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Review

Advancement in carbon nanotubes: basics, biomedical applications and toxicity

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Abstract

Objectives Carbon nanotubes (CNTs) have attracted much attention by researchers worldwide in recent years for their small dimensions and unique architecture, and for having immense potential in nanomedicine as biocompatible and supportive substrates, as a novel tool for the delivery of therapeutic molecules including peptides, RNA and DNA, and also as sensors, actuators and composites.

Key findings CNTs have been employed in the development of molecular electronic, composite materials and others due to their unique atomic structure, high surface area-to-volume ratio and excellent electronic, mechanical and thermal properties. Recently they have been exploited as novel nanocarriers in drug delivery systems and biomedical applications. Their larger inner volume as compared with the dimensions of the tube and easy immobilization of their outer surface with biocompatible materials make CNTs a superior nanomaterial for drug delivery. Literature reveals that CNTs are versatile carriers for controlled and targeted drug delivery, especially for cancer cells, because of their cell membrane penetrability.

Summary This review enlightens the biomedical application of CNTs with special emphasis on utilization in controlled and targeted drug delivery, as a diagnostics tool and other possible uses in therapeutic systems. The review also focuses on the toxicity aspects of CNTs, and revealed that genotoxic potential, mutagenic and carcinogenic effects of different types of CNTs must be explored and overcome by formulating safe biomaterial for drug delivery. The review also describes the regulatory aspects and clinical and market status of CNTs.

Keywords biomedical application; carbon nanotubes; nanocarrier; nanomedicine; targeted drug delivery

Introduction

Nanotechnology is a branch of science that is based on the applied principles of physics, electronics, engineering and material science at a molecular or submicron level. The term 'nanotechnology' is derived from a Greek word 'nano' meaning 'dwarf', hence it relates to materials of very small size ranges (0.1–100 nm).[1] It covers an entire range of composite materials, including nanoparticles, nanoemulsions, dendrimers, quantum dots, nanocells, Xpclad nanoparticles, etc. Among these, carbon nanotubes (CNTs) have evolved as a novel nanocarrier system having a wide variety of applications. As the name indicates, these are hollow tube-shape substances of a polymeric nature made from monomers upon polymerization to give a tube-shaped structure. CNTs were discovered by Iijima in 1991[2] as an allotrope of carbon which rolled into cylindrical single-walled tubes. Further, multi-walled CNTs were made from graphitic rods by subjecting them to a discharge of an electric arc.[2,3] The higher surface area, conductivity, high tensile strength, typical length-to-diameter ratio up to 28 000 000:1 as well as potentially greater absorption abilities due to their cylindrical structure make them a novel nanomaterial for drug delivery and biomedical application.[4]

CNTs are basically made up of pure carbon and belong to the family of fullerenes (C_{60}), and are available in various geometrical shapes such as spherical, ellipsoidal or tubes.[5,6] Nowadays several members of fullerenes are being synthesized and categorized

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as a specific family according their carbon number as C_{20}, C_{30}, C_{36}, C_{70} and C_{78} and are called graphenes. CNTs are graphenes with a hollow cylindrical tube-like structure, where sp^2 hybridized carbon atoms are arranged in a specific pattern to form hexagonal structural units.[7] This imparts higher C–C bond stiffness, tensile strength of 150 Gpa (Giga Pascal) and Young's modulus of approximately 1, which is a measure of nanotube stiffness. Chemically, CNTs originate from synthetic graphites on exposure to an electric arc or laser beam source.[8,9]

Recently, CNTs have been considered as an ideal nanocarrier in the field of nanomedicine, which is an applicative field that uses concepts of nanotechnology, biology and medicine. The various applications of these include: controlled drug delivery; targeted delivery of drug molecules to a specific site, to cancer cells; delivery of bionanotechnology products; as an additive to improve the solubility of poorly water soluble drugs; vaccines delivery, hormone and enzyme delivery; and as a nanofluidic device in drug delivery.[10] CNTs have also been used in several biomedical applications, such as diagnostic tools, like nanosensors, nanorobots, nanoprobes and actuators for identifying various diseases. The literature survey on CNTs suggests that this novel nanocarrier has multiple biomedical applications. However, proven success in uses for different purposes may not translate to the use of CNTs in medicine for humans. For this purpose, it must be proven safe, albeit little data on cytotoxicity and limited control over functionalized-CNT behaviour have put a question mark on its success. Therefore, this review documents the advancement in biomedical application of these novel nanotubes as carriers, and also highlights the toxicity and regulatory aspects and current market status of CNTs.

Classification of CNTs

Nanotubes are mainly classified into two types depending upon the structure: (1) single-walled carbon nanotubes (SWNTs) and (2) multi-walled carbon nanotubes (MWNTs).[9] In SWNTs only one sheet of graphene is arranged to give a cylindrical structure which is one atom thick, having a radius of up to 1 nm. The SWNTs are closed at both ends with cap-like structures during the process of synthesis and the rings form ends by C–C bonds.[11] MWNTs consist of a few layers of graphene sheets (2–10), more than one atom thick, having an external diameter of >10 nm (Table 1). Also the SWNTs are structurally different from MWNTs by having different basic arrangements of the carbon atoms to give three different structural configurations: armchair arrangement where the chiral vector is characterized by the presence of chairs perpendicular to the tube axis; zigzag arrangement where the tube is characterized by having a V-shape perpendicular to the tube axis; and chiral or helical arrangement, which is unidentical from the above two types of arrangement. The degree of chirality in CNTs is a representative measure of their electrical as well as conductivity properties and helps in designing a wide variety of nanoelectronics instruments. Apart from this, chirality also determines the diameter of nanotubes, and metallic or semimetallic characteristics.[7,12]

MWNTs are of two types depending upon the pattern of arrangement of the graphitic sheets. One is a Russian-doll model structural arrangement where graphite sheets are arranged in concentric layers, for example a sheet of (0, 8) SWNT enclaved within a large diameter (0, 10) SWNT. The second model is known as a parchment model in which a single sheet of graphite is rolled around itself, resembling a scroll of parchment or a rolled newspaper.[13] Apart from these, there is another type of nanotube resembling SWNTs, known as double-walled nanotubes (DWNTs), having structural similarity with SWNTs; they are of considerable interest in the pharmaceutical field.[14]

In addition CNTs can be classified into three different prototypic structures depending upon their shapes (Figure 1). These includes carbon nanohorns (CNHs), nanobuds and nanotorus.

CNHs are identical to CNTs in structure, prepared from graphites by a laser ablation method.[15] These are single walled nanomaterials made from sheets of graphene (2–3 nm diameter) with the tip capped by a five-member ring. Unlike CNTs, a CNH resembles a horn-like structure having a cone shape with one broad open end and a narrow open end. They look like petals of a dahlia and are often described as 'dahlia-like' aggregates.[15,16] These have the unique property of changing diameter as their length increases. These have several applications in drug delivery and are used in drug delivery to cancer cells.[17–20] Nanotorus are theoretically described carbon nanotubes of structures bent into a torus (doughnut shape).[21] Carbon nanobuds are newly

Table 1 Basic differences between single-walled and multi-walled carbon nanotubes

Properties	SWNTs	MWNTs
Organoleptic property		
Appearance	Granular or fluffy black powder, sometimes with a shiny metallic appearance	Mostly granular and fluffy black powder
Appearance on electron microscopy	Appears as aggregated bundles	Aggregated bundles
Qualitative analysis using Raman spectroscopy	Gives characteristic peaks	There are no such characteristic peaks observed
Solubility study	Soluble in water and ethanol, form aggregates soon after sonication	Quite soluble in water but form slightly transluscent dispersions
Electrical property	Good electrical conductivity	Bad conductors of electricity

MWNTs, multi-walled carbon nanotubes; SWNTs, single-walled carbon nanotubes.

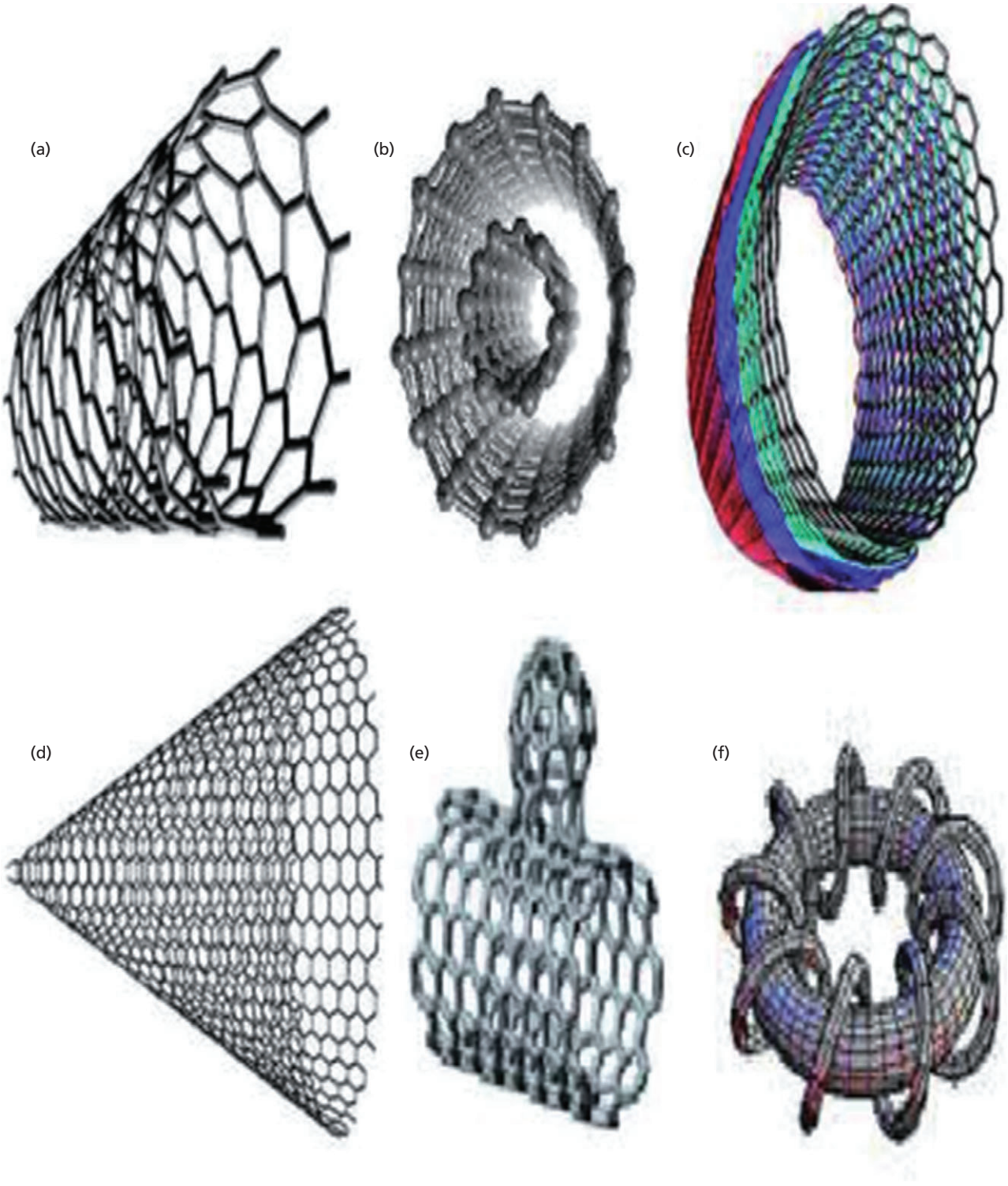

Figure 1 Types of carbon nanotubes: (a) SWNT, single-walled carbon nanotube; (b) DWCNT, double-walled carbon nanotube; (c) MWNT, multi-walled carbon nanotube; (d) carbon nanohorn; (e) carbon nanobud; (f) carbon nanotorus.

discovered materials synthesized from CNTs and fullerenes having properties intermediate between them. They have a shape and structure similar to CNTs, but are characterized by the presence of an external outgrowth (bud) on the external surface.

Apart from these, CNTs are also classified in several ways depending upon their structural modifications, method of preparation and solubility properties, for example functionalized CNTs, solvent dispersed CNTs, surfactant assisted CNTs, nanotubes assisted with biomolecules.[22]

Table 2 Comparison of various techniques for preparation of carbon nanotubes

	EAD technique	LA technique	CVD technique	HiPCO technique
Advantages	CNTs prepared from this method have higher Young's modulus with least defects as compared with other methods	As compared with EAD technique, LA can yield both SWNTs and MWNTs	The most economical method for production of CNTs, forming nanotubes with least production variables	Used for the catalytic production of SWNTs, and helps in production of nanotubes of any diameter
Disadvantages	Inability to produce SWNT/MWNTs with uniform diameter, and mostly a time-intensive method	CNTs obtained are very narrow in diameter and form tangled ropes and bundles along with impurities	This method is found to have broad spectrum of advantages, hence advantages outweigh disadvantages	A costly technique
Method of choice	Not useful for industrial scale	Mostly used for laboratory scale and rarely for industrial scale	This is regarded as the method of choice for industrial scale	Promising for bulk production of carbon nanotubes

CNTs, carbon nanotubes; CVD, chemical vapor deposition method; EAD, electric arc discharge technique; HiPCO, high pressure carbon monoxide; LA, laser ablation technique; MWNTs, multi-walled carbon nanotubes; SWNTs, single-walled carbon nanotubes.

Methods of preparation of CNTs

CNTs are widely accepted nanocarriers in the field of drug delivery and in biomedical application. For pharmaceutical use, the CNTs produced must be of good quality, free from impurities and carbonaceous matter and should not have damaged structures.[2]

CNTs can be synthesized naturally by heating carbon black and graphites in a controlled flame environment. However, nanotubes produced by this method are mostly irregular in size, shape, mechanical strength, quality and purity because of the uncontrollable natural environment.[18] Nowadays CNTs are synthesized by artificially developed methods of considerable interest to the pharmaceutical scientist, which include electric arc discharge (EAD), laser ablation technique (LA) and catalytic chemical vapour deposition (CVD) methods. In addition, several other techniques such as plasma enhanced chemical vapour deposition (PE-CVD)[8] and high pressure carbon monoxide disproportionation process (HiPCO) technique are of recent interest. The HiPCO technique can be used for the catalytic production of SWNTs in a continuous-flow gas phase, using carbon monoxide (CO) as the carbon feedstock and $Fe(CO)_5$ (iron pentacarbonyl) as the iron-containing catalyst precursor. The size and diameter distribution of the nanotubes can be roughly selected by controlling the pressure of the CO. This process is promising for bulk production of CNTs.[23] Different methods of preparation produce CNTs with different physical and mechanical properties. The type of CNTs produced, solubility, mechanical properties, quality, purity and yield usually differ from one method to another (Table 2).

