion seem to play a critically important role in the biodistribution and toxicokinetic profile of CNT. It will very much depend on the specific biomedical application to decide what will be the preferred pharmacological pattern of choice.

References

[1] H. Ali-Boucetta, K.T. Al-Jamal, D. McCarthy, M. Prato, A. Bianco, K. Kostarelos, Multiwalled carbon nanotube–doxorubicin supramolecular complexes for cancer therapeutics, Chem. Commun. (2008) 459–461.

[2] A. Bianco, K. Kostarelos, C.D. Partidos, M. Prato, Biomedical applications of functionalised carbon nanotubes, Chem. Commun. 5 (2005) 571–577.

[3] S.Y. Hong, G. Tobias, K.T. Al-Jamal, B. Ballesteros, H. Ali-Boucetta, S. Lozano-Perez, P.D. Nellist, R.B. Sim, C. Finucane, S.J. Mather, M.L.H. Green, K. Kostarelos, B.G. Davis, Filled and glycosylated carbon nanotubes for in vivo radioemitter localization and imaging, Nat. Mater. 9 (2010) 485–490.

[4] K. Kostarelos, Carbon nanotubes: fibrillar pharmacology, Nat. Mater. 9 (2010) 793–795.

[5] K. Kostarelos, A. Bianco, M. Prato, Promises, facts and challenges for carbon nanotubes in imaging and therapeutics, Nat. Nanotechnol. 4 (2009) 627–633.

[6] J. Podesta, T.K. Al-Jamal, M.A. Herrero, B. Tian, H. Ali-Boucetta, V. Hegde, A. Bianco, M. Prato, K. Kostarelos, Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model, Small (2009) 1176–1185.

[7] L. Lacerda, M.A. Herrero, K. Venner, A. Bianco, M. Prato, K. Kostarelos, Carbon-nanotube shape and individualization critical for renal excretion, Small 4 (2008) 1130–1132.

[8] K.T. Al-Jamal, A. Nunes, L. Methven, H. Ali-Boucetta, S. Li, F.M. Toma, M.A. Herrero, W.T. Al-Jamal, H.M.M. ten Eikelder, J. Foster, S. Mather, M. Prato, A. Bianco, K. Kostarelos, Degree of chemical functionalization of carbon nanotubes determines tissue distribution and excretion profile, Angew. Chem. Int. Ed. 51 (2012) 6389–6393.

[9] M.R. McDevitt, D. Chattopadhyay, J.S. Jaggi, R.D. Finn, P.B. Zanzonico, C. Villa, D. Rey, J. Mendenhall, C.A. Batt, J.T. Njardarson, D.A. Scheinberg, PET imaging of soluble yttrium-86-labeled carbon nanotubes in mice, PLoS One 2 (2007) e907.

[10] M.R. McDevitt, D. Chattopadhyay, B.J. Kappel, J.S. Jaggi, S.R. Schiffman, C. Antczak, J.T. Njardarson, R. Brentjens, D.A. Scheinberg, Tumor targeting with antibody-functionalized, radiolabeled carbon nanotubes, J. Nucl. Med. 48 (2007) 1180–1189.

[11] Z. Liu, C. Davis, W. Cai, L. He, X. Chen, H. Dai, Circulation and long-term fate of functionalized, biocompatible single-walled carbon nanotubes in mice probed by Raman spectroscopy, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 1410–1415.

[12] R. El-Sayed, M. Eita, Å. Barrefelt, F. Ye, H. Jain, M. Fares, A. Lundin, M. Crona, K. Abu-Salah, M. Muhammed, M. Hassan, Thermostable luciferase from Luciola cruciate for imaging of carbon nanotubes and carbon nanotubes carrying doxorubicin using in vivo imaging system, Nano Lett. 13 (2013) 1393–1398.

[13] A. Bianco, K. Kostarelos, M. Prato, Making carbon nanotubes biocompatible and biodegradable, Chem. Commun. 47 (2011).

[14] C. Samorì, H. Ali-Boucetta, R. Sainz, C. Guo, F.M. Toma, C. Fabbro, T. da Ros, M. Prato, K. Kostarelos, A. Bianco, Enhanced anticancer activity of multi-walled carbon nanotube–methotrexate conjugates using cleavable linkers, Chem. Commun. 46 (2010) 1494–1496.

[15] N.W.S. Kam, M. O'Connell, J.A. Wisdom, H. Dai, Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 11600–11605.

[16] P. Cherukuri, C.J. Gannon, T.K. Leeuw, H.K. Schmidt, R.E. Smalley, S.A. Curley, R.B. Weisman, Mammalian pharmacokinetics of carbon nanotubes using intrinsic near-infrared fluorescence, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 18882–18886.

[17] S.-t. Yang, W. Guo, Y. Lin, X.-y. Deng, H.-f. Wang, H.-f. Sun, Y.-f. Liu, X. Wang, W. Wang, M. Chen, Y.-p. Huang, Y.-P. Sun, Biodistribution of pristine single-walled carbon nanotubes in vivo, J. Phys. Chem. C 111 (2007) 17761–17764.

[18] Z. Liu, W. Cai, L. He, N. Nakayama, K. Chen, X. Sun, X. Chen, H. Dai, In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice, Nat. Nanotechnol. 2 (2007) 47–52.

[19] G. Prencipe, S.M. Tabakman, K. Welsher, Z. Liu, A.P. Goodwin, L. Zhang, J. Henry, H. Dai, PEG branched polymer for functionalization of nanomaterials with ultralong blood circulation, J. Am. Chem. Soc. 131 (2009) 4783–4787.

[20] X. Liu, H. Tao, K. Yang, S. Zhang, S.-T. Lee, Z. Liu, Optimization of surface chemistry on single-walled carbon nanotubes for in vivo photothermal ablation of tumors, Biomaterials 32 (2011) 144–151.

[21] R. Singh, D. Pantarotto, L. Lacerda, G. Pastorin, C. Klumpp, M. Prato, A. Bianco, K. Kostarelos, Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 3357–3362.

[22] J. Guo, X. Zhang, Q. Li, W. Li, Biodistribution of functionalized multiwall carbon nanotubes in mice, Nucl. Med. Biol. 34 (2007) 579–583.

[23] H. Wang, J. Wang, X. Deng, H. Sun, Z. Shi, Z. Gu, Y. Liu, Y. Zhao, Biodistribution of carbon single-wall carbon nanotubes in mice, J. Nanosci. Nanotechnol. 4 (2004) 1019–1024.

[24] X. Deng, G. Jia, H. Wang, H. Sun, X. Wang, S. Yang, T. Wang, Y. Liu, Translocation and fate of multi-walled carbon nanotubes in vivo, Carbon 45 (2007) 1419–1424.

[25] J. Wang, X. Deng, S. Yang, H. Wang, Y. Zhao, Y. Liu, Rapid translocation and pharmacokinetics of hydroxylated single-walled carbon nanotubes in mice, Nanotoxicology 2 (2008) 28–32.

[26] X. Deng, S. Yang, H. Nie, H. Wang, Y. Liu, A generally adoptable radiotracing method for tracking carbon nanotubes in animals, Nanotechnology 19 (2008) 075101.

[27] S.T. Yang, K.A. Shiral Fernando, J.-H. Liu, J. Wang, H.-F. Sun, Y. Liu, M. Chen, Y. Huang, X. Wang, H. Wang, Y.-P. Sun, Covalently PEGylated carbon nanotubes with stealth character in vivo, Small 4 (2008) 940–944.

[28] L. Lacerda, A. Soundararajan, G. Pastorin, K. Al-Jamal, M.A. Herrero, A. Bao, D. Emfietzoglou, S. Mather, W.T. Phillips, M. Prato, A. Bianco, B. Goins, K. Kostarelos,

Dynamic imaging of functionalised multi-walled carbon nanotube systemic circulation & urinary excretion, Adv. Mater. 20 (2) (2008) 225–230.
[29] L. Lacerda, H. Ali-Boucetta, M.A. Herrero, G. Pastorin, A. Bianco, M. Prato, K. Kostarelos, Tissue histology and physiology following intravenous administration of different types of functionalized multiwalled carbon nanotubes, Nanomedicine 3 (2008) 149–161.
[30] A. Ruggiero, C.H. Villa, E. Bander, D.A. Rey, M. Bergkvist, C.A. Batt, K. Manova-Todorova, W.M. Deen, D.A. Scheinberg, M.R. McDevitt, Paradoxical glomerular filtration of carbon nanotubes, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 12369–12374.
[31] S. Jain, V.S. Thakare, M. Das, C. Godugu, A.K. Jain, R. Mathur, K. Chuttani, A.K. Mishra, Toxicity of multiwalled carbon nanotubes with end defects critically depends on their functionalization density, Chem. Res. Toxicol. 24 (2011) 2028–2039.
[32] J.V. Jokerst, T. Lobovkina, R.N. Zare, S.S. Gambhir, Nanoparticle PEGylation for imaging and therapy, Nanomedicine 6 (2011) 715–728.
[33] Z. Liu, K. Chen, C. Davis, S. Sherlock, Q. Cao, X. Chen, H. Dai, Drug delivery with carbon nanotubes for in vivo cancer treatment, Cancer Res. 68 (2008) 6652–6660.
[34] Z. Liu, X. Sun, N. Nakayama-Ratchford, H. Dai, Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery, ACS Nano 1 (2007) 50–56.
[35] A.A. Bhirde, S. Patel, A.A. Sousa, V. Patel, A.A. Molinolo, Y. Ji, R.D. Leapman, J.S. Gutkind, J.F. Rusling, Distribution and clearance of PEG-single-walled carbon nanotube cancer drug delivery vehicles in mice, Nanomedicine 5 (2010) 1535–1546.
[36] D. Zhang, X. Deng, Z. Ji, X. Shen, L. Dong, M. Wu, T. Gu, Y. Liu, Long-term hepatotoxicity of polyethylene-glycol functionalized multi-walled carbon nanotubes in mice, Nanotechnology 21 (2010) 1–10.
[37] S.-T. Yang, Y.-W. Wang, J.-H. Liu, H. Wang, Biodistribution of multi-walled carbon nanotubes functionalized by hydroxyl terminated poly(ethylene glycol) in mice, J. Radioanal. Nucl. Chem. 295 (2013) 1181–1186.
[38] S.-T. Yang, K.A.S. Fernando, J.-H. Liu, J. Wang, H.-F. Sun, Y. Liu, M. Chen, Y. Huang, X. Wang, H. Wang, Y.-P. Sun, Covalently PEGylated carbon nanotubes with stealth character in vivo, Small 4 (2008) 940–944.
[39] A.-C. Faure, S. Dufort, V. Josserand, P. Perriat, J.-L. Coll, S. Roux, O. Tillement, Control of the in vivo biodistribution of hybrid nanoparticles with different poly(ethylene glycol) coatings, Small 5 (2009) 2565–2575.
[40] F.A. Murphy, C.A. Poland, R. Duffin, K.T. Al-Jamal, H. Ali-Boucetta, A. Nunes, F. Byrne, A. Prina-Mello, Y. Volkov, S. Li, S.J. Mather, A. Bianco, M. Prato, W. MacNee, W.A. Wallace, K. Kostarelos, K. Donaldson, Length-dependent retention of carbon nanotubes in the pleural space of mice initiates sustained inflammation and progressive fibrosis on the parietal pleura, Am. J. Pathol. 178 (2011) 2587–2600.
[41] Y. Sato, A. Yokoyama, K. Shibata, Y. Akimoto, S. Ogino, Y. Nodasaka, T. Kohgo, K. Tamura, T. Akasaka, M. Uo, K. Motomiya, B. Jeyadevan, M. Ishiguro, R. Hatakeyama, F. Watari, K. Tohji, Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell line THP-1 in vitro and subcutaneous tissue of rats in vivo, Mol. Biosyst. 1 (2005) 176–182.
[42] S. Koyama, Y.A. Kim, T. Hayashi, K. Takeuchi, C. Fujii, N. Kuroiwa, H. Koyama, T. Tsukahara, M. Endo, In vivo immunological toxicity in mice of carbon nanotubes with impurities, Carbon 47 (2009) 1365–1372.
[43] J.C. Carrero-Sanchez, A.L. Elias, R. Mancilla, G. Arrellin, H. Terrones, J.P. Laclette, M. Terrones, Biocompatibility and toxicological studies of carbon nanotubes doped with nitrogen, Nano Lett. 6 (2006) 1609–1616.
[44] A. Radomski, P. Jurasz, D. Alonso-Escolano, M. Drews, M. Morandi, T. Malinsky, M. Radomski, Nanoparticle-induced platelet aggregation and vascular thrombosis, Br. J. Pharmacol. 146 (2005) 882–893.
[45] M.L. Schipper, N. Nakayama-Ratchford, C.R. Davis, N.W.S. Kam, P. Chu, Z. Liu, X. Sun, H. Dai, S.S. Gambhir, A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice, Nat. Nanotechnol. 3 (2008) 216–221.
[46] S.T. Yang, X. Wang, G. Jia, Y. Gu, T. Wang, H. Nie, C. Ge, H. Wang, Y. Liu, Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice, Toxicol. Lett. 181 (2008) 182–189.
[47] X. Deng, F. Wu, Z. Liu, M. Luo, L. Li, Q. Ni, Z. Jiao, M. Wu, Y. Liu, The splenic toxicity of water soluble multi-walled carbon nanotubes in mice, Carbon 47 (2009) 1421–1428.
[48] A.D. Maynard, P.A. Baron, M. Foley, A.A. Shvedova, E.R. Kisin, V. Castranova, Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material, J. Toxicol. Environ. Health A 67 (2004) 87–107.
[49] J.G. Li, W.X. Li, J.Y. Xu, X.Q. Cai, R.L. Liu, Y.J. Li, Q.F. Zhao, Q.N. Li, Comparative study of pathological lesions induced by multiwalled carbon nanotubes in lungs of mice by intratracheal instillation and inhalation, Environ. Toxicol. 22 (2007) 415–421.
[50] L.A. Mitchell, J. Gao, R.V. Wal, A. Gigliotti, S.W. Burchiel, J.D. McDonald, Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes, Toxicol. Sci. 100 (2007) 203–214.
[51] L.A. Mitchell, F.T. Lauer, S.W. Burchiel, J.D. McDonald, Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice, Nat. Nanotechnol. 4 (2009) 451–456.
[52] D.B. Warheit, B.R. Laurence, K.L. Reed, D.H. Roach, G.A.M. Reynolds, T.R. Webb, Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats, Toxicol. Sci. 77 (2004) 117–125.
[53] C.W. Lam, J.T. James, R. McCluskey, R.L. Hunter, Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation, Toxicol. Sci. 77 (2004) 126–134.
[54] A. Huczko, H. Lange, E. Calko, H. Grubek-Jaworska, P. Droszcz, Physiological testing of carbon nanotubes: are they asbestos-like? Fuller. Sci. Technol. 9 (2001) 251–254.
[55] A. Huczko, H. Lange, M. Bystrzejewski, P. Baranowski, H. Grubek-Jaworska, P. Nejman, T. Przybylowski, K. Czuminska, J. Glapinski, D.R.M. Walton, H.W. Kroto, Pulmonary toxicity of 1-D nanocarbon materials, Fuller. Nanotubes Carbon Nanostruct. 13 (2005) 141–145.
[56] D. Elgrabli, S. Abella-Gallart, F. Robidel, F. Rogerieux, J. Boczkowski, G. Lacroix, Induction of apoptosis and absence of inflammation in rat lung after intratracheal instillation of multiwalled carbon nanotubes, Toxicology 253 (2008) 131–136.
[57] Z. Li, T. Hulderman, R. Salmen, R. Chapman, S.S. Leonard, S.H. Young, A. Shvedova, M.I. Luster, P.P. Simeonova, Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes, Environ. Health Perspect. 115 (2007) 377–382.
[58] A. Erdely, T. Hulderman, R. Salmen, A. Liston, P.C. Zeidler-Erdely, D. Schwegler-Berry, V. Castranova, S. Koyama, Y.-A. Kim, M. Endo, P.P. Simeonova, Cross-talk between lung and systemic circulation during carbon nanotube respiratory exposure. Potential biomarkers, Nano Lett. 9 (2009) 36–43.
[59] A. Huczko, H. Lange, Carbon nanotubes: experimental evidence for a null risk of skin irritation and allergy, Fuller. Sci. Technol. 9 (2001) 247–250.
[60] J.T. Robinson, G. Hong, Y. Liang, B. Zhang, O.K. Yaghi, H. Dai, In vivo fluorescence imaging in the second near-infrared window with long circulating carbon nanotubes capable of ultrahigh tumor uptake, J. Am. Chem. Soc. 134 (2012) 10664–10669.