Expert Opinion

informa
healthcare

Carbon nanotubes in drug delivery: focus on infectious diseases

Yitzhak Rosen[†] & Noel M Elman[‡]

[†]*Superior NanoBioSystems LLC, 11722 Newbridge Court, Reston, Virginia, 20191, 385 N St., Southwest, Washington DC, 20024-2903, USA and* [‡]*Massachusetts Institute of Technology, Materials Science and Engineering Department, 77 Massachusetts Avenue, Room 12-002A, Cambridge, Massachusetts, USA*

Carbon nanotubes have the potential to address the challenges of combating infectious agents by both minimizing toxicity by dose reduction of standard therapeutics and allowing a multiple payload capacity to achieve both targeted activity and combating infectious strains, resistant strains in particular. One of their unique characteristics is the network of carbon atoms in the nanometer scale, allowing the creation of nano-channels via cellular membranes. This review focuses on the characterization, development, integration and application of carbon nanotubes as nanocarrier-based delivery systems and their appropriate design for achieving the desired drug delivery results in the different areas of infectious diseases. While a more extensive toxicological and pharmacological profile must be obtained, this review will focus on existing research and pre-clinical data concerning the potential use of carbon nanotubes.

Keywords: carbon nanotubes, drug delivery, functionalization, infectious diseases, multi-walled, nanodevices, nanotechnology, resistant, single-walled

Expert Opin. Drug Deliv. (2009) 6(5):517-530

1. Introduction

Carbon nanotubes (CNTs), first defined by Iijima in the early 1990s, are a new family of highly innovative nanomaterials that are being investigated for their potential in medicine [1-5,9,10]. CNTs consist of a network of carbon atoms in the nanometer scale which has great tensile strength as well as high electrical and thermal conductivity. CNTs are considered ideal nano-materials for several applications, which range from ultra-strong fibers to field emission displays. CNTs have recently generated great interest in medicine, where suitably modified CNTs can serve a variety of systems such as vaccine delivery systems or protein transporters [4-10].

CNTs exhibit superb physical and electrical characteristics. CNTs possess high current carrying capacity, excellent thermal conductivity, low thermal expansion coefficients and are less susceptible to electromigration when compared to other conventional materials at that scale, including copper, tungsten and aluminum. In addition, CNTs exhibit superior electrical and mechanical characteristics, which will potentially be implemented as future solutions for lab-on-chip applications [10,11-13].

Encouraging feasibility studies have shown the ability of CNTs to be used as delivery systems for genes, peptides, oligonucleotides, antimicrobial agents and cytotoxic drug molecules [14-21]. CNTs have delivered these macromolecules successfully inside cells. Moreover, one study by Ali-Boucetta demonstrated that multi-walled carbon nanotubes in aqueous dispersions using block copolymers can form supramolecular complexes with aromatic chromophore and chemotherapeutic agents such as doxorubicin via stacking and thus enhance their cytotoxicity [22].

10.1517/17425240902865579 © 2009 Informa UK Ltd ISSN 1742-5247

Table 1 indicates some of the advantages and disadvantages of CNTs in nano-medicine. It should be noted that the toxicological profiles of CNTs have been a subject of debate for potential use in clinical applications [23-25].

One of the most urgently needed applications for innovation is in the area of infectious diseases. Infectious diseases represent a highly dynamic arena where medications quickly lose their efficacy due to the emergence of resistant strains. The time required to introduce new medications is far longer than the development of resistant strains, which renders current treatments ineffective. In addition, several potential innovative treatments are unfeasible due to incompatible toxicological profiles. Moreover, conventional treatments demonstrate limited penetration and control to the targeted region [10,26-31].

The frequency and spectrum of antimicrobial and antifungal resistant infections have alarmingly increased in both the hospital and the community. Certain infections have essentially become untreatable, and have begun to occur as epidemics both in first world countries and the developing world. The increasing frequency of drug resistance has been attributed to combinations of microbial fungal and viral characteristics, selective pressures of antimicrobial and antifungal use, and societal and technological changes that enhance the transmission of drug-resistant micro-organisms and viruses. The increase in resistance mechanisms has resulted in a significant rise in morbidity, mortality and healthcare costs. Prevention and control of these infections will require the use of new antimicrobial, antifungal and antiviral agents, prudent use of existing agents, novel vaccines and enhanced public health efforts to reduce transmission [26-32].

The aim of this paper is to give an overview of the recent developments of CNTs for the implementation as potential drug delivery systems. Several applications will be discussed that focus on a number of areas in the field of infectious diseases. The paper will also discuss the existing toxicological and pharmacological profiles of CNTs.

2. Functionalized carbon nanotubes (*f*-CNTs)

New trends in CNTs allow for chemical functionalization in order to control chemical properties, including solubility and toxicity. CNTs can be employed in several biological applications, among which drug delivery appears to be particularly promising [6,10,33-35]. It has been shown by Singh *et al.* that the toxicity profile of carbon nanotubes can be altered by functionalization, solubility, and biocompability [36]. The properties indicating the differences between functionalized CNTs and micro/nanoparticles can be seen in Table 2.

Several functionalization approaches have been investigated for modification of CNTs. CNTs can be oxidized using strong acids, resulting in the reduction of their length while generating carboxylic groups, which increase their dispersibility in aqueous solutions [13-16,37-39]. Alternatively, additional reactions to the CNT external walls and tips make them soluble in water. For example, one of the processes is to use the 1,3 dipolar cyclo-addition reaction, which allows the CNTs to be water dispersible and soluble, thereby potentially rendering them compatible in a biological milieu [13,14,40-42]. A structural drawing of functionalized CNTs can be seen in Figure 1. Solubility under physiological conditions is therefore one of the key requirements to make CNTs biocompatible and potentially viable for future clinical applications [37-44]. Functionalized CNTs (*f*-CNTs) can be potentially used as vehicles for gene delivery, as they can linked to a wide variety of active molecules, including peptides, proteins, nucleic acids and other therapeutic agents [40,43-48].

It has been shown by Kostarelos *et al.* that various types of *f*-CNTs can be internalized by a wide range of cells, which include both prokaryotic and mammalian types, and can intracellularly traffic through different cellular barriers. Transportation to the perinuclear region has been observed within a few hours following contact with cells, even in conditions which inhibit endocytosis. The functionalization performed by this group involved the 1,3-dipolar cyclo-addition of azomethineylides which allows the insertion of amino functions around the sidewalls and at the tips of the CNTs, thereby allowing the tubes to be highly soluble in aqueous environments [49]. It has been suggested by Lacerda *et al.* (2008) that each functionalization method is probably producing CNTs with different characteristics. It has been suggested that this will lead to differences in the mechanism of CNT metabolism, degradation or dissolution, clearance and bioaccumulation [50]. The suggested potential use of CNTs as non-viral vectors for gene delivery is due their attractive physiochemical and electronic properties, as well as from very preliminary *in vivo* studies that demonstrated efficient mammalian cell internalization. Although such models have shown a great promise for gene delivery, as well as DNA and siRNA transfection models, further investigation and fundamental research work is required to understand the capabilities of CNTs in terms of functionalization properties, biocompatibility and toxicological profiles.

Lacerda *et al.* (2008) investigated the use of functionalized CNTs and demonstrated preliminary *in vivo* results for the delivery of DNA and siRNA using non-covalent functionalization for both SWCNTs and MWCNTs. Dai *et al.* (2009) investigated a number of non-covalent configurations for functionalization of SWCNTs, including a biotin–streptavidin system and adsorption properties, using surfactants and poly(ethyl) glycol to further investigate surface properties for binding functional groups as well as proteins onto CNTs. Therefore, CNTs have a great versatility for functionalization using both covalent and non-covalent bonds.

With respect to other forms of functionalization, Liu *et al.* introduced the concept of partitioning nanotubes, which involves imparting multiple chemical species with different functionalities on the same nanotube. These species include polyethylene glycol (PEG), drugs and fluorescent tags. Moreover, large surface areas have been demonstrated to exist for

Table 1. Properties and parameters that determine the advantages and disadvantages of carbon nanotubes in nanomedicine.

Pros	Cons
High stability *in vivo* because of their mechanical properties	Non-biodegradable
Large surface area available for multiple functionalization	Large surface area for protein opsonization
Capacity to easily pass biological barriers leading to novel biocompatible delivery systems	Insolubility of as-produced materials – functionalization is required to render the material compatible in physiological conditions
Unique electrical and conducting properties for the development of new devices for diagnostics	Strong tendency to aggregate
Empty internal space for encapsulation and transport of therapeutic and imaging molecules	Limited data on tolerance by healthy tissues
Bulk production associated with low costs	Extremely high variety of carbon nanotube types – standardization difficult

Table 2. Characteristics of functionalized carbon nanotubes in comparison with microparticles and nanoparticles.

