Review

Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics

Cédric Klumpp [a,b], Kostas Kostarelos [c], Maurizio Prato [b], Alberto Bianco [a,*]

[a] *Institute of Molecular and Cellular Biology, UPR 9021 CNRS, 15 Rue René Descartes, 67084 Strasbourg, France*
[b] *Department of Pharmaceutical Sciences, University of Trieste, 34127 Trieste, Italy*
[c] *Centre for Drug Delivery Research, The School of Pharmacy, University of London, London WC1N 1AX, UK*

Abstract

Functionalized carbon nanotubes (*f*-CNT) are emerging as a new family of nanovectors for the delivery of different types of therapeutic molecules. The application of CNT in the field of carrier-mediated delivery has become possible after the recent discovery of their capacity to penetrate into the cells. CNT can be loaded with active molecules by forming stable covalent bonds or supramolecular assemblies based on noncovalent interactions. Once the cargos are carried into various cells, tissues and organs they are able to express their biological function. In this review, we will describe the potential of *f*-CNT to deliver different types of therapeutic molecules.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Carbon nanotube; Vector; Vaccine; Gene; Drug delivery; Peptide

Contents

1. Introduction

In the past decade, the rediscovery of carbon nanotubes has opened new frontiers in the field of nanotechnology and nanoscience [1]. The unique structural, mechanical and

electronic properties of CNT were initially exploited in the field of materials science [2]. Soon after this new types of nanostructures started to be used in interaction mainly with proteins aiming to develop efficient biosensors [3–8]. The combination of nanotubes with proteins and other natural products including nucleic acids and polysaccharides paved the way for the compatibility of such materials with biological systems. However, dispersibility of CNT in aqueous media is a fundamental prerequisite to study their biological properties. CNT are practically insoluble in any type of solvent and only the recent development of strategies for linking chemical moieties on the tubes has facilitated their applicability.

The possibility to form complexes between CNT and different types of polymers or to modify the CNT sidewalls by organic functionalization drastically increased the characteristics of solubility of CNT. Depending on the type of strategy (covalent or noncovalent) and moiety attached to or interacting with the tubes, solubility can be modulated in different solvents. As a consequence, a wide range of applications has been envisaged including the use of CNT as substrates for neuronal growth [9–11], supports for adhesion of liposaccharides to mimic cell membrane [12], ion channel blockers [13] and delivery systems [14,15]. This review will focus on the application of carbon nanotubes for drug delivery. Initially, CNT characteristics and strategies for solubilization will be described. Then, the application as new carrier systems for drugs, peptides, proteins and nucleic acids will be discussed.

2. Carbon nanotubes

2.1. Structure and characteristics

Carbon nanotubes were discovered in the late 1950s, but they have been reconsidered and studied thoroughly only during the last 15 years [16,17]. They represent, along with fullerenes, the third allotropic crystalline form of carbon. CNT are basically a rolled sheet of graphite terminated by two end caps similar to a half C_{60}. Two main types of nanotubes exist: (i) the single-walled carbon nanotubes (SWNT) which are composed by a rolled monolayered graphene sheet, and (ii) the multi-walled carbon nanotubes (MWNT) which possess several graphitic concentric layers. The distance between each layer of a MWNT is about 0.34 nm. When a nanotube contains only two layers, it is referenced as double-walled carbon nanotube (DWNT). The diameter varies from 0.4 to 2 nm for SWNT and from 1.4 to 100 nm for MWNT, while the length can reach several micrometers for both types. The high electron density created by their aromatic structure makes them easily observable by transmission electron microscopy. SWNT can be seen mainly in bundles because of the strong van der Waals interactions, whereas MWNT are mainly monodispersed (Fig. 1). CNT exhibit different electrical properties: SWNT can be either semi-conducting or metallic while MWNT are only semi-conducting [18].

There are several methods to produce CNT including arc discharge, laser ablation and chemical vapor deposition [19]. A
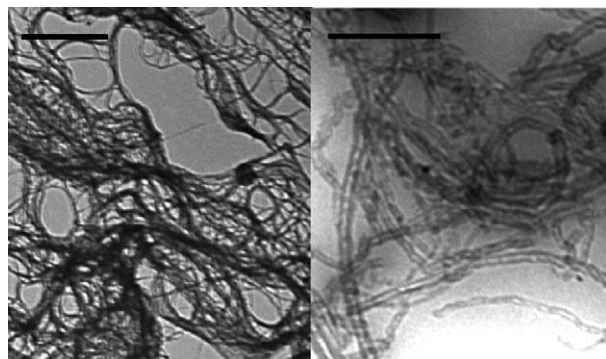

Fig. 1. TEM images of single- (left) and multi-walled (right) carbon nanotubes. While SWNT are present as bundles of different diameter and length, due to their strong aggregation tendency, MWNT can be instead observed as isolated entities. The scale bars correspond to 1 μm and 250 nm, respectively.

subcategory of the latter is the HiPCO (High-Pressure CO Conversion) process, widely used because it can be easily scaled-up to industrial production affording long length, controllable diameter and acceptable CNT purity [20]. However, a further purification is always needed before any further use. As-prepared nanotubes typically contain up to 30% metal catalyst (mainly iron and nickel particles) and some amorphous carbon and nanoparticles residual of the production procedure.

2.2. Purification of carbon nanotubes

One of the most commonly used technique for the purification and eventual solubilization of CNT is the oxidation using strong acid treatments which permit removal of a large part of the metallic impurities. Nevertheless, this methodology is not devoid of consequences for the tubes. Indeed, strong acid conditions cut the tubes in shorter pieces and generate carboxylic functions at the tips and around the sidewalls where the curvatures of the tubes present a higher strain. To avoid this, alternative methods have been explored including GPC, chromatography, centrifugation, filtration or other chemical derivatization techniques [21–26]. These purification techniques increase also the nanotube solubility making them easier to separate from the insoluble impurities.

2.3. Solubilization of carbon nanotubes

CNT are materials practically insoluble, or hardly dispersed, in any kind of solvent. To integrate the nanotube technology with the biological milieu, the solubility of the tubes especially in aqueous solutions must be improved. Several ways of dispersion and solubilization have been explored and can be basically divided in two main approaches. One procedure consists on the noncovalent functionalization of CNT with surfactants, nucleic acids, peptides, polymers and oligomers [6,8,27–33]. The advantage of this process is the preservation of the electronic structure of the nanotube aromatic surface. This property is of fundamental importance for the use of nanotubes as biosensors. The second methodology is based on CNT covalent functionalization [34–38]. First, CNT are cut and oxidized to generate a certain number of carboxylic groups

subsequently derivatized with different types of molecules. Alternatively, CNT sidewalls can be directly functionalized by addition reactions. The introduction of moieties on the tube external surface creates repulsion between the single tubes allowing them to easily disperse into the solvent.

