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Functionalized carbon nanotubes and their promising applications in therapeutics and diagnostics

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15.1 INTRODUCTION

Carbon nanotubes (CNTs), a sub-family of fullerenes (or bucky balls), are unique sp^2 hybridized pseudo-one-dimensional carbon allotropes with a C–C distance of approximately 1.4 Å. A high aspect ratio, high surface area, rich surface chemistry, neutral electrostatic potential, extremely high drug cargo ability, and excellent material properties, such as ultimate electrical and thermal conductivities and mechanical strength, make them an attractive tool for biomedical applications (Lu et al., 2009). They are regarded as a third allotropic form of carbon with a typical length-to-diameter ratio of up to 28,000,000:1. Individual CNTs differ from each other in the number of carbon atoms, such as C_{20} , C_{30} , C_{36} , C_{70} , and C_{78} , and each of these individual members is called “graphene.” These hollow cylindrical tubes are thus arranged in a specific pattern to form hexagonal structural units. This is primarily responsible for a high tensile strength of 150 Gpa which in turn leads to higher C–C bond stiffness of the network (Awasthi et al., 2005).

15.2 ORIGIN AND HISTORICAL PERSPECTIVE OF CNT

The existence of CNTs dates back to 1952, when these nanofilaments were observed by two Russian scientists, LV Radushkevich and VM Lukyanovich. Their discovery went unnoticed as the article was published in Russian with a low circulation. Subsequently, Bacon in 1956, while investigating the properties of carbon fibers in parma, observed CNTs in his samples. He later presented his observations in 1960 wherein he described these carbon nanowhiskers as scroll-like structures (Bacon, 1960). In the 1970s, CNTs were produced and imaged during the production of carbon fibers by pyrolysis of benzene and ferrocene at 1000 °C. These were referred to as single-walled nanotubes (Obelin and Endo, 1976). Following this discovery, a group of Soviet scientists published the chemical and structural properties of CNTs produced by thermocatalytical disproportionation of carbon monoxide. Howard G Tennent was granted a US patent in 1987 for Hyperion Catalysis in the production of “cylindrical discrete carbon fibrils” with a constant diameter between 3.5 and 70 nm (Tennent, 1987).

The experimental evidence of CNTs, however, came into the picture in 1991, when a Japanese microscopist, S Iijima, observed multiwalled carbon nanotubes (MWCNTs) in his TEM studies. He described them as allotropes of carbon with a hollow cylindrical-tube-shaped structure (Iijima, 1991). Two years later Iijima and coworkers, and Bethune and coworkers, independently observed single-walled carbon nanotubes (SWCNTs; Iijima and Ichihashi, 1993; Bethune et al., 1993). Since then research on CNTs has accelerated and they have been investigated in diverse fields and arenas.

15.3 CLASSIFICATION OF CNTs

CNTs can be broadly classified into two types based on their wall structure, that is, SWCNTs and MWCNTs (Foldvari and Bagonluri, 2008). In SWCNTs, a single graphene sheet is folded to form a cylindrical closed structure, whereas in case of MWCNTs, around 3–5 sheets of single-walled nanotubes are rolled upon each other to form a multilayered structure. In addition, the SWCNTs may further be categorized based on the differences in the arrangements of their carbon atoms. The armchair arrangement is typically characterized by the chairs perpendicular to the tube axis, whereas a V-shape perpendicular to the tube axis is characteristic of the zigzag arrangement. The unique electrical, conductive properties and metallic characteristics are primarily determined by their degree of chirality. This forms the basis of designing a variety of nanoelectronic and diagnostic instruments (Tessonnier and Su, 2011). Beside structural differences, the CNTs also differ from each other dimensionally. A coaxial structure, containing two concentric graphene

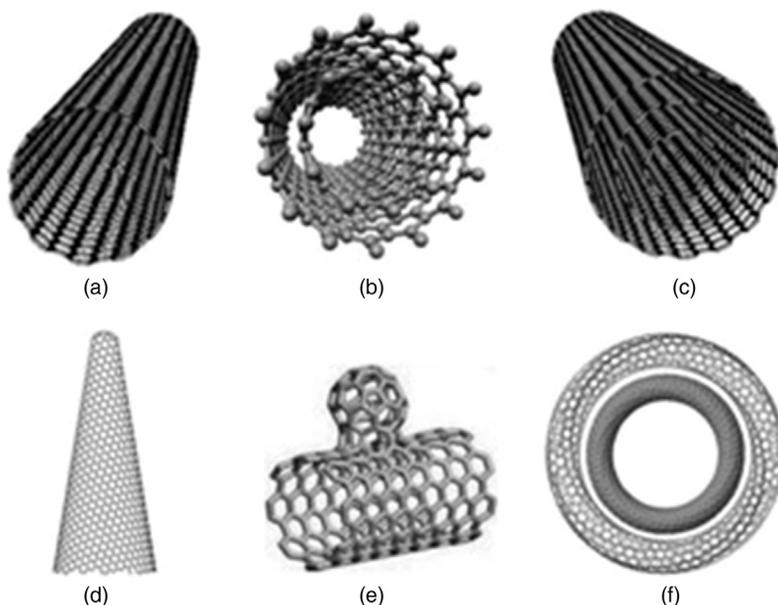


FIGURE 15.1

Various types of CNTs employed in drug delivery.

cylinders with higher thermal and chemical stability than single-walled nanotubes has been explored recently. These are termed double-walled CNTs. Apart from this, several variants in CNT shapes have been seen including carbon nanohorns (CNHs), nanobuds, and nanotorus, which have enormous applications in drug delivery due to the suitability of their characteristic modified structure. [Figure 15.1](#) diagrammatically depicts the various types of CNTs employed in drug delivery.

Further, CNTs are also classified depending upon their methods of preparation, structural modifications, and solubility properties, such as functionalized CNTs, CNTs dispersed in solvent, surfactant-coated CNTs, and conjugated CNTs, which may include drugs, monoclonal antibodies, ligands, etc.

15.4 METHODS FOR PREPARATION OF CNTs

Heating carbon black and graphites in a controlled flame environment has been successfully utilized for the preparation of CNTs for the last two decades ([Iijima, 1991](#)). However, nanotubes synthesized by this method are irregular in size, shape, mechanical strength, quality, and purity owing to their uncontrollable

natural environment. Of late, a number of artificially developed methods have been extensively utilized for their synthesis, including catalytic chemical vapor deposition (CVD), electric arc discharge, and laser ablation (Awasthi et al., 2005) as depicted in Figures 15.2–15.4 respectively, and Table 15.1.