	Functionalized carbon nanotubes	Micro/nanoparticles
Shape	Tubular form	Spherical form
Manufacturing	Easy fabrication and processing	Difficult large-scale production
Modification (i)	Functionalization with different groups	Functionalization with different groups
Modification (ii)	Molecules can be adsorbed or linked to the external walls. There is the potential to insert them into the tube cavity	Molecules can be attached or adsorbed at the surface or encapsulated
Modification (iii)	Good control of conjugation	Low loading capacity
Routes of administration	Potential administration via all routes	Administration via different routes (mucosal, systemic, transcutaneous)
Biocompatibility/biodegradability	Biocompatible/non-biodegradable	Biocompatible/biodegradable
Cell uptake	Good	Good
Cytotoxicity	Low toxicity (tested only *in vitro*)	Low toxicity
Immunogenicity	Absent	Absent
Adjuvant effect	Absent	Absent
Storage	Not tested	Instability of the active compounds into the spheres
Controlled release	Potential (possibility of tailor-made deliveiy according to the needs)	Critical to control drug release rates
Active delivery of molecules	Oligodeoxynucleotide, plasmid DNA	Oligodeoxynucleotide, plasmid DNA, antigens

supramolecular chemistry on single-walled carbon nanotubes (SWCNTs) pre-functionalized non-covalently or covalently by common surfactant or acid-oxidation routes according to Liu *et al.* [51].

Pristine single-walled CNTs are extremely hydrophobic tubes of hexagonic carbon (graphene), whereas multi-walled CNTs have several concentric graphene tubes [37]. Some studies reveal that CNT hydrophobicity and aggregation tendencies makes them harmful to cultured living cells [37,52,53]. Single-walled CNTs have diameters as small as 0.4 nm and lengths of up to few micrometers, whereas multi-walled CNTs can have diameters of up to 100 nm and lengths that range from micrometers up to several millimeters [37,54-60] This type of CNTs are shown in **Figure 2**.

Different methods have been proposed to deal with the solubility of CNTs. Covalent modifications by the organic functionalization of end-groups and sidewalls of CNTs have been among the most commonly proposed methods. Water soluble nano-hybrids have been created by starch, or oligomers such as poly(vinylpyrrolidone), which are able to wrap and transport SWCNTs into aqueous buffers. Oxidized CNTs exposed to strong acids to create hydroxyl and carboxyl groups at their ends allows coupling to a variety of biomolecules as well as an increase in dispersion in aqueous solutions [37-44,54-60].

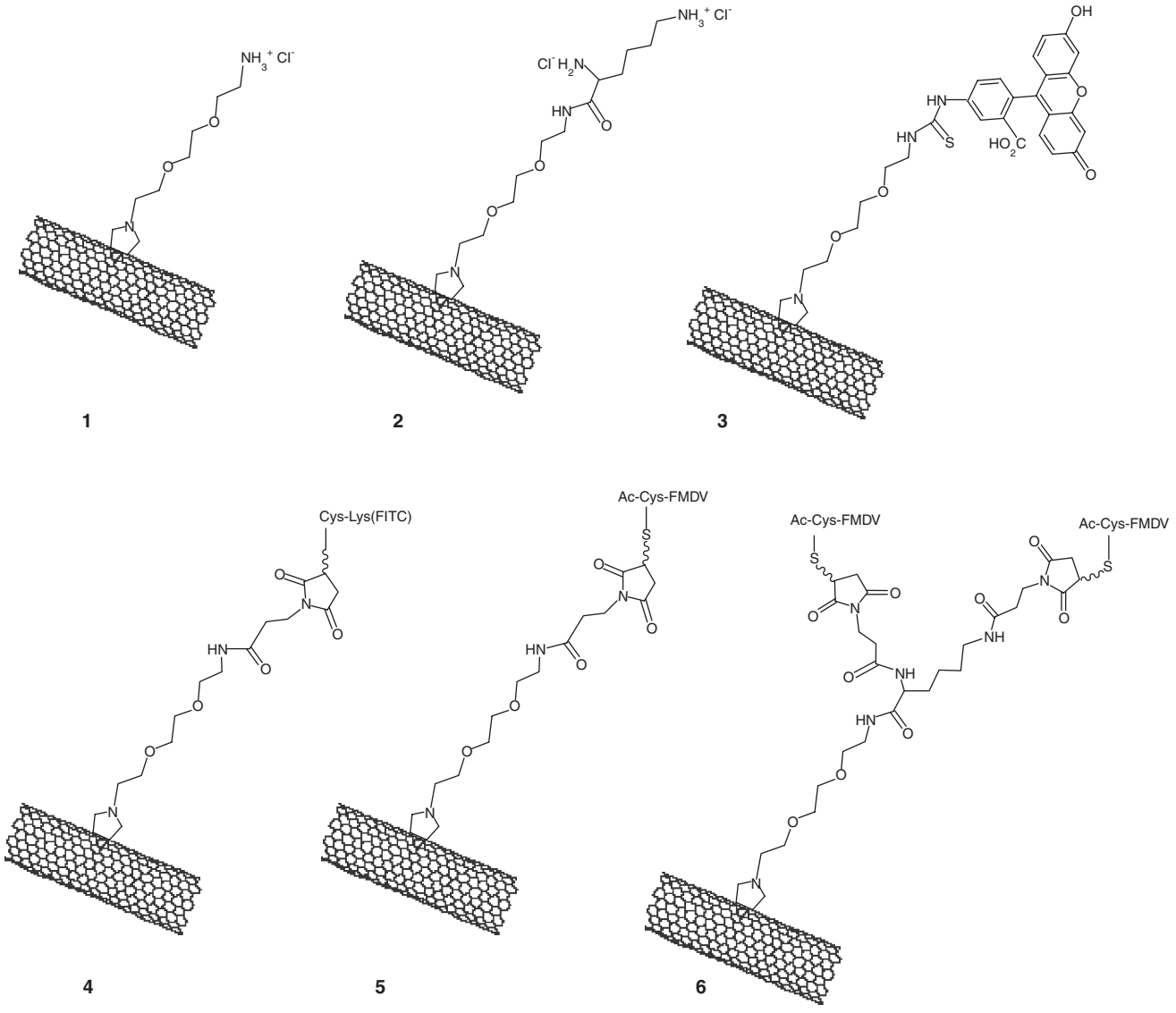

Figure 1. Structural drawings of functionalized carbon nanotubes.

3. CNTs and bacteria

Bacterial infections present a challenge in infectious disease therapeutics. Their constant modification mechanisms have been a major challenge for the research and development of antibiotics. Gene modification and transfers from different sources have been an important method of resistance mechanism. Antibiotic inactivation or modification such as the use of β-lactamases to disrupt the β-lactamase ring of penicillin is a common resistance mechanism. Alteration of the antibiotic binding site is another mechanism, which causes resistance to antibiotics seen in the ubiquitous Methicillin Resistant Staphylococcus Aureus (MRSA) strains in hospitals. An alternative metabolic pathway is involved in some of the sulfonamide-resistant bacteria which do not require para-aminobenzoic acid like their counterparts [26-32].

Cirz *et al.* demonstrated that inhibiting a mutation could serve as a novel therapeutic strategy for combating the evolution of antibiotic resistance. By interfering with the activity of the protease LexA, pathogenic *Escherichia coli* have been demonstrated to be unable to evolve resistance *in vivo* to ciprofloxacin or rifampicin. This corroborates the need to develop a drug delivery system, which can introduce such a targeted activity [61,62].

Another focus of current research is that of host defense peptides. These antimicrobial peptides are endogenous peptide antibiotics, playing an essential part in the immune system. They are involved in the direct killing of bacteria as well as having roles in antiviral and immunomodulatory functions. Delivery of similar peptides, which is difficult with current delivery modalities, will have an important impact in combating infections, particularly in patients where these peptides are scant in production [63-64].