In the electric arc discharge method, two different types of electrodes (anode and cathode) are used. CNTs are produced at the end of the anode, which consists of pure graphite. The nanotubes are produced by high voltage beams (around 100 amp) of electrons produced by the electric arc, which bombards the graphite surface. The electric arc is a plasmon setup made across CNTs resulting in the formation of CNTs on a substrate.[8,24,25]

In the laser ablation technique, the nanotubes are produced by allowing a specific spectrum of laser beam to strike on the graphitic target using transition metal as catalyst, which produces both SWNTs and MWNTs. This method uses two different laser sources, as primary laser and secondary laser beam. The initial bombardment is done with the primary laser followed by a secondary laser beam to finally produce CNTs of high quality. This method has the advantage of producing nanotubes desired for particular applications.[26–28] However, this method has the drawback of being time consuming and costly.

The catalytic chemical vapour deposition (CVD) technique works on a principle completely different from the above methods. Here, the feed material used is present in the form of a mixed vapour phase (vaporized carbon along with an inert gas). This feed material is passed through a hot furnace where it decomposes to give CNTs deposited on the surface of a substrate. The substrate is made by embedding nanometre-sized nickel or cobalt particles, or a combination of both, as a catalyst on its surface and is generally heated to approximately 700°C.[26,29,30] The variables, including nanotube diameter and tensile strength, depend on the size of the metal particles. These can be controlled by masked deposition of the metal, by annealing or by plasma etching of a metal layer. For commercial production, the nano-sized metal particles are mixed with MgO or Al_2O_3 to increase catalyst support and increase the surface area for higher yield.[31] Additionally, several newer techniques namely plasma-enhanced chemical vapour deposition (PECVD), CoMoCat process, thermal CVD, laser-assisted CVD and high pressure CVD have been developed for high quality CNT production.[32–36]

Characterization of CNTs

CNTs used in biomedical and drug delivery applications need to be characterized extensively to determine their fundamental properties.[37] The characteristic properties include diametric size, shape, purity, solubility, electromechanical properties and thermal conductivity. Several instrumental and analytical techniques have been developed for characterizing nanotubes: scanning electron microscopy

Table 3 A brief account of various techniques for characterization of carbon nanotubes

Instrumental methods of analysis	Characteristics properties	Advantages	Disadvantages	References
Thermo gravimetric analysis (TGA)	Quantitative determination of the amount of carbon and non-carbon matter in CNTs, helps in assessment of purity, thermal stability and nanotubes homogeneity	Easy estimation of quality is possible	Unable to identify the metallic impurities present in CNT sample	[212,213]
Transmission electron microscopy (TEM)	(a) Determines the morphology (b) Qualitative assessment of purity (c) Allows understanding of the structural arrangement of CNT–drug composites and also identifies the CNTs after cellular uptake	Provides qualitative information on size, shape and structure of CNTs, and amount of impurities	(a) Unable to identify metallic impurities (b) Cannot differentiate between SWNTs and MWNTs	[52,117,214,215]
Scanning electron microscopy (SEM)	For preliminary evaluation of CNTs	Provides information on CNT morphology as well as on metallic impurities	Unable to identify the unreacted catalysts and carbonaceous impurities	[212,216,217]
Scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX)	Most widely useful method for routine estimation of metallic content in CNT	–	–	[218]
Raman spectroscopy	(a) Novel technique for characterization and evaluation of SWNTs (b) Gives information about radial breathing mode (RBM) of nanotubes, which includes various vibrational transitions like radial movements, expansions and contractions	Gives RBM peaks, which help in distinguishing SWNTs from other carbonaceous material and also identifies the dispersion state of SWNTs in solution	Unable to evaluate the impurities like graphites, fullerenes, amorphous carbon	[6,218–220]
H^1 NMR	Provides information on the presence of functional groups on CNTs by assigning characteristic peaks. Determines diameter of nanotubes	Helpful in quick monitoring of CNT functionalization	–	[221]
IR spectroscopy	Acts as an qualitative tool for identification of functional groups, helps in assessing the effect of functionalization on CNT properties	A complementary technique to NMR to confirm the presence of bonds between CNTs and attached groups	–	[222,223]

CNTs, carbon nanotubes; IR, Infra-red spectroscopy; MWNTs, multi-walled nanotubes; NMR, nuclear magnetic resonance; SWNTs, single-walled nanotubes.

(SEM), transmission electron microscopy (TEM), thermogravimetric analysis (TGA), infrared spectroscopy (IR), nuclear magnetic resonance (NMR), Raman spectroscopy and atomic force microscopy (AFM) (Table 3). Each of the techniques has its own identity and advantages to help in determining CNT features. These methods have been used for several years, but there is no standard procedure developed in industry and no strict regulation.[7,38] The purity of the CNTs is the most important property, because commercially available CNTs are mostly contentious. Hence safety data sheets are used which help in indicating the purity of CNTs.

Applications of CNTs

As described earlier, CNTs have emerged as efficient drug delivery carriers in the biomedical and drug delivery field. Several applications of CNTs are listed below.

CNTs in controlled drug delivery

In the 21st century, the concept of practising safe and effective medicine with a high therapeutic potential is of great importance. Inspired by the ‘magic bullet’ concept proposed by Paul Erlich, devices for drug delivery to a targeted site in a controlled manner were developed and are still under

extensive research.[39] Various types of carrier systems already used for controlled drug delivery include polymeric systems, dissolution controlled, diffusion controlled and osmotic pressure controlled systems, hydrogels, liposomes, microspheres and nanoparticles.[40,41] Recently CNTs have attracted much attention due to their ability to deliver drug molecules to a specific site in a controlled manner.[17,42–44] They are used in the controlled release of drugs as well as delivery of genetic material such as DNA, genes and antibodies. Drugs or biomolecules can be loaded inside the hollow tube or can be directly attached to the walls of CNTs. It has been postulated that the tubes develop a combined Van der Waals force as well as a hydrophobic interaction force, where the Van der Waals force is consistently more important for insertion of drug molecules.[45] The special characteristic that makes nanotubes promising drug delivery carriers is their hollow monolithic structure having an outer and inner core, which can be modified by the method of functionalization with desired groups on the outer and inner areas. This helps in insertion of required drug molecules in the inner core environment while the outer surface can be modified for achieving biocompatibility and biodegradation.[46] Functionalization is a process of chemical synthesis where desired functional groups can be introduced onto the wall of CNTs for various applications. The functionalized CNTs can be used for the purpose of enhanced biocompatibility within the body, enhancement of the encapsulation tendency and solubility, and multimodal drug delivery and imaging.[47–50]

Several studies on the fate of nanotubes in the body suggested that CNTs loaded with drug molecules could easily pass into cells and also have the tendency to enter the cell nucleus, thus both cellular and nuclear levels of targeted delivery can be achieved.[51] Pantoratto *et al*.[52,53] examined the cellular and nuclear uptake of CNTs by TEM, which showed better uptake of CNTs with targeted action after functionalization due to the attachment of surface functional groups on cellular structures. They suggested that the most important factor that affects the fate of a CNT in the body is its dimension. Hence CNTs must be of nano size to prevent cellular opsonization as a harmful microbial intruder.[54]

In 2009, Yang and coworkers found that amine-functionalized mesoporous silica nanotubes (NH_2-MSNTs), synthesized from needle-like nanoparticles of calcium carbonate ($CaCO_3$) as inorganic templates and post-modification with 3-aminopropyltriethoxysilane via a sol–gel route having additional functionalization with blue fluorescent CdS quantum dots, were able to deliver the anti-inflammatory drug ibuprofen in controlled manner.[55] Furthermore, ibuprofen release in simulated body fluid revealed that the drug was released from amine-functionalized systems at a significantly lower rate compared with that from amine-free systems and the incorporation of CdS quantum dots had almost no effect on ibuprofen release.

Similarly, Zhang *et al*.[56] prepared nanohybrid hydrogels for controlled drug delivery. These nanohybrid hydrogels were obtained from the hydrogen bond self-assembly of poly (methacrylic acid) networks and carboxyl-functionalized multi-walled carbon nanotubes (MWNT-COOH). Such hydrogels showed low micropore densities and large mesh sizes with an increase in MWNT-COOH content. Upon contact with water, these nanogels swelled due to pH responsiveness and produced controlled release profiles of delivery for theophylline and aminophylline by modulating pH values.

Recently, a new generation of nanomaterials known as 'smart bio-nanotubes' was developed by the University of California.[57,58] The smart bio-nanotubes were in a trilayered structure made with the help of a microtubular protein called tubulin coated with a bilayer of lipid followed by coat of tubulin protein in the form of rings or spirals where proteins had either open ends (negatively overcharged) or closed ends (positively overcharged with lipid caps). The important formulation variable for smart bio-nanotubes that regulate the release profile is thickness of protein lipid versus protein coats, which leads to a basis for controlled release of drug molecules (Table 4).

CNTs in targeted drug delivery

Nanotubes are being investigated as a good carrier for targeted delivery of drug molecules to various parts of body that could help in treating several diseases. Many techniques have been applied to achieve targeting of drug molecules.

Targeted drug delivery with chitosan–nanotube complexes

Chitosan is a polymer of marine origin, derived from molluscs and prawns. Chemically, it is a polysaccharide which has several applications, with a wide application in controlled drug delivery because of its safe and nontoxic nature. The basic mechanism through which this system achieves its targeted action is its mucoadhesive nature. When the chitosan is functionalized on the surface of CNTs, the cells become attached to the sidewalls of the nanotubes, resulting in the desired targeted release to the cells, with improved drug absorption. Animal studies of targeted drug delivery revealed that the CNT–chitosan complexes had better release and targeting properties than chitosan-free CNTs. The system may have several applications in drug delivery, transmucosal drug delivery, delivery of peptides and nucleic acids to targeted cells, and it was found to have a broad spectrum of applications in drug delivery to the lungs for the treatment of lung cancers.[59]

Targeted delivery with functionalized CNTs

Functionalized nanotubes are a growing area of research for targeting malignant cells by virtue of better uptake by a specific population of malignant cells without affecting other collateral healthy tissues, which is a basic guiding principle of chemotherapy and targeted delivery.[60,61] Additionally, functionalized nanotubes are most preciously used for targeted delivery of nucleic acids, proteins, antibodies, drugs and other therapeutics agents to their site of execution.[62] CNTs use for targeted delivery is widely accepted in treating malignant disorders such as choriocarcinoma, Burkitt's lymphoma, carcinoma of cervix, breast cancer, and testicular tumors. For example, methotrexate in cancer chemotherapy usually shows a low level of cellular uptake due to improper absorption from the gastrointestinal tract.[63] However

Table 4 Overview of applications of carbon nanotubes in drug delivery

Drug	Indication	Functionalization of nanotubes	Inferences	References
Methotrexate	Cancer	Double functionalization made on its side wall by amino groups by 1,3-dipolar cycloaddition reaction	Better cellular uptake of drug	[47]
		Methotrexate attached on nanotube surface by conjugation with fluorescein molecules	Significant reduction in drug-related unwanted toxicity achieved	[110]
Paclitaxel	Breast cell carcinoma	Nanotubes pegylated with PEG (polyethylene glycol) moiety on its side wall surface in the form of small branches sprouting from centre	Bioavailability improved due to increased retention time of drug in blood vessels	[95]
Doxorubicin	Cancer	Drug given with PEG to form water-dispersible nanohorns	Achieves significant retardation of tumour growth due to prolonged retention in tumour cell	[19,105]
		Derivatization of single-walled carbon nanotubes (SWNTs) by attaching carboxylate groups and coated with polysaccharides to achieve pH-independent solubility of drug	Cell proliferations inhibited due to increased damage to nuclear DNA of malignant cells and enhanced water solubility	[96]
		Functionalization made by hydrophilic functional groups	Solubility increased due to functionalized partitioning	[43]
Ibuprofen	Anti-inflammatory action	Drug molecules encapsulated in nanotubes made up of $CaCO_3$ nanoparticles, functionalized by 3-aminopropyltriethoxysilane with CdS quantum dots	Comparative study of these functionalized nanotubes with functionalization-free ones showed a significantly slower release rate and thus a controlled drug delivery	[55]
Curcumin	Breast cancer treatment	Single-walled nanotubes loaded with curcumin	Significant reduction in the size of breast cancer	[224]
Combretastatin	Anti-angiogenic agent	Single-walled nanotubes loaded with combretastatin	Considerable reduction in the growth of cancer cells	[224]
Cisplatin	Cancer of ovary, testis, lung	Nanotubes functionalized with ammonium groups and folic acid for site-specific targeting to folate receptors expressed on malignant cells	Better cellular uptake of drug due to increased circulation time in blood	[99]
		Encapsulated in nanotubes, known as nanocapsules	Reduced vital organ-related adverse effects on delivering via carbon nanotube system	[10]
Polyoxometalate (POM)	Cancer	POM attached with surface of SWNTs and complexed with chitosan	Initial burst release of POMs from chitosan-modified carbon nanotubes (CNT/chitosan complex) as compared with functionalization-free CNTs, increased stability of POM in physiologically relevant pH environment and showed high loading capacity and prolonged drug release	[97]
Carboplatin	Cancer	CNTs filled with carboplatin	In-vitro studies showed higher efficacy of drug-filled CNTs with reduced growth of bladder cancer cells as compared with nanotube-free drugs	[98]
Mitomycin-C, oxaliplatin	Colorectal cancer	Multi-walled nanotubes induce hyperthermia when exposed to infra-red radiation and consequently improve cellular uptake of drugs due to cell membrane opening	Better uptake of drugs leads to mitigation of colorectal cancer	[89]
Dexamethasone	Anti-inflammatory	Drug loaded in oxidized single-walled nanohorns	During in-vitro studies, sustained release profile observed in mouse bone marrow stromal ST2 cells due to induction of alkaline phosphatase level in mouse osteoblastic MC3T3-E1 cells	[225]
Fluoroscein	Imaging	Selective functionalization	Solubility increased due to partitioning	[113]

Table 4 *(Continued)*

Drug	Indication	Functionalization of nanotubes	Inferences	References
Cellulose	Excipient	CNTs used special culture media along with multi-walled nanotubes	Better yield in productivity	[162]
Amphotericin B	Antifungal	Amphotericin B attached on nanotube surface by conjugation with fluorescein molecules	Reduced viable growth of fungus along with significant reduction of drug-related unwanted toxicity	[109]
Delivery of siRNA and more potent RNAi by SWNTs	Gene therapy	Nanotube-biomolecule conjugates with the incorporation of cleavable bonds to enable controlled molecular release from nanotube surfaces	Better efficacious delivery of these agents in a controlled manner	[47]
Theophylline	Asthma	Nanohybrid gel mediated delivery system formed from hydrogen bond self-assembly of poly (methacrylic acid) (PMAA) networks and carboxyl-functionalized multi-walled carbon nanotubes (MWNT-COOH)	Controlled release profile of drug delivery to lungs.	[56]

methotrexate administration using CNTs double functionalized by amino groups showed better cellular uptake. The functionalization was performed on MWNTs by 1,3-dipolar cycloaddition reaction of azomethine ylides which introduced amino groups in the orthogonal positions. Finally, methotrexate was attached with amino groups at the external surface. On administration, when the carriers reached the targeted tissues, cleavage of bonds took place to yield the drug molecules, with better cellular uptake.[64] Several covalent functionalizations of CNTs with anticancer, antiviral and antibacterial drugs have been employed and are of considerable interest for research.[65,66]

Recent findings have shown that drug-loaded functionalized CNTs face some problems in release of their contents. Hence to overcome this problem, Kulamarva *et al*.[67] encapsulated the CNTs in a novel membrane microcapsule made up of an alginate–poly-L-lysine-alginate (APA) membrane to form a polymeric membrane targeted drug delivery device. The nanotubes were either embedded in the core or attached to the surface of the alginate capsules. When such systems were delivered to the body they showed a promising drug release profile, safely and effectively, due to protection from the external harsh environment provided to the nanotubes by the polymeric membrane.

