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Two Faces of Carbon Nanotube: Toxicities and Pharmaceutical Applications

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ABSTRACT: In the field of nanotechnology, carbon nanotube (CNT) is gaining importance for the delivery of therapeutic agents and diagnosis of diseases. CNT is emerging as an efficient nanocarrier system with cylindrical nanostructure. Due to its nanoscale dimensions, CNTs have a high cell-penetration quality that allows its use in site-specific targeting. Another aspect of the utilization of CNT lies in its hollow structure through which an active moiety can be delivered in a controlled manner via CNTs' nano channels. Despite these positive aspects of CNT, scientists are still working to improve its biocompatibility and solubility and eliminating toxicity in vivo, which are creating problems with the use of CNTs. Therefore, functionalization becomes an important aspect to be studied because it decreases the toxicity of CNTs and make them nonimmunogenic. In this review, different functionalization techniques of CNTs and their biomedical applications—in particular for cancer therapy to date—are reviewed in detail to present the potential of this nanovector.

Key Words: carbon nanotubes, single-walled carbon nanotube, multiwalled carbon nanotube, functionalization, arc discharge technique, laser ablation technique, chemical vapor deposition technique, cancer targeting, biosensing

I. INTRODUCTION

Bionanotechnology acts as an interface between nanoscale materials and biological systems. The main objective is the application of nano-sized carriers in the biological systems for the purpose of diagnosis and treatment of diseases. Carbon nanotube (CNT), a well-ordered hexagonally arranged nanostructure product, is a carbon allotrope and made up of graphite. The length:diametater ratio is more than 10^6. Its diameter is in the nanometer range whereas its length ranges from nanometers to several millimeters.[1,2] Focusing on its chemistry, which is quite similar to graphite, carbons in CNTs possess sp^2 bonding that is linked to another 3 neighboring atoms; therefore, it can be called *graphite sheet* (also known as *graphene*).[3] Carbon–carbon sp^2 bonding provides excellent mechanical strength to CNT because sp^2 bonding is stronger than sp and sp^3 bonding. For instance, CNTs can have Young (elastic) modulus—the measure of stiffness of an elastic material and a quantity used to characterize materials—more than 1 TPa and a tensile strength of 63 GPa.[4–6] CNTs are classified into 2 categories: single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs). This classification is broadly discussed in Table 1. Re-

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cently, CNTs have become preferred for use in biomedical applications because of their unique structural, dimensional, electrical, mechanical, and optical properties.[7,8] A high length:diameter ratio renders CNTs with a large surface area, due to which many biological entities can be attached to its surface, and makes them best for medicinal applications. CNTs can also carry electric current up to 10^4 times greater than normal metal, depending on the chiral vector, the vector perpendicular to the direction in which the graphite sheet is rolled to form a nanotube cylinder. Therefore, CNTs can be metallic or semiconducting. Electrical properties of CNTs basically rely upon the peculiar electronic structure of graphite.[9,10] It has extremely low electrical resistance. The cause of resistance is obstruction of electrons within the structure, when electrons collide with defects (impure atoms or a defect in the crystal) in the structure. Because of the small size of CNTs, electrons do not get many opportunities for collisions. This special feature provides CNT the ability of biosensing. Various techniques have been discussed for the production of CNTs, for example, the arc discharge technique,[11] the laser ablation technique,[12] and the chemical vapor deposition technique,[13] which are represented as flowcharts in Figs. 1, 2, and 3, respectively. The differences and characteristics of these techniques are summarized in Table 2. During synthesis, some graphite soot is formed because of vaporization and is deposited on the surface of CNTs, which acts as impurity for the biological systems. Another source of impurities are metal catalyst residues (mostly first-row transition metals such as nickel and cobalt), which are used during synthesis, and smaller fullerenes remain on the CNT's surface. To remove these toxic materials/impurities, purification is carried out.[14] Besides impurity, another problem is CNT's solubility, which is of major concern with respect to biocompatibility and biodegradability.[15]

II. BIODISTRIBUTION OF CARBON NANOTUBES

The biodistribution and pharmacokinetics of CNTs are based chiefly on their physicochemical features such as size, shape, aggregation, chemical composition, surface