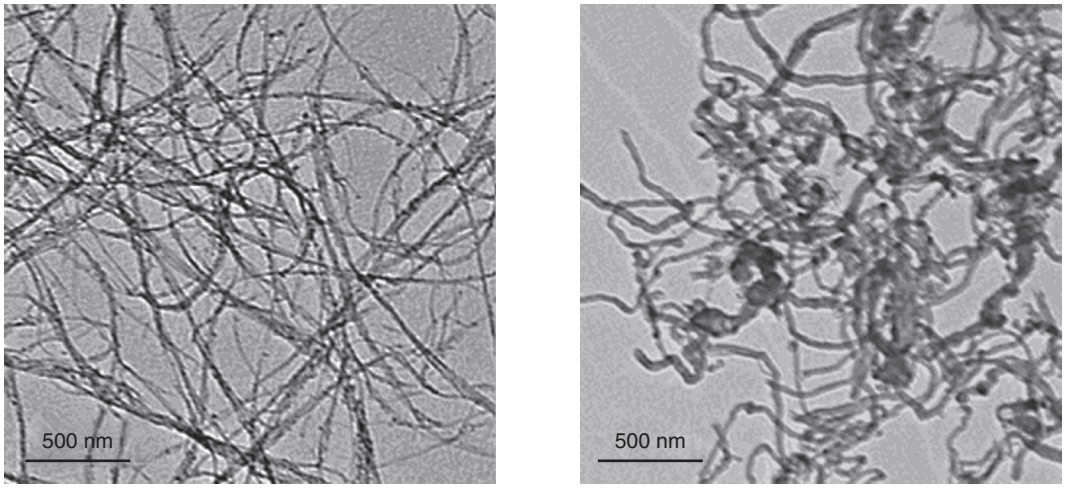

Figure 2. Transmission electron microscopy (TEM) photographs of pristine SWNT (left) and MWNT (right). These images were taken on pristine samples purchased from Carbon Nanotechnology Inc. (SWNT) and Nanostructured & Amorphous Materials Inc. (MWNT).
SWNT: Single-walled carbon nanotubes; MWNT: Multi-walled carbon nanotubes.

Interestingly, the potential for CNTs can also be applicable for mycobacterium infections, such as tuberculosis, with resistance strains in particular. These bacterial infections also produce resistance strains which require careful administration of therapeutic agents to decrease the threat of the emergence of resistant strains [31,32].

3.1 Carbon nanotubes and antibacterial effects

It has been demonstrated that CNTs exhibit antibacterial effects, and that the dimensional characteristics of the CNTs play a significant role [17,65-69]. An increased surface area leads to an increased opportunity for interaction with living cells. Single-walled CNTs (SWCNTs) exhibit significant cytotoxicity to human and animal cells. Multi-walled CNTs (MWCNTs) exhibit mild effects on the same type of cells. It should be noted that the results of cytotoxicity of CNTs can be contradictory to efficacy, as functionalization and differing purities can affect the toxicity [70-78].

It has been shown that a large population of *E. coli* cells was inactivated following direct contact with highly purified SWCNTs. SWCNTs were deposited without any culture medium. It is still unclear as to what specific mechanisms were involved in the process [69].

A recent article by Elimelech *et al.* shows that CNTs exhibit antibacterial effects and that the most likely CNT cytotoxicity mechanism is cell membrane damage by direct contact with CNTs [66]. Gene expression data showed that in the presence of both MWCNTs and SWCNTs, *E. coli* expresses high levels of stress-related gene products, with the quantity and magnitude of expression being much higher in the presence of SWCNTs. Experiments with well-characterized SWCNTs and MWCNTs demonstrated that SWCNTs are much more toxic to bacteria than MWCNTs, and may have the potential to be used as alternative agents to combat microbial infections resistant to traditional antibiotic treatments [67].

Kang *et al.* also demonstrated that SWCNTs exhibit a much stronger antibacterial activity than MWCNTs, thereby suggesting that the CNTs' size plays an important role in the process [67]. It has been suggested by Kang *et al.* that this may be attributed to smaller nanotubes due to the following reasons:

- Increased facilitation of partitioning and partial penetration into the cell wall.
- Increased interaction with the cell membranes due to increased contact area.
- Unique chemical and electronic properties translated into greater chemical reactivity.

Several cytotoxic mechanisms have been suggested concerning CNTs. Disruption of intracellular metabolic pathways, oxidative stress, physical membrane damage causing rupture and the generation of reactive oxygen species and oxidative stress have been among the proposed cytotoxic mechanisms. Human cells, incubated with CBTs, induce the production of tumor necrosis factor-alpha (TNF-α) and oxidative stress, leading to the production of nuclear factor-kappa B (NF-κB). Interestingly, there has been no direct experimental evidence of oxidative stress related to CNT-derived free radicals. Instead, CNTs have been shown to have free radical scavenging activity [67,70-78].

3.2 Nanoscale electroporation

Electroporation is a technique in which biomembranes are permeabilized by pulsed fields of several kV cm^{-1} amplitude and microsecond duration, which typically requires high electric field pulses. These pulses can lead to irreversible permeability and cell lysis [79-81].

CNTs have been demonstrated to target bacterial cells and allow a subsequent plasmid delivery using different microwave pulse durations which create temporary nanochannels across the cell envelope. This effect allows miniaturization of electroporation systems to the nanoscale, and provides selective targeting, as well as electroporation of cell organelles in eukaryotic cells and prokaryotic microorganisms [79-81].

It has been shown that exposure of *E. coli* cells to short microwave irradiation and low concentrations of CNTs had no detrimental effect on cell morphology and viability. The permeabilized sites reseal spontaneously, allowing the cells to continue to grow.

A system demonstrated by Rojas-Chapana *et al.* involving electroporation and MWCNTs, has been used to target the bacterial surface and deliver plasmids through temporary nano-channels across the cell envelope [80,81]. The potential use of such plasmid delivery developed more thoroughly for *in vivo* human studies may hold promise for the treatment of micro-abscesses and tumors situated in hard to reach areas, or for enhancing specific treatment in exposed areas [31,32].

3.3 CNTs as plasmid DNA vectors

Delivering genes requires an efficient delivery system. Currently, both viral and non-viral systems have been demonstrated. Viral delivery, albeit achieving high levels of gene expression, has several noteworthy disadvantages such as induction of immunogenic reactions, induction of inflammatory reactions which render transient transgene expression and potential oncogenic effects [31,32,82,83].

Non-viral vectors have the potential to overcome some of these disadvantages. Non-viral vectors can be assembled in cell-free systems from well-defined components. This construction gives them a superior advantage over viral vectors in terms of safety and manufacturing. Non-viral vectors still require a minimum therapeutic level of gene expression [13,14,31,32,82-84].

Cationic molecules such as various lipids, polylysine, protamine sulfate and cationic dendrimers have been used to condense DNA and form complexes, which have the ability to enhance the efficiency of gene transfer. These condensates commonly have a spherical morphology. The molecular interactions between DNA and the cationic component have an important impact on the number of biological processes relevant for gene expression. These processes include the following: enhancement of cell membrane interactions due to electrostatic forces; increased endocytosis; and improved trafficking to the nuclei [15].

CNTs can be functionalized in a similar way to gene delivery vehicles [13,14] (e.g., ammonium-functionalized SWCNTs and MWCNTs, and lysine functionalized SWCNTs with plasmid DNAs). It has been shown by Singh *et al.* that the three types of cationic carbon nanotubes are able to condense DNA and successfully deliver plasmid-DNA to cells, leading to a subsequent gene expression [14,15]. These results are important in particular for infectious agents which carry resistant genes, such as antibiotic-resistant bacteria [31,32]. Several noteworthy points should be made when considering the development of such vectors. Most cation and polycation DNA complexes transfect *in vitro* and *in vivo* at a variety of charge ratios, thereby requiring an optimization of ratios according to Singh *et al.* Factors such as complex size, surface charge, DNA topology and degree of condensation play an important role in determining what charge ratio is needed for optimal gene transfer. Interestingly, DNA complexed with MWCNTs shows a tighter association than that of SWCNTs. Highly condensed DNA is more resistant to serum inhibition. Tightly bound DNA may, however, be unable to detach from the nanotubes [14,15].

Much of the research conducted with DNA complexes implemented single-stranded DNA (ssDNA). This work was performed to increase the solubility and reduce the polydispersity of nanotubes in aqueous solutions. It would be beneficial to explore the different DNAs and possibly RNAs seen in virus vectors such as negative and positive strands and research their interactions with CNTs [31,32].

4. Viral infections

The treatment of many of the viral infections remains a challenge. A targeted novel approach of delivering antisense therapy and other entities which are typically too problematic to deliver may be instrumental. Therefore achieving a combination of targeted delivery of oligonucleotides, other genetic disrupters and other viral targets not normally feasible with current systems may be advantageous [31,32,85-102].