Targeted delivery with the help of nanotube-based antibody therapy

Earlier, the concept of antibody-mediated drug delivery was found to be the best approach for targeted drug delivery. However, loss of specificity of antibodies on binding with drug molecules is its biggest disadvantage.[68] Nanotubes do not alter the specificity of antibodies on attachment and can deliver the drug at the targeted site. McDevit *et al*.[69] delivered anticancer agents using SWNTs covalently functionalized by monoclonal antibodies for tumour targeting, and made radiation ion chelates and fluorescent compounds for diagnostic imaging to identify the site of target achieved. The drug molecules to be delivered with CNTs must have similar properties, with both targeting antibodies as well as nanotubes, because the antigenicity of antibodies must remain after attachment of the drug to the nanotubes and, subsequently, nanotubes with antibodies for their desired site of action. Therefore, they should match with each other and need to be compatible. These CNTs are capable of delivering several drug molecules (such as taxol) and more than one type of drug to the tumour site in different nanotubes attached to the surface of a single antibody. Ashcroft *et al*.[70] revealed that using a specific type of single skin cancer antibody (ZME-108) enabled delivery of more than 40 nanotube-loaded drugs to the targeted site. This approach has the advantage of delivering both hydrophilic and lipophilic drugs. Welsher *et al*.[71] reported that SWNTs with PEG functionalization and conjugated with the monoclonal antibody rituxan were able to target selectively the CD20 cell surface receptor on B-cells with little nonspecific binding to negative T-cells, and with Herceptin to recognize HER2/neu positive breast cancer cells. This antibody mediated approach has thus proved to be an ideal alternative in targeting drug molecules to cancer cells (Figure 2).

Nanotubes in lymphatic targeting

Lymphatic targeting is required for curing lymphatic diseases along with targeting drug molecules to the reticuloendothelial system (liver, spleen, kidney, etc.). Previously, many approaches have been tried for drug targeting to the lymphatic system (such as controlled-release microspheres, magnetic microspheres, etc). The major drawback of microspheres is their larger size, which causes capillary blockade and leads to 'chemoembolism'-like problems.[72] Inspired by the earlier concept, researchers have now shown that lymphatic targeting of drugs with the use of magnetic nanotubes proves to be better accepted.[73,74] Yang *et al*.[75] suggested that controlling the size of CNTs could allow effective take up into the lymphatics. Functionalization of CNTs with folic acid and entrapped magnetic nanoparticles along with drugs showed better delivery to cancer cells in the lymph nodes. Using an externally placed magnet, the MWNTs could be retained in the targeted draining lymph nodes for several days and

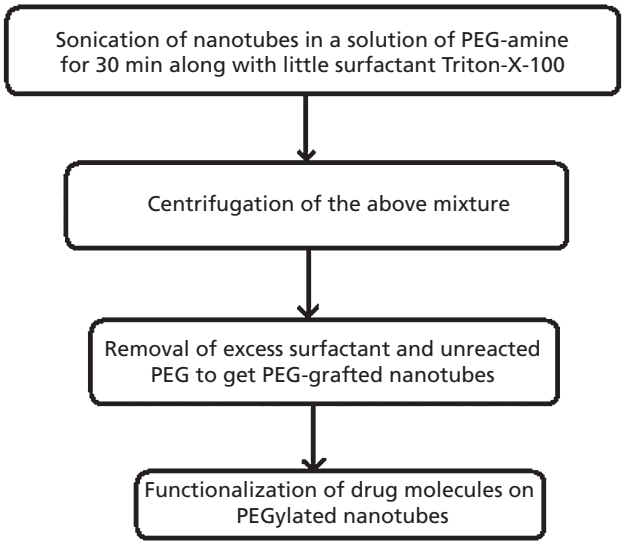

Figure 2 Steps involve in PEG functionalization of carbon nanotubes (CNTs).

continuously released chemotherapeutic drugs. Selective killing of tumour cells overexpressing the folate receptor in the lymph nodes was achieved; the folate receptor is overexpressed across a broad spectrum of human tumours.

Nanotubes in brain targeting

The blood–brain barrier (BBB) restricts the entry of substances so as to maintain the internal milieu of the brain. Many diseases remain untreated because of restriction in entry of therapeutic substances.[76] The restriction in entry of therapeutic molecules to the brain is not only because of the BBB but also due to the presence of enzymes that restrict entry by degrading the neuropharmaceutical agents, food nutrients and minerals.[77–79] Conventional drug delivery systems that release drugs into the general circulation fail to deliver drugs effectively into the brain. Diseases that directly affect the central nervous ststem include Alzheimer's disease, dementia, parkinsonism, mood disorder, AIDS and viral and bacterial meningitis. These brain disorders require the development of an effective targeted delivery system. Therefore, drug delivery systems for the brain need to be re-configured. CNTs have attracted much attention for delivery of drug molecules to the brain, having an ability to cross the BBB.[44] Kateb *et al*.[80] reported that MWNTs are quite effective in delivering neuropharmaceutical agents to the inner environment of brain microglial cells. Similar findings were reported by VanHandel *et al*.[81] They administered MWNTs by intratumoral injection to GL261 murine intracranial glioma cells for 24 h. Observation showed that 75% of MWNTs were taken up by macrophages of brain tumour cells. These findings suggested that the CNT-loaded drug can invade the BBB effectively compared with existing techniques. Additionally, nanotubes also have a potential role in treating neurodegenerative disorders due to their magnetic properties. Nanotubes in combination with nerve growth factors help in enabling specific cells to differentiate into neurons and thus treat neurological disorders.[82]

Nanotubes in ocular drug targeting

CNTs have also been used for ocular delivery of therapeutic agents. They are found to help in local targeting of drug molecules to the retinal site. They also have the ability to cross the blood–retinal barrier, which is a semi-permeable tough membrane restricting the entry of drugs such as antibiotics, anticholinergics and mydriatics.[44] However, little research has been done so far and thus this area needs to be further explored for ocular delivery.

CNTs in cancer therapy

It is very difficult to treat malignant cells or eradicate them from the body without 'spillover' to the normal cells.[83] In more than 99% of cases chemotherapy destroys cancer cells along with killing of normal cells, with serious side effects. Nanotubes thus help greatly in treating the cancer cells in a safe manner.[84,85] Chemotherapeutic agents delivered with CNTs help in achieving better uptake by malignant cells without affecting collateral tissues.[86] Consequently, nanotubes potentially lower the dose of drug by localizing its distribution at the tumour site only. In addition to chemotherapy, nanotubes also act as carriers for gene delivery, in treatment of carcinoma of cells.[48,87] Recently, Stanford University disclosed the newer applicative advantages of CNTs that have their own cancer-curing property.[60] Results showed that nanotubes exposed to an infrared (IR) light source tend to heat up to 70°C to 160°C in a very few seconds (<120 s), and when placed at a tumour site easily destroy malignant cells of a specific population and seem to act like a tumoricidal agent. MWNTs specially prepared by the CoMoCAT process are of considerable interest for their use in chemotherapy due to their quick infrared absorbing properties. Specifically, CoMoCAT nanotubes with a uniform size (about 0.81 nm) and a narrow absorption peak at 980 nm are ideal candidates for such a novel approach. In a study for achieving better targeting ability, such nanotubes were attached to a tumour marker, folic acid, which bound to its specific folate receptor, and a radiation of specific wavelength with a 980 nm laser caused programmed death of malignant cells. Several works describe this process of cancer treatment as 'photothermal therapy for cancer treatment'.[60,88] Levi-Polyachenko and group[89] conducted an experiment on MWNTs and showed that nanotubes exposed to IR radiation for a period of < 2 s caused hyperthermia (heating of cellular environment) up to 42°C. This study showed that MWNTs exposed to strong IR wavelengths (700–1100 nm) caused hyperthermia (42°C) in peritoneal cells for up to 2 h, which helps in the treatment of colorectal carcinoma. Drugs like mitomycin C or oxaliplatin given to colorectal cancer cells lead to effective reduction of the malignant cell population due to hyperthermia-mediated increased cellular uptake of drugs by increased cell membrane permeability. Similarly Torti *et al*.[90] reported that MWNTs doped with nitrogen gas induce thermal ablation causing death of cancer cells upon irradiation with an infrared beam. This technique is used in the treatment of kidney tumour cells. It was concluded that the anti-tumour activity of CNTs might be due to heat transduction, which leads to cellular cytotoxicity. Several variables were reported that make this technique effective, including the length of the

nanotube. The longer the CNT, the more effective would be the therapy as less time of exposure would be required with a minimum dose of radiation due to the larger surface area.[91,92]

Similar to the above findings, MWNTs coated with DNA showed better tumoricidal action than non-DNA-encased MWNTs. Guenzel[93] studied the tumoricidal action of DNA-coated nanotubes on 12 prostate cancer affected mice which received treatment with DNA-encased MWNTs, non DNA-encased MWNTs and MWNTs with and without laser beam separately. The results showed a better tumour cure rate with DNA-encased MWNTs within 70 s from a laser beam of 3 W power for up to six days. The suggested underlying basic mechanism might be that increased heat production around two- to three-fold of the threshold leads to death of malignant cells. This approach has an advantage over simple radiation/heat therapy to treat human tumours because of the selective tumoricidal action of DNA-encased nanotubes. Apart from the above, amino-functionalized MWNTs complexed with siRNA showed successful suppression of tumour in a human lung carcinoma model *in vivo*.[94] It is not only MWNTs—researchers have also worked extensively with SWNTs for delivery of many anti-cancer agents: paclitaxel,[95] doxorubicin,[96] polyoxometalate,[97] carboplatin,[98] cisplatin,[99] taxoids[100] and siRNA.[101,102] It is the functionalization of CNTs which made easy attachment of an even layer of drug molecules or bioactive agents for tumour targeting.[95,96] SWNT–siRNA complex or siRNA attached with functionalized SWNTs also produced effective and prolonged suppression of tumour growth compared with the older techniques of siRNA delivery.[101,102] (Table 4).

Wang and coworkers[102] observed that functionalized SWNTs are best in reducing the progression of human myelogenous leukaemia. The disorder is usually caused by a critical mediator cyclin A_2 found in human body cells; a higher level is found in tumour cells and this plays a critical role in disrupting DNA replication, transcription and cell cycle regulation. Overexpression of cyclin A_2 leads to development of several types of cancer, including leukaemia. Hence reduction or suppression of cyclin A_2 level in the body is a suggested measure to prevent tumour progression. Functionalized SWNTs carrying small interfering siRNA into K562 myelogenous leukaemia cells showed inhibition of cyclin A_2-dependent development of leukaemia, inhibition of cell proliferation and high apoptosis. This provides a new site of application for the field of nanotubes apart from chronic myelogenous leukaemia-related chemotherapy, towards reduction of multidrug resistance during chemotherapy of several diseases. Apart from the application of nanotubes in delivering pharmaceuticals and nucleic acids to malignant cells, recent literature recounts their important use in radiotherapy for treating cancer by increasing the rate of oxygen uptake to malignant cells as compared with the normal rate of uptake. This makes the radiotherapy more effective.[103,104] Apart from the CNTs, carbon nanohorns (CNHs) are now under investigation for exploring their application in chemotherapy. Water-soluble CNHs have been used for delivery of anti-cancer agents, such as doxorubicin administered intratumorally to human non-small cell lung cancer cell (NCI-H460)-bearing mice, and showed significant retardation of tumour growth associated with prolonged doxorubicin retention in the tumour cavity.[19,105,106]

CNTs in imaging

Nanotubes play a vital role in diagnostic procedures by helping in the imaging of organs and help in identifying the site of action of drugs in targeted delivery systems.[107] CNTs are shown to have greater potential to act as a contrast agent in imaging and identification of cancer cells.[108] Methotrexate (an anti-cancer agent), when given with fluoroscein probe functionalized nanotubes showed better visibility in the body due to the fluorescence produced by the drug-carrying probe on its surface.[109] Other examples of drugs, such as amphotericin B (an antifungal used in dermatophytosis), were used for their action on reticuloendothelial organs along with considerable reduction of their unwanted toxic effects. CNTs functionalized with fluorescent compounds are used as a radio-opaque substance which produces images of the desired in-vivo organs. On administration of functionalized nanoformulations into the body, the nanoformulations are attach on the specific site for which they have been designed and can be studied with special imaging techniques such as gamma scintigraphy, radiolabelling.[44]

Recent developments have shown that nanoimaging of several body parts can be done by administering nanotubes encapsulating miniaturized video systems in the form of a pill. This system can be easily swallowed, which helps in imaging the disease area in a nanoscale segment of a particular tissue or organ, where techniques such as endoscopy and colonoscopy fails due to their macro size imaging.[54]

CNTs in solubility enhancement

CNTs prepared synthetically are hydrophobic in nature and thus in soluble in water. Therefore, they create a problem in drug delivery and targeting. However, functionalization introduces the required functional groups by covalent functionalization on their sidewalls and tips to make them hydrophilic.[110,111] These functionalized nano carriers are able to deliver several hydrophobic biomolecules (proteins, peptides, nucleic acids, enzymes) to the target site.[112] Several poorly water-soluble drug molecules can be delivered, such as doxorubicin, fluorescein, etc. In addition to primary functionalization of nanotubes, secondary or double functionalization can be done by coupling with amino acids and bioactive peptides, allowing enhanced solubility characteristics. This forms the basis for the treatment of several diseases, like cancers, and vaccine and gene delivery where the carrier system enters damaged cells and releases enzymes for accelerating the autodestruction signaling for malignant cells or may repair the cells for their normal functions.[49] The basic mechanism by which they improved solubility as well as release is by 'functionalized-partitioning'.[43,113]

CNTs as a drug delivery nanocapsule

Nanocapsules are the small nano-sized particulate system which acts as a carrier for loading drug molecules. They are usually transported to a targeted tumour site and release their contents on change in environmental conditions in

close proximity to the targeted area.[114] A major advantage of the nanocapsule delivery system is the protection of the drug from the external environment. After release, it enters into the cell and is prevented from being ejected by attaching with the help of specialized surface structures such as ligands, antibodies and functional groups.[10] Though nanocapsules have some similarity with nanoparticles, they differ in the sense that nanoparticles are hollow spherical particles while nanocapsules are core-containing solid particles.[115] Therefore, CNTs are identical to nanocapsules in their property of encapsulation and their capability of not being ejected from the targeted cell, which makes them an ideal drug delivery carrier for the treatment of diseases like cancer, tuberculosis and bacterial and viral disease where the chance of drug resistance is more common due to efflux of drug molecules from cells by the action of of P-glycoprotein.[51,116,117]

CNTs as a tool in drug discovery

In the 21st century, pharmaceutical drug discovery is a most powerful area of research in finding new drug molecules for therapeutic use. The major drawback in drug discovery is time consumption due to the trial and error involved in the methods. CNTs are now used for drug discovery in the form of sensors along with several advanced information technology systems such as data mining and computer-aided drug design for identification of genes and genetic material for drug discovery and development.[118,119] Functionalized CNTs also have great importance in drug discovery.[109]

CNTs for biomedical application

Nanotubes have become of interest in their application as an accessory tool for various allied fields along with biomedical uses.[120–122] The applications of CNTs as biomedical tools are discussed in the following sections.