Additionally, several newer techniques, plasma-enhanced CVD, thermal CVD, laser-assisted CVD, high-pressure CVD, cobalt-molybdenum catalytic (CoMoCat) process, and high-pressure carbon monoxide (HiPCO) disproportionation process have been developed for high-quality CNT production (Beg et al., 2011). Different methods of preparation produce CNTs with different physical and mechanical properties. The types of methods usually differ from one another in terms of the type of CNTs produced, solubility, mechanical properties, quality, purity, and yield.

Table 15.1 Artificially Developed Methods Utilized for the CNT Synthesis

Method	Technique	Reference
Chemical vapor deposition method	Feed material, in the form of a mixed vapor phase, is passed through a hot furnace, where it decomposes to give CNTs deposited on the surface of a substrate	Kumar and Ando (2010)
Electric arc discharge method	Nanotubes are produced by high-voltage beams (around 100 A) of electrons produced by the electric arc, which bombards the graphite surface	Awasthi et al. (2005)
Laser ablation technique	The substrate is made by embedding nanosized nickel or cobalt particles, or a combination of both as a catalyst on its surface, and is generally heated to approximately 700 °C	Arepalli (2004)

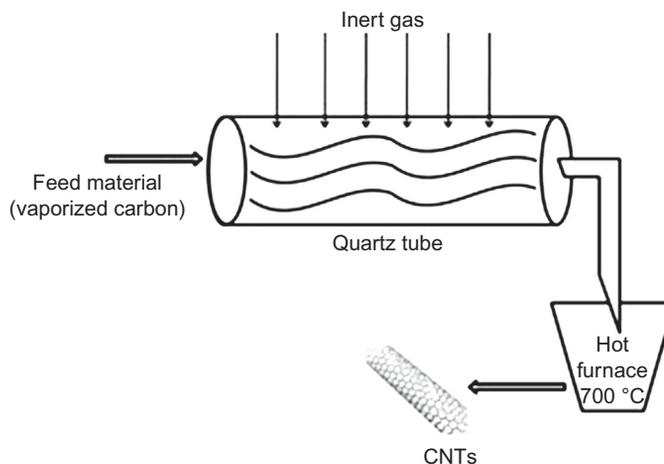
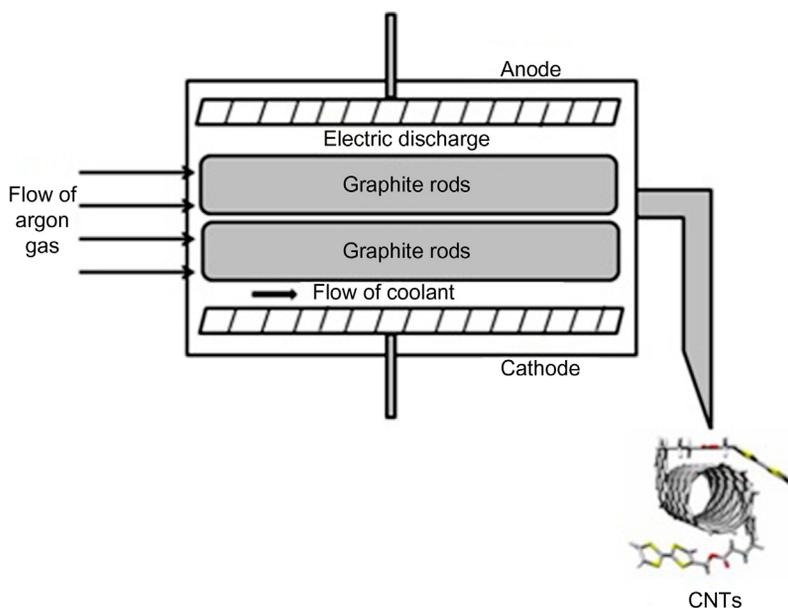
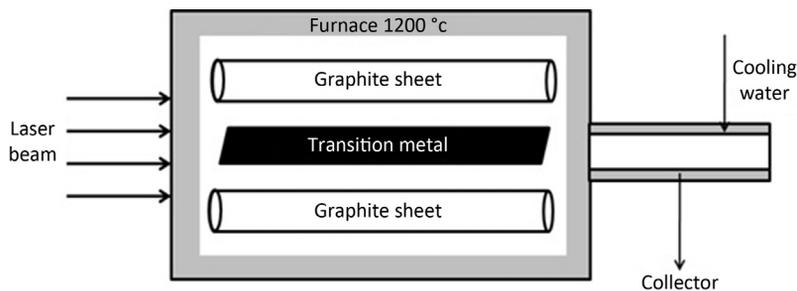


FIGURE 15.2

Chemical vapor deposition technique for the preparation of CNTs.

**FIGURE 15.3**

Electric arc discharge method for the production of CNTs.

**FIGURE 15.4**

Laser ablation technique for the preparation of CNTs.

15.5 FUNCTIONALIZATION OF CNTs

Modification of CNTs by introduction of a drug molecule or ligand onto the walls and/or sides of CNTs is referred to as CNT functionalization (Lay et al., 2011). This functionalization may be utilized for their enhanced biocompatibility, enhanced encapsulation tendency, and multimodal drug delivery and imaging

(Liu et al., 2009). Several studies on the fate of nanotubes in the body have suggested that the functionalized CNTs loaded with drug molecules could easily pass into the cells and further into the cell nucleus, thus attaining targeted drug delivery both at cellular and nuclear levels (Mehra et al., 2015). Functionalization can be of two types: noncovalent (or adsorption) or covalent.

15.5.1 NONCOVALENT FUNCTIONALIZATION

Many small, as well as large, drug molecules can be adsorbed noncovalently onto the surfaces of CNTs. In this manner, CNTs act as nanoreservoirs to adsorb the drug molecules by host–guest interaction. Forces that govern such adsorption are the hydrophobic and π – π stacking interactions between the chains of the adsorbed molecules and the surfaces of CNTs. In the case of lipophilic drug moieties, the hydrophobic forces are the main driving forces for the loading of such drugs into or onto CNTs. The presence of charge on the nanotube surface due to chemical treatment can enable the adsorption of the charged molecules through ionic interactions (Liu et al., 2010; Chen et al., 2002). Noncovalent functionalization of CNTs is particularly attractive as it offers the possibility of attaching chemical modifications without affecting the electronic network of the tubes. Such functionalization can be achieved simply by exposing the CNTs to vapors containing functionalization species that noncovalently bond to the nanotube surface. Using this functionalization process, surfactants, polymers, and biomolecules like DNA, siRNA, proteins, and peptides can be successfully loaded onto the surfaces of CNTs (Figure 15.5).

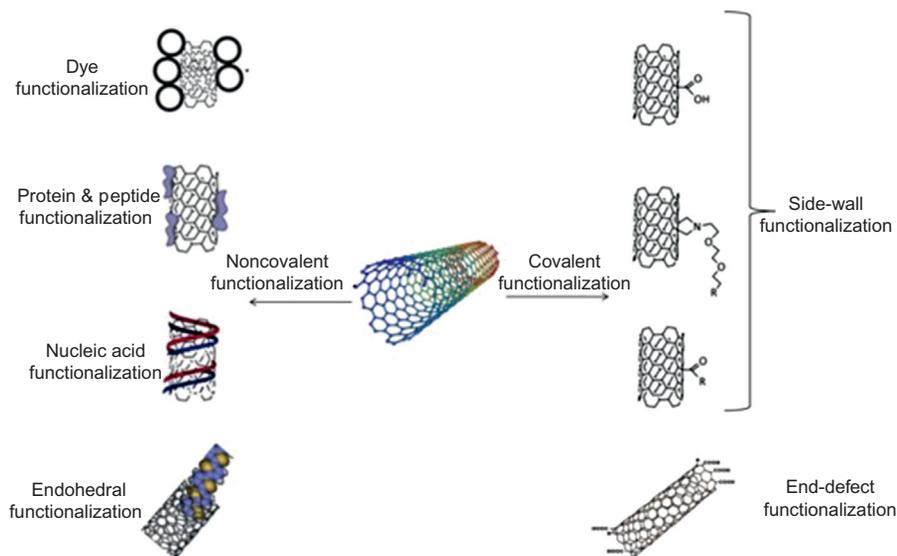


FIGURE 15.5

Different types of functionalization on CNTs.

15.5.2 COVALENT FUNCTIONALIZATION

Covalent functionalization gives the more secure conjunction of drugs or functional groups (Balasubramanian and Burghard, 2005). In order to achieve such functionalization, CNTs can be oxidized using strong acids, resulting in the reduction of their length while generating carboxylic groups, thus increasing their dispersibility in aqueous solutions. Alternatively, addition reactions of hydrophilic groups to the CNTs external walls and tips can also make them soluble in water. To achieve such a type of functionalization with therapeutic molecules like methotrexate, chemical reactions like 1,3-cycloaddition can be employed. Complete control over such chemo- or region-selective additions, however, is somewhat tricky to achieve, as it involves particular groups such as cyclic compounds, halogens, arynes, or carbenes. Moreover, such reactions often require extreme conditions for covalent bonding. Further, characterization of such functionalized nanotubes to determine the precise functionalization location and mode of addition are also very difficult. It may broadly be classified as end-defect functionalization or side-wall functionalization. End-defect functionalization involves oxidation at the “end” tips, whereas covalent binding of surfactants, proteins, and peptides on the surface of CNTs is referred to as side-wall functionalization. Figure 15.5 schematically represents the types of functionalization possible on the CNT surface.

15.6 CHARACTERIZATION OF CNTs

CNTs used in pharmaceutical applications need to be characterized extensively to determine their fundamental properties. The characteristic properties include diametric size, shape, purity, solubility, electromechanical properties, and thermal conductivity (Foldvari and Bagonluri, 2008). Quite a few techniques are being employed for characterizing these nanotubes, each with its pros and cons. The preliminary investigation of the morphology and impurities present in the CNT structure are presented by the use of scanning electron microscopy. Transmission electron microscopy gives elucidation of the structural arrangement of CNT drug composites and provides qualitative information on size, shape, and structure of CNTs. Atomic force microscopy provides a three-dimensional (3D) surface profile desirable for determining the surface topology of CNTs. Other commonly employed techniques include thermogravimetric analysis, infrared spectroscopy, nuclear magnetic resonance (NMR), Raman spectroscopy, H^1 -NMR, and dynamic light scattering.

15.7 CELLULAR TRAFFICKING OF CNTs

Lately, functionalized CNTs have been exploited for the delivery of biomolecules and drugs to desired sites. Their ability to readily cross the plasma membrane for the transport of cargo molecules renders them as interesting nanovehicles.

To achieve a desired response cellular internalization needs to be extensively studied. Scientists across the globe have proposed different pathways for its cellular internalization. This internalization by the cell also depends on the physical properties, surface charge, and chemical functionalization of these CNTs. The other parameters influencing its interactions include degree of dispersion and formation of supramolecular complexes (Lacerda et al., 2006). Two most commonly understood mechanisms are described below.

15.7.1 DIRECT CYTOPLASMIC TRANSLOCATION

Referred to as insertion, passive diffusion, or nanoneedle mechanism, this process is quite instantaneous and CNTs diffuse across the lipid bilayer in a noninvasive manner. The uniqueness of CNTs in terms of high aspect ratio, cylindrical shape, and elongated form favors translocation across the barriers. The two-step process involves accommodation of the CNTs on the lipidic bilayer followed by transmembrane configuration (Lopez et al., 2004).

15.7.2 RECEPTOR-MEDIATED ENDOCYTOSIS

Well-individualized MWCNTs have been shown to cross cell membranes by means of an energy-dependent process employing the use of ATP. Some scientists have also reported that CNTs of approximately 100 nm are able to fit into caveolae and clathrin vesicles, while larger-sized CNTs are taken up by means of the macro-pinocytosis pathway (Mu et al., 2009). Figure 15.6 clearly demonstrates the receptor-mediated endocytosis mechanism for surface-engineered CNTs.

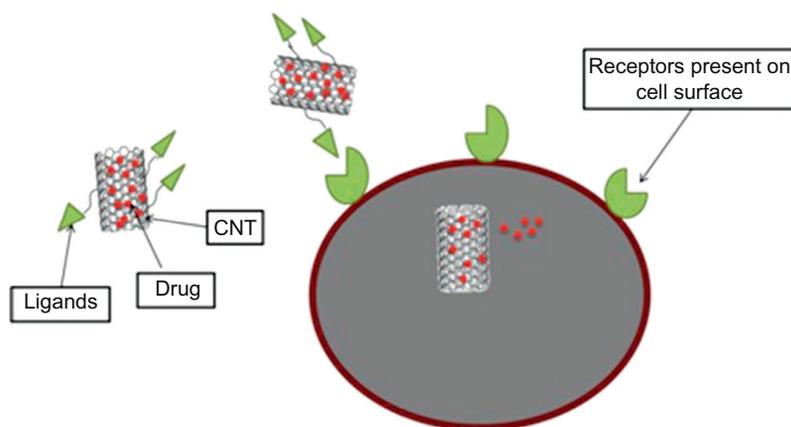


FIGURE 15.6

The receptor-mediated endocytosis mechanism for surface-engineered CNTs.

15.8 APPLICATIONS OF CNTs

CNTs have recently emerged as efficient carriers in the arena of drug delivery. [Table 15.2](#) provides a bird's eye view on the applications of CNTs in the delivery of various therapeutic agents against diseases like cancer, and genetic and infectious disorders. An explicit account of diverse drug-delivery applications of CNTs is enumerated below.

15.8.1 DRUG DELIVERY WITH CNTs

The unique capability of CNTs and their functional counterparts to penetrate into cells makes them interesting vehicles for the delivery of small-molecule drugs. In addition to their ability to carry one or more therapeutic agents, their recognition capacity, optical imaging signals, and targeted delivery have also been exploited for the successful development of these delivery systems. Both hydrophilic and hydrophobic molecules can be bound to CNT by means of amide/ester linkages. In addition, various polymers are also grafted to their surface. Researchers have also reported that PEGylated MWCNTs may be exploited as an efficient drug carrier to overcome multidrug resistance (MDR). These MWCNTs can target and specifically accumulate in MDR tumor cells. Besides, these MDR cells cannot remove intracellular MWCNTs thereby effecting more efficient drug delivery.

15.8.2 TARGETED DELIVERY WITH CNTs

Functionalized nanotubes have been employed on a large scale for targeted delivery of nucleic acids, proteins, antibodies, drugs, and other therapeutic agents to their respective sites of action. The use of CNTs for targeted delivery is primarily accepted in treating various malignant disorders, which include choriocarcinoma, carcinoma of the cervix, breast cancer, prostate cancer, brain gliomas, and testicular tumors ([Thakare et al., 2010](#)). As drug-loaded functionalized CNTs encounter problems in the release of their drug contents, these have been encapsulated in novel membrane microcapsules made up of an alginate–poly-L-lysine–alginate membrane ([Degim et al., 2010](#)). Excellent drug-release profile and enhanced safety and effectiveness, owing to protection from the external harsh environment, have been exploited for their use in targeted delivery. In addition, functionalization of chitosan on their surface enhances cell attachment to the sidewalls of the nanotubes, resulting in the desired targeted release to the cells, and improved drug absorption. Such systems have significant potential for the delivery of drugs, peptides, and nucleic acids.

15.8.2.1 Nanotube-based antibody therapy

Antibody-mediated drug delivery, despite being a popular drug-delivery technique, suffers from a major hiccup of antibody specificity on binding with drug molecules.

Table 15.2 Diverse Biomedical Applications of Carbon Nanotubes (CNTs)

CNTs	Drug Molecule	Inference of Study	Reference
MWCNT–taxol complexes	Taxanes	Sustain release of taxol without an initial burst over 60 days at an average rate of 0.003–0.0073 mg per day	Kim et al. (2015)
Hydroxyapatite–carbon nanotube (CNT) composites	Hydroxyapatite	Exhibited better mechanical properties, excellent hemocompatibility	Mukherjee et al. (2015)
Poly-L-lactic acid matrix MWCNT	Poly-L-lactic acid matrix	Support neuronal growth and differentiation, interface with cells and to mimic the neural environment	Scapin et al. (2014)
Silver MWCNTs	Silver	Potential to increase the target-specific antibacterial activity; retains optimal biocompatibility	Seo et al. (2014)
Hydroxyapatite–magnetite–MWCNT nanocomposite	Hydroxyapatite–magnetite	Nanomaterials as bone-specific systems for controlled drug delivery	Pistone et al. (2014)
DOX/DEX MWCNTs	Doxorubicin (DOX)	DOX/DEX MWCNTs found less hemolytic and more cytotoxic	Lodhi et al. (2013)
Angiopeptide-conjugated PEGylated oxidized MWCNTs	Doxorubicin	Angiopeptide-conjugated PEGylated is a dual-targeting carrier to deliver DOX for brain tumor	Ren et al. (2012)
AmB/mannose MWCNTs	Amphotericin B (Vinoth et al., 2015)	AmB-CNT showed better targeting efficiency to macrophages with reduced toxicity	Pruthi et al. (2012)
Hyaluronate-tethered MWCNTs	Doxorubicin	The tumor-growth-inhibitory effect of HA MWCNTs-DOX showed fivefold anticancer activity	Datir et al. (2012)
DOX-FA-MN MWCNT	Doxorubicin	This showed high drug loading, pH-dependent release	Lu et al. (2011)
CNT-PEI and CNT-pyridinium MWCNTs	siRNA	CNT-PEI and CNT-pyridinium did not show any added value over PEI	Varkouhi et al. (2011)
Azomethine ylide (1,3-dipolar cycloaddition) MWCNTs	Methotrexate (MTX)	Cytotoxic activity of CNT conjugate was strongly dependent on the presence and type of linker	Samori et al. (2009)
Diaminotriethylene glycol MWCNT	10-Hydroxycamptothecin (HCPT)	Enhanced antitumor activity, long circulation and high tumor burden	Wu et al. (2009)
Pluronic F 127 stabilized MWCNTs	Doxorubicin	Enhanced cytotoxicity of DOX-MWCNT complex over free DOX	Ali-Boucetta et al. (2008)