4.1 Delivery of antisense therapy

Antisense therapy delivery has been suggested as a promising tool for infectious diseases. The rapid degradation of antisense nucleic acids and poor diffusion across the cell membrane represent the main impediments. Antisense oligonucleotides research is considered a very promising technology for use in the development of drugs with both high target specificity and reduced side effects. Studies have demonstrated antisense inhibition of viral gene expression in biochemical assays, in cultured cells and in animal models [85-102]. Currently, there is ongoing antisense oligonucleotides research targeting human cytomegalovirus and human immunodeficiency virus, which is being evaluated in clinical trials. Cui *et al.* demonstrated the use of single-walled carbon nanotubes as a delivery system for transporting antisense myc into HL-60 cells [103].

One of the key challenges in gene therapy is the lipophilic nature of biological membranes, which restricts the intracellular delivery of external entities. SWCNTs do have the ability to cross cell membranes. Krajcik *et al.* chemically functionalized SWCNTs with hexamethylenediamine (HMDA) and poly(diallyldimethylammonium) chloride (PDDA) to obtain a combined material that was able to bind to negatively charged siRNA by electrostatic interactions. In preliminary studies, negligible cytotoxic effects were observed using PDDA-HMDA-SWCNTs on isolated rat heart cells at concentrations up to 10 mg/L. This drug delivery system, developed by Krajcik *et al.*, loaded with extracellular signal-regulated kinase (ERK) siRNA

was able to cross the cell membrane and to suppress expression of the ERK target proteins in primary cardiomyocytes by about 75% [13].

Such a drug delivery system implemented with SWCNTs and siRNA may be also viable for treatment of the hepatitis C virus (HCV) infection, which represents a major cause of chronic liver diseases and hepatocellular carcinoma. Current treatments have not demonstrated promising results, thus demanding innovative treatments [85-102]. One promising antiviral strategy implemented against HCV is the use of RNA interference (RNAi), a specific gene silencing process mediated by small interfering RNA (siRNA) duplexes. Chevalier *et al.* identified one siRNA, targeting a sequence that is highly conserved across all genotypes and forms a critical pseudo knot structure involved in translation, and considered it as one of the most promising therapeutic candidates among the siRNAs assessed. If siRNAs can be delivered with an acceptable toxicological and pharmacological profile, potentially effective treatments may be demonstrated for HCV [104]. A highly efficient delivery of siRNA by SWCNTs, which allows a more potent RNAi compared to conventional transfection agents, was demonstrated by Kam *et al.* [35]. The authors describe a novel functionalization scheme for SWCNTs which can afford nano-tube biomolecule conjugates with the incorporation of cleavable bonds to enable controlled molecular releasing from nanotube surfaces [35,44]. A highly efficient delivery of siRNA by SWNTs and achievement of a more potent RNAi functionality than a widely used conventional transfection agent was demonstrated [35].

5. Fungal infectious

Fungal infections are problematic for many reasons, one of which is that fungal cells share similar characteristics to eukaryotic cells. Antifungals such as Amphotericin B, a very potent antifungal agent, have numerous side effects greatly impeding their widespread use. Liposomal constituents have been implemented. There is still a limitation for targeted delivery and dose reduction as this agent has numerous side effects [31,32,102]. For example, Amphotericin B is highly toxic at 10 μg/mL, reaching a 40% cell mortality [102].

5.1 Antifungal delivery

Several formulations have been developed for Amphotericin B due to its renal toxicity and the fact that Amphotericin B is eliminated unchanged by the liver and the kidneys. The lipid formulations include liposomal Amphotericin B, Amphotericin B colloidal dispersion and Amphotericin B lipid complex, all of which have significant differences in their plasma pharmacokinetics. These differences can be attributed to the diverse disposition of the lipid moieties, while liberated Amphotericin B displays a pharmacokinetic behavior independent from the lipid formulation applied [31,32,42,105].

Several advantages make CNTs an ideal candidate for drug delivery of antifungal agents. Amphotericin B carried by CNTs preserved a very high antifungal activity. It has been shown that conjugated Amphotericin B with CNTs against candida is more potent than the drug alone. In addition, it was shown that the internalization of Amphotericin B linked to the nanotubes was dose dependent. Wu *et al.* showed that functionalized CNTs behave as nano-needles, with the ability to pass through the cell membrane without causing cell death [42]. Another advantage of CNTs suggested by Wu *et al.* is the prevention of aggregation phenomena presented typically by Amphotericin B in solution, which may increase its toxicity effects in cells. It was also demonstrated that conjugated Amphotericin B linked to CNTs in concentrations of up to10 μg/mL was not toxic. Functionalized CNTs with Amphotericin B showed activity even on strains resistant to Amphotericin B by an unclear mechanism [42].

6. Prions

Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders affecting humans and animals. The causative agent of TSEs is believed to be a prion protein (PrP), which is an abnormal, transmissible agent that is able to induce abnormal folding of normal cellular prion proteins in the brain, leading to brain damage and the characteristic signs and symptoms of the disease. Some of these distinguishing characteristics include long incubation periods, spongiform changes associated with neuronal loss and a failure to induce inflammatory response. These diseases are usually rapidly progressive and are always fatal. Unfortunately, early treatment is impossible in human patients since this disease can only be detected after the onset of neurological symptoms, even in familial cases. Prions are thought to consist mainly or entirely of misfolded PrP, a constitutively expressed host protein. Prions associated with the same PrP sequence may occur in the form of different strains; the strain phenotype is believed to be encoded by the conformation of the PrP [106-108].

According to Toupet *et al.*, the classical drug therapy has faced serious difficulties and the time has come to examine radically different strategies such as innovative gene therapies. Toupet *et al.* demonstrated that chronic injections of dominant negative lentiviral vectors into the brain may be a promising approach for a curative treatment of prion diseases [108]. Similarly, a targeted drug delivery system composed of carbon nanotubes implementing similar content without the use of a viral vector may have the potential of achieving similar results. Neurodegenerative diseases require therapeutic agents to be widely spread in the brain [108]. Carbon nanotubes, with their delivery abilities, may have the potential to address these issues [2,3,6,7,11].

7. Vaccines

In vaccinations, a critical component is the retention of antigen conformation, which is necessary to induce an antibody response with the right specificity. An important component

of vaccinations is the development of new and effective delivery approaches to administer protective antigens [16].

7.1 Enhancing neutralizing antibody responses in vaccines

Enhancing the neutralizing antibody response is an important method of potentially increasing the efficacy of a vaccine. Pantarotto *et al.* assessed virus-specific neutralizing antibody responses to a vaccine delivery system using covalent linkage to a neutralizing B cell epitope from the Foot and Mouth Diseases Virus (FMDV) to mono- and bis-derivatized CNTs as a vaccine preparation. The mono-derivatized CNTs elicited high levels of virus neutralizing antibodies. The CNT–peptide conjugates elicited antibodies of the IgG isotype, thereby suggesting that this system with ovalbumin is sampled by antigen presenting cells (APCs). The bystander cellular help provided by the ovalbumin-specific T Helper cells assists the B cells to produce the necessary antibodies targeted for the specified FMDV peptides [16].

Several advantages have been demonstrated using CNTs as vaccine delivery systems. First, CNTs themselves did not show any detectable immunogenicity. Second, the nanotubes displayed the B cell epitope with retained conformational characteristics.

Third, organic modification of CNTs allows the generation of multiple sites for attachment of bioactive molecules, thereby allowing generation of desired immune responses [16].

8. Toxicity of CNTs

Single-walled carbon nanotubes (SWCNTs) have been shown to be acutely toxic in a number of cell types [70-75]. CNTs can be harmful to cells in a time- and dose-dependent manner. This has been seen for pristine SWCNTs on the proliferation of HEK293 kidney epithelial cells and pristine MWCNTs on skin epithelial cells [37].