Diagnostic tools

Diagnostic tests are those which help in determining the level of diseases, pathological conditions, etc. Nanotubes can assist such diagnosis, where they are used in the form of artificial smart intelligence devices such as nanosensors and nanorobots. These nanosensors and nanorobots are nano-size devices meant for implantation in the body.[123,124]

Nanosensors are devices that detect small physiological changes qualitatively and quantitatively. There are various types of nanosensor that have been studied for application in biomedical devices. CNTs have several physical properties of which the temperature coefficient is exploited in designing pressure nanosensors. This works on the concept that when temperature increases by two orders of magnitude, this leads to twice the rate of pressure developed, which is detected by a pressure-sensing device.

Liu and Dai[125] discovered an advanced form of pressure-sensing device, called a piezoelectric pressure sensor, made up of CNTs. These are grown on SWNT surfaces suspended in square polysilicon membranes. They work by the mechanism that when uniform pressure is applied on the membrane this leads to a change in resistance in the SWNTs. It has several applications including use in patient monitoring, hospital beds, respiratory devices, inhalers, eye surgery and kidney dialysis machines.[126,127]

During eye surgery they help to reduce operation difficulties by reducing fluid turnover and control the vacuum that is used to remove the fluid. In addition to this they are quite useful in hospital bed-ridden patients and burn victims for preparing mattresses consisting of sensors that regulate a series of inflatable air-filled chambers that provide a smooth sensation to the patient with less friction, pain and burning and promote healing. Pressure sensors can also be used for detecting sleep apnoea where, if there is no movement in sleep for a certain period, the sleeper is awakened by an alarm.[127] Likewise, pressure sensors also help in monitoring the operation of dialysis systems by measuring the inlet and outlet pressures of both the blood and the solution during kidney dialysis.[128] Recent advances in pressure sensor devices led to the development of intelligent pressure sensing systems which play a vital role in manufacturing portable respiratory devices, including both diagnostic devices (e.g. spirometers, ergometers and plethysmographs) and therapeutic equipments (e.g. ventilators, humidifiers, nebulizers and oxygenometers). They serve patients with disorders of asthma, sleep apnoea and chronic obstructive pulmonary disease.[126]

Biosensors are the nanosized materialistic devices which have applications in detecting biological disorders of the human body. Sotiropoulou and Chaniotakis[129] used CNTs as an immobilization matrix for the development of an amperometric biosensor. The biosensor was developed by growing aligned MWNTs on platinum substrates.

CNT-based nanobiosensors are now used to detect DNA sequences in the body and help in the detection of very specific pieces of DNA related to cancer production, and identification of genes and biomolecules such as antibodies associated with human autoimmune diseases.[130–132] These sensors have also been used during space missions due to their advantage of transdermal administration through skin.[133] Other important applications of biosensors include glucose sensing and blood pressure monitoring. Use of CNTs as chemical sensors also has potential applications in liquid chemistry for the detection of sodium, potassium and other mineral elements in blood samples and measurement of pH value.[134] Due to their small size and less power consumption, nanotubes are used in various other biomedical applications, such as monitoring of pulse, temperature, repair of damaged cells, monitoring the activity of the heart, heart beat regulation, treatment of retinal diseases and in cochlear impairment-related hearing problems.[135,136]

CNTs can also be used as flow sensors for precise measurements of respiratory gases.[137] Ghosh *et al*.[138] showed that when liquid flows on the surface of bundles of SWNTs they induce a voltage in the direction of fluid flow, which helps in designing micromachines, such as heart pacemakers, that do not need heavy battery packs or recharging.

Early cancer detection could reduce disease progression to other parts of the body. Therefore, diagnostic procedures must be sensitive to detect malignant cells so that cancer can be cured in its early stages by radiation or other chemotherapeutic treatment. Among several types of cancers, prostate cancer can be a cause of death due to delayed diagnosis. Recently, nanotube-based bionanosensors have been helpful

in detecting such type of cancers in their early stage. Small needle cellular bionanosensors are devices made up of nanotube constructs that have the sensitivity to differentiate between metastatic and non-metastatic cancer when inserted directly into blood by detecting change in hormonal levels. Initial testing with a cell biosensor showed that a nanotube electrode can characterize different solutions of LNCaP prostate cancer cells.[139] The nano biosensor device basically consists of three different parts, a responsive material, a nanoneedle system and a coil antenna. Responsive materials are said to be the heart of the sensor, which help in sensing and detecting the difference between cancer and non-cancer cells. The responsive material is made up of inductors and solenoids prepared from nanowires and coiled nanotubes. Nanotubes provides real selectivity to the sensor in detecting cells and thus their sensitivity can be further enhanced by coating or functionalization with gold material, by bioconjugation using antibodies, or with special receptors for easy detection. MWNTs act as a telescope and measure the change in electrical resistance by the strain produced due to electrochemical bond expansion and this change in resistance sends signals to the detector and amplifier. Nanowire responsive materials have an intrinsic ability to sense and respond to external stimuli. Nanoneedle systems help in the injection of the sensor into the body and the coil antenna helps in transmitting the signals recorded by nanotubes. In addition to these, other parts include a power-generating system and piezoelectric nanobelts.

Similar to nanosensors, nanorobots have shown application in the build up of immunity by protecting from harmful bacteria and viruses, etc., in protection of skin by curing diseases, protection from teeth decay, surgical procedures for preparing nanosyringes, electromechanical nanothermometers and nanojet engines.[140] Another advanced application of nanorobots is to protect the skin from external unwanted stimuli by removing pathogens and accelerating the new skin recovery process by optimal moisturization due to its self-intelligence system. Similarly, a mouthwash containing such a system has been shown to protect teeth from bacteria and to lift foods and plaques.[123]

Surgical aids

Besides the application of nanotubes in diagnosis, they are now used in many surgical operating procedures. In earlier days surgical procedures were carried out using large-size macro-scale instruments that were quite difficult to handle and hence made the surgical procedures more complicated. The major disadvantages of these macro instruments were difficulty of handling by physician/surgeon, chances of larger wounds leading to severe pain, scarring of patients and higher operating time, which may result in fatigue of the surgeon and could lead to errors in surgery. Additionally such types of instrument are unable to operate in sophisticated procedures like ophthalmic, ear and nasal surgery and in colonoscopy. Hence, to overcome the above difficulties, nanosized small artificially intelligent equipment is of considerable interest.[141] Being a nano-size material, nanotubes are now used for preparing the nanoscale surgical instrument devices which have now made surgery easier and more sophisticated. The nanosized equipment, including forceps, scalpels and grippers, have sensor-type embedded systems, which guide the physician to cut a particular area of tissue.[44,142,143] Nanorobots are also used in surgery for several diseases including oesophageal, colorectal, gynaecological, prostatic, cardiac, liver, gallbladder and bypass surgery.[141]

Nanoprobes

Briefly, nanoprobes are devices that are designed to access information from a remote or unknown region of a body cavity like the stomach, uterus, ear or heart. There have been several reports on the use of CNTs in preparing probes.[144–147] For example, Stevens *et al*. [147] used the nanotubes and attached them with an atomic force microscope (AFM) tip made up of silicon or silicon nitride by electric arc-discharge method and were able to image a protein filament using these tips. The high mechanical robustness and low buckling force of CNTs help in reducing the force exerted on the sample during imaging and so could be applied for imaging soft materials like biological samples.[148]

Nanotweezers

Nanotweezers are instruments that have the capacity to modify cell physiology. Basically they are a kind of probe that is driven by electrostatic interaction between two nanotubes on a probe tip. They work on the principle of balancing elastic restoring force with electrostatic force.[149] Nanotweezers are used for manipulation and modification of biological systems, like changing the physiology, shape, size and morphology of the cell, DNA sequence and assembling cellular structures, and have many other applications in treatment of diseases.[136,150]

Actuators

CNTs have now emerged as an excellent material for the manufacturing of actuators. These are widely used nowadays in the field of biomedical engineering where they are used in preparing biomedical equipment such as artificial limbs, artificial ocular ciliary muscles, irises, optical display units and pulsating hearts.[151] Baughman *et al*.[152] described the use of CNTs for preparing actuators. Nanotubes are found to be the best material for actuators because of their excellent physicochemical properties such as the ability to withstand higher stress-strain forces, electromechanical properties and higher electrochemical coefficients. The variables that affect the actuator property of CNTs include the type of nanotube used (SWNT/MWNT), size, method of preparation, purity and chirality.[153] Actuators work by converting electrical energy to mechanical energy. After energy conversion, the actuator utilizes mechanical energy in performing useful work. Baughman *et al*.[152] showed that CNTs, after conversion of electrical energy into mechanical energy, behave like an artificial muscle which generates higher mechanical stress compared with natural muscle. Both the single-walled and multi-walled nanotubes were found to be good candidates for several biomedical surgical devices.[154]

Nanofluidic systems in drug delivery

Nanotubes are also employed in delivering drug through injectable routes. Generally this route is considered to be a route of emergency; hence drug formulations that are given by

this route must favour certain conditions, such as particle size control, syringeability, sterility, zeta-potential and apyrogenicity, for their safe and effective fate in the body. The most vital problem seen with this route is the chance of blockage of blood vessels due to large particle size that leads to tissue toxicity. Hence, nanotubes are designed as a small nanofluidic system carrying drugs for injectable use, which make safer drug delivery possible. Among the nanotubes, MWNTs are most widely used because of their special properties such as higher mechanical strength. These systems are shown to have wide application in drug delivery through intravenous routes in chemotherapy for tumour targeting and also in treating diseases like lupus, diabetes and AIDS.[155,156]

CNTs as quantum dots for therapeutic purpose

Quantum dots (Q-dots) are defined as nanoparticles with size in the range of 2–10 nm. Chemically these are semiconductor and diode substances capable of emitting light of various colours. Due to their light-emitting property, they have potential application in imaging of various body parts like X-rays.[157] In recent years, research revealed that CNTs can also generate Q-dots, or may behave like a Q-dot, due to their mechanism of coulomb blockade and hence the phenomena of electron quantization leads to formation of such types of light-emitting nanoparticles.[158] Q-dots emerged as a special tool in imaging because of their continuous light-emitting characteristics and this does not fade when exposed to UV light. In addition, Q-dots also help in drug targeting and diagnosing the site of action of the drug molecules attached on it. Example include the delivery of antisense agents and Q-dots attached on MWNTs.[159] Bhirde and co-scientists[160] delivered cisplatin and epidermal growth factor (EGF) attached on SWNTs specifically to target squamous cell cancer and compared them with the non-targeted control SWNT–cisplatin without EGF. Imaging studies in head and neck squamous carcinoma cells (HNSCC) overexpressing EGF receptors (EGFR) using Q-dot luminescence and confocal microscopy showed that SWNT-Qdot–EGF bioconjugates internalized rapidly into the cancer cells and vice-versa. Also, regression of tumour growth was rapid in mice treated with targeted SWNT–cisplatin–EGF relative to non targeted SWNT–cisplatin.

CNTs as a medium for biotechnological/ microbiological products

Microbiological products include substances derived from microbial origin. However, the natural process of preparing these products is quite time consuming and costly. Recently, nanotubes have emerged as an excellent carrier for producing such products by acting as a growing medium for microorganisms. Czaja *et al*.[161] used this method in the production of cellulose which was found to have several biomedical applications. Cellulose is generally produced by the bacteria *Gluconobactrium xylinum*, but the rate of production does not fulfill the rate of demand. For this purpose, MWNTs were used with Hestrin and Schramm (HS) medium and produced higher yield than conventional methods of production.[162] Tissue engineering is a field of biotechnology that helps in regular monitoring and evaluation of engineered tissues along with other biomaterial for improving growth function. Nanotubes help a lot in tissue engineering by tracking of cells, sensing of microenvironments, in delivery of transfection agents and in scaffolding of them. In addition they help in examination of the engineered tissue by optical means, magnetic resonancing and by radiotracer contrast agents.[163]

CNTs in vaccine delivery

Vaccines are biological substances used for imparting immunization against foreign disease-causing pathogenic microorganisms. They usually impart active immunity to the human body by inducing antibody production in a most natural way.[164] Major problems associated with vaccine delivery include improper absorption, chances of antigen-induced hypersensitivity, anaphylactic reactions and hypersensitivity due to vaccine adjuvants. Several novel approaches have been tried for improving vaccine delivery, including liposomes, microspheres, nanoparticles and bone delivery systems. Similarly, CNTs have also been tried for vaccine delivery, where they help in improving vaccine action due to their adjuvant-like action in increasing the body to antigenic exposure.[164,165] CNTs, when conjugated with antigenic peptides, can act as a new system for safe and effective delivery of synthetic vaccines.[109]

CNTs in gene therapy

Gene therapy can be improved by using CNTs that help in the replacement of damaged or missing genes. The major problem associated with gene delivery is the complication of DNA passing through the cell membrane.[166,167] CNTs help in transportation of DNA into cells.[168–170] Researchers have made nanotubes with dendrimers grafted onto the surface for treating gene defects by delivering the grafted genes.[171] Recently, the potential of CNTs as matrices to support and stimulate neural growth has been reported.[8] Prato *et al*.[109] reported ammonium-functionalized CNTs as a vector for gene-encoding nucleic acids and plasmid DNA to demonstrated the enhancement of gene therapeutic capacity in comparison with DNA alone. Yang *et al*.[172] delivered siRNA complexed with SWNTs for efficient gene delivery. The siRNA and SWNT complex can be easily taken up by splenic immune recognizing cells such as CD11c+ cells, CD11b+ cells and Gr-1+CD11b+ cells to induce the immune response for the particular gene.

Wu *et al*.[173] reported that when single-stranded DNA bound to SWNTs, DNA probes were protected from enzymatic cleavage and interference from nucleic acid binding proteins. Study showed that an SWNT modified DNA probe could target a specific mRNA inside living cells and cause increased self-delivery capability and intracellular biostability compared with free DNA probes. Hence, this new conjugate provides great potential for applications in the field of genetic engineering.

CNTs in anti-sporal applications

Wang *et al*.[174] at Clemson University reported application of nanotubes in treating anthrax infection. Earlier, during civil wars, anthrax spores were used as a biological weapon. Their common mode of transmission is through inhalation which causes respiratory problems. CNTs have been shown to help in protection against bioterrorism. Galactose-a, b and

mannose-c sugar functionalized SWNTs molecules stick on the spore surface to form a coat and neutralize their toxicity and prevent infection. The basic mechanism of disinfection is prevention from inhalation and absorption from the bronchiole surface. Study showed that nanotubes play several identical roles in disease treatment by minimizing toxicity with dose reduction of standard therapeutics and allowing a multiple payload capacity to achieve both targeted activity and combating infectious strains and resistant strains.[82,175]

Toxicity of CNTs

Nanomaterials have unique properties in comparison with bulk materials, such as a high surface area to volume ratio that also leads to unique mechanisms of toxicity from xenobiotics. In general, researchers found that the toxicity originated from the nanomaterial size and surface area, composition and shape.[176] Reduction of size leads to an exponential increase in surface area relative to volume, consequently making the nanomaterial surface more reactive towards itself (aggregation) and to its surrounding environment (biological components).[177–179] Accumulation of nano-sized material may also cause increased uptake into tissues, which alters the critical biological function of cells. Recent studies on the toxicity of CNTs performed by nanotechnologists are presented in Table 5.

Recently, Qu *et al*.[180] demonstrated temporary organ injury in lungs and heart due to delayed clearance of carboxylated MWNTs in mice. The delayed clearance was a result of the strong tendency of MWNTs to agglomerate. Persistent accumulation of agglomerated MWNTs in the lungs even caused inflammatory responses. Another group studied the influences of SWNTs with different degrees of agglomeration on primary cultures derived from chicken embryonic spinal cord (SPC) or dorsal root ganglia (DRG).[181] SWNTs' significant decrease in DNA content was more pronounced if cells were exposed to highly agglomerated SWNTs as compared with better dispersed SWNT-bundles. In addition, cell-based ELISA tests showed that the amount of glial cells was reduced in both peripheral nervous system (PNS) and central nervous system (CNS) derived cultures. This suggested that, on SWNTs introduction at high concentrations, it is likely that adverse effects on glial cells and neurons might occur.

CNTs were shown to impair cell viability, which could be caused by the strong tendency of CNTs to agglomerate.[182,183] Bottini *et al*.[182] compared the toxicity of pristine and oxidized MWNTs on human T cells and found that the MWNTs were more toxic and induced massive loss of cell viability through programmed cell death. Chiaretti *et al*.[184] disclosed that MWNTs on intraperitoneal administration produce neither significant neurovegetative nor behavioural effects up to 10 mg/kg of body weight. Similarly, neither modification of immunoglobulins nor of humoral immunity was observed. Rotoli *et al*.[185] described an alteration of the permeability of human airway epithelial cells after treatment with MWNTs, whereas, Poland *et al*.[186] revealed that MWNTs resulted in asbestos-like, length-dependent, pathogenic behaviour on introduction into the abdominal cavity of mice. Shvedova *et al*.[187] investigated the adverse effects of SWNTs using a cell culture of immortalized human epidermal keratinocytes (HaCaT). They observed oxidative stress and cellular toxicity due to formation of free radicals, accumulation of peroxidative products, antioxidant depletion and loss of cell viability. Exposure to SWNTs also resulted in ultrastructural and morphological changes in cultured skin cells. Metal traces available in nanotubes are also another causative agent for toxicity. Pulskamp *et al*.[188] did not observe any acute toxicity on cell viability (WST-1, PI-staining) upon incubation with all CNT products. However, increase in ROS was observed due to the presence of metal particles as well as metalloids in commercial nanotubes, while treatment with a highly ultra-purified form of CNTs had no biological adverse effects. Thus, it could be concluded that presence of metal in commercial nanotubes might be a major toxicity inducer.

Genotoxic potential and mutagenic and carcinogenic effects of different types of CNTs were reported recently but very little work has been published. This could be primary genotoxicity (direct interactions of particles with the cells) or secondary genotoxicity (generation of an excess of reactive oxygen species, ROS).[189] Zhu *et al*.[190] developed MWNTs and studied their genotoxic effects. They reported DNA damage through ROS and also an increase in the mutation frequency in mice. Similarly, Muller *et al*.[191] found genotoxic effects, such as increase in micronuclei frequency, in rats during *in vivo* as well as *in vitro* studies. Also, SWNT treatment did not show mutagenic effects in *Salmonella typhimurium* YG1024 and YG1029 strains and caused a non-significant micronuclei increase in the V79 lung fibroblast cell line.[192] However, the genotoxic effect of MWNTs is not universal. Dissimilar results were reported by Szendi and Varga.[189] However, Takagi *et al*.[193] suggested a carcinogenic effect in peritoneal cells after intraperitoneal injection MWNTs on p53 heterozygous mice and observed mesothelioma in peritoneal cells. Similar to the above findings, Sakamoto *et al*.[194] observed that the carcinogenetic potential of MWNTs in scrotal cells led to the development of mesothelioma in rats, confirming the toxicity profile of MWNTs. There is much in the literature reporting the carcinogenicity of MWNTs in different organs of humans and animals. Such toxicity includes pulmonary toxicity, lung inflammation, granulomas, fibrotic reactions and mutations in epithelial cells and cardiopulmonary toxicity.[195,196]

Recently many conflicting reports concerning the safety and biocompatibility of carbon nanotubes (CNTs) have been published. Long-term accumulation and low toxicity of SWNTs in intravenously exposed mice was reported by Yang *et al*.[197] However, no acute toxicity was reported by Zeni *et al*.[198] on administration of SWNTs. Similarly, other authors have reported low or no cytotoxic effects due to exposure to CNTs.[188,197–199] Huczko and Lange[200] reported null risk of skin irritation and allergy on dermatological trials of CNTs. Recently, Bardi *et al*.[201] suggested that PF127-coated MWNTs do not induce apoptosis of cortical neurons on administration to mouse cerebral cortex. Moreover, the presence of MWNTs can reduce PF127 toxicity. The mutagenic effect of MWNTs of small surface/volume ratio, high diameter and less than 0.1% of metal contaminants have been evaluated for mutagenic activity by the bacterial reverse mutation assay (Ames test).[202] These MWNTs were devoid of mutagenic effect in the bacterial cellular systems tested in that

Table 5 A brief account of the toxicity of carbon nanotubes during in-vitro and in-vivo studies with different models

Reference No.	Objective	Model	Toxicity	Inference
Inoue *et al.*[226]	Examined the effects of pulmonary exposure to MWNTs on allergic airway inflammation *in vivo* and their cellular mechanisms *in vitro*	Mice	MWNT aggravated allergen induced airway inflammation, MWNT with allergen amplified lung protein levels of Th cytokines and chemokines compared with allergen alone, significantly increased allergen (OVA)-specific syngeneic T-cell proliferation	MWNT may become one of the important environmental risk factors of allergic asthma
Qu *et al.*[180]	Established the relationship between the agglomeration propensity and the CNTs behaviour and toxicity *in vivo* for carboxylated-CNTs	Mice	No acute general toxicity in mice, but delayed clearance of MWNT-COOH generated temporary organ injury in lungs and heart	Delayed clearance was the result of in-vivo agglomeration of CNT
Tong *et al.*[196]	Tested cardiopulmonary toxicity on mice for acid functionalization (AF)-SWNT and carbon black particles	Mice	Intrapulmonary instillation of (AF)-SWNTs enhanced cardiac ischaemia/reperfusion injury and caused myocardial degeneration, no significant ischaemia/reperfusion injury on exposure to non-functionalized SWNTs or ultrafine carbon particles	Might be due to enhanced translocation, or via systemic vascular response generated in association with the AF-SWNT-mediated pulmonary injury
Bardi *et al.*[201]	Investigated MWNTs coated with Pluronic F127 (PF127) surfactant toxicity and of their dispersion factors in the brain	Mouse	Did not cause degeneration in the mouse cerebral cortex	PF127-coated MWNTs do not induce apoptosis of cortical neurons
Belyanskaya *et al.*[181]	Analysed the influences of SWNTs with different degrees of agglomeration on primary cultures derived from chicken embryonic spinal cord (SPC) or dorsal root ganglia (DRG)	Chicken embryonic spinal cord or dorsal root ganglion	Significantly decreased the overall DNA content on 30 mg/ml SWNT treatment, reduced the amount of glial cells in both PNS and CNS derived cultures	Level of toxicity depends on the agglomeration state of the CNTs
Vittorio *et al.*[203]	Investigated the effect of various physicochemical features of MWNTs on toxicity and biocompatibility with cultured human neuroblastoma cells by using MTT, WST-1, Hoechst and oxidative stress assays	Human neuroblastoma cells	Loss of cell viability was minimal for pure MWNTs (99% purity), but cell proliferation was decreased significantly for 97% purity MWNTs and AT- MWNTs (97% purity, surface oxidation 8%); no intracellular ROS were detected	Purity, concentration and functionalization affects the cell viability, 5–10 μg/ml MWNTs was supposed to be ideal for for gene and drug therapy against cancer
Sotto *et al.*[202]	Evaluated mutagenic effect of MWNTs by the bacterial reverse mutation assay (Ames test) on *Salmonella typhimurium* TA 98 and TA 100 strains, and on *Escherichia coli* WP2*uvr*A strain	Bacteria	No mutagenic effect in the bacterial cellular systems was observed, not even in the presence of the metabolic activator	Exhibited different biological activities and toxicities in relation to their physico-chemical characteristics, size, shape, crystallinity and presence of metal traces
Kishore *et al.*, 2009[227]	Investigated ocular and dermal irritation potential of MWNT *in vitro* and *in vivo*	Rabbit	Reversible conjunctival redness and discharge was observed in acute eye irritation toxicity studies, whereas MWNTs were non-irritant to skin *in vivo*; non-irritant to eye and skin on in-vitro studies	Efforts were made to improve the predictability of in-vitro assays to assess the in-vivo ocular and dermal toxicity of MWNTs

Table 5 *(Continued)*

Reference No.	Objective	Model	Toxicity	Inference
Poland *et al.*[186]	Studied MWNTs for asbestos-like pathogenicity in a pilot study	Mice	Showed asbestos-like, length-dependent, pathogenic behaviour, inflammation and the formation of lesions was observed in mesothelial lining	For long-term avoidance of toxicity, further research is needed
Yang *et al.*[197]	Studied long-term accumulation and toxicity of intravenously injected SWNTs in the main organs (such as liver, lung and spleen)	Mice	Histological observations demonstrated slight inflammation in lung, but the serum immunological indicators (CH 50 level and TNF-α level) remained unchanged, No apoptosis observed	Decreasing GSH and increasing MDA level suggest that the toxicity of SWNTs might be due to oxidative stress
Soto *et al.*[177]	Investigated cytotoxicity of an array of commercially manufactured inorganic nanoparticulate materials, naturally occurring mineral chrysotile asbestos and MWNT aggregates and black carbon aggregates	Murine alveolar macrophage cell line and human macrophage and epithelial lung cell lines	Varying degrees of cytotoxicity for all cell lines was observed and the general trends were similar for both the murine and human macrophage cell lines	In-vitro cytotoxicity study of CNTs may predict the potential respiratory health risks in humans
Pulskamp *et al.*[188]	Studied toxic effects of SWNT, MWNT, SWNT-AT on NR8383 and compared with carbon black and quartz as reference particles	Human A549 lung cells	No acute toxicity on cell viability; none of the CNTs induced the inflammatory mediators NO, TNF-α and IL-8	Metal traces in the commercial nanotubes are responsible for the biological effects
Zhu *et al.*[190]	Assessed the DNA damage response to MWNTs in mouse embryonic stem (ES) cells	Mouse ES cells	Accumulate and induce apoptosis in mouse ES cells and activate the tumor suppressor protein p53, increased the mutation frequency by 2-fold compared with the spontaneous mutation frequency	Genotoxicity/cytotoxicity of nanomaterials needs more careful study
Wick *et al.*[178]	Investigated CNTs at various degrees of agglomeration using an in-vitro cytotoxicity study	Human MSTO-211H cells	Asbestos was more cytotoxic than CNT-bundles; rope-like agglomerates of CNTs induced more pronounced cytotoxic effects than asbestos fibres at the same concentrations	Agglomeration plays a role in the cytotoxic effect of nanomaterials
Bottini *et al.*[182]	Compared the toxicity of pristine and oxidized MWNTs	Human T cells	MWNTs are more toxic and induce massive loss of cell viability	Concentration-dependent toxicity occurred and thus careful toxicity studies need to be undertaken
Shvedova *et al.*[187]	Investigated adverse effects of SWNT using a cell culture	Immortalized human epidermal keratinocytes (HaCaT)	Oxidative stress and cellular toxicity were indicated by formation of free radicals, accumulation of peroxidative products, antioxidant depletion, and loss of cell viability	Cell viability was due to accumulation in cells

CNS, central nervous system; CNTs, carbon nanotubes; GSH, glutathione; MDA, malondialdehyde; MWNTs, multi-walled carbon nanotubes; PNS, peripheral nervous system; SWNTs, single-walled carbon nanotubes; SWNT-AT, single-walled carbon nanotube-acid treated.

they did not significantly increase the number of revertant colonies. Thus, it was concluded that the MWNTs studied appeared to be devoid of mutagenic effects and the lack of mutagenicity might be related to their structure or to their purity.[202] Correspondingly, Vittorio *et al*.[203] studied the influence of purity and surface oxidation on the cytotoxicity of multi-walled CNTs in human neuroblastoma cells. In-vitro studies corroborated that cell viability was not affected and apoptosis and ROS were not induced in the SH-SY5Y cells after a three-day incubation period with three different types of CNT dispersed in Pluronic F127 solution. Purity of MWNTs and the concentration was found to affect the cell viability and proliferation. Results indicated that a concentration of 5–10 μg/ml MWNTs and 99% purity seem ideal for studies on the design and development of artificial MWNT nanovectors for gene and drug therapy against cancer. We suggest studying the manuscript of Smart *et al*.[204] for better understanding of CNT toxicity.

The present knowledge of CNT toxicity is inadequate and contradictory, and thus still requires more extensive toxicity, safety and efficacy studies on animal models and in humans. The effects of CNTs' aggregation, size, length, functionalization, metal impurities and polymers on safety require more thorough research. Functionalization of SWNTs and MWNTs and its effects on aggregation and consequently genotoxicity needs to be evaluated.

Regulatory aspects of CNTs

Awareness of nanotechnology has dramatically risen in recent years among lawmakers, regulators and environmental activists. However, the question of whether, and how well, to regulate nanotechnology is not new. To date increasing interest in the field of development and nanotechnology applications of nanomaterials has had an impact on development of strict regulatory norms in their production and use in animals as well as in humans. The major guideline involves the related environmental health and safety issues of existing nanomaterial. As per the reports of the US Environmental Protection Agency (EPA), CNTs require major regulatory concern over toxicity as well as environmental safety.[205]

CNTs can enter the human body after inhalation, dermal exposure or ingestion. They have a high bioavailability and can penetrate the bloodstream, digestive tract, surface tissues and blood–brain barrier without difficulty. Depending on whether nanomaterials are toxic or not, exposure to nanomaterials could trigger negative health effects. In contrast, carbon nanotubes can be coated and functionalized to prevent them from physical aggregation problems, resulting in long circulation in the air being inhaled and deposition deep into the respiratory tract. Studies in rodents and in cells have shown that ultrafine CNTs also escape detection and clearance by macrophages to a greater extent than fine particles, and may to a greater degree travel from alveolar regions to the blood circulation, where they may migrate to vital organs of the body. Ultrafine particles can produce greater inflammatory effects via free radical generation due to their small particle size and shape, surface charge, coatings and functionality, leading to toxicity, surface reactivity and influence on the cytotoxicity. CNTs could also facilitate transport of toxins deeper into the soil due to their larger surface area, bind with pollutants and transport them through the soil causing pollutants to be absorbed faster and deeper than normal, leading to formation of possibly newer toxic compounds due to strong catalytic actions.

Another significant regulatory concern is the potential effects of bioaccumulation due to the high bioavailability of nanotubes. A recent study performed in Rice University and Georgia Institute of Technology reported that CNTs formed aggregates that lead to alteration of their general physicochemical properties.[206] Thus, they suggested further consideration of bulk behaviour of these nanomaterials and their counterparts in developing regulatory norms. Apart from these, a definitive knowledge of the development and framework of regulatory guidelines for the manufacture and handling of these nanomaterials will help in marketing in the near future.

Clinical trials and market status of CNTs

Despite the several regulatory issues regarding production, handling, toxicity and environmental safety of CNTs, they are still accepted in the field of nanotechnology due to their immense biomedical and drug delivery potential. Hence as successful as the alliance has been seen in the use of these nanomaterials, to address and solve many problems in the laboratory, the ultimate measure of the program's success lies in the translation of research discoveries to the clinic. To date several products based on carbon nanotubes have reached the clinical trial stage while several are already being marketed.

Recently, Ensysce Biosciences Inc. received regulatory approval for the development and conducting a clinical trial on SWNTs for siRNA delivery into tumours for treatment of cancer cells.[207] Similarly, Calando Pharmaceuticals, Inc. has received clinical approval to carry out Phase-1 clinical trials on its patented nanoparticle-based drug delivery technology for transfer and delivery of small interfering ribonucleic acid (siRNA) for its own first therapeutic candidate, IT-101. Also, Tego BioSciences Corporation (Tego) has obtained its first approval on patented technology based on the antioxidant properties of fullerenes, a member of the CNT family, and signed agreements with Bronx Project, Inc. (TBP) for development and commercialization of the patented product carboxylated fullerenes (C3) in the treatment of Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, brain trauma and schizophrenia. Nanotopes are a bioactive fibre system used to regenerate specific tissues, including neurons, vasculature, bone, myocardium, cartilage and spinal cord, and for the treatment of peripheral arterial, which is a nanotube-based product developed by Nanotope, Inc.[208]

Thus, for better monitoring of outcomes of clinical trials on nanomaterials for their safe use, several global regulatory authorities have come into the frontline, such as the US FDA, the EMEA and the TGA. In a recent report, the FDA has approved clinical trials on CNTs for screening of their imaging property in colorectal cancers.[209]

In addition to the clinical-trial stage, several CNT-based products are being marketed, such as Graphistrength by Arkema Corporation used to optimize the performance of

CNTs in polymer matrices such as thermoplastic, elastomeric and thermosetting plastics.[210] While many MWNT products have been developed, such as Sizicyl, EpoCyl, PregCyl and Nanocyl, by Nanocyl S.A. for sizing of fibres, MWNTs containing 15 graphene sheets (Baytubes by Bayer Material Science) are used in polymer matrix to strengthen the material.[211] However, to date no CNT-based drug delivery system has received approval for marketing.

Conclusions

Nano delivery systems hold great potential to overcome many of the present obstacles of drug delivery, of which carbon nanotubes (CNTs) have been proposed and actively explored as multipurpose innovative carriers for drug delivery and diagnostic applications. They are extremely versatile nanocarriers that can be included in numerous different fields because of their great material properties. They have many unique physical, mechanical and electronic properties. These distinct and exceptional properties have made it possible to exploit CNTs for numerous applications, such as sensors, probes, actuators, composites, nanoelectronics devices and drug delivery systems within biomedical applications. As a result, in a very short time, CNTs appear to have drawn the attention of nanotechnologists from both industry and academia.

In the last two decades, remarkable work has been done in the field of CNTs, including biomedical application. Functionalization of CNTs has opened new perspectives in the study of their biological properties. Attachment of an organic moiety to nanosized tubes has made possible their use in diagnostics for imaging as well as for targeting purposes, especially in cancer therapy and infectious disease treatment. Nanotube drug delivery holds future promise for high treatment efficacy combined with minimal side effects for cancer therapy with low drug doses. In addition, it is the most promising non-viral nanocarrier in human gene therapy and in nucleic acid, peptide, vaccine and protein delivery. As much work is under progress, it is expected that plenty of applications of CNTs will be explored in the near future.

Even though CNTs are playing a larger and most promising role in the field of nanomedicine, more research is required to guarantee safety in drug delivery. Toxicity studies are critical to establish the full in-vivo potential of CNTs for drug delivery before their actual application and marketing. Physiological, physicochemical and molecular processes need to be considered for understanding of clinical and pre-clinical toxicity of CNTs. As there have been conflicting results regarding toxicity, more comprehensive studies are required. Regardless of the knowledge gained in recent years in nanotoxicology, scientists still are not able to precisely anticipate the behaviour and biokinetics of CNTs. Cytotoxicity and genotoxicity are major concerns, and antigenicity remains poorly characterized and understood. Strict nano regulation is compulsory and needs amending to cover environmental, health, pharmaceutical and safety issues. The commercialization of CNTs requires clear-headed and thoughtful environmental, health and safety research and meaningful and open discussion of broader societal impacts of pharmaceutical and toxicological issues.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. *Drug Discov Today* 2003; 8: 1112–1120.
2. Iijima S. Helical microtubules of graphitic carbon. *Nature* 1991; 354: 56–58.
3. Carbon nanotube science and technology: history. *A Carbon Nanotube*. [online] 2009; http://www.personal.reading.ac.uk/~scsharip/tubes.htm#History (accessed 13 January 2009).
4. Boczkowski J, Lanone S. Potential uses of carbon nanotubes in the medical field: how worried should patients be? *Nanomedicine* 2007; 2: 407–410.
5. Kroto H *et al*. C60: buckminsterfullerene. *Nature* 1985; 318: 162–163.
6. Zheng LX *et al*. Ultralong single-wall carbon nanotubes. *Nat Mater* 2004; 3: 673–676.
7. Dresselhaus MS *et al*. Electronic, thermal and mechanical properties of carbon nanotubes. *Phil Trans A Math Phys Eng Sci* 2004; 362: 2065–2098.
8. Awasthi K *et al*. Synthesis of carbon nanotubes. *J Nanosci Nanotechnol* 2005; 5: 1616–1636.
9. Foldavari M, Bagonluri M. Carbon nanotubes as functional excipients for nanomedicines: I. Pharmaceutical properties. *Nanomed Nanotechnol Biol Med* 2008; 4: 173–182.
10. Hilder TA, Hill JM. Theoretical comparison of nanotube materials for drug delivery. *Micro Nano Lett* 2008; 3: 18–24.
11. Joselevich E. Electronic structure and chemical reactivity of carbon nanotubes: a chemist's view. *Chem Phys* 2004; 5: 619–624.
12. Schonenberger C. Multiwall carbon nanotubes. *Physics world* Article: [online] 2000; http://physicsworld.com/cws/article/print/606 (accessed 2 June 2000).
13. Flahaut E *et al*. Gram-Scale CCVD synthesis of double-walled carbon nanotubes. *Chem Commun* 2003; 12: 1442–1443.
14. Danailov D *et al*. Bending properties of carbon nanotubes encapsulating solid nanowires. *J Nanosci Nanotechnol* 2002; 2: 503–507.
15. Iijima S *et al*. Nano-aggregates of single-walled graphitic carbon nano-horns. *Chem Phys Lett* 1999; 309: 165–170.
16. Zhang Y *et al*. Heterostructures of single-walled carbon nanotubes and carbide nanorods. *Science* 1999; 285: 1719–1722.
17. Shiba K *et al*. Carbon nanohorns as a novel drug carrier. *Nippon Rinsho* 2006; 64: 239–246.
18. Nasibulin AG *et al*. A novel hybrid carbon material. *Nat Nanotechnol* 2007; 2: 156–161.
19. Murakami T *et al*. Water-dispersed single-wall carbon nanohorns as drug carriers for local cancer chemotherapy. *Nanomedicine* 2008; 3: 453–463.
20. Ajima K *et al*. Carbon nanohorns as anticancer drug carriers. *Mol Pharmacol* 2005; 2: 475–480.
21. Liu L *et al*. Colossal paramagnetic moments in metallic carbon nanotori. *Phys Rev Lett* 2002; 88: 217206.
22. Brian M. Practical applications of carbon nanotubes in medicine. *Basic Biotechnology* ejournal. [online] 2009; MMG: 445.

http://ejournal.vudat.msu.edu/index.php/mmg445/article/viewFile/151/113 (accessed 13 January 2009).
23. Bronikowski MJ *et al*. Gas-phase production of carbon single-walled nanotubes from carbon monoxide via the HiPCO process: a parametric study. *J Vac Sci Technol A* 2001; 19: 1800–1805.
24. Wang Y *et al*. The effect of catalyst concentration on the synthesis of single walled carbon nanotubes. *Spectrochim Acta A Mol Biomol Spectrosc* 2002; 58: 2089–2095.
25. Ajayan PM *et al*. Nanotubes in a flash-ignition and reconstruction. *Science* 2002; 296: 705.
26. Nagy B *et al*. On the growth mechanism of single walled carbon nanotubes by catalytic carbon vapour deposition on supported metal catalysts. *J Nanosci Nanotechnol* 2004; 4: 326–345.
27. Thess A *et al*. Crystalline ropes of metallic carbon nanotubes. *Science* 1996; 273: 483–487.
28. Conceicao J *et al*. Photoelectron spectroscopy of transition-metal clusters: correlation of valence electronic structure to reactivity. *Phys Rev B Condens Matter* 1995; 51: 4668–4671.
29. Jose-Yacaman M. Catalytic growth of carbon microtubules with fullerene structure. *Appl Phys Lett* 1993; 273: 483–487.
30. Abdulkareem AS *et al*. Synthesis of carbon nanotubes by swirled floating catalyst chemical vapour deposition method. *J Nanosci Nanotechnol* 2007; 7: 3233–3238.
31. Inami N *et al*. Synthesis-condition dependence of carbon nanotube growth by alcohol catalytic chemical vapor deposition method. *Sci Technol Adv Mater* 2007; 8: 292–295.
32. Choi EC *et al*. Synthesis of carbon nanotubes on diamond-like carbon by the hot filament plasma-enhanced chemical vapor deposition method. *Micron* 2009; 40: 612–616.
33. Kleinsorge B *et al*. Growth of aligned carbon nanofibres over large areas using colloidal catalysts at low temperatures. *Chem Commun* 2004; 10: 1416–1417.
34. Vajtai R *et al*. Controlled growth of carbon nanotubes. *Phil Trans A Math Phys Eng Sci* 2004; 362: 2143–2160.
35. Baddour C, Briens C. Carbon nanotube synthesis: a review. *Int J Chem Reactor Eng* 2005; 3: R3–R9.
36. Ose-Yacaman M. Catalytic growth of carbon microtubules with fullerene structure. *Appl Phys Lett* 1993; 62: 657–659.
37. Dresselhaus MS *et al*. Carbon nanotubes. *Physicsworld.com*. [online] 2009; 1761. http://physicsworld.com/cws/article/print/1761 (accessed 13 January 2009).
38. Dresselhaus MS, Dresselhaus G. Single walled nanotubes raman spectroscopy. *Acc Chem Res* 2002; 35: 1070–1078.
39. Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. *Pharm Res* 2006; 23: 1417–1450.
40. Park K. *Controlled Drug Delivery: Challenges and Strategies*. Washington, DC: American Chemical Society, 1997.
41. Hui HW *et al*. Design and fabrication of oral controlled release drug delivery systems. In: Robinson JR, Lee VHL, eds. *Controlled Drug Delivery: Fundamentals and Applications*. New York: Marcel Dekker, 1987: 373–432.
42. Riggio C. Combination of polymer technology and carbon nanotube array for the development of an effective drug delivery system at cellular level. *Nanoscale Res Lett* 2009; 4: 668–673.
43. Liu Z *et al*. Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery. *ACS Nano* 2007; 1: 50–56.
44. Sinha N, Yeow JTW. Carbon nanotubes for biomedical applications. *IEEE Trans Nanobioscience* 2005; 4: 180–195.
45. Gao H *et al*. Spontaneous insertion of DNA oilgonucleotide into carbon nanotubes. *Nano Lett* 2003; 3: 471–473.
46. Mitchell DT *et al*. Smart nanotubes for bioseparation and biocatalysis. *J Am Chem Soc* 2001; 124: 11864–11865.
47. Pastorin G *et al*. Double functionalization of carbon nanotubes for multi modal drug delivery. *Chem Commun* 2006; 1182–1184.
48. Kam NW, Dai H. Carbon nanotubes as intracellular protein transporters: generality and biological functionality. *J Am Chem Soc* 2005; 127: 6021–6026.
49. Singh R *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: 4388–4396.
50. Pantarotto D *et al*. Functionalized carbon nanotubes for plasmid DNA gene delivery. *Angew Chem Int Ed Engl* 2004; 43: 5242–5246.
51. Martin CR, Kohli P. The emerging field of nanotube biotechnology. *Nat Rev Drug Discov* 2003; 2: 29–37.
52. Pantoratto D *et al*. Functionalized carbon nanotubes for plasmid DNA gene delivery. *Angew Chem Int Ed Engl* 2004; 43: 5242–5246.
53. Pantarotto D *et al*. Translocation of bioactive peptides across cell membranes by carbon nanotubes. *Chem Commun* 2004; 1: 16–17.
54. Penman D. Carbon nanotubes show drug delivery promise. *New scientist.com* [online] 2003; http://www.new scientist.com/article. ns? id=dn4485 & print=true (accessed 6 August 2009).
55. Yang YJ *et al*. Fluorescent mesoporous silica nanotubes incorporating CdS quantum dots for controlled release of ibuprofen. *Acta Biomater* 2009; 5: 3488–3496.
56. Zhang CH *et al*. Preparation and theophylline delivery applications of novel PMAA/MWNT-COOH nanohybrid hydrogels. *J Biomater Sci Polym Ed* 2009; 20: 1119–1135.
57. 'Smart' bio-nanotubes developed; may help in drug delivery. *Science Daily* [online] 3 August 2005; http://www.sciencedaily.com/releases/2005/08/050802182805.htm (accessed 11 August 2009).
58. Leonard S. Titanium nanotubes offer implant integration, drug delivery. *Qmed Beta*. [online] 17 May 2009; http://www.qmed.com/mpmn/article/1923/titanium-nanotubes-offer-implant-integration-drug-delivery (accessed 11 June 2010).
59. Mohapatra SS, Kumar A. Method of drug delivery by carbon nanotube-chitosan nanocomplexes. US20080214494.
60. Kam NW *et al*. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc Natl Acad Sci USA* 2005; 102: 11600–11605.
61. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer* 2005; 5: 161–171.
62. Prakash S, Kulamarva AG. Recent advances in drug delivery: potential and limitations of carbon nanotubes. *Recent Pat Drug Deliv Formul* 2007; 1: 214–221.
63. Quintana A *et al*. Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor. *Pharm Res* 2002; 19: 1310–1316.
64. Pantarotto D *et al*. Synthesis, structural characterization, and immunological properties of carbon nanotubes functionalized with peptides. *J Am Chem Soc* 2003; 125: 6160–6164.
65. Wu W *et al*. Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes. *Angew Chem Int Ed Engl* 2005; 44: 6358–6362.
66. Special nanotubes may be used as a vehicle for treating neurodegenerative disorders. *Science daily*: Science news [online] 2009; http://www.sciencedaily.com/releases/2009/01/090114212227.htm (accessed 28 July 2009).
67. Kulamarva A *et al*. Microcapsule carbon nanotube devices for therapeutic applications. *Nanotechnology* 2009; 20: 025612.