2.4. Noncovalent functionalization of carbon nanotubes

The noncovalent dispersion of CNT in solution allows preservation of their aromatic structure and thus their electronic characteristics. The dispersion procedures usually involving ultrasonication, centrifugation and filtration are quick and easy. Hydrophobic or $\pi-\pi$ interactions are often evoked as likely responsible for noncovalent stabilization. Nowadays, three classes of molecules are mainly used for CNT dispersion. Surfactants are used because they are easily available and low-cost. Polymers and biopolymers (nucleic acids and peptides) are also very efficient in the dispersion process.

2.4.1. Surfactants

A series of anionic, cationic and nonionic surfactants have been already proposed to disperse nanotubes. Sodium dodecyl sulfate (SDS) and Triton X-100 were used to obtain CNT suspensions up to 0.1 and 0.5 mg/mL, respectively [27]. However, the stability of this suspension was no longer than 1 week. A better result was obtained by using sodium dodecyl-benzene sulfonate (SDBS), which was able to provide stability over one month reaching 10 mg/mL concentration of the suspension. The combination of $\pi-\pi$ interactions of aromatic moieties between CNT and SDBS and the long lipid chains of the SDBS increases the stability of the complex. Atomic force microscopy (AFM) and electronic transmission microscopy (TEM) studies of SDS/CNT dispersions showed that CNT are mainly present as individual tubes uniformly covered by the surfactant [28]. The types of amphiphilic molecules with long lipid chains are able to form a half-cylinder perpendicular or tilted around the tubes in a micelle-like arrangement [6]. Triton-X instead mainly interacts by π-stacking. Another approach for the adsorption/dispersion of CNT via π interaction resides on the use of 1-pyrenebutanoic acid activated as succinimidyl ester, which promptly reacts with the amino groups present in the proteins like ferritin or streptavidin [39]. The solubility of CNT was between 0.1 and 0.7 mg/mL, which is rather low but acceptable for biological use. Although surfactants may be efficient in the solubilization of CNT, they are known to permeabilize plasma membranes and have a toxicity profile of their own. Therefore, the implications stemming from use of surfactants interacting with biological systems can limit the possible biomedical applications of such surfactant-stabilized CNT complexes.

2.4.2. Polymers

Polymers are widely used for example as molecular carriers for drug delivery [40]. In the solubilization of CNT they represent a good alternative to surfactants although they do not have a better dispersion efficiency [28]. The mechanism of dispersion is based in this case on wrapping of the polymer around the tubes. Star et al. [41] have used for example a substituted poly(metaphenylenevinylene) to suspend SWNT in organic solvents. The polymer wraps around ropes of nanotubes. The driving force of the phenomenon is likely to be the steric repulsion of the polymer. Once the polymer is attached to the surface of the nanotube, it offers a sufficient repulsive potential stabilizing the dispersion [30]. In the case of nonionic polymers, based on poly(oxyehtylene) copolymers, the efficiency of the dispersion is instead due to their hydrophilic counterpart. For particularly high molecular weight polymers, the suspendability is enhanced as the steric stabilization is increased by a wider coverage of the surface [28]. In a similar approach, CNT were dispersed by using cationic copolymers [31]. The nanotubes were covered by the hydrophobic backbone of the polymer while the positive tetraalkylammonium groups were exposed at the surface to display water solubility. These types of fluorescent polymers have also been employed to study the interaction with mammalian cells. Poly(vinylpyrrolidone) was conjugated with various fluorescent dyes. CNT were suspended in 1% SDS and mixed with the fluorescent polymers to form supramolecular complexes, which were found to have potential applications as new molecular probes [42].

2.4.3. Biopolymers

The solubilization of CNT with biological components is certainly more appropriate towards integration of this new type of material with living systems. Self-assembly processes similar to $\pi-\pi$ interactions typical of double-stranded DNA can be for example exploited to disperse the nanotubes. Nucleic acids are certainly ideal candidates to form supramolecular complexes based on π-stacking between the aromatic bases and the CNT surface. Indeed, Zeng et al. [33,43] have described an easy way to solubilize carbon nanotubes by simple sonication in the presence of a single-strand DNA. A molecular modeling study was performed to explain the formation of the hybrids exerted by DNA wrapping and subsequent CNT debundling. The DNA–nanotube complexes displayed solubility in the range of mg/ml, and their good stability permitted the purification using ion-exchange chromatography. Amphiphilic peptides belong to another class of biopolymers that efficiently disperse CNT [44]. The presence of amino acids like tryptophan, phenylalanine, tyrosine and histidine into the peptide sequence plays a key role on the solubilization process in water. These peptides could be selected from phage-display peptide libraries [8] or by design [44–46]. The design of highly specific peptides able to wrap around the nanotubes represent an interesting way to assure solubility and may even provide a useful tool for size-separation. More recently, cyclic peptides were also proven to have similar capabilities [47].

2.5. Covalent functionalization of carbon nanotubes

The alternative way to render CNT soluble into a wide range of solvents is the modification of their sidewalls and tips by organic functionalization [34–36,48]. The aqueous solubility

can be assured for example by a covalent attachment of hydrophilic moieties. Two main strategies are currently used to attach functional groups to CNT (Fig. 2). The first one consists in the oxidative treatment using strong acid solutions. The variation of the type of acid, its concentration and the reaction conditions (temperature, sonication) generated cut tubes covered by carboxylic functions at their tips and at the defect points [49]. The carboxylates were then used to incorporate a variety of other groups to improve CNT solubility. The introduction occurred via COOH activation using thionyl chloride or carbodiimide. Similarly, oxidized CNT were solubilized by direct heating in the presence of amino polymers [50,51]. The second type of covalent functionalization is based on the addition reactions to CNT. By exploiting the chemistry of fullerenes, 1,3-dipolar cycloaddition of azomethine ylides, aryl diazonium salt addition or reductive alkylation using lithium and alkyl halides have been successfully employed to CNT [22,34,38,52,53]. Such direct sidewall modification of CNT permitted the incorporation of different functional groups on the nanotube which could be further derivatized [54]. The covalent bond presents the advantage of being more robust during manipulation and processing in comparison to the noncovalent dispersion. Nevertheless, both covalent and noncovalent functionalization of CNT have been exploited for the application of such materials in the field of drug delivery.