It has been observed that the physical form of carbon has a major impact on toxicity. CNTs are more toxic than similar chemical compounds of carbon in the form of amorphous carbon black, a material non-toxic even at the highest tested concentrations of 400 µg/mL. Direct observation of cellular uptake of SWCNTs has been demonstrated using transmission electron microscopy and confocal microscopy to image the translocation of SWCNTs into both stained and unstained human cells. Untreated CNTs were proven to exhibit a high toxicity concentration, as they can enter the cytoplasm and localize within the cell nucleus, causing cell mortality in a dose-dependent manner [37]. Liu *et al.* have shown that PEGylated SWCNTs show relatively long blood circulation times and a low uptake by the reticulo-endothelial system [109]. While Liu *et al.* suggest that drug molecules carried into the reticulo-endothelial system are released from SWCNTs and excreted via the biliary pathway without any obvious toxic effects to normal organs in a system that involves delivery of the chemotherapeutic agent paclitaxel in mice via a water soluble conjugate of SWCNT–paclitaxel, further studies are needed before clinical studies in humans are undertaken [110]. Moreover, Lacerda *et al.* concluded that toxicological and pharmacological profiles of CNT systems used as therapeutics will have to be better elucidated prior to undertaking any clinical studies [50].

Acute and chronic toxicity of functionalized SWCNTs when injected into the bloodstream of mice was examined by Schipper *et al.* Their results indicated no evidence of toxicity over 4 months by their survival, clinical and laboratory parameters. Necroscopy and tissue histology upon killing showed only age-related changes. In addition, using histology and Raman microscopic mapping it was possible to observe that these nano-tubes persisted within liver and spleen macrophages for 4 months without apparent toxicity [111].

The tissue distribution and blood clearance rates of intravenously administered CNT radiotracers study in mice by Singh *et al.* revealed several important results related to toxicity and pharmacological profiles. Urine excretion studies using *f*-SWCNT and *f*-MWCNTs followed by electron microscopy analyses showed that both were excreted as intact nanotubes. Systemic blood circulation clearance was through the renal excretion route. It was observed that *f*-SWCNTs were not retained in any of the reticulo-endothelial system organs. A rapid blood clearance and half-life of 3 h were observed for *f*-SWCNTs [36].

A renal clearance of CNTs study by Lacerda *et al.* demonstrated several important findings. One observation was the localization of CNT dispersions in the glomerular capillaries, which were not able translocate through the kidney glomerular filtration barrier when these dispersions were not adequately individualized, or had aggregated *in vivo*. MWCNTs were observed translocating both at 5 min and 30 min post-injection and it has been suggested that MWCNTs are capable of reorientation while in the blood circulation. It has been suggested that the longitudinal nanotube dimension does not appear to be critical for renal clearance, according to Lacerda *et al.* Moreover, the authors suggest that a fine balance exists between CNT shape, backbone structure, surface character and a degree of individualization, which will determine the pharmacological and excretion profile of these fibrous nano-materials *in vivo* [112].

A recent study conducted by Takagi *et al.* (2006) demonstrated that the structural properties of CNTs contribute significantly to the toxicological profile. The mouse model studied demonstrated that MWCNTs that are 10 – 20 microns in length are carcinogenic, typically inducing mesothelioma, of a similar toxicity to asbestos [77]. A more prudent approach to handling CNTs should be utilized, especially when investigating CNTs as potential new delivery vectors. In assessing the toxicity of CNTs, not only the functionalization of the CNTs and chemical degradation should be considered, but also the physical properties and structural dimensions.

8.1 Lung toxicity of CNTs

Several *in vivo* studies on rodents have indicated that CNTs have the ability to induce inflammation, granulomas, fibrosis,

as well as biochemical and toxicological changes in the lungs. In addition, complex interactions involving pathophysiological mechanisms such as inflammation and oxidative stress can act synergistically and amplify pulmonary toxicity when exposed to SWCNTs [113].

It has been shown by Lacerda *et al.* that the high degree of functionalization responsible for adequate individualization of nanotubes, rather than the nature of the functional groups, is the critical factor limiting tissue accumulation and normal tissue physiology at least within the first 24 h after administration. Moreover, it has been shown that the higher degree of functionalization of MWCNT-NH_3+, the less the accumulation in tissues. Lacerda *et al.* demonstrated an accumulation mostly in the lung and liver in dark clusters of purified MWCNTs coated with autologous serum proteins prior to injection of the mice [113].

Poland *et al.* have demonstrated that the exposure of the mesothelial lining of the body cavity of mice to long MWCNTs results in asbestos-like, length-dependent pathological manifestations. These manifestations include inflammation and granulomas. Since both CNTs and asbestos have similar fiber needle shapes, the major concern is the development of mesothelioma, a devastating malignant tumor, which can be caused by asbestos. Poland *et al.* suggest that scrutiny is needed before introducing CNTs to the market [114].

MWCNTs have been documented to form fibrous or rod-shaped particles of around 10 – 20 micrometers in length with an aspect ratio of more than three. Fibrous particles of this dimension range, including asbestos and other man-made fibers, are reported to be carcinogenic, inducing the highly malignant tumor mesothelioma. MWCNTs have been reported to induce mesothelioma along with a positive control, crocidolite (blue asbestos), when administered intraperitoneally to p53 heterozygous mice, which have already been reported to be sensitive to asbestos. Takagi *et al.* suggests that their results may raise the possibility that carbon-made fibrous or rod-shaped micrometer particles may share the carcinogenic mechanisms postulated for asbestos [77].

Warheit *et al.* demonstrated that pulmonary exposures to SWCNTs in rats produced a non-dose-dependent series of multifocal granulomas. These granulomas showed evidence of being a foreign tissue body reaction and were non-uniform in distribution and not progressive beyond one-month post-exposure. However, Warheit *et al.* suggest that the observation of SWCNT-induced multifocal granulomas is inconsistent with several findings. These findings, according to the authors, include a lack of lung toxicity by assessing lavage parameters, a lack of lung toxicity by measuring cell proliferation parameters, an apparent lack of a dose–response relationship, a non-uniform distribution of lesions, the paradigm of dust-related lung toxicity effects, as well as the possible regression of effects over time [71].

In a study conducted by Lam *et al.*, pulmonary toxicity was assessed and compared in intratracheally instilled mice with concentration ranges of 0.1 – 0.5 mg of CNTs, a carbon black negative control, or a quartz positive control, and killed at 7 days or 90 days [23,78]. Lam *et al.* demonstrated that all nanotube products induced dose-dependent epithelioid granulomas and, in some cases, interstitial inflammation in the animals of the 7-day groups. In the 90-day studies, the observed lesions persisted and were more pronounced. In addition, the lungs of some animals revealed peribronchial inflammation and necrosis that had extended into the alveolar septa. Lam *et al.* concluded in their mouse model that if carbon nanotubes reach the lungs, they are much more toxic than carbon black, and can even be more toxic than quartz, which is considered a serious occupational health hazard in chronic inhalation exposures. Since geometry and the surface chemistry of particulates can play an important role in causing lung toxicity, these two parameters must be taken into account for future animal studies [28,115].

8.2 Immunotoxicity of CNTs

Several important effects of CNTs on the immune system have been documented. Dumortier *et al.* assessed the effects of *f*-CNTs – pristine single-walled carbon nanotubes (SWCNTs) – on the immune system, following the 1,3-dipolar cyclo-addition reaction and the oxidation/amidation treatments. Their results showed that both types of *f*-CNTs are taken up by B and T lymphocytes as well as macrophages *in vitro*, without affecting cell viability. In addition, they showed that making *f*-CNT a highly water soluble compound did not influence the functional activity of immunoregulatory cells. Moreover, their results showed that *f*-CNTs having a reduced solubility and forming stable water suspensions preserved lymphocytes' functionality, albeit provoking the secretion of proinflammatory cytokines by macrophages [19].

Oxidized CNTs are more toxic than hydrophobic pristine CNTs. Oxidized CNTs are considered more suitable for biological applications. Oxidized CNTs can be dispersed in aqueous solution and achieve higher concentrations of free CNTs at similar weight per volume values [37,52,53]. Bottini *et al.* calculated that the less toxic amount of 40 µg/mL of CNTs under their experimental conditions is equal to an estimated 10^6 individual CNTs per cell. This calculation is based on an average length of approximately one micrometer and a diameter of 40 nm, having an average molecular mass of $5x10^{-9}$ [37].

CNTs, specifically MWCNTs both pristine and oxidized, can cause a time-dependent decrease in the viability of Jurkat T leukemia cells. At 400 µg/mL, oxidized CNTs cause a loss of more than 80% of the cells within 5 days, while pristine CNTs killed less than half of this number of cells. Amorphorphous carbon, in comparison, at 400 µg/mL had a minimal effect on cell viability [37].