TABLE 1. List of Differences between Single-walled Nanotubes and Multiwalled Nanotubes

Basis of Differences	Single-walled Nanotubes	Multiwalled Nanotubes
Diameter (nm)	0.5–1.5	>100
Specific surface area (m^2/g)	300–600	40–300
Graphene layer	Single grapheme sheet is rolled	Multiple sheets are rolled
Characterization	Evaluation is easy	Has a more complex structure
Flexibility	Can be twisted easily	Cannot be twisted easily
Accumulation in body	Less accumulation	More accumulation
Purity	Impure	Pure
Synthesis	Synthesized with the use of metal catalyst and graphite	Synthesized with pure graphite

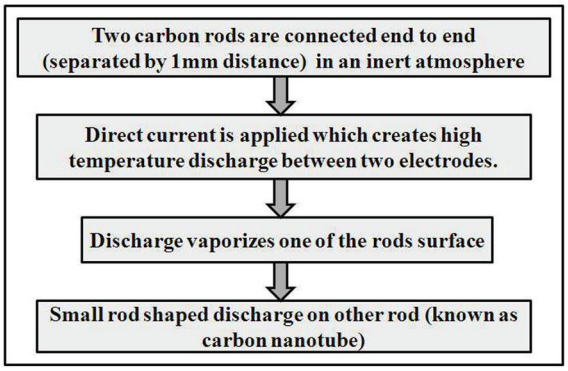

Figure 1. Flowchart depicting the arc discharge method for carbon nanotube production.

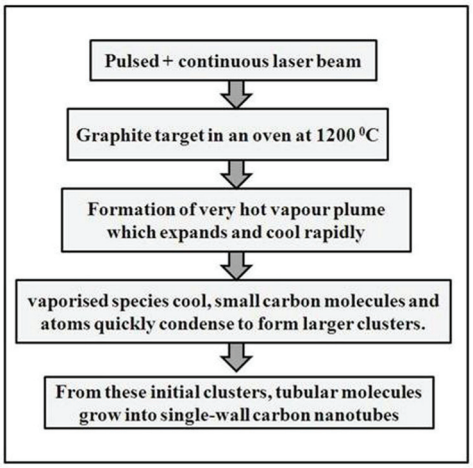

Figure 2. Representation of the laser ablation method for carbon nanotube production.

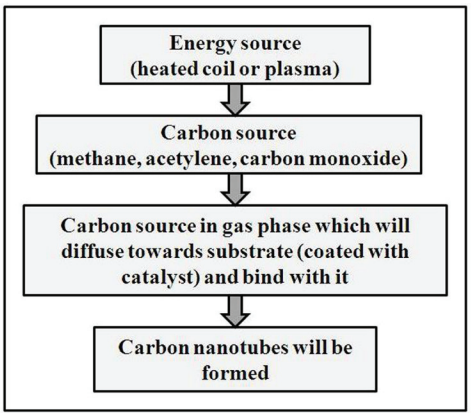

Figure 3. Flowchart depicting the chemical vapor deposition method for carbon nanotube production.

TABLE 2. Critical Differences Found in the Different Methods of Preparation of Carbon Nanotubes

	Arc Discharge Method	Chemical Vapor Deposition Method	Laser Ablation Method
Yield (%)	30–90	20–100	≤70
SWNT	Short tubes with a diameter of 0.6–1.4 nm	Long tubes with a diameter of 0.6–4 nm	Long bundles of tube with individual diameters of 1–2 nm
MWNT	Short tubes with an inner diameter of 1–3 nm and an outer diameter approximately 10 nm	Long tubes with a diameter of 10–240 nm	Synthesis is possible but because of its cost it is used less often
Defects	Needs a lot of purification	Often is riddled with defects	Fewer defects but technique is expensive

MWNT, multiwalled nanotube; SWNT, single-walled nanotube.