FITC-MWCNTs and oxidized MWCNTs	Amphotericin B	Less toxicity, potential to enter the cell by a spontaneous mechanism	Wu et al. (2005)
F-SWCNTs-COS-GTX-p53	Glilotoxin (GTX)	The F-SWCNTs-COS-GTX-p53 was found to be most effective delivery vehicle with effective apoptosis potential	Bhatnagar et al. (2014)
Sgc8c-Aptamer SWCNTs	Daunorubicin	Dau-aptamer-SWCNT complex was able to selectively target Molt-4 cells compared to nontarget cells	Taghdisi et al. (2010)
EGP SWCNTs-cisplatin-EGF	Cisplatin	PEG-SWCNTs loaded with both cisplatin and EGF, inhibit growth of squamous cell tumors	Bhirde et al. (2010)
Folic acid (FA)-chitosan (CHI)/alginate (ALG) SWCNTs	Doxorubicin	CNTs complex found to be much more effective than free DOX due to targeting based on FA and released of DOX at lysosomal pH	Zhang et al. (2009)
DSPE-PEG 5000-PTX SWCNTs	Paclitaxel (PTX)	Water-soluble SWCNT-PTX formulation is cremophor-free and less toxic	Liu et al. (2008)
PL-PEG-RGD SWCNT and FITC annexin V SWCNTs	Doxorubicin	Enhanced uptake of DOX in case of integrin-positive U87 MG using RGD-based targeting relative to integrin-negative MCF-7 cells	Liu et al. (2007)
PL-PEG SWCNTs	Cisplatin prodrug conjugate	Cytotoxicity of the free platinum (Awasthi et al., 2005) complex increases by >100-fold	Feazell et al. (2007)

MWCNTs, multiwalled carbon nanotubes; SWCNTs, single-walled carbon nanotubes; fCNTs, functionalized carbon nanotubes; FITC, fluorescence isothiocyanate; PEI, polyethyleneimine; PL, phospholipid; PEG, poly(ethylene glycol); RGD, arginine-glycine-aspartic acid; NGR, Asn-Gly-Arg; HCPT, hydroxycamptothecin; PL-PEG-FA, phospholipid polyethylene glycol-folic acid; PEI, polyethleneimine; NIR, near infrared; EGF, epidermal growth factor; siRNA, small interfering ribonucleic acid; GL, glycyrrhizinic acid.

Nanotubes, in this regard, are at a specific advantage as these do not alter this antibody specificity and can deliver the drug at the targeted site (Prakash and Kulamarva, 2007). Monoclonal antibody-functionalized SWCNTs have been employed for tumor targeting and for diagnostic imaging. An important prerequisite is the compatibility of the CNTs with the targeting antibodies as well as nanotubes. Also, the antigenicity of antibodies must remain unaltered after their attachment to the nanotubes and subsequently to their desired site of action. In addition, this approach can be employed for the delivery of both hydrophilic and lipophilic drugs. One of the functionalization techniques, that is, PEGylation, has recently gained immense importance owing to its enhanced biocompatibility, solubility, and reduced toxicity (Bottini et al., 2011). In addition, monoclonal antibodies on SWCNTs functionalized with PEG have the distinct ability to target the CD20 cell surface receptor on B-cells selectively. This antibody-mediated approach has thus been proved to be an ideal alternative in targeting drug molecules to cancer cells.

15.8.2.2 Lymphatic targeting

Lymphatic targeting is essential to protect the body against a range of infectious diseases and malignant disorders. In addition, it helps in targeting drug molecules to the reticuloendothelial system. Over the past several years, various approaches have been attempted for lymphatic targeting of the drug molecules, for example, controlled-release and magnetic microspheres. These systems, however, cause capillary blockade and chemoembolism owing to their large size. Researchers have now shown that lymphatic targeting of drugs with the use of magnetic nanotubes is better accepted and employed for lymphatic targeting. Because of their chemical and mechanical stability, incorporation, and controlled drug release, these CNTs can be chemically modified to achieve the desired targeting effect. In addition, Yang et al. (2008) indicated that the size specificity allows for effective uptake into the lymphatics. CNTs, functionalized with folic acid in conjugation with magnetic nanoparticles, have shown better targeting ability to cancer cells in the lymph nodes. In addition, these MWCNTs can be retained at the target site by means of an externally placed magnetic field.

15.8.2.3 Brain targeting

Brain targeting of drug molecules is hampered by the impermeable blood–brain barrier, which restricts the entry of substances so as to maintain the internal milieu of the brain. In addition, the presence of enzymes in the brain degrades the few neuropharmaceutical agents that manage to cross the impermeable barrier. Conventional drug-delivery systems for that matter, fail to deliver drugs effectively into the brain. Of late, CNTs have been exploited for the brain targeting of drug molecules, primarily because of their inherent ability to cross the impermeable barrier (Yang et al., 2010). In this regard, MWCNTs are quite effective in delivering these agents to the inner environment of brain microglial cells. Recently, Au/CNT hybrid nanomaterials have been exploited for detection of

dopamine and ascorbic acid owing to the biocompatibility of AuNPs and unique electronic properties and ease of surface modification of CNTs (Vinoth et al., 2015). Additionally, magnetic properties bestowed by these nanotubes have been exploited for treating neurodegenerative disorders. In combination with nerve growth factors, these CNTs also enable cells to differentiate into neurons and, thus, provide effective management for neurodegenerative disorders.

15.8.2.4 Ocular drug targeting

The potential of CNTs has also been used for ocular drug delivery. These help in specific and local targeting of drug molecules to the retinal site. Examples of drugs which have been delivered for ocular delivery of drugs include antibiotics, anticholinergics, mydriatics, etc. (Sinha and Yeow, 2005). However, limited research work has been carried out in this area so far, thus calling for further exploration of CNT applications in ocular delivery.

15.8.2.5 Cancer targeting

The rampant spread of cancer in society has posed a great challenge for scientists across the globe. The biggest challenge, therefore, is to treat malignant cells without their “spillover” to neighboring cells. Conventional chemotherapy has the disadvantage of nonspecificity and in more than 99% of cases normal cells are destroyed along with the cancerous tissues. CNTs, in this regard, have been investigated for *in vivo* distribution and highly effective tumor targeting for cancer therapy in mice. Investigations are being done on the biodistribution of radio-labeled SWCNTs in mice by *in vivo* positron emission tomography, *ex vivo* biodistribution, and Raman spectroscopy. It has been found that SWCNTs that are functionalized with phospholipids bearing polyethylene glycol (PEG) are surprisingly stable *in vivo*. CNTs loaded with chemotherapeutic agents have been shown to achieve relatively higher uptake by cancerous tissue without affecting collateral tissues. Consequently, nanotubes may also be beneficial in dosage reduction by localizing dose distribution at the tumor site. This can further be fortified by functionalizing the drug-containing CNTs with an antibody molecule and targeting it to the antigen of cancer cells. Recently, CNTs have been reported to exhibit their own cancer-curing properties as well when exposed to an infrared light source (Zhou et al., 2009). Such heated tubes, especially MWCNTs, when placed at a tumor site, specifically destroy malignant cells and seem to act as a tumoricidal agent.

Attachment of CNTs to folic acid, a tumor marker, has also been used for programmed cell death. This process of cancer treatment is generally referred to as photothermal therapy. Similarly, MWCNTs doped with nitrogen gas have been found to induce thermal ablation causing apoptosis (i.e., programmed death) of cancer cells upon irradiation with an infrared beam (Torti et al., 2007). It can thus be concluded that the antitumor activity of CNTs might be due to heat transduction, which leads to less cellular cytotoxicity (Figure 15.7).

Literature reports also suggest increased tumoricidal activity of MWCNTs when functionalized with DNA and siRNA. SWCNT–siRNA complexes have

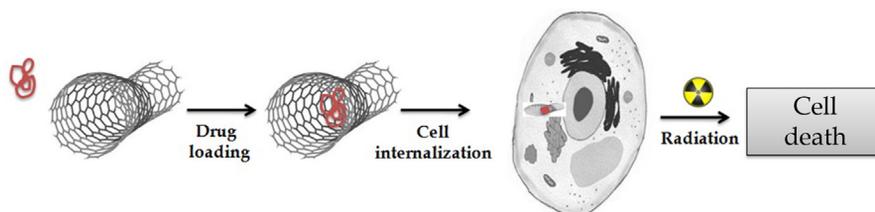


FIGURE 15.7

Hyperthermia as an adjuvant therapy in cancer treatment.

been demonstrated to have effective and prolonged suppression of tumor growth in comparison to earlier available tools for siRNA delivery. It has been observed that functionalized SWCNTs are an effective solution in reducing the progression of human myelogenous leukemia.

Apart from the delivery of pharmaceuticals and/or nucleic acids to malignant cells, recent investigations have proved their importance in radiotherapy for cancer treatment by increasing the rate of oxygen uptake to malignant cells, thereby making the therapy more efficacious. Apart from the CNTs, CNHs are under investigation for exploring their application in chemotherapy. Water-soluble CNHs have also been screened for the delivery of anticancer agents with some promising results (Murakami et al., 2006).

15.8.3 CNTs IN TISSUE AND NERVE REGENERATION

The application of CNTs as reinforcing agents has been mainly attributed to their excellent and unique electrical and mechanical properties (Erik and Tsu-Wei, 2002). CNTs may be the best tissue engineering candidate among numerous other materials of natural or synthetic origin for tissue scaffolds. In addition, they are less dense, highly flexible, and have a very high Young's modulus representing good stiffness. These properties are utilized to make lighter scaffolds with very high strength (Iijima et al., 1996). It has also been shown by various researchers that the material surface-free energies also play an important role in influencing cell adhesion, leading to greater tissue regeneration. Surface functionalization of these CNTs further imparts to them biocompatibility and biodegradability, ideal for their use in the body.

15.8.4 CNTs IN CONTROLLED DRUG DELIVERY

Controlled delivery of drugs and genetic material such as DNA, genes, and/or antibodies has challenged pharmaceutical scientists for years. Of late, CNTs have attracted much attention due to their ability to deliver drug molecules to a specific site in a controlled manner (Luo et al., 2011). Studies carried out by Yang et al. (2009) on amine-functionalized mesoporous silica nanotubes (NH₂-MSNTs), with additional

functionalization by CdS quantum dots (QDs), have been found shown to deliver anti-inflammatory drugs in a controlled manner. Furthermore, carboxyl functionalized MWCNTs (MWCNT-COOH) along with nanohybrid hydrogels have also been employed as controlled drug-delivery vehicles. Such systems show low micropore densities and large mesh sizes with an increase in MWCNT-COOH content. The pH responsiveness conferred by the unique combination upon contact with water produces controlled drug-release profiles. Recently, “smart bio-nanotubes,” a new generation of nanomaterials, have been developed. These trilayered structures are able to regulate the rate of drug release by tailoring the thicknesses of protein lipid and protein coats.

15.8.5 CNTs IN TRANSDERMAL DRUG DELIVERY

Highly efficient electrophoretic and iontophoretic pumping has been accomplished through functionalized CNT membrane due to the high charge density, dramatically quick flow of drugs through the CNT cores, and small pore dimensions (Degim et al., 2010). CNT membranes are usually integrated with the drug molecule to obtain a switchable transdermal delivery device. These highly energy-efficient programmable devices offer minimal skin irritation without any skin barrier disruption. Utilizing this concept, MWCNTs for transdermal delivery of nicotine have been fabricated through the CVD approach.

15.8.6 CNTs IN VACCINE DELIVERY

Improper absorption, antigen-induced hypersensitivity, anaphylactic reactions, and hypersensitivity due to vaccine adjuvant are some of the serious drawbacks associated with vaccine delivery. Among the new approaches suggested for delivery of vaccines, CNTs have also been tried for vaccine delivery, where these help in improving vaccine action due to their adjuvant action (Bianco et al., 2005). In addition, CNTs, when conjugated with antigenic peptides, can act as a new system for safe and effective delivery of synthetic vaccines.

15.8.7 CNTs IN GENE DELIVERY

Gene delivery or gene therapy is an approach in which a defective gene, which is the cause of some chronic hereditary disease, is corrected by introducing a DNA molecule into the cell nucleus. CNTs have been quite widely exploited for improving gene delivery owing to their capability of replacement of damaged/missing genes, and transportation of DNA into cells (Bianco, 2004). Attempts have been made for treating gene defects by transporting grafted genes with the help of nanotubes (Pan et al., 2009). Ammonium-functionalized CNTs have been demonstrated to enhance gene therapy in comparison with DNA alone (Prato et al., 2008). Research has also indicated that siRNA and SWCNT 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. It has also been observed that when single-stranded DNA is bound to SWCNTs, DNA probes are protected from enzymatic cleavage and interference from nucleic-acid-binding proteins. Studies have shown that an SWCNT-modified DNA probe can target a specific mRNA inside living cells, thus causing increased self-delivery capability and intracellular biostability, compared with free DNA probes. In contrast to the traditional methods available for gene delivery, CNTs provide distinct advantages like enhanced capability to penetrate into the cells owing to their needle-like shape, hydrophobic surface, and their electrical properties. In addition, their capacity to achieve controlled release, influence on transitions of DNA/siRNA, and ability to monitor the therapeutic effects provides this conjugate with great potential for applications in the field of genetic engineering (Cheung et al., 2010).