68. Singh R, Lillard JW Jr. Nanoparticles based targeted delivery. *Exp Mol Pathol* 2009; 86: 215–223.
69. McDevit MR *et al*. Tumor targeting with antibody-functionalized radiolabelled carbon nanotubes. *J Nucl Med* 2007; 48: 1180–1189.
70. Ashcroft JM *et al*. Fullerene (C60) immunoconjugates: interaction of water-soluble C60 derivatives with the murine anti-gp240 melanoma antibody. *Chem Commun* 2006; 3004–3006.
71. Welsher K *et al*. Selective probing and imaging of cells with single walled carbon nanotubes as near-infrared fluorescent molecules. *Nano Lett* 2008; 8: 586–590.
72. Kasab AC *et al*. Rifampicin carrying polyhydroxybutyrate microspheres as a potential chemoembolization agent. *J Biomater Sci Polym Ed* 1997; 8: 947–961.
73. Yang F *et al*. Pilot study of targeting magnetic carbon nanotubes to lymph nodes. *Nanomedicine* 2009; 4: 317–330.
74. Bystrzejewski M *et al*. Carbon encapsulated magnetic nanoparticles for biomedical applications: thermal stability studies. *Biomol Eng* 2007; 24: 555–558.
75. Yang F *et al*. Magnetic lymphatic targeting drug delivery system using carbon nanotubes. *Med Hypotheses* 2008; 70: 765–767.
76. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx*^{R} 2005; 2: 3–14.
77. Pardridge WM. Blood-brain barrier delivery. *Drug Discov Today* 2007; 12: 54–61.
78. Hunyh GH *et al*. Barriers to carrier mediated drug and gene delivery to brain tumors. *J Control Release* 2006; 110: 236–259.
79. Rautioa J, Chikhale PJ. Drug delivery systems for brain tumor therapy. *Curr Pharm Des* 2004; 10: 1341–1353.
80. Kateb B *et al*. Internalization of MWNTs by microglia: possible application in immunotherapy of brain tumors. *Neuroimage* 2007; 37(Suppl. 1): S9–S17.
81. VanHandel M *et al*. Selective uptake of multi-walled carbon nanotubes by tumor macrophages in a murine glioma model. *J Neuroimmunol* 2009; 208: 3–9.
82. Rosen Y, Elman NM. Carbon nanotubes in drug delivery: focus on infectious diseases. *Expert Opin Drug Deliv* 2009; 6: 517–530.
83. Jingyi C *et al*. Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery. *J Am Chem Soc* 2008; 130: 16778–16785.
84. Sharon G. MIT uses nanotubes to help fight cancer. *Computerworld IDG*. [online] 2009; 15idg. http://www.nytimes.com/external/idg/2008/12/15/15idg-MIT-uses-nanotu.html (accessed 13 July 2009).
85. Nanotubes the 'bomb diggity' for cancer treatment? *Inpharma Weekly*. [online] 2009; http://web.ebscohost.com/ehost/detail? (accessed 27 October 2009).
86. Liz K. Carbon nanotubes pass through body fast. *Nanotechweb org*. [online] 2009; 24233. http://nanotechweb.org/cws/article/tech/24223 (accessed 12 January 2009).
87. Gannon CJ *et al*. Carbon nanotube-enhanced thermal destruction of cancer cells in a non-invasive radiofrequency field. *Cancer* 2007; 2654.
88. Zhou F *et al*. Cancer photothermal therapy in the near-infrared region by using single-walled carbon nanotubes. *J Biomed Opt* 2009; 14: 021009.
89. Levi-Polyachenko NH *et al*. Rapid photo thermal intracellular drug delivery using multiwalled carbon nanotubes. *Mol Pharm* 2009; 6: 1092–1099.
90. Torti SV *et al*. Thermal ablation therapeutics based on CN(x) multi-walled nanotubes. *Int J Nanomedicine* 2007; 2: 707–714.
91. Burke A *et al*. Long-term survival following a single treatment of kidney tumors with multiwalled carbon nanotubes and near-infrared radiation. *Proc Natl Acad Sci USA* 2009; 106: 12897–12902.
92. Zach Z. Killing cancer with red-hot nanotubes. *Discover Magazine*. [online] 2005; http://web.ebscohost.com/ehost/ (accessed 27 June 2009).
93. Guenzel JDN. A-coated nanotubes help kill tumors without harm to surrounding tissue. *Eureka alert*. [online] 2009; http://www.eurekalert.org/pub_releases/2009-08/wfub-dnh081909.php (accessed 19 August 2009).
94. Podesta JE *et al*. Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model. *Small* 2009; 5: 1176–1185.
95. Liu Z *et al*. Drug delivery with carbon nanotubes for *in vivo* cancer treatment. *Cancer Res* 2008; 68: 6652–6660.
96. Zhang X *et al*. Targeted delivery and controlled release of doxorubicin to cancer cells using modified single wall carbon nanotubes. *Biomaterials* 2009; 30: 6041–6047.
97. Zhao Q *et al*. Carbon-nanotube-assisted high loading and controlled release of polyoxometalates in biodegradable multilayer thin films. *Nanotechnology* 2009; 20: 105101.
98. Hampel S *et al*. Carbon nanotubes filled with a chemotherapeutic agent: a nanocarrier mediates inhibition of tumor cell growth. *Nanomedicine* 2008; 3: 175–182.
99. Dhar S *et al*. Targeted single-wall carbon nanotube-mediated Pt(IV) prodrug delivery using folate as a homing device. *J Am Chem Soc* 2008; 130: 11467–11476.
100. Chen J *et al*. Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery. *J Am Chem Soc* 2008; 130: 16778–16785.
101. Zhang Z *et al*. Delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes suppresses tumor growth. *Clin Cancer Res* 2006; 12: 4933–4939.
102. Wang X *et al*. Targeted RNA interference of cyclin A2 mediated by functionalized single-walled carbon nanotubes induces proliferation arrest and apoptosis in chronic myelogenous leukemia K562 cells. *Chem Med Chem* 2008; 3: 940–945.
103. Yang J *et al*. Oxygen adsorption by carbon nanotubes and its application in radiotherapy. *IET Nanobiotechnol* 2007; 1: 10–14.
104. Ou Z *et al*. Functional single-walled carbon nanotubes based on an integrin alpha v beta 3 monoclonal antibody for highly efficient cancer cell targeting. *Nanotechnology* 2009; 20: 105102.
105. Murakami T *et al*. Solubilization of single-wall carbon nanohorns using a PEG-doxorubicin conjugate. *Mol Pharm* 2006; 3: 407–414.
106. Ajima K *et al*. Enhancement of *in vivo* anticancer effects of cisplatin by incorporation inside single-wall carbon nanohorns. *ACS Nano* 2008; 2: 2057–2064.
107. Pramanik M *et al*. Single-walled carbon nanotubes as a multimodal-thermoacoustic and photoacoustic-contrast agent. *J Biomed Opt* 2009; 14: 034018.
108. Schulz MJ *et al*. On beating cancer (with nanotechnology). *Nano werk spotlight* [online] 2007; http://www.nanowerk.com/spotlight/spotid=3115.php (accessed 31 October 2007).
109. Prato M *et al*. Functionalized carbon nanotubes in drug design and discovery. *Acc Chem Res* 2008; 41: 60–68.
110. Dyke CA, Tour JM. Overcoming the insolubility of carbon nanotubes through high degrees of sidewall functionalization. *Chem Eur J* 2004; 10: 812.
111. Tasis D *et al*. Soluble carbon nanotubes. *Chem Eur J* 2003; 9: 4000–4008.

112. Bianco A *et al*. Biomedical applications of functionalized carbon nanotubes. *Chem Commun* 2005; 5: 571–577.
113. Gao Y *et al*. Temperature-sensitive and highly water-soluble titanate nanotubes. *Polymer* 2009; 50: 2572–2577.
114. Klumpp C *et al*. Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. *Biochim Biophys Acta* 2006; 1758: 404–412.
115. LaVan DA *et al*. Small-scale systems for *in vivo* drug delivery. *Nat Biotechnol* 2003; 21: 1184–1191.
116. Gasparc R *et al*. Template synthesis of nano test tubes. *Nano Lett* 2004; 4: 513.
117. Kim BM *et al*. Filling nanotubes with particles. *Nano Lett* 2005; 5: 873–878.
118. Jorgensen WL. The many roles of computation in drug discovery. *Science* 2004; 303: 1813–1818.
119. Briefing paper: Inst. Neurosci. Mental health and addiction. *CIHR*. [online] 2003;http://www.regenerativemedicine.ca/nanomed/Nanomedicine%20Taxonomy%20 (Feb%2003) (accessed 15 September 2009).
120. Guo X, Xu H. Research and development of biomedical application of carbon nanotubes and related composites. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* 2006; 23: 438–441.
121. Kramer K *et al*. Multifunctional carbon nano-tubes for biomedical applications (CARBIO). A project of the European Marie Curie Network. *Urologe A* 2007; 46: 1248.
122. Mehra NK *et al*. Challenges in the use of carbon nanotubes for biomedical applications. *Crit Rev Ther Drug Carrier Syst* 2008; 25: 169–206.
123. Bhargava A. Nanorobots: medicine of the future. *IEEE* [online] July 1999; http://www.ewh.ieee.org/r10/Bombay/news 3/Page4.html (accessed 6 July 2009).
124. Polizu S *et al*. Applications of carbon nanotubes-based biomaterials in biomedical nanotechnology. *J Nanosci Nanotechnol* 2006; 6: 1883–1904.
125. Liu J, Dai H. Design, fabrication, and testing of piezoresistive pressure sensors using carbon nanotubes. [online] 2002; http://www.nnf.cornell.edu/2002reu/Liu.pdf (accessed 11 June 2010).
126. Romero M *et al*. Pressure sensing systems for medical devices. *Med Dev Diag Ind Mag* [online] 2000; http://www.devicelink.com/mddi/archive/00/10/004.html (accessed 12 October 2009).
127. Joseph H *et al*. MEMS in the medical world. *Sens Mag* 1997; 14: 47–51.
128. Sakai K. Artificial kidney engineering-dialysis membrane and dialyzer for blood purification. *J Chem Eng Jpn* 1997; 30: 587–599.
129. Sotiropoulou S, Chaniotakis NA. Carbon nanotube array-based biosensor. *Anal Bioanal Chem* 2003; 375: 103–105.
130. Wang J *et al*. Ultrasensitive electrical biosensing of proteins and DNA: carbon-nanotube derived amplification of the recognition and transduction events. *J Am Chem Soc* 2004; 126: 3010–3011.
131. Xu Y *et al*. Electrochemical impedance detection of DNA hybridization based on the formation of M-DNA on ploypyrrole/carbon nanotube modified electrode. *Anal Chim Acta* 2004; 516: 19–27.
132. He P, Dai L. Aligned carbon nanotube-DNA electrochemical sensors. *Chem Commun* 2004; 3: 348–349.
133. Galaasen OP. Nanomedicine and the future of healthcare. *Med Technol* [online] 2002; http://plausible.custompublish.com/cparticle54173-5911.html (accessed 3 August 2009).
134. Adrian P. Nanosensors targeted at the right markets could generate big business opportunities. *Sens Bus Dig* [online] 2003; http://www.sensorsmag.com/resources/businessdigest/sbd0703.shtml (accessed 19 October 2009).
135. Shandas R, Lanning C. Development and evaluation of implantable sensors for monitoring function of prosthetic heart valves: *in vitro* studies. *Med Biol Eng Comput* 2003; 41: 416–424.
136. Shultts MC *et al*. A telemetry instrumentation system for monitoring multiple subcutaneously implanted glucose sensors. *MEEE Trans Biomed Eng* 1994; 41: 937–972.
137. Liao KJ *et al*. Experimental studies on flow velocity sensors based on multiwalled carbon nanotubes. *Microfab Technol* 2003; 4: 57.
138. Ghosh S *et al*. Carbon nanotube flow sensors. *Science* 2003; 299: 1042–1044.
139. Schulz MJ *et al*. Electrochemical impedance measurement of prostate cancer cells using carbon nanotube array electrodes in a microfluidic channel. *Nanotechnology* 2007; 18: 465505.
140. Popov AM *et al*. Biocompatibility and applications of carbon nanotubes in medical nanorobots. *Int J Nanomedicine* 2007; 2: 361–372.
141. Cavalcanti A *et al*. Nanorobots for laparoscopic cancer surgery. *6th IEEE/ACIS 2007 International Conference on Computer and Information Science (ICIS 2007)*. IEEE Computer Society, 2007: 738–743.
142. Mamalis AG. Recent advances in nanotechnology. *J Mat Process Technol* 2007; 181: 52–58.
143. Harutyunyan AR *et al*. Carbon nanotubes for medical applications. *Eur Cell Mater* 2002; 3(Suppl. 2): 84–87.
144. Stevens RMD *et al*. Carbon nanotube scanning probe for imaging in aqueous environment. *IEEE Trans Nanobioscience* 2004; 3: 56–60.
145. Nguyen CV *et al*. High lateral resolution imaging with sharpened tip of multi walled carbon nanotube probe. *J Phys Chem B* 2004; 108: 2816–2821.
146. Emirov YN *et al*. Making carbon nanotube probes for high aspect ratio scanning probe metrology. *Proc SPIE* 2003; 5038: 493–495.
147. Stevens RMD *et al*. Carbon nanotubes as probes for atomic force microscopy. *Nanotechnology* 2000; 11: 1–5.
148. Baughman RH *et al*. Carbon nanotubes: the route toward applications. *Science* 2002; 297: 787–792.
149. Akita S *et al*. Nanotweezers consisting of carbon nanotubes operating in an atomic force microscope. *Appl Phys Lett* 2001; 79: 1691–1693.
150. Kim P, Lieber CM. Nanotube nanotweezers. *Science* 1999; 286: 2148–2150.
151. Vohrer U *et al*. Carbon nanotube sheets for use as artificial muscles. *Carbon* 2000; 42: 316–319.
152. Baughman RH *et al*. Carbon nanotube actuators. *Science* 1999; 284: 408–410.
153. Kiernan G *et al*. Characterization of nanotube based artificial muscles materials. *Proc SPIE* 2002; 4876: 775–782.
154. Gao M *et al*. Electrochemical properties of aligned nanotube arrays: Basis of new electrochemical actuators. *Proc SPIE* 2000; 3987: 18–24.
155. Megaridis CM *et al*. Attoliter fluid experiment in individual closed-end carbon nanotubes: liquid film and fluid interface dynamics. *Phys Fluids* 2002; 14: L5–L8.
156. Gogotsi Y *et al*. In situ multiphase fluid experiments in hydrothermal carbon nanotubes. *Appl Phys Lett* 2001; 79: 1021–1023.
157. Lim YT *et al*. Selection of quantum dot wavelengths for biomedical assays and imaging. *Mol Imaging* 2003; 2: 50–64.
158. Tans SJ *et al*. Individual single-wall carbon nanotubes as quantum wires. *Nature* 1997; 386: 474–477.
159. Jia N *et al*. Intracellular delivery of quantum dots tagged antisense oligodeoxynucleotides by functionalized multiwalled carbon nanotubes. *Nano Lett* 2007; 7: 2976–2980.