3. Drug delivery by carbon nanotubes

Drug delivery systems are continuously being developed to improve the pharmacological profile and the therapeutic properties of administered drugs [55]. A wide variety of delivery systems are currently available [56]. The application of f-CNT as new nanovectors for drug delivery was apparent

immediately after the first demonstration of the capacity of this material to penetrate into cells. We demonstrated that fluorescently labeled CNT were uptaken by various cell types (Fig. 3, CNT 1). CNT were functionalized using the 1,3-dipolar cycloaddition reaction and further modified with fluorescein isothiocyanate or a fluorescent peptide [57]. These conjugates were tracked into the cytoplasm and the nucleus of 3T3 or 3T6 fibroblasts, respectively (Fig. 4). In a similar work, Dai and Wender functionalized oxidized carbon nanotubes with biotin and formed a complex with a fluorescent streptavidin. CNT covered by the protein were found in the endosomes of CHO and 3T3 cells [58]. The mechanism of penetration is not yet completely elucidated. Two routes of internalization have been proposed. It has been found that f-CNT penetrate following a passive diffusion across the lipid bilayer similar to a "nanoneedle" able to perforate the cell membrane without causing cell death [59,60]. Alternatively, when CNT were used to deliver proteins by adsorbing them onto their external surface, they seem to be uptaken by endocytosis [58,61].

It is highly probable that the type of molecules covalently or noncovalently attached to the external walls of the tubes play a critical role in the process of transport into the cells. This capacity of CNT to cross the cell membrane was recently exploited to deliver small organic molecules. We have shown that CNT functionalized with amphotericin B (AmB) (Fig. 3, CNT 2), one of the most effective antibiotic molecules for the treatment of chronic fungal infections, are rapidly internalized by mammalian cells with a reduced toxicity in comparison to the drug administered alone [62]. At the highest doses, more than 40% of cells died by effect of free AmB whereas all mammalian cells remained alive upon treatment with AmB covalently linked to CNT. Interestingly, the evaluation of the antifungal activity against different types of microorganisms showed that the effect of the AmB is enhanced when
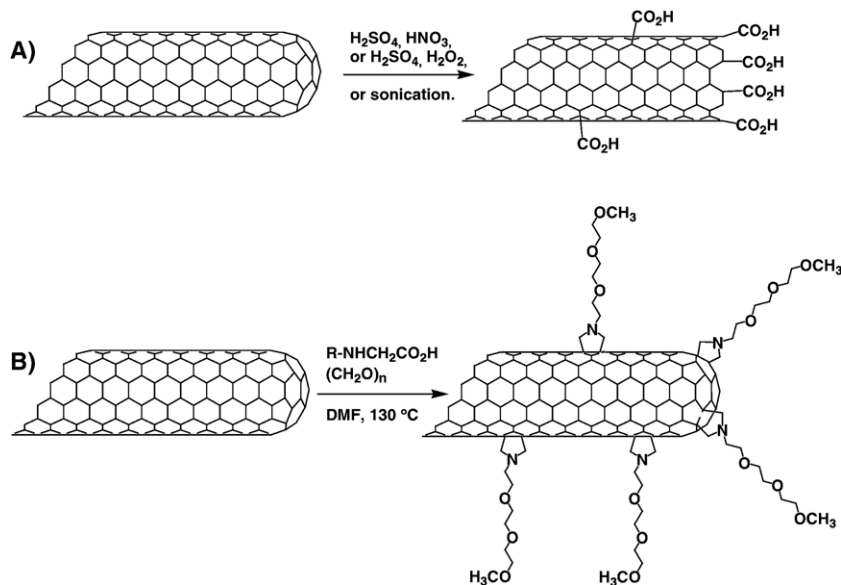

Fig. 2. Organic functionalization of carbon nanotubes. (A) Pristine CNT can be treated with acid to cut them and form carboxylic groups at the tips and the sidewalls. (B) CNT undergo the 1,3 dipolar cycloaddition by reacting an α-amino acid derivative with *para*formalaldehyde to add solubilizing moieties around the external surface. R═CH₃O(CH₂CH₂O)₂CH₂CH₂–.
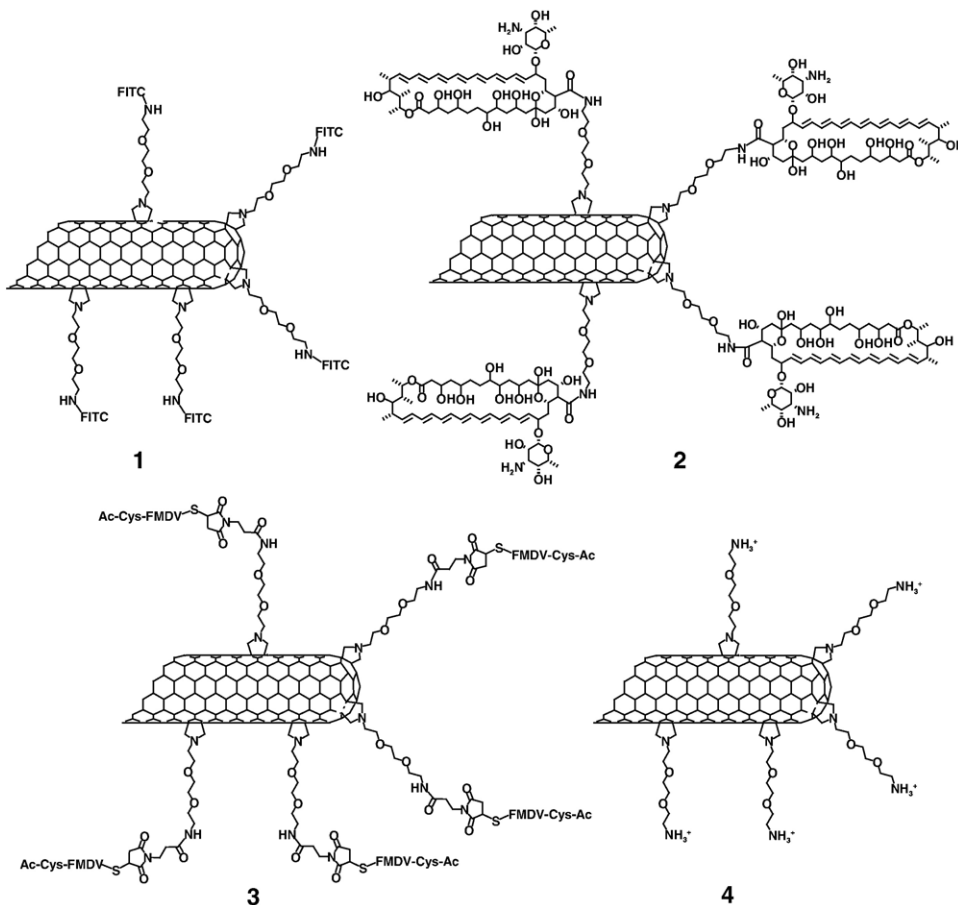
Fig. 3. Molecular structures of *f*-CNT capable to penetrate into the cells and display a biological function.

conjugated to the tubes (Fig. 5). In another approach, Hosmane and coworkers prepared a SWNT substituted with a carborane cage for neutron boron capture therapy. After the administration of the conjugates the concentration of the boron atoms was mostly detected in tumor cells rather than in blood or other organs, however the mechanism for this is not yet understood [63]. Very recently, the property of CNT to adsorb near-infrared irradiation was exploited to kill cancer cells. Pristine SWNT were wrapped with a poly(ethylene glycol) (PEG) modified with a phospholipid (PL) moiety and folic acid (FA). Because tumor cells are known to overexpress folate receptors, the PL-PEG-FA/SWNT construct was only internalized inside cancer cells, which were then destroyed by using a laser wavelength of

808 nm. Laser pulses induced local heating and consequently death only of those tumor cells that had uptaken the CNT [64].

f-CNT were also successfully used to deliver and present antigenic peptide to the immune system. We prepared a peptide-CNT conjugate containing a B cell epitope from VP1 protein of foot-and-mouth disease virus (FMDV) (Fig. 3, CNT **3**). The peptide attached to SWNT could be recognized by specific polyclonal and monoclonal antipeptide antibodies as assessed by ELISA test and surface plasmon resonance (SPR). This result suggested that the peptide covalently linked to CNT
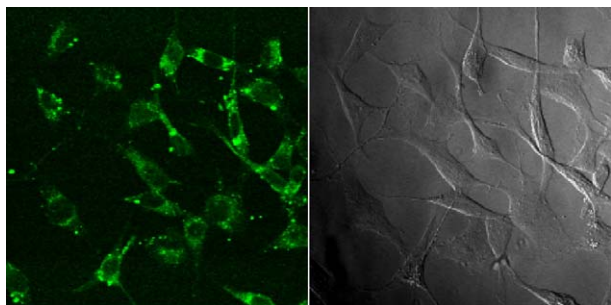

Fig. 4. Confocal microscopy images of 3T6 cells incubated with fluorescent CNT **1**.
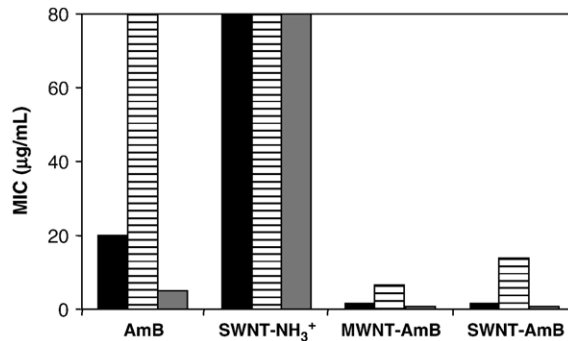

Fig. 5. Antifungal activity of AmB conjugated to carbon nanotubes (CNT **2**). The MIC corresponds to the lowest concentration of compound that inhibits visible growth on the pathogens. *Candida parapsilosis* (black bar); *Candida Albicans* (dashed bar); *Cryptococcus neoformans* (grey bar).

was able to adopt the correct native conformation and CNT were efficient supports for the multipresentation of peptides to the immune system [65]. In addition, mice were immunized with the peptide-functionalized CNT eliciting high antibody responses with virus neutralizing capacity [66]. Importantly, CNT devoid of the peptide moiety were not immunogenic, since antibodies against CNT were not detected. These results point out the potential of CNT to deliver peptide-based synthetic vaccines.

4. Delivery of nucleic acids by carbon nanotubes

The use of *f*-CNT as a new carrier system for nucleic acids is another area of research currently under investigation. The most commonly used DNA carriers are based on viral vectors (retrovirus, lentivirus or adenovirus), liposomes, cationic lipids, polymers and nanoparticles [67]. Although viral vectors are very efficient in gene expression, their use is limited because of the concerns about their safety. Indeed, they can provoke severe, undesired immune responses. Nonviral vectors offer various alternatives regarding the size and type of vectors, they are chemically controllable, and display a reduced immunogenicity. However, they often present low gene expression efficiency rates because of their poor capability in reaching and crossing the nuclear membrane. Generally, the development of a new vector for therapeutic gene transfer requires protection of DNA from degradation, good membrane penetration and low immunogenicity. In this context, CNT seem to be very promising because they do not inherently trigger an immune response [66]. Ammonium-functionalized CNT were used to condense plasmid DNA expressing β-galactosidase (pBgal) (Fig. 3, CNT **4**) [59,68]. The complexes based on positive/negative charge interactions were characterized using different techniques including TEM, SPR, scanning electron microscopy (SEM), gel electrophoresis and picogreen dye exchange. The capacity of these complexes to induce gene transfer was measured on CHO cells. Levels of gene expression 5 to 10 times higher than using the plasmid DNA alone were detected. The efficiency of *f*-CNT on DNA transfection was very recently improved by conjugating poly(ethyleneimine) (PEI) onto the external surface of the nanotubes [69]. PEI was grafted to CNT and the conjugates were condensed with a plasmid expressing the luciferase marker gene. The levels of gene expression were comparable to that of PEI alone.

Recently, Cai et al. have designed an alternative physical method of gene transfer, appropriate only for in vitro and ex vivo applications, called the nanotube "spearing" method capable of inducing cell internalization of plasmid DNA [60]. Nanotubes grown from plasma-enhanced chemical vapor deposition contained nickel particle catalysts entrapped into their tips, allowing them to respond to a magnetic field. The tubes were functionalized with a DNA strain containing the sequence coding for the enhanced green fluorescent protein. Dividing and non dividing cells like Bal17, B-lymphoma, ex vivo B and primary neurons were grown on a substrate and incubated with magnetic pDNA/CNT. A rotating magnetic

field first drove the nanotubes to mechanically spear the cells. In a subsequent step, a static magnetic field pulled the tubes into the cells. The cells were efficiently transfected as confirmed by fluorescent microscopy measurements. The efficiency was equal to a viral approach even for nondividing cells, such as primary B cells and neurons which are generally more difficult to transfect.

The use of ammonium functionalized CNT was not limited to complex pDNA. In fact, short oligodeoxynulceotide (ODN) sequences were able to interact with positive charged CNT and form stable complexes. In particular, ODN CPG motifs have been condensed to this type of nanotubes and the immunostimulation properties of these complexes were evaluated [70]. High ratios of *f*-CNT over a minimum immunostimulatory dose of a specific ODN CpG increased its immunopotentiating activity in vitro and it decreased the secretion of proinflammatory cytokine interleukine-6. Lu et al. have studied the translocation of the non encoding RNA polymer poly(rU) across MCF7 breast cancer cells using oxidized carbon nanotubes [71]. The complex was labeled with tritiated thymidine and was detected inside the cytoplasm as well as in the nucleus.

In a different approach, SWNT were interacted with a single-stranded (ss) DNA sequence labeled with the Cy3 dye [64]. The ssDNA was transported into HeLa cells by the nanotubes via endocytosis. Once entrapped into the endosomes the DNA/SWNT complex was liberated by applying a near-infrared radiation for a short time. Following the release from the tubes into the cytoplasm, the ssDNA can freely diffuse towards the nuclear membrane and eventually express the encoded protein. Similarly, short interference RNA (siRNA) for gene silencing were adsorbed onto CNT after being covalently linked to a PEG using a cleavable disulfide bond [72]. The siRNA/CNT complexes penetrated into the cell and the enzymatic and acid conditions of the endosomal/lysosomal compartments allowed the release of siRNA into the cytosol. After crossing the nuclear membrane, siRNA carried by SWNT achieved efficient gene silencing in comparison to the commercially available transfection agent lipofectamine.

Overall, the results described above highlight the capacity of *f*-CNT to form stable complexes with different types of nucleic acids and their potential use in gene therapy and genetic vaccination.

5. Toxicity

As graphite has been associated with increased dermatitis and keratosis, investigation is needed to determine the potential danger of exposure to CNT. Studies so far have focused on the effects of pristine CNT on different cell lines like human epidermal keratinocytes, HEK293 human embryonic kidney cells, human acute monocytic leukemia cell lines, human T cells and alveolar macrophages [73–79]. When SWNT exposure was studied on keratinocytes, oxidative stress and cellular toxicity were detected from the presence of free radicals and peroxides, leading to antioxidant depletion and loss of cell viability [79]. Inflammatory responses were also

reported on the same cell line by MWNT [78]. The mechanism is likely to be due to the production of reactive oxygen species, leading to the activation of the nuclear transcription factor-κB [77]. Moreover, CNT can activate the human serum complement system via the classical and the alternative pathway, generating proinflammatory peptides [80]. Studies on the biocompatibility of SWNT on HEK293 human embryonic kidney cells showed a cell growth inhibition via induction of apoptosis and decrease of cell adhesion ability [73]. Pristine nanotubes usually contain transition metal catalysts like iron or nickel, which could explain the generation of free radicals, however, in the case of the MWNT no iron was detected before cellular interaction.

A comparative study on the toxicity of pristine and oxidized MWNT onto human Jurkat T leukemia cells has shown that the latter were more toxic [74]. CNT tend to aggregate in ropes, decreasing in aqueous solubility and increasing in cytotoxicity. The type of nanotubes used in such studies is therefore extremely important. Comparison of cytotoxicity induced by different carbon materials in alveolar macrophages showed that SWNT provoke the highest toxic effect, followed by MWNT, quartz and fullerenes [76]. A study on the effect of the CNT length on cytotoxicity showed that the inflammatory response was higher for the CNT of 825 nm in comparison to that of 220 nm [75]. CNT may lead to toxicological side-effects in the case of biological and biomedical applications, however up to this date this seems mainly the case for non-fuctionalized, insoluble material. It has been previously shown that cytotoxicity of water-soluble fullerene derivatives is improved as the degree of surface modification is increased [81]. Thus, it is reasonable to anticipate that functionalized and water-soluble nanotubes will be less cytotoxic. Different types of functionalized, soluble CNT have already been studied in various laboratories reporting no significant cell damage by using SWNT-RNA polymer hybrids with a concentration up to 1 mg/mL with MCF7 breast cancer cells [71]; 90% of fibroblast survival following incubation with 5 μM fluorescein functionalized SWNT [57]; and no toxicity for CHO and 3T3 cells interacting with protein-functionalized SWNT [58].

Another important aspect that plays a critical role in determining CNT toxicity is related to their bioavailability and deposition in lungs following inhalation. Lam et al. described a dose-dependent formation of epitheloid granulomas and some interstitial inflammations [82]. This study showed that nanotubes were much more toxic than carbon black and quartz. Warheit and co-workers have determined that SWNT exposure in rats produced dose-independent series of multifocal granulomas [83]. Recently, ^{125}I-labelled SWNT were radiotraced in mice following intraperitoneal administration [84]. The SWNT used in this study were water-soluble and compatible with physiological fluid. They were shown to be distributed among different tissues. Their excretion was mainly through urine, and bone accumulation was reported after 18 days without any concomitant toxicity.

In summary, pristine CNT have been found to be cytotoxic to various mammalian cell lines in vitro and to the skin and lungs in vivo. However, pristine nanotubes tend to aggregate

due to their insolubility, therefore such toxicological responses are not surprising. Significant efforts have been made to improve the nanotube solubility and a remarkable reduction on toxicity has been reported using such functionalized CNT. The toxicological profile of CNT will depend on many parameters such as the type of nanotubes, the presence of impurities, the length of the tubes, the type of functionalization and the molecular nature of the conjugated groups. It is still early to establish a general toxicological profile for this type of material and more systematic in vitro and in vivo investigations using biologically compatible CNT are necessary to achieve that.

6. Conclusions

Great efforts in the development of carbon nanotubes as advanced nanomaterials have only very recently begun to translate into potentially useful tools for biomedical applications. Proof-of-principle studies in our laboratories and those of others have clearly shown that carbon nanotubes, following appropriate surface modification to render them water soluble (i.e. biologically compatible), hold great promise as nanovectors for the delivery of a variety of therapeutic and diagnostic agents. The mechanisms of CNT interaction with mammalian cells and their compartments is crucial in further developing and optimizing the capabilities of carbon nanotube carrier systems, particularly if future clinical utilization is envisaged. Equally so, the interaction of CNT nanovectors with physiological components, including blood, tissues, interstitial fluid, the complement system, reticoloendothelial system etc., and the ensuing toxicological profiles obtained, will determine the clinical fate of these developing nanomedicinal tools. Nevertheless, carbon nanotubes today represent a class of emerging nanovectors that are capable to intracellularly deliver biologically functional peptides, proteins, nucleic acids and small molecules covalently or noncovalently attached on their surface. Future studies will determine the opportunities as well as the limitations that these novel nanovectors hold towards their clinical realization.

Acknowledgements

References

[1] R.H. Baughman, A.A. Zakhidov, W.A. De Heer, Carbon nanotubes—The route toward applications, Science 297 (2002) 787–792.

[2] Special issue on carbon nanotubes, Acc. Chem. Res. 35 (2002) 997–1113.

[3] F. Balavoine, C. Richard, C. Mioskowski, P. Schultz, V. Mallouh, T.W. Ebbesen, Helical crystallization of proteins on carbon nanotubes: a first step towards the development of new biosensors, Angew. Chem., Int. Ed. 38 (1999) 1912–1915.

[4] E. Bekyarova, Y. Ni, E.B. Malarkey, V. Montana, J.L. McWilliams, R.C. Haddon, V. Parpura, Applications of carbon nanotubes in biotechnology and biomedicine, J. Biomed. Nanotechnol. 1 (2005) 3–17.

[5] Y. Lin, S. Taylor, H. Li, K.A.S. Fernando, L. Qu, W. Wang, L. Gu, B. Zhou, Y.-P. Sun, Advances toward bioapplications of carbon nanotubes, J. Mater. Chem. 14 (2004) 527–541.

[6] C. Richard, F. Balavoine, C. Mioskowski, P. Schultz, T.W. Ebbesen, Supramolecutar self-assembly of lipid derivatives on carbon nanotubes, Science 300 (2003) 775–778.

[7] J. Wang, M. Musameh, Y. Lin, Solubilization of carbon nanotubes by nafion toward the preparation of amperometric biosensors, J. Am. Chem. Soc. 125 (2003) 2408–2409.

[8] S. Wang, D.F. Delduco, S.R. Lustig, H. Wang, K.N. Parker, N.W. Rizzo, S. Subramoney, A. Jagota, E.S. Humphreys, S.-Y. Chung, Y.-M. Chiang, Peptides with selective affinity for carbon nanotubes, Nat. Mater. 2 (2003) 196–200.

[9] V. Lovat, D. Pantarotto, L. Lagostena, G. Spalluto, M. Prato, L. Ballerini, B. Cacciari, M. Grandolfo, M. Righi, Carbon nanotube substrates boost neuronal electrical signaling, Nano Lett. 5 (2005) 1107–1110.

[10] H. Hu, R.C. Haddon, Y. Ni, V. Montana, V. Parpura, Chemically functionalized carbon nanotubes as substrates for neuronal growth, Nano Lett. 4 (2004) 507–511.

[11] M.P. Mattson, R.C. Haddon, A.M. Rao, Molecular functionalization of carbon nanotubes and use as substrates for neuronal growth, J. Mol. Neurosci. 14 (2000) 175–182.

[12] X. Chen, G.S. Lee, A. Zettl, C.R. Bertozzi, Biomimetic engineering of carbon nanotubes by using cell surface mucin mimics, Angew. Chem., Int. Ed. 43 (2004) 6112–6116.

[13] K.H. Park, M. Chhowalla, Z. Iqbal, F. Sesti, Single-walled carbon nanotubes are a new class of ion channel blockers, J. Biol. Chem. 278 (2003) 50212–50216.

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

[15] A. Bianco, M. Prato, Can carbon nanotubes be considered useful tools for biological applications? Adv. Mater. 15 (2003) 1765–1768.

[16] R. Bacon, Growth, structure, and properties of graphite whiskers, J. Appl. Phys. 31 (1960) 284.

[17] S. Iijima, Helical microtubules of graphitic carbon, Nature 354 (1991) 56–58.

[18] V.N. Popov, Carbon nanotubes: properties and application, Mater. Sci. Eng. R43 (2004) 61–102.

[19] C.E. Baddour, C. Briens, Carbon nanotube synthesis: a review, Int. J. Chem. React. Eng. 3 (2005) R3.

[20] P. Nikolaev, M.J. Bronikowski, R.K. Bradley, F. Rohmund, D.T. Colbert, K.A. Smith, R.E. Smalley, Gas-phase catalytic growth of single-walled carbon nanotubes from carbon monoxide, Chem. Phys. Lett. 313 (1999) 91–97.

[21] M.S. Arnold, S.I. Stupp, M.C. Hersam, Enrichment of single-walled carbon nanotubes by diameter in density gradients, Nano Lett. 5 (2005) 713–718.

[22] C.A. Dyke, M.P. Stewart, J.M. Tour, Separation of single-walled carbon nanotubes on silica gel. Materials morphology and raman excitation wavelength affect data interpretation, J. Am. Chem. Soc. 127 (2005) 4497–4509.

[23] V. Georgakilas, D. Voulgaris, E. Vázquez, M. Prato, D.M. Guldi, A. Kukovecz, H. Kuzmany, Purification of hipco carbon nanotubes via organic functionalization, J. Am. Chem. Soc. 124 (2002) 14318–14319.

[24] B. Zhao, H. Hu, S. Niyogi, M.E. Itkis, M.A. Hamon, P. Bhowmik, M.S. Meier, R.C. Haddon, Chromatographic purification and properties of soluble single-walled carbon nanotubes, J. Am. Chem. Soc. 123 (2001) 11673–11677.

[25] S. Niyogi, H. Hu, M.A. Hamon, P. Bhowmik, B. Zhao, S.M. Rozenzhak, J. Chen, M.E. Itkis, M.S. Meier, R.C. Haddon, Chromatographic purification of soluble single-walled carbon nanotubes (s-SWNTs), J. Am. Chem. Soc. 123 (2001) 733–734.

[26] B.Z. Tang, H. Xu, Preparation, alignment, and optical properties of soluble poly(phenylacetylene)-wrapped carbon nanotubes, Macromolecules 32 (1999) 2569–2576.

[27] M.F. Islam, E. Rojas, D.M. Bergey, A.T. Johnson, A.G. Yodh, High weight fraction surfactant solubilization of single-wall carbon nanotubes in water, Nano Lett. 3 (2003) 269–273.

[28] V.C. Moore, M.S. Strano, E.H. Haroz, R.H. Hauge, R.E. Smalley, J. Schmidt, Y. Talmon, Individually suspended single-walled carbon nanotubes in various surfactants, Nano Lett. 3 (2003) 1379–1382.

[29] P. Petrov, F. Stassin, C. Pagnoulle, R. Jérôme, Noncovalent functionalization of multi-walled carbon nanotubes by pyrene containing polymers, Chem. Commun. 9 (2003) 2904–2905.

[30] R. Shvartzman-Cohen, E. Nativ-Roth, E. Yerushalmi-Rozen, E. Baskaran, I. Szleifer, Y. Levi-Kalisman, Selective dispersion of single-walled carbon nanotubes in the presence of polymers: the role of molecular and colloidal length scales, J. Am. Chem. Soc. 126 (2004) 14850–14857.

[31] V.A. Sinani, N.A. Kotov, A.A. Yaroslavov, A.A. Rakhnyanskaya, M.K. Gheith, J.P. Wicksted, K. Sun, A.A. Mamedov, Aqueous dispersions of single-wall and multiwall carbon nanotubes with designed amphiphilic polycations, J. Am. Chem. Soc. 127 (2005) 3463–3472.

[32] N. Nakashima, S. Okuzono, H. Murakami, T. Nakai, K. Yoshikawa, DNA dissolves single-walled carbon nanotubes in water, Chem. Lett. 32 (2003) 456–457.

[33] M. Zheng, A. Jagota, E.D. Semke, B.A. Diner, R.S. McLean, S.R. Lustig, R.E. Richardson, N.G. Tassi, DNA-assisted dispersion and separation of carbon nanotubes, Nat. Mater. 2 (2003) 338–342.

[34] N. Tagmatarchis, M. Prato, Functionalization of carbon nanotubes via 1,3-dipolar cycloadditions, J. Mater. Chem. 14 (2004) 437–439.

[35] A. Hirsch, Functionalization of single-walled carbon nanotubes, Angew. Chem., Int. Ed. 41 (2002) 1853–1859.

[36] C.A. Dyke, J.M. Tour, Overcoming the insolubility of carbon nanotubes through high degrees of sidewall functionalization, Chem. Eur. J. 10 (2004) 812–817.

[37] J.L. Stevens, A.Y. Huang, H. Peng, I.W. Chiang, V.N. Khabashesku, J.L. Margrave, Sidewall amino-functionalization of single-walled carbon nanotubes through fluorination and subsequent reactions with terminal diamines, Nano Lett. 3 (2003) 331–336.

[38] J.L. Hudson, M.J. Casavant, J.M. Tour, Water-soluble, exfoliated, nonroping single-wall carbon nanotubes, J. Am. Chem. Soc. 126 (2004) 11158–11159.

[39] R.J. Chen, Y. Zhang, D. Wang, H. Dai, Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization, J. Am. Chem. Soc. 123 (2001) 3838–3839.

[40] T. Minko, Soluble polymer conjugates for drug delivery, Curr. Drug Discov. Technol. 2 (2005) 15–20.

[41] A. Star, J.F. Stoddart, D. Steuerman, M. Diehl, A. Boukai, E.W. Wong, X. Yang, S.-W. Chung, H. Choi, J.R. Heath, Preparation and properties of polymer- wrapped single-walled carbon nanotubes, Angew. Chem., Int. Ed. 40 (2001) 1721–1725.

[42] V.V. Didenko, V.C. Moore, D.S. Baskin, R.E. Smalley, Visualization of individual single-walled carbon nanotubes by fluorescent polymer wrapping, Nano Lett. 5 (2005) 1563–1567.

[43] M. Zheng, A. Jagota, B.A. Diner, R.S. McLean, G.B. Onoa, E.D. Semke, D.J. Watts, M.S. Strano, P. Barone, M. Usrey, A.P. Santos, S.G. Chou, M.S. Dresselhaus, G.G. Samsonidze, Structure-based carbon nanotube sorting by sequence-dependent DNA assembly, Science 302 (2003) 1545–1548.

[44] G.R. Dieckmann, J. Razal, G.M. Giordano, I.H. Musselman, R.H. Baughman, A.B. Dalton, E. Muñoz, P.A. Johnson, R.K. Draper, J. Chen, Controlled assembly of carbon nanotubes by designed amphiphilic peptide helices, J. Am. Chem. Soc. 125 (2003) 1770–1777.

[45] V. Zorbas, A. Ortiz-Acevedo, G.R. Dieckmann, R.K. Draper, R.H. Baughman, I.H. Musselman, A.B. Dalton, M.M. Yoshida, M. Jose-Yacaman, Preparation and characterization of individual peptide-wrapped single-walled carbon nanotubes, J. Am. Chem. Soc. 126 (2004) 7222–7227.

[46] A.B. Dalton, J.M. Razal, R.H. Baughman, R.K. Draper, I.H. Musselman, G.R. Dieckmann, A. Ortiz-Acevedo, V. Zorbas, E. Brunner, W.M. Sampson, S. Collins, M.M. Yoshida, M. Jose-Yacaman, Hierarchical self-assembly of peptide-coated carbon nanotubes, Adv. Funct. Mater. 14 (2004) 1147–1151.

[47] A. Ortiz-Acevedo, H. Xie, V. Zorbas, W.M. Sampson, A.B. Dalton, R.H. Baughman, R.K. Draper, I.H. Musselman, G.R. Dieckmann, Diameter-selective solubilization of single-walled carbon nanotubes by reversible cyclic peptides, J. Am. Chem. Soc. 127 (2005) 9512–9517.

[48] K. Balasubramanian, M. Burghard, Chemically functionalized carbon nanotubes, Small 1 (2005) 180–192.

[49] K.J. Ziegler, Z. Gu, H. Peng, E.L. Flor, R.H. Hauge, R.E. Smalley, Controlled oxidative cutting of single-walled carbon nanotubes, J. Am. Chem. Soc. 127 (2005) 1541–1547.

[50] J. Chen, A.M. Rao, S. Lyuksyutov, M.E. Itkis, M.A. Hamon, H. Hu, R.W. Cohn, P.C. Eklund, D.T. Colbert, R.E. Smalley, R.C. Haddon, Dissolution of full-length single-walled carbon nanotubes, J. Phys. Chem., B 105 (2001) 2525–2528.

[51] Y. Lin, A.M. Rao, B. Sadanadan, E.A. Kenik, Y.-P. Sun, Functionalizing multiple-walled carbon nanotubes with aminopolymers, J. Phys. Chem., B 106 (2002) 1294–1298.

[52] C.G.R. Heald, G.G. Wildgoose, R.G. Compton, L. Jiang, T.G.J. Jones, Chemical derivatisation of multiwalled carbon nanotubes using diazonium salts, Chem. Phys. Chem. 5 (2004) 1794–1799.

[53] F. Liang, A.K. Sadana, A. Peera, J. Chattopadhyay, Z. Gu, R.H. Hauge, W.E. Billups, A convenient route to functionalized carbon nanotubes, Nano Lett. 4 (2004) 1257–1260.

[54] K.M. Lee, L. Li, L. Dai, Asymmetric end-functionalization of multi-walled carbon nanotubes, J. Am. Chem. Soc. 127 (2005) 4122–4123.

[55] T.M. Allen, P.R. Cullis, Drug delivery systems: entering the mainstream, Science 303 (2004) 1818–1822.

[56] C.W. Pouton, L.W. Seymour, Key issues in non-viral gene delivery, Adv. Drug. Deliv. Rev. 46 (2000) 187–203.

[57] D. Pantarotto, J.-P. Briand, M. Prato, A. Bianco, Translocation of bioactive peptides across cell membranes by carbon nanotubes, Chem. Commun. (2004) 16–17.

[58] N.W.S. Kam, T.C. Jessop, P.A. Wender, H. Dai, Nanotube molecular transporters: internalization of carbon nanotube–protein conjugates into mammalian cells, J. Am. Chem. Soc. 126 (2004) 6850–6851.

[59] D. Pantarotto, R. Singh, D. McCarthy, M. Erhardt, J.-P. Briand, M. Prato, K. Kostarelos, A. Bianco, Functionalized carbon nanotubes for plasmid DNA gene delivery, Angew. Chem., Int. Ed. 43 (2004) 5242–5246.

[60] D. Cai, Z. Huang, D. Carnahan, J.M. Mataraza, T.C. Chiles, Z.-H. Qin, J. Huang, K. Kempa, Z. Ren, Highly efficient molecular delivery into mammalian cells using carbon nanotube spearing, Nat. Methods 2 (2005) 449–454.

[61] N.W.S. Kam, H. Dai, Carbon nanotubes as intracellular protein transporters: generality and biological functionality, J. Am. Chem. Soc. 127 (2005) 6021–6026.

[62] W. Wu, S. Wieckowski, G. Pastorin, M. Benincasa, C. Klumpp, J.-P. Briand, R. Gennaro, M. Prato, A. Bianco, Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes, Angew. Chem., Int. Ed. 44 (2005) 6358–6362.

[63] Z. Yinghuai, A.T. Peng, K. Carpenter, J.A. Maguire, N.S. Hosmane, M. Takagaki, Substituted carborane-appended water-soluble single-wall carbon nanotubes: new approach to boron neutron capture therapy drug delivery, J. Am. Chem. Soc. 127 (2005) 9875–9880.

[64] 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.

[65] D. Pantarotto, C.D. Partidos, R. Graff, J. Hoebeke, J.-P. Briand, M. Prato, A. Bianco, Synthesis, structural characterization, and immunological properties of carbon nanotubes functionalized with peptides, J. Am. Chem. Soc. 125 (2003) 6160–6164.

[66] D. Pantarotto, C.D. Partidos, J. Hoebeke, F. Brown, E. Kramer, J.-P. Briand, S. Muller, M. Prato, A. Bianco, Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses, Chem. Biol. 10 (2003) 961–966.

[67] P.J. Carter, R.J. Samulski, Adeno-associated viral vectors as gene delivery vehicles, Int. J. Mol. Med. 6 (2000) 17–27.

[68] R. Singh, D. Pantarotto, D. McCarthy, O. Chaloin, J. Hoebeke, C.D. Partidos, J.-P. Briand, M. Prato, A. Bianco, K. Kostarelos, Binding and condensation of plasmid DNA onto functionalized carbon nanotubes: toward the construction of nanotube-based gene delivery vectors, J. Am. Chem. Soc. 127 (2005) 4388–4396.

[69] Y. Liu, D.-C. Wu, W.-D. Zhang, C.-B. He, S.H. Goh, X. Jiang, T.S. Chung, K.W. Leong, Polyethylenimine-grafted multiwalled carbon nanotubes for secure noncovalent immobilization and efficient delivery of DNA, Angew. Chem., Int. Ed. 44 (2005) 4782–4785.

[70] A. Bianco, J. Hoebeke, S. Godefroy, O. Chaloin, D. Pantarotto, J.-P. Briand, S. Muller, M. Prato, C.D. Partidos, Cationic carbon nanotubes bind to cpg oligodeoxynucleotides and enhance their immunostimulatory properties, J. Am. Chem. Soc. 127 (2005) 58–59.

[71] Q. Lu, J.M. Moore, A.M. Rao, L.L. Larcom, P.C. Ke, G. Huang, A.S. Mount, RNA polymer translocation with single-walled carbon nanotubes, Nano Lett. 4 (2004) 2473–2477.

[72] N.W.S. Kam, Z. Liu, H. Dai, Functionalization of carbon nanotubes via cleavable disulfide bonds for efficient intracellular delivery of sirna and potent gene silencing, J. Am. Chem. Soc. 127 (2005) 12492–12493.

[73] D. Cui, F. Tian, M. Wang, H. Gao, C.S. Ozkan, Effect of single wall carbon nanotubes on human HEK293 cells, Toxicol. Lett. 155 (2005) 73–85.

[74] M. Bottini, S. Bruckner, K. Nika, N. Bottini, S. Bellucci, A. Magrini, A. Bergamaschi, T. Mustelin, Multi-walled carbon nanotubes induce T lymphocyte apoptosis, Toxicol. Lett. 160 (2006) 121–126.

[75] 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. Watarib, K. Tohji, Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell THP-1 in vitro and subcutaneous tissue of rats in vivo, Mol. BioSyst. 1 (2005) 176–183.

[76] G. Jia, H. Wang, L. Yan, X. Wang, R. Pei, T. Yan, Y. Zhao, X. Guo, Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene, Environ. Sci. Technol. 39 (2005) 1378–1383.

[77] S.K. Manna, S. Sarkar, J. Barr, K. Wise, E.V. Barrera, O. Jejelowo, A.C. Rice-Ficht, G.T. Ramesh, Single-walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-κB in human keratinocytes, Nano Lett. 5 (2005) 1676–1684.

[78] N.A. Monteiro-Riviere, R.J. Nemanich, A.O. Inman, Y.Y. Wang, J.E. Riviere, Multi-walled carbon nanotube interactions with human epidermal keratinocytes, Toxicol. Lett. 155 (2005) 377–384.

[79] A.A. Shvedova, V. Castranova, E.R. Kisin, D. Schwegler-Berry, A.R. Murray, V.Z. Gandelsman, A. Maynard, P. Baron, Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells, J. Toxicol. Environ. Health, Part A 66 (2003) 1909–1926.

[80] C. Salvador-Morales, E. Flahaut, E. Sim, J. Sloan, M.L.H. Green, R.B. Sim, Complement activation and protein adsorption by carbon nanotubes, Mol. Immunol. 43 (2006) 193–201.

[81] C.M. Sayes, J.D. Fortner, W. Guo, D. Lyon, A.M. Boyd, K.D. Ausman, Y.J. Tao, B. Sitharaman, L.J. Wilson, J.B. Hughes, J.L. West, V.L. Colvin, The differential cytotoxicity of water-soluble fullerenes, Nano Lett. 4 (2004) 1881–1887.

[82] 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.

[83] 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.

[84] 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. Nanotech. 4 (2004) 1019–1024.