15.8.8 TREATMENT OF INFECTIOUS DISEASES

Infectious diseases, such as tuberculosis, leishmaniasis, severe acute respiratory syndrome, and flu (swine, bird, and avian), have always been a critical public health issue with global concerns. These infectious agents show a high level of resistance against numerous antiviral and antibacterial drugs. Recently, functionalized CNTs have shown promising outcomes in the treatment of these diseases owing to their ability to easily conjugate drugs like amphotericin B (Vinoth et al., 2015), dapson, etc. (Mehra et al., 2015). Conjugation of AmB to these *f*-CNTs has been shown to have reduced toxicity and enhanced antimycotic efficiency. In addition, their targeted deliveries to macrophage cells have also been indicated by researchers. Furthermore, CNTs themselves might possess antimicrobial activity as bacteria, such as *Escherichia coli* may be absorbed onto the surface of the CNTs. The CNT might induce oxidation of the intercellular oxidant glutathione, resulting in increased oxidative stress on the bacterial cells and thus eventual cell death.

15.8.9 CNTs AS ANTIOXIDANTS

The potential role of CNTs as antioxidants is still an emerging area of research. CNTs, and in particular carboxylated SWCNTs, have been reported as antioxidants in nature and may possess useful biomedical applications in preventing chronic ailments, aging, and in food preservation as well. Such potential calls for more investigations of different forms of CNTs to develop their precious effect as free radical scavengers.

15.8.10 CNTs IN ANTITUMOR IMMUNOTHERAPY

Antitumor immunotherapy consists of stimulating the patient's immune system to act against malignant tumor cells. Studies have shown that CNTs can be effectively used as carriers for a cancer vaccine or a therapeutic antibody as a drug.

15.9 ROLE OF CNTs IN DIAGNOSTICS

Biosensors in the field of diagnostics have made an enormous impact on basic scientific research and healthcare. These detect chemical, physical or biological quantity and transduce it in the form of a signal. Fast electron transfer rate, wide potential window, flexible surface chemistry, and good biocompatibility of CNTs give them excellent property for their use in biosensors. For example, CNTs have been effectively coupled with glucose-oxidase biosensors for blood sugar control in diabetic patients. Various mechanisms and properties of CNTs have been utilized in construction of these molecular machineries. Figure 15.8 elucidates different sensing mechanisms of CNT-based biosensors.

Lately, research has revealed that CNTs can also generate QDs, or may behave like QDs. Basically, these are semiconductor nanocrystals with nanosize range. They are diode substances capable of emitting light of various colors. Due to their light-emitting property, they have potential applications in imaging various body parts (Lim et al., 2003). CNTs are capable of mimicking these light-emitting nanoparticles. Cisplatin and epidermal growth factors (EGFs) have been attached to SWCNTs, specifically to target squamous cell cancer (Bhirde et al., 2009). Also, SWCNT-QD–EGF bioconjugates have been shown to possess better cell internalization properties compared to plain CNTs.

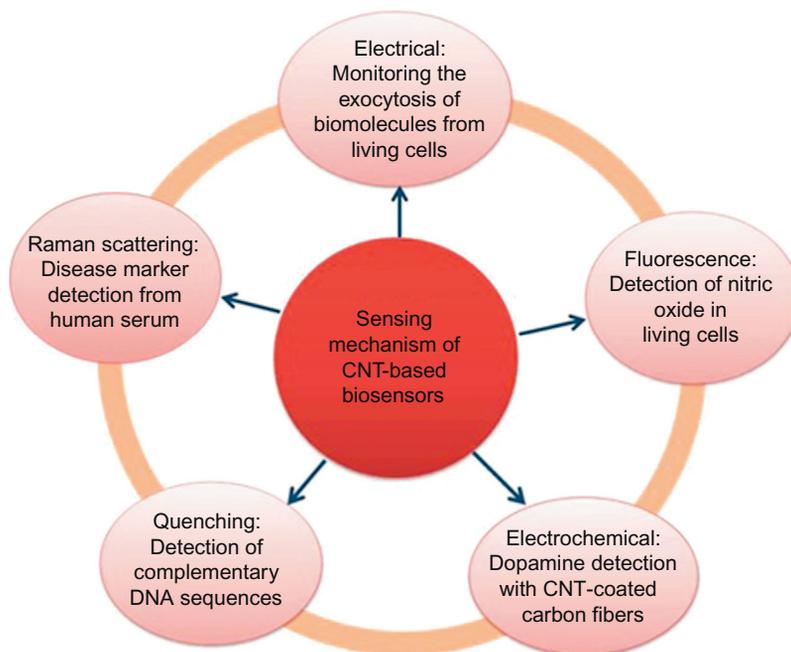


FIGURE 15.8

Sensing mechanism of CNT-based biosensors.

15.10 TOXICITY CONSIDERATION OF CNTs

In comparison to bulk materials, nanotubes have unique properties including an exponentially high aspect ratio that leads to toxicity. Reduction of size consequently makes the nanomaterial surface more reactive on itself (i.e., aggregation) and its surrounding environment (i.e., biological components). Accumulation of nanosized material may also cause increased uptake into tissues, thus influencing the critical biological function of cells. Besides, metal traces available in nanotubes are also another causative agent for toxicity (Beg et al., 2011).

Exposure to CNTs causes multifunctional defects in various body organs and organ systems. Early systemic exposure of MWCNTs causes temporary organ injury, particularly to lungs and heart, attributable largely to their delayed clearance from the body due to strong agglomeration. Persistent accumulation of agglomerated MWCNTs in the lungs initiates inflammatory events (Takagi et al., 2008). Similarly, SWCNTs have been found to affect the CNS due to a high degree of accumulation in the spinal cord or dorsal root ganglia. Furthermore, SWCNTs significantly decrease the DNA content when the cells were exposed to disperse SWCNT bundles due to their accumulation in the nuclear material. Also, exposure to SWCNTs has resulted in ultrastructural and morphological changes in cultured skin cells (Yacobi et al., 2007). Comparative evaluation of toxicity studies has revealed that MWCNTs are more toxic as compared to SWCNTs, and induce massive loss of cell viability through programmed cell death. This toxicity of MWCNTs is primarily due to oxidative stress, cellular toxicity due to formation of free radicals, accumulation of peroxidative products, antioxidant depletion, and loss of cell viability as depicted in Figure 15.9. CNTs also exhibit primary genotoxicity due to the direct interaction of particles with cells, or secondary genotoxicity due to the generation of an excess of ROS. MWCNTs have genotoxicity effects due to DNA damage through ROS, leading to increased mutation frequencies (Sargent et al., 2010).

Recently, researchers have warranted the elucidation of an alternative, nonoxidative stress-mediated pathway of cellular damage. Physical interference of CNT with cellular and extracellular constituents is also one of the potentially relevant mechanisms of damage. This physical interference may cause alterations of vital cellular processes, leading to various degrees of cellular injury, and in some cases even to cell death.

Despite the aforementioned literature reports, the present knowledge of CNT toxicity is inadequate and contradictory, thus still requiring more extensive toxicity, safety, and efficacy studies on animal models including humans. Also, effects of CNT aggregation, size, length, functionalization, metal impurities, and polymers on safety require more extensive research. Functionalization of SWCNTs and MWCNTs and its effects on aggregation and consequently genotoxicity also need to be evaluated (Uo et al., 2011).

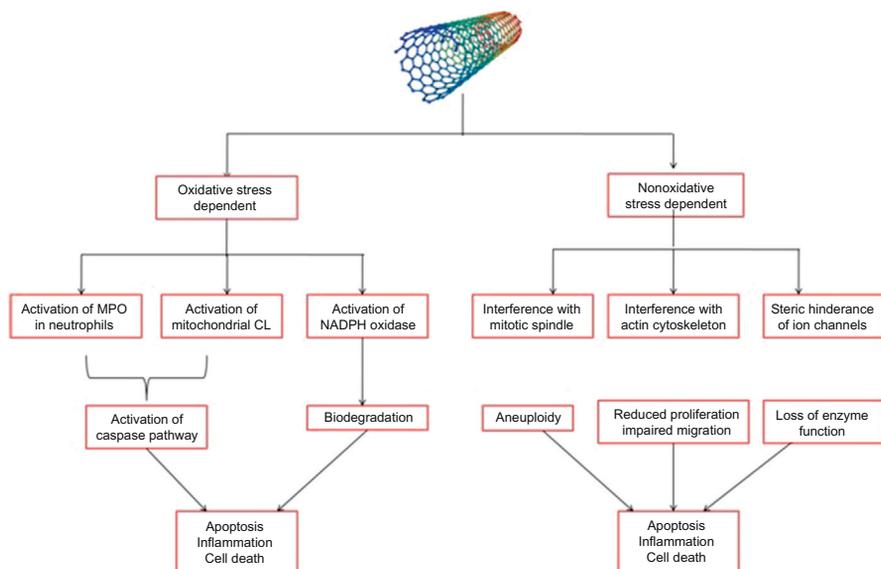


FIGURE 15.9

Schematic figure depicting pathways of cellular toxicity induced by CNTs.

15.11 BIODISTRIBUTION OF CNTs

The biodistribution of CNTs is not significantly influenced by the route of administration. Researchers have shown that these CNTs distribute quickly throughout the whole body, with preferential organs being the stomach, kidneys, and bone. Approximately 94% of CNTs are shown to excrete into the urine and 6% in the feces. Recently, analyses of urine samples by workers have revealed that both SWCNT and MWCNT are excreted as intact nanotubes. The preferred sites of accumulation were found to be the stomach, kidneys, or bones, but no tissue damage or distress was reported. Functionalizations of these SWCNTs have also been shown to prolong the SWCNT blood circulation, reduced uptake in the reticuloendothelial system, and complete clearance from major organs (Shvedova et al., 2009).

15.12 REGULATORY CONSIDERATIONS

Awareness of nanotechnology has dramatically risen in recent years among lawmakers, regulators, and environmental activists. Accordingly, 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. As per the reports of the US Environmental Protection Agency (Arepalli, 2004), CNTs require major regulatory concern over toxicity as well as environmental safety (Lewinski, 2005). France is in the process of publishing a series of technical guidance documents related to nanotechnologies including CNTs as FR1, FR2, and FR3. Similarly, the UK Government has committed toward EHS research, primarily with new studies on safety issues of specific nanomaterials, in particular nanosilver and CNTs with the United States as UK2, UK3, UK4, and OECD1. Of late, the National Environmental Policy Act (NEPA) of United States has issued certain regulations for CNTs and other nanomaterials. The primary considerations for regulating these nanomaterials include product quality assessment (i.e., quality control and manufacturing) and product safety assessment (i.e., biodistribution, clearance, metabolism, and toxicity).

15.13 CONCLUSIONS

CNTs have been proposed and explored as multipurpose innovative carriers for drug delivery and diagnostic applications. As a consequence, in a very short time span, CNTs have drawn the attention of nanotechnologists, from industry as well as academia. In the last two decades, remarkable work has been carried out on the use of CNTs for biomedical applications. Functionalization of CNTs has further opened new perspectives in the application of CNTs in drug delivery. Attachment of organic moiety to nanosized tubes has facilitated their use for diagnostic as well as targeting purposes, especially in cancer therapy and infectious disease treatment.

However, despite their promising role in nanomedicine, CNTs still require extensive research investigations to guarantee their safety profile in drug delivery. Toxicity studies, therefore, are critical to establish the full *in vivo* potential of CNTs for drug delivery before their real application and marketing. Physiological, physicochemical, and molecular processes need to be considered for understanding of clinical and preclinical toxicity of CNTs. Regardless of knowledge gained in recent years on nanotoxicology; scientists have not yet been able to precisely forecast the behavior and biokinetics of CNTs. Furthermore, before the pharmaceutical commercialization of CNTs, strict regulations are mandatory taking into their ambit the environmental, health and safety issues. However, as alarmingly high numbers of reports are piling up, it can be rationally anticipated that CNTs have a golden future in drug delivery.

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