MWCNTs were also assessed for apoptosis of T cells and signal transduction by the T-cell antigen receptor by Bottini *et al.* Both pristine and oxidized CNTs caused apoptosis in a dose- and time-dependent fashion. Oxidized CNTs appeared to be more toxic in terms of apoptosis than pristine CNTs in this particular study [37]. It should be noted, however, that batches

Table 3. Potential applications and issues related to CNTs for treatment of diseases.

Type of CNT	Pathogen/disease type	Potential efficacy of treatment
SWCNTs	Mycobacterium, *E. Coli*, *Staphylococcus Aureus* [17,65-69] [69-78]	*In vitro* – very high. Further *in vitro* models required to establish the toxicity of treatment for eventual *in vivo* model
CNTs	Fungal infections [42]	*In vitro* – very high. *In vivo* – potentially high – concern with very high toxicity level for *in vivo* applications
SWCNTs	Hepatitis C virus [35,104]	*In vitro* – high. *In vivo* – potentially high – concern with toxicity level
CNTs, SWCNTs	Prions [2,3,6,7,11].	*In vitro* – potentially high. Further *in vitro* and *in vivo* models required, as well as for toxicity studies
f-CNTs (maleimido-derivatized water soluble)	FMDV [16]	Preliminary study very promising, showing high potential. Further *in vitro* and *in vivo* studies required for efficacy, as well as for toxicity
f-CNTs (antisense, siRNA)	Cancer [31,32,82,83].	*In vitro* – potentially high. Basic animal models required to obtain a preliminary understanding of potential

of pristine CNTs can show impurities, with metallic and amorphous carbon nanoparticles causing severe toxic effects [116]. As for signal transduction, 40 μg/mL of oxidized CNTs had a small stimulatory effect on the basal level of intracellular tyrosine phosphorylation but virtually no effect on the receptor-induced increase in this phosphorylation [37].

Several recommendations have been made by Bottini *et al.* concerning the use of this type of CNTs. First, CNTs should be used at concentrations much lower than 40 μg/mL, or 1 ng/cell. Second, cell viability should be carefully followed with all new forms of CNTs. It has been suggested that cell toxicity will depend also on the physical form, diameter, length and nature of attached molecules or nano-materials [37].

9. Conclusion

This review describes the potential application of carbon nanotubes in infectious diseases, and in resistant strains in particular. The ability to carry multiple payloads potentially allows carbon nanotubes to be used both as a targeted therapy and combinational therapy for resistant strains, as well as a powerful vaccine delivery agent. Functionalization allows them to be soluble in aqueous solution. Further research and development is needed to accurately delineate their toxicological and pharmacological profiles.

10. Expert opinion

Infectious diseases clearly provide a challenge with standard therapeutic measures. First, the emerging resistance mechanisms of many infectious entities takes place much faster than the introduction of new therapeutic agents, that require many years of research and development. Despite the cautious use of these agents, emerging resistance still takes place, albeit at a relatively slower pace. In addition, several of the agents have problematic side effects, such as Amphotericin B. Therefore, there is a strong need for developing delivery systems that will be able to carry several types of payloads, combating resistance mechanisms and at the same time be able to target specific areas of interest to avoid several of the deleterious effects associated with high doses.

There are still a number of toxicity issues that need to be resolved. Residence time, degradation mechanisms and clearance are regarded as unresolved issues. In addition, interactions with DNA, siRNA and other biological components are still unclear in terms of the potentially effective clinical applications. Deposits of CNTs in a variety of tissues still have unknown side effects. This may be a major impediment to their introduction into human subjects. **Table 3** summarizes some of the potential applications and issues related to CNTs for the treatment of diseases.

A necessary interdisciplinary approach by both nano-material engineering and medical sciences is needed to address these issues. First, a more comprehensive toxicity profile is needed. Second, a more thorough understanding of the advantages of CNTs over biodegradable nanomaterials is required in order to assess the relevant medical indications. Third, the interactions of CNTs with clearance and detoxification mechanisms seen in the kidneys and liver respectively must be delineated in order to corroborate whether a desired toxicity profile can be achieved.

CNTs may have the potential to drastically change how drug delivery is conducted regarding infectious diseases. A main reason for this is the ability of CNTs to carry macromolecules unable to pass through the cellular membrane by themselves. In the field of vaccination, multiple conjugate

payloads may offer a significant advantage in the production of desired neutralizing antibodies and other related immune responses. In the field of antifungals, CNTs may be able to limit the highly deleterious effects of these agents as they are targeted to fungal cells, which carry many similarities to human eukaryotic cells. In the field of viruses, CNTs may offer the ability to directly provide an innovative and potentially effective treatment against viruses, rather than simply limit their proliferation. For emerging resistant strains by a variety of agents, CNTs have the potential to carry multiple and targeted payloads, which are able to overcome typical resistant mechanisms, for example, point mutations.

Declaration of interest

The authors declare that this work was performed independently under their own auspices. No institutional funding was requested for this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Iijima S. Helical microtubules of graphitic carbon. Nature 1991;354:56-8
2. Bianco A. Carbon nanotubes for the delivery of therapeutic molecules. Expert Opin Drug Deliv 2004;1(1):57-65

•• **Excellent review of the applications of carbon nanotubes.**

3. Prato M, Kostarelos K, Bianco A. Functionalized carbon nanotubes in drug design and discovery. Acc Chem Res 2008;41(1):60-8

• **Provides a well-rounded overview of carbon nanotubes in drug design and discovery.**

4. Pantarotto D, Partidos CD, Graff R, et al. Synthesis, structural characterization, and immunological properties of carbon nanotubes functionalized with peptides. J Am Chem Soc 2003;125(20):6160-4
5. Tohji K, Akimoto Y, Shinoda K, et al. Kenichiro shibata preparation of carbon nanotubes for biomedical applications: length control, surface modification, and biocompatibility [abstract 585]. 205th Meeting, 2004 The Electrochemical Society, Inc
6. Bianco A, Kostarelos K, Prato M. Opportunities and challenges of carbon-based nanomaterials for cancer therapy. Expert Opin Drug Deliv 2008;5(3):331-42

•• **Excellent review on new carbon-based nanomaterials and their application in cancer.**

7. Escorcia FE, McDevitt MR, Villa CH, Scheinberg DA. Targeted nanomaterials for radiotherapy. Nanomed 2007;2(6):805-15
8. Angelikopoulos P, Bock H. Directed self-assembly of surfactants in carbon nanotube materials. J Phys Chem B 2008;112(44):13793-801
9. Awasthi K, Srivastava A, Srivastava ON. Synthesis of carbon nanotubes. J Nanosci Nanotechnol 2005;5(10):1616-36
10. Thostenson ET, Ren ZF, Chou TW. Advances in the science and technology of carbon nanotubes and their composites: a review. Composites Sci Technol 2001;61(13):1899-912
11. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. Curr Opin Chem Biol 2005;9(6):674-9
12. Saito R, Dresselhaus G, Dresselhaus MS. Physical properties of carbon nanotubes. Imperial College Press. London, UK, 1998
13. Krajcik R, Jung A, Hirsch A, et al. Functionalization of carbon nanotubes enables non-covalent binding and intracellular delivery of small interfering RNA for efficient knock-down of genes. Biochem Biophys Res Commun 2008;369(2):595-602
14. Pantarotto D, Singh R, McCarthy D, et al. Functionalized carbon nanotubes for plasmid DNA gene delivery. Angew Chem Int Ed Engl 2004;43(39):5242-6
15. Singh R, Pantarotto D, McCarthy D, et al. Binding and condensation of plasmid DNA onto functionalized carbon nanotubes: toward the construction of nanotube-based gene delivery vectors. J Am Chem Soc 2005;127(12):4388-96
16. Pantarotto D, Partidos CD, Hoebeke J, et al. Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses. Chem Biol 2003;10(10):961-6

• **Promising research in the area of vaccine delivery.**

17. Yacoby I, Benhar I. Antibacterial nanomedicine. Nanomed 2008;3(3):329-41
18. Chakravarty P, Marches R, Zimmerman NS, et al. Thermal ablation of tumor cells with antibody-functionalized single-walled carbon nanotubes. Proc Natl Acad Sci USA 2008;105(25):8697-702
19. Dumortier H, Lacotte S, Pastorin G, et al. Functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells. Nano Lett 2006;6(7):1522-8
20. McDevitt MR, Chattopadhyay D, Kappel BJ, et al. Tumor targeting with antibody functionalized, radiolabeled carbon nanotubes. J Nucl Med 2007;48:1180-9
21. McDevitt MR, Chattopadhyay D, Jaggi JS, et al. PET imaging of soluble yttrium-86-labeled carbon nanotubes in mice. PLoS ONE 2007;2(9):e907
22. Ali-boucetta H, Al-jamal KT, Mccarthy D, et al. Multiwalled carbon nanotube-doxorubicin supramolecular complexes for cancer therapeutics. Chem Commun 2008;(4):459-61
23. Lam CW, James JT, McCluskey R, et al. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. Crit Rev Toxicol 2006;36(3):189-217
24. Borrelli I. Nanotubes and occupational medicine. Article in Italian. G Ital Med Lav Ergon 2007;29(3 Suppl):851-2
25. Magrez A, Kasas S, Salicio V, et al. Cellular toxicity of carbon-based nanomaterials. Nano Lett 2006;6(6):1121-5
26. Cohen ML. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 1992;257(5073):1050-5
27. Aarestrup FM, Wegener HC, Collignon P. Resistance in bacteria of the food chain: epidemiology and control strategies.

Expert Rev Anti Infect Ther 2008;6(5):733-50

28. Banerjee R, Schecter GF, Flood J, Porco TC. Extensively drug-resistant tuberculosis: new strains, new challenges. Expert Rev Anti Infect Ther 2008;6(5):713-24
29. Pitout JD. Multiresistant Enterobacteriaceae: new threat of an old problem. Expert Rev Anti Infect Ther 2008;6(5):657-69
30. Arias CA, Murray BE. Emergence and management of drug-resistant enterococcal infections. Expert Rev Anti Infect Ther 2008;6(5):637-55
31. Mandell GL, Bennett JE, Dolin R. Principles and practice of infectious diseases. Sixth edition. Churchill Livingstone. Various pages. 2004
32. Harrison's principles of internal medicine. Sixteenth edition. In: Kasper D, Braunwald E, Hauser S, Longo D, Jameson JL, Fauci AS, editors, McGraw-Hill Professional 2004, various pages
33. Georgakilas V, Kordatos K, Prato M, et al. Organic functionalization of carbon nanotubes. J Am Chem Soc 2002;124(5):760-1
34. Pastorin G, Wu W, Wieckowski S, et al. Double functionalization of carbon nanotubes for multimodal drug delivery. Chem Commun 2006;(11):1182-4
35. Kam NW, Liu Z, Dai H. Functionalization of carbon nanotubes via cleavable disulfide bonds for efficient intracellular delivery of siRNA and potent gene silencing. J Am Chem Soc 2005;127(36):12492-3
36. Singh R, Pantarotto D, Lacerda L, et al. Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. Proc Natl Acad Sci USA 2006;103(9):3357-62
37. Bottini M, Bruckner S, Nika K, et al. Multi-walled carbon nanotubes induce T lymphocyte apoptosis. Toxicol Lett 2006;160(2):121-6
38. Liu J, Rinzler AG, Dai H, et al. Fullerene pipes. Science 1998;280(5367):1253-6
39. Bottini M, Tautz L, Huynh H, et al. Covalent decoration of multi-walled carbon nanotubes with silica nanoparticles. Chem Commun 2005;(6):758-60
40. Georgakilas V, Tagmatarchis N, Pantarotto D, et al. Amino acid functionalisation of water soluble carbon nanotubes. Chem Commun 2002;(24):3050-1
41. Isobe H, Tanaka T, Maeda R, et al. Preparation, purification, characterization, and cytotoxicity assessment of water-soluble, transition-metal-free carbon nanotube aggregates. Angew Chem Int Ed Engl 2006;45(40):6676-80
42. Wu W, Wieckowski S, Pastorin G, et al. Targeted delivery of Amphotericin B to cells by using functionalized carbon nanotubes. Angew Chem Int Ed Engl 2005;44(39):6358-62
43. Pantarotto D, Briand JP, Prato M, Bianco A. Translocation of bioactive peptides across cell membranes by carbon nanotubes. Chem Commun 2004;(1):16-7
44. Shi Kam NW, Jessop TC, Wender PA, Dai H. Nanotube molecular transporters: internalization of carbon nanotube-protein conjugates into Mammalian cells. J Am Chem Soc 2004;126(22):6850-1
45. Tasis D, Tagmatarchis N, Bianco A, Prato M. Chemistry of carbon nanotubes. Chem Rev 2006;106(3):1105-36
46. Tasis D, Tagmatarchis N, Georgakilas V, Prato M. Soluble carbon nanotubes. Chemistry 2003;9(17):4000-8
47. Fu K, Sun YP. Dispersion and solubilization of carbon nanotubes. J Nanosci Nanotechnol 2003;3(5):351-64
48. Chang LW, Lue JT. Electrical conductance in a single carbon nanofiber. J Nanosci Nanotechnol 2005;5(10):1672-6
49. Kostarelos K, Lacerda L, Pastorin G, et al. Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. Nat Nanotechnol 2007;2(2):108-13
50. Lacerda L, Bianco A, Prato M, Kostarelos K. Carbon nanotube cell translocation and delivery of nucleic acids in vitro and in vivo. J Mater Chem 2008;(18):17-22
51. Liu Z, Sun X, Nakayama-ratchford N, Dai H. Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery. ACS Nano 2007;1(1):50-6
52. Cui D, Tian F, Ozkan CS, et al. Effect of single wall carbon nanotubes on human HEK293 cells. Toxicol Lett 2005;155(1):73-85
53. Monteiro-Riviere NA, Nemanich RJ, Inman AO, et al. Multi walled carbon nanotube interactions with human epidermal keratinocytes. Toxicol Lett 2005;155(3):377-84
54. Huang XM, Caldwell R, Huang L, et al. Hone Controlled placement of individual carbon nanotubes. J Nano Lett 2005;5(7):1515-8
55. Du Z, Yu YL, Chen XW, Wang JH. The isolation of basic proteins by solid-phase extraction with multiwalled carbon nanotubes. Chemistry 2007;13(34):9679-85
56. Salvador-Morales C, Basiuk EV, Basiuk VA, et al. Effects of covalent functionalization on the biocompatibility characteristics of multi-walled carbon nanotubes. J Nanosci Nanotechnol 2008;8(5):2347-56
57. Zou J, Ji B, Feng XQ, Gao H. Molecular-dynamic studies of carbon-water-carbon composite nanotubes. Small 2006;2(11):1348-55
58. Pillai SK, Ray SS, Moodley M. Purification of single-walled carbon nanotubes. J Nanosci Nanotechnol 2007;7(9):3011-47
59. Reilly RM. Carbon nanotubes: potential benefits and risks of nanotechnology in nuclear medicine. J Nucl Med 2007;48(7):1039-42
60. Star A, Steuerman DW, Heath JR, Stoddart JF. Starched carbon nanotubes. Angew Chem Int Ed Engl 2002;41(14):2508-12
61. Cirz RT, Chin JK, Andes DR, et al. Inhibition of mutation and combating the evolution of antibiotic resistance. PLoS Biol 2005;3(6):e176
62. Cohen ML. Epidemiology of drug resistance – implications for a postantimicrobial era. Science 1992;257(5073):1050-5
63. Bowdish DM, Davidson DJ, Hancock RE. A re-evaluation of the role of host defence peptides in mammalian immunity. Curr Protein Pept Sci 2005;6(1):35-51
64. Agerberth B, Gudmundsson GH. Host antimicrobial defence peptides in human disease. Curr Top Microbiol Immunol 2006;306:67-90
65. Brady-Estevez AS, Kang S, Elimelech M. A single-walled carbon nanotube filter for

removal of viral and bacterial pathogens. Small 2008;(4):81-484

66. Elimelech M. Carbon nanotubes kill bacteria. Mater Technol 2007;(3):79-179

67. Kang S, Herzberg M, Rodrigues DF, Elimelech M. Antibacterial effects of carbon nanotubes: size does matter. Langmuir 2008;24(13):6409-13

• **Important research paper indicating antibacterial effects and physical determinants.**

68. Kang S, Mauter MS, Elimelech M. Physicochemical determinants of multiwalled carbon nanotube bacterial cytotoxicity. Environ Sci Technol 2008;42(19):7528-34

69. Kang S, Pinault M, Pfefferle LD, Elimelech M. Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir 2007;23(17):8670-3

70. Muller J, Huaux F, Moreau N, et al. Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharmacol 2005;207(3):221-31

71. Warheit DB, Laurence BR, Reed KL, et al. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. Toxicol Sci 2004;77(1):117-25

72. Medina C, Santos-Martinez MJ, Radomski A, et al. Nanoparticles: pharmacological and toxicological significance. Br J Pharmacol 2007;150(5):552-8 [Epub 2007 Jan 22]

73. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. Int J Nanomedicine 2008;3(2):133-49

74. Lanone S, Boczkowski J. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. Curr Mol Med 2006;6(6):651-63

75. Tian F, Cui D, Schwarz H, et al. Cytotoxicity of single-wall carbon nanotubes on human fibroblasts. Toxicol In Vitro 2006;20(7):1202-12

76. Wörle-Knirsch JM, Pulskamp K, Krug HF. Oops they did it again! Carbon nanotubes hoax scientists in viability assays. Nano Lett 2006;6(6):1261-8

77. Takagi A, Hirose A, Nishimura T, et al. Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. J Toxicol Sci 2008;33(1):105-16

78. Lam CW, James JT, McCluskey R, Hunter RL. Pulmonary toxicity of single-wall arbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol ci 2004;77(1):126-34

79. Joshi RP, Schoenbach KH. Mechanism for membrane electroporation rreversibility under high-intensity, ultrashort electrical pulse conditions. Phys Rev E Stat Nonlin Soft Matter Phys 2002;66(5 Pt 1):052901

80. Rojas-Chapana JA, Correa-duarte MA, Ren ZF, et al. Enhanced introduction of gold nanoparticles into vital acidothiobacillus ferrooxidans by carbon nanotube-based microwave electroporation. Nano Lett 2004;4(5):985-8

81. Rojas-Chapana J, Troszczynska J, Firkowska I, et al. Multiwalled carbon nanotubes for plasmid delivery into Escherichia coli cells. Lab Chip 2005;5(5):536-9

82. Walther W, Stein U. Viral vectors for gene transfer: a review of their use in the treatment of human diseases. Drugs 2000;60(2):249-71

83. Bokhoven M, Stephen SL, Knight S, et al. Insertional gene activation by lentiviral and gammaretroviral vectors. J Virol 2008

84. Itaka K, Kataoka K. Recent development of non-viral gene delivery systems with virus-like structures and mechanisms. Eur J Pharm Biopharm 2008

85. Zein NN, Rakela J, Krawitt EL, et al. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative Study Group. Ann Intern Med 1996;125(8):634-9

86. Sharara AI, Hunt CM, Hamilton JD. Hepatitis C. Ann Intern Med 1996;125(8):658-68

87. Resnick RH, Koff R. Hepatitis C-related hepatocellular carcinoma. Prevalence and significance. Arch Intern Med 1993;153(14):1672-7

88. Cholongitas E, Papatheodoridis GV. Review article: novel therapeutic options for chronic hepatitis C. Aliment Pharmacol Ther 2008;27(10):866-84

89. Zhang H, Hanecak R, Brown-Driver V, et al. Antisense oligonucleotide inhibition of hepatitis C virus (HCV) gene expression in livers of mice infected with an HCV-vaccinia virus recombinant. Antimicrob Agents Chemother 1999;43(2):347-53

90. Agrawal S, Lisziewicz J. Potential for HIV-1 treatment with antisense oligonucleotides. J Biotechnol 1994;Healthcare 1:167-82

91. Alt M, Renz R, Hofschneider PH, et al. Specific inhibition of hepatitis C viral gene expression by antisense phosphorothioate oligodeoxynucleotides. Hepatology 1995;22(3):707-17

92. Anderson KP, Fox MC, Brown-Driver V, et al. Inhibition of human cytomegalovirus immediate-early gene expression by an antisense oligonucleotide complementary to immediate-early RNA. Antimicrob Agents Chemother 1996;40(9):2004-11

93. Azad RF, Driver VB, Tanaka K, et al. Antiviral activity of a phosphorothioate oligonucleotide complementary to RNA of the human cytomegalovirus major immediate-early region. Antimicrob Agents Chemother 1993;37(9):1945-54

94. Offensperger WB, Offensperger S, Walter E, et al. In vivo inhibition of duck hepatitis B virus replication and gene expression by phosphorothioate modified antisense oligodeoxynucleotides. EMBO J 1993;12(3):1257-62

95. Raviprakash K, Liu K, Matteucci M, et al. Inhibition of dengue virus by novel, modified antisense oligonucleotides. J Virol 1995;69(1):69-74

96. Vidalin O, Major ME, Rayner B, et al. In vitro inhibition of hepatitis C virus gene expression by chemically modified antisense oligodeoxynucleotides. Antimicrob Agents Chemother 1996;40(10):2337-44

97. Bennett CF, Kornbrust D, Henry S, et al. An ICAM-1 antisense oligonucleotide prevents and reverses dextran sulfate sodium-induced colitis in mice. J Pharmacol Exp Ther 1997;280(2):988-1000

98. Crooke ST. Therapeutic applications of oligonucleotides. Annu Rev Pharmcol Toxicol 1992;32:329-76

99. Crooke ST. Oligonucleotide therapeutics. Fifth edition. In: Wolff ME, editor, Burger's medicinal chemistry and drug discovery, John Wiley & Sons, Inc., New York, N.Y. 1995 vol. 1. p. 863-900

100. Dean N, McKay R, Miraglia L, et al. Inhibition of growth of human tumor cell lines in nude mice by an antisense of

oligonucleotide inhibitor of protein kinase C-alpha expression. Cancer Res 1996;56(15):3499-507

101. Monia BP, Johnston JF, Geiger T, et al. Antitumor activity of a phosphorothioate antisense oligodeoxynucleotide targeted against C-raf kinase. Nat Med 1996;2(6):668-75
102. Higgins KA, Perez JR, Coleman TA, et al. Antisense inhibition of the p65 subunit of NFkappa B blocks tumorigenicity and causes tumor regression. Proc Natl Acad Sci USA 1993;90(21):9901-5
103. Cui D, Tian F, Coyer SR, et al. Effects of antisense-myc-conjugated single-walled carbon nanotubes on HL-60 cells. J Nanosci Nanotechnol 2007 ;7(4-5):1639-46
104. Chevalier C, Saulnier A, Benureau Y, et al. Inhibition of hepatitis C virus infection in cell culture by small interfering RNAs. Mol Ther 2007;15(8):1452-62
105. Bellmann R. Clinical pharmacokinetics of systemically administered antimycotics. Curr Clin Pharmacol 2007;2(1):37-58
106. Aguzzi A. Unraveling prion strains with cell biology and organic chemistry. Proc Natl Acad Sci USA 2008;105(1):11-2
107. Center for Disease Control Website: Available from: www.cdc.gov/ncidod/dvrd/prions/
108. Toupet K, Compan V, Crozet C, et al. Effective gene therapy in a mouse model of prion diseases. PLoS ONE 2008;3(7):e2773
109. Liu Z, Cai W, He L, et al. In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice. Nat Nanotechnol 2007;2(1):47-52
110. Liu Z, Chen K, Davis C, et al. Drug delivery with carbon nanotubes for in vivo cancer treatment. Cancer Res 2008;68(16):6652-60
111. Schipper ML, Nakayama-Ratchford N, Davis CR, et al. A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. Nat Nanotechnol 2008;3(4):216-21
112. Lacerda L, Herrero MA, Venner K, et al. Carbon nanotube shape and individualization critical for renal excretion. Small 2008;4(8):1130-2
113. Lacerda L, Ali-Boucetta H, Herrero MA, et al. Tissue histology and physiology following intravenous administration of different types of functionalized multiwalled carbon nanotubes. Nanomed 2008;3(2):149-61
114. Poland CA, Duffin R, Kinloch I, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol 2008;3(7):423-8
115. Lippmann M. Nature of exposure to chrysotile. Ann Occup Hyg 1994;38(4):459-67, 408
116. Kostas Kostarelos. The good, the bad and ugly nanomaterials in biology – learning from the carbon nanotube experience. 2nd International Conference on Nanotoxicology, Zurich, Switzerland. September 7–10, 2008, page 6, section 1.1

Affiliation

Yitzhak Rosen[†1] & Noel M Elman[2]
[†]Author for correspondence
[1]Superior NanoBioSystems LLC,
11722 Newbridge Court,
Reston, Virginia,
20191, 385 N St.,
Southwest, Washington DC,
20024-2903, USA
Tel: +1 301 909 7976;
E-mail: yitzhakrosen@yahoo.com
[2]Massachusetts Institute of Technology,
Materials Science Engineering Department,
Cambridge, Massachusetts, USA