160. Bhirde AA *et al*. Targeted killing of cancer cells *in vivo* and *in vitro* with EGF-directed carbon nanotube-based drug delivery. *ACS Nano* 2009; 3: 307–316.
161. Czaja WK *et al*. The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules* 2007; 8: 1–12.
162. Park W-II *et al*. Synthesis of bacterial celluloses in multiwalled carbon nanotube dispersed medium. *Carbohydr Polym* 2009; 77: 457–463.
163. Harrison BS, Atala A. Carbon nanotube applications for tissue engineering. *Biomaterials* 2007; 28: 344–353.
164. Ada GL. The traditional vaccines: an overview. In: Levine MM *et al*., ed. *New Generation Vaccines*. New York: Marcel Dekker, 1997: 13–23.
165. Bianco A *et al*. Applications of carbon nanotubes in drug delivery. *Curr Opin Chem Biol* 2005; 9: 674–679.
166. Carbon nanotubes offer a new approach to gene therapy. *The Medical News: from News-Medical.net* [online] 2004; http://www.news-medical.net/news/2004/10/11/5469.aspx (accessed 11 October 2004).
167. Pan B *et al*. Synthesis and characterization of polyamidoamine dendrimer-coated multi-walled carbon nanotubes and their application in gene delivery systems. *Nanotechnology* 2009; 20: 125101.
168. Zhang M, Gorski W. Electrochemical sensing platform based on the carbon nanotubes/redox mediators-biopolymer system. *J Am Chem Soc* 2005; 127: 2058–2059.
169. Zhang M, Gorski W. Electrochemical sensing based on redox mediation at carbon nanotubes. *Anal Chem* 2005; 77: 3960–3965.
170. Svistunenko DA. Reaction of haem containing proteins and enzymes with hydroperoxides: the radical view. *Biochim Biophys Acta* 2005; 1707: 127–155.
171. Bi-feng P *et al*. Design of dendrimer modified carbon nanotubes for gene delivery. *Chin J Cancer Res* 2007; 19: 1–6.
172. Yang R *et al*. Single-walled carbon nanotubes-mediated *in vivo* and *in vitro* delivery of siRNA into antigen-presenting cells. *Gene Ther* 2006; 13: 1714–1723.
173. Wu Y *et al*. Carbon nanotubes protect DNA strands during cellular delivery. *ACS Nano* 2008; 2: 2023–2028.
174. Wang H *et al*. Unique aggregation of anthrax (Bacillus anthracis) spores by sugar coated single-walled carbon nanotubes. *J Am Chem Soc* 2006; 128: 13364–13365.
175. Hilder TA, Hill JM. Modeling the loading and unloading of drugs into nanotubes. *Small* 2009; 5: 300–308.
176. Lanone S, Boczkowski J. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. *Curr Mol Med* 2006; 6: 651–666.
177. Soto K *et al*. Cytotoxic effects of aggregated nanomaterials. *Acta Biomater* 2007; 3: 351–358.
178. Wick P *et al*. The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicol Lett* 2007; 168: 121–131.
179. Fraczek A *et al*. Comparative *in vivo* biocompatibility study of single- and multi-wall carbon nanotubes. *Acta Biomater* 2008; 4: 1593–1602.
180. Qu G *et al*. The effect of multiwalled carbon nanotube agglomeration on their accumulation in and damage to organs in mice. *Carbon* 2009; 47: 2060–2069.
181. Belyanskaya L *et al*. Effects of carbon nanotubes on primary neurons and glial cells. *Neurotoxicol* 2009; 30: 702–711.
182. Bottini M *et al*. Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicol Lett* 2006; 160: 121–126.
183. Davoren E *et al*. *In vitro* toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicol In Vitro* 2007; 21: 438–448.
184. Chiaretti M *et al*. Carbon nanotubes toxicology and effects on metabolism and immunological modification *in vitro* and *in vivo*. *J Phys Condens Matter* 2008; 20: 10.
185. Rotoli BM *et al*. Non-functionalized multi-walled carbon nanotubes alter the paracellular permeability of human airway epithelial cells. *Toxicol Lett* 2008; 178: 95–102.
186. Poland CA *et al*. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nanotechnol* 2008; 3: 423–428.
187. Shvedova AA *et al*. Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J Toxicol Environ Health A* 2003; 66: 1909–1926.
188. Pulskamp K *et al*. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol Lett* 2007; 168: 58–74.
189. Szendi K, Varga C. Lack of genotoxicity of carbon nanotubes in a pilot study. *Anticancer Res* 2008; 28: 349–352.
190. Zhu L *et al*. DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. *Nano Lett* 2007; 7: 3592–3597.
191. Muller J *et al*. Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells. *Carcinogenesis* 2008; 29: 427–433.
192. Kisin ER *et al*. Single-walled carbon nanotubes: geno and cytotoxic effects in lung fibroblast V79 cells. *J Toxicol Environ Health A* 2007; 70: 2071–2079.
193. Takagi A *et al*. Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. *J Toxicol Sci* 2008; 33: 105–116.
194. Sakamoto Y *et al*. Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. *J Toxicol Sci* 2009; 34: 65–76.
195. Muller J *et al*. Absence of carcinogenic response to multiwall carbon nanotubes in a 2-year bioassay in the peritoneal cavity of the rat. *Toxicol Sci* 2009; 110: 442–448.
196. Tong H *et al*. Influence of acid functionalization on the cardiopulmonary toxicity of carbon nanotubes and carbon black particles in mice. *Toxicol Appl Pharmacol* 2009; 239: 224–232.
197. Yang S *et al*. Long-term accumulation and low toxicity of single-walled carbon nanotubes in intravenously exposed mice. *Toxicol Lett* 2008; 181: 182–189.
198. Zeni O *et al*. Cytotoxicity investigation on cultured human blood cells treated with single-wall carbon nanotubes. *Sensor* 2008; 8: 488–499.
199. Yacobi NR *et al*. Nanoparticle effects on rat alveolar epithelial cell monolayer barrier properties. *Toxicol In Vitro* 2007; 21: 1373–1381.
200. Huczko A, Lange H. Carbon nanotubes: experimental evidence for a null risk of skin irritation and allergy. *Fuller Sci Technol* 2001; 9: 247–250.
201. Bardi G *et al*. Pluronic-coated carbon nanotubes do not induce degeneration of cortical neurons *in vivo* and *in vitro*. *Nanomedicine* 2009; 5: 96–104.
202. Sotto AD *et al*. Multi-walled carbon nanotubes: lack of mutagenic activity in the bacterial reverse mutation assay. *Toxicol Lett* 2009; 184: 192–197.
203. Vittorio O *et al*. Influence of purity and surface oxidation on cytotoxicity of multiwalled carbon nanotubes with human neuroblastoma cells. *Nanomed Nanotechnol Biol Med* 2009; 5: 524–531.
204. Smart SK *et al*. The biocompatibility of carbon nanotubes. *Carbon* 2006; 44: 1034–1047.
205. Pelley J, Saner M. International approaches to the regulatory governance of nanotechnology. *Regulatory*

Governance Initiatives. [online] April 2009; http://www.regulatorygovernance.ca (accessed 13 June 2010).
206. Lewinski N. Nanotechnology policy and environmental regulatory issues. *J Eng Public Pol* 2005; 9: 1–37.
207. Ensysce receives funding for carbon nanotube therapeutics for siRNA delivery. *Nano werk* [online] June 11 2010; http://www.nanowerk.com/news/newsid=16674.php (accessed 13 June 2010).
208. Arrowhead Research Corporation (ARWR.O). [online] 11 June 2010; http://www.reuters.com/finance/stocks/companyProfile?rpc=66&symbol=ARWR.O (accessed 13 June 2010).
209. Nanotechnology in clinical trials. *NCI Alliance for nanotechnology in cancer*. National Cancer Research Institute. [online] 2010; http://nano.cancer.gov/learn/now/clinicaltrials.asp (accessed 14 June 2010).
210. Arkema receives EPA approval to market its carbon nanotubes in the United States. *Nano werk* [online] June 2 2010; http://www.nanowerk.com/news/newsid=16535.php (accessed 13 June 2010).
211. Multi wall carbon nanotubes modified to resolve dispersion problems. *Plastermart.com* [online] 2010; http://www.plastemart.com/upload/Literature/Multi-wall-carbon-nanotubes-MWCNT-modified-dispersion-problems.asp?LiteratureID=1346 (accessed 13 June 2010).
212. Itkis ME *et al*. Comparison of analytical techniques for evaluation of single-walled carbon nanotubes. *J Am Chem Soc* 2005; 127: 3439–3448.
213. McKee GS, Vecciho KS. Thermogravimetric analysis synthesis variation effects on CVD generated multiwalled carbon nanotubes. *J Phys Chem B* 2006; 110: 1179–1186.
214. Georgakilas V *et al*. Purification of HiPCO carbon nanotubes via organic functionalization. *J Am Chem Soc* 2002; 124: 14318–14319.
215. Pantoratto D *et al*. Synthesis, structural characterization and immunological properties of carbon nanotubes functionalized with peptides. *J Am Chem Soc* 2003; 125: 6160–6164.
216. Li PH *et al*. Synthesis of cactus top decorated aligned carbon nanotubes and their third order nonlinear optical properties. *J Nanosci Nanotechnol* 2006; 6: 990–995.
217. Odom TW *et al*. Single-walled nanotubes from fundamental studies to new device concepts. *Ann N Y Acad Sci* 2002; 960: 203–215.
218. Sanchez S *et al*. Carbon nanotubes/polysulfone screen printed electrochemical immunosensor. *Biosens Bioelectron* 2007; 23: 332–340.
219. Heller D *et al*. Using Raman spectroscopy to elucidate the aggregation state of single-walled carbon nanotubes. *J Phys Chem B* 2004; 108: 6905–6909.
220. Eklund P *et al*. Vibrational modes of carbon nanotubes; spectroscopy & theory. *Carbon* 2004; 33: 6905–6909.
221. Rao AM *et al*. Diameter-selective Raman scattering from vibrational modes in carbon nanotubes. *Science* 1997; 275: 187–191.
222. Georgakilas V *et al*. Organic functionalization of carbon nanotubes. *J Am Chem Soc* 2002; 124: 760–761.
223. Karajanagi SS *et al*. Structure and function of enzyme adsorbed onto single-walled carbon nanotubes. *Langmuir* 2004; 20: 11594–11599.
224. Vinogradov SV *et al*. Curcumin-Combretastatin nanocells as breast cancer cytotoxic and antiangiogenic agent. [online] 2008; http://www.stormingmedia.us/cat/sub/subcat21-1.html?perpage=200 (accessed 24 September 2008).
225. Murakami T *et al*. Drug-loaded carbon nanohorns: adsorption and release of dexamethasone *in vitro*. *Mol Pharm* 2004; 1: 399–405.
226. Inoue K *et al*. Effects of multi-walled carbon nanotubes on a murine allergic airway inflammation model. *Toxicol Appl Pharmacol* 2009; 237: 306–316.
227. Kishore AS *et al*. Assessment of the dermal and ocular irritation potential of multi-walled carbon nanotubes by using in vitro and in vivo methods. *Toxicol Lett* 2009; 191: 268–274.