functionalization, and solubility.[16,17] The liver, spleen, and kidney are the major organs responsible for metabolism of nanoparticles, if administered via an intravenous (IV) route.[18] Most studies are performed using water-soluble CNTs. Wang et al[19] studied the biodistribution of CNTs by administering them by different routes such as intraperitoneal (IP), subcutaneous (SC), oral, and IV. They have employed 125-iodine–labeled, multiple hydroxylated SWNT (125I-SWNT-OH), functionalized by oxidation of the nanotubes, and radiotraced their distribution in mice. This study testified that CNT biodistribution was not significantly affected by the administration route and that 125I-SWNT-OH distributes quickly throughout the whole body. The stomach, kidneys, and bones were the preferred organs for accumulation. From the safety point of view, 94% of nanotubes were excreted into the urine and 6% in the feces. No tissue damage or distress was reported in the study.[19]

Many studies were performed using functionalized CNTs with different surface modifications. In one research study, the effect on biodistribution and blood circulation half-lives were studied with CNTs functionalized with the chelating molecule diethylenetriaminepentaacetic (DTPA) acid (using 100% and 60% surface functionalization with DTPA; the remaining 40% were amino functions) and radiolabeled with 111-indium ([111In] DTPA-CNT). The biodistribution profiles showed an affinity for kidneys, muscle, skin, bone, and blood 30 min after administration. CNTs were found to be cleared from all tissues, and CNTs with 100% DTPA were found to be excreted via the renal route into the bladder and urine after IV administration.[20] Liu et al[21] performed in vivo distribution of CNTs in mice. They carried out polyethylene glycol functionalization (PEGylation) of SWNTs. Long blood circulation times and low uptake by the reticuloendothelial system (RES) was exhibited by efficiently PEGylated SWNTs. When SWNTs were coated with polyethylene glycol (PEG) chains linked to an arginine–glycine–aspartic acid (RGD) peptide, efficient targeting of integrin positive tumor was ob-

served in mice.[21] Functionalization also modified the interaction of CNTs with cells by conjugating a different functional group to it; that is, paclitaxel when conjugated with SWNTs seems to remain confined more in the intestines and liver, whereas its conjugation with PEG restrict its localization, often in the lung.[22] Similar to paclitaxel, rituximab when conjugated with CNTs was found in the liver compared with when rituximab was used alone. Conclusively, the concentration of CNTs never reached zero with IV or intraperitoneal injection, even after chronic studies.[23] From a drug delivery point of view, where constant drug concentration in the blood is required, functionalized CNTs (f-CNTs) showed fast clearance. More research needs to be carried out that focuses mainly on the biodistribution pattern of CNTs in the body and their clearance.[20,24,25]

III. TOXICITIES ASSOCIATED WITH CARBON NANOTUBES

Apart from the view of CNT's incomparable features and their application in biological/therapeutic systems, one more aspect comes into picture: CNT's negative impact on human health and the environment,[26,27] as shown in Fig. 4. Harmful effects of CNTs arise from structural features such as a large surface area and intrinsic toxicity of the surface. The nano size of CNTs poses significant health problems such as pulmonary toxicity, genotoxicity, and cytotoxicity/cellular toxicity.[28]

III.A. Pulmonary Toxicity

CNTs can easily enter the respiratory tract via inhalation.[29] Distribution in various body organs of CNTs after inhalation is shown in Fig. 5. Several studies have been carried out to evaluate the health risk of CNTs, especially in the lungs[30–34] (Table 3). Donaldson

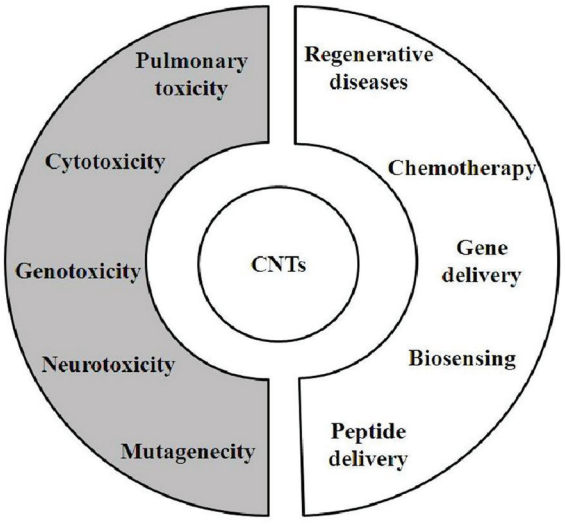

Figure 4. Chart showing the positive and negative aspects of carbon nanotubes (CNTs).

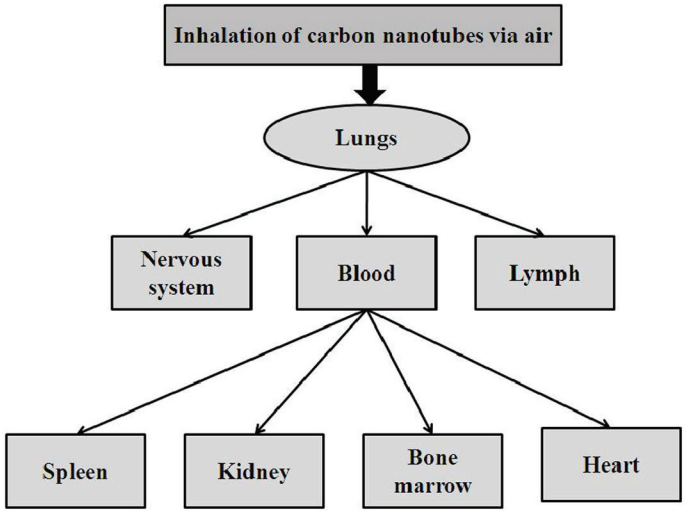

Figure 5. Schematic representation of distribution of carbon nanotubes in body organs after inhalation.

et al[35] demonstrated that the structural characteristics of CNTs, such as the fiber shape, length, and the aggregation status, affect their local deposition in the lungs. Upon exposure to CNTs, the immunologic response also is influenced.[35] Warheit[36] carried out a study on SWNT toxicity after administering them intratracheally in rats. He observed lung granuloma formation as well as 15% mortality in rats after exposure of SWNTs (0.5 mg/kg intratracheally) due to increased blockage of airways. Pulmonary exposure

TABLE 3. Compilation of Work Concerning the Pulmonary Toxicity of Carbon Nanotubes

Purpose of Study	Results	Reference
To analyze the SWNT's pulmonary toxicity comprising different amounts and kinds of residual catalytic metals on B6C3F1 mice	Interstitial granulomas and pulmonary injuries	30
To investigate the effect of structural properties on the toxicity of MWNTs on Wistar rats	Acute pulmonary toxicity	31
To explore the pulmonary inflammatory reactions to aspired SWNTs on C57BL/6 mice	Induced acute inflammation and profibrotic responses	32
To observe toxicity of SWNTs on aquatic animals (rainbow trout)	Respiratory toxicity, enlarged mucocytes on the gills, elevated ventilation rates	33
To explore the potential toxicity of MWNTs on humans using Sprague-Dawley rats	Caused prominent protein exudation and granulomas on the peritoneal side of the diaphragm, produced inflammatory and fibrotic reactions	34

MWNTs, multiwalled nanotubes; SWNTs, single-walled nanotubes.

to SWNTs in rats produced multifocal mononuclear granulomas.[36] Alveolar macrophage activation and severe pulmonary granuloma formation was observed by Chou et al[37] after intratracheal instillation of SWNTs (0.5 mg) into male imprinting control region mice (8 weeks old). Stoker et al[38] evaluated the risks of CNTs on the human respiratory system using a co-culture of normal human fibroblasts and normal bronchial epithelial cells. Increased production of nitrous oxide and reduced viability of cells was observed upon incubation of different concentrations of aqueous solutions of SWNTs (length:diameter ratio is 500 nm:10 nm).[38] Constant exposure to CNTs via inhalation is a serious health hazard, as explained by Lam and coworkers.[30] They explored the pulmonary toxicity of SWNTs in mice by suspending pristine SWNTs in heat-inactivated mouse serum. After a single intratracheal instillation of SWNT dispersion, histopathologic studies of lungs at 7 and 90 days showed that the epithelial granulomas and interstitial inflammation at day 7 developed to peribronchial inflammation and necrosis at day 90.[30] Another research group explained the dose dependence and time course of pulmonary responses upon exposure to CNTs. They exposed mice to pharyngeal aspiration of purified pristine SWNTs, which caused acute inflammation, progressive fibrosis, and formation of granulomas. In addition, an increase in protein levels was confirmed, namely lactate dehydrogenase and γ-glutamyl transferase activities in bronchioalveolar lavage fluid, and the effect on normal pulmonary function was unrelenting, whereas bacterial clearance was diminished.[39] A persistent accumulation of CNTs aggregates in lung was reported in mice after intratracheal or pharyngeal instillation of an SWNT suspension, which was followed by the rapid formation of pulmonary granulomatous and fibrous tissues at the site and lead to cardiovascular toxicity.[40]

III.B. Neurotoxicity

Some scientists have reported neurotoxicity of CNTs on an in vitro model of PC12 cells (a common model for neurotoxicity study). Wang et al[41] investigated the effect of 2 types of commercially available SWNTs on PC12 cells and found reduced mitochondrial membrane potential with enhanced levels of lipid peroxide. Findings concluded that SWNTs give rise to oxidative stress in the nervous system in vivo and may stimulate the occurrence of neurodegenerative disorders by causing cell injuries.[41] Likewise, another group has investigated and compared the concentration-dependent cytotoxicity of SWNTs and PEGylated SWNTs in neuronal PC12 cells at the biochemical, cellular, and gene expression levels. The evaluation parameter was generation of reactive oxygen species (ROS). The results exposed that PEGylated SWNTs were less cytotoxic than uncoated SWNTs, and the authors further concluded that surface functionalization of SWNTs decreases ROS-mediated toxicologic response in vitro.[42]

III.C. Cytotoxicity/Cellular Toxicity

The hydrophobic surfaces of CNTs cause them to form agglomerates and lead to cytotoxicity. Wick et al[43] performed an experiment to observe the CNT's cytotoxicity on

human MSTO-211H cells by using CNTs at different degrees of agglomeration. They compared CNT agglomerates formed by the conventional purification technique, well-dispersed CNT bundles (in noncytotoxic polyoxyethylene sorbitan monooleate) with asbestos as reference. Results concluded that CNT agglomerates were more toxic than well-dispersed CNT bundles and they induce cytotoxicity equivalent to asbestos at a similar concentration.[43] Thus, one can interpret the effect of agglomeration, which can be a cause of toxicity in biological systems. Regarding skin irritation of CNTs, Huczko and Lange[44] conducted 2 routine dermatological tests and concluded that no special precautions need to be taken during the handling of CNTs.[44] In another study, CNTs were found to be a potential dermatological hazard. The researchers incubated purified MWNTs with human epidermal keratinocyte (HEK) cells for up to 48 hours and found that CNTs were localized in the cells with the production of proinflammatory cytokine (interleukin 8) and decreased cell viability in a time- and dose-dependent manner.[45] Likewise, Jia et al[46] explored the effect of different carbonaceous nanomaterials on the cytotoxicity of alveolar macrophages. They exposed alveolar macrophages to SWNTs, MWNTs, and C60 fullerenes for 6 hours. The study showed that SWNTs displayed the most cytotoxic response, even if both SWNTs and MWNTs demonstrated decreased cell viability and impaired phagocytic function.[46] Murr et al[47] reported asbestos-like toxicity in RAW-264.7 cells upon in vitro exposure to SWNTs or MWNTs using an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Raja et al[48] compared the impact of SWNTs and activated carbon alone in rat aortic smooth muscle cells (SMCs) over a 3.5-day time course. They observed that SWNTs were found to be more inhibitory to SMC growth than activated carbon, which indicates its high cytotoxicity.[48] Tamura et al[49] carried out a concise exploration of the cytotoxic effect of purified CNTs on neutrophils isolated from human blood. After contact with the cells for 1 h, a significant increase in superoxide anion and tumor necrosis factor-α production compared with controls was observed; at the same time, cell viability was noticeably diminished.[49] Moreover, SWNTs declined HEK293 cells by inducing apoptosis and decreasing cellular adhesion ability, as observed by Cui et al.[50] They cultured HEK293 cells in media containing different concentrations of SWNTs, ranging from 0.78 to 200 mg/ml. Adhesion ability and protein secretion was tested, which demonstrated cytotoxicity.[50]

III.D. Genotoxicity

The studies cited earlier raise a major concern for carrying out studies on genotoxicity of CNTs. Still, in vivo and in vitro studies for the genotoxic potential of CNTs are limited. Researchers had found some evidence for the genotoxicity of CNTs, which is summarized in Table 4. Kisin et al[51] observed genotoxic potential of purified SWNTs by 3 different tests: the comet assay and micronucleus (MN) test in a lung fibroblast (V79) cell line, and the Salmonella gene mutation assay in YG1024/YG1029 strains. Analysis showed DNA damage in terms of strand breaks but did not generate significant micronucleus induction in Chinese hamster lung fibroblast cells. The results clearly pointed out that SWNT-exposed V79 cells showed a considerable time- and concen-

TABLE 4. Summary of Evidence of Genotoxicity of Carbon Nanotubes

Study Purpose	Results	Conclusions	Reference
To examine the genotoxic effects of purified SWNTs (0.23wt.% Fe) using 3 different test systems: the comet assay, the micronucleus test in a lung fibroblast (V79) cell line, and the Salmonella gene mutation assay in YG1024/ YG1029 strains	Dose concentration-dependent increase in the frequency of DNA damage (mainly strand breaks)	SWNT-exposed V79 cells indicated a significant time-and concentration-dependent loss of viability	51
To assess the genotoxicity and mutagenicity of SWNTs (2% iron) and C(60) fullerenes in comparison with carbon black in the FE1-MetaMT mouse lung epithelial cell line	Oxidation of purines detected by significant generation of formamidopyrimidine-DNA glycosylase–sensitive sites	SWNT and C(60) are less genotoxic in vitro than carbon black particles	52
To compare the toxicity of different metal oxide particles to that of carbon nanoparticles and MWNTs using human lung epithelial cell line A549	The carbon nanotubes showed cytotoxic effects and caused DNA damage in the lowest dose tested	CNTs were found to be cytotoxic	55
To explore the effect of MWNTs in mouse embryonic stem cells	Significant DNA damage and enhanced mutagenic frequency	CNTs were found to be cytotoxic	129

CNTs, carbon nanotubes; MWNTs, multiwalled nanotubes; SWNTs, single-walled nanotubes.

tration-dependent loss of viability. This recommends that SWNT-induced loss of cells might obstruct accurate evaluation of genotoxicity responses. In addition, the negative response in the MN assay may be due to a low degree of SWNT uptake by V79 cells.[51] Likewise, Jacobsen et al[52] assessed genotoxicity and mutagenicity of SWNTs in the FE1-MetaMT mouse lung epithelial cell line. SWNT exposure resulted in oxidation of purines, which was identified by the significant generation of formamidopyrimidine DNA glycosylase-sensitive sites. After treatment with SWNTs, 630 lesions per diploid cell were observed.[52] Vinzents et al[53] have found oxidation of the DNA base by CNTs compared with environmental factors. In addition, damage to DNA and increased mutation frequency was observed by Zhu et al[54] in mouse embryonic stem cells on exposure to MWNTs. Moreover, Karlsson et al[55] displayed a significant enhancement in DNA

damage by MWNTs in A549 type II epithelial cells. Takagi et al[56] observed intraperitoneally administered MWNT-induced mesothelioma in p53 heterozygous (+/−) mice. The p53(+/−) mouse is recommended as a good model to use to evaluate carcinogenicity. Still, various studies are ongoing to evaluate the genotoxicity potential of CNTs in humans.[56]

IV. FUNCTIONALIZATION OF CARBON NANOTUBES: MAKING CARBON NANOTUBES THE HOLY GRAIL

Previously discussed life-threatening toxicities remove the zeal of applying CNTs in pharmaceutical/medical systems.[57] However, this statement is synonymous with nonfunctionalized CNTs. Various recent advancements had been made to reduce the adverse effects of CNTs without affecting its structural properties.[58] This effort of reducing/eliminating the barriers between CNTs and the pharmaceutical/biological system is known as *functionalization*. It is an approach to the modification of the surface of CNTs and integrates them into the medical field.[59] One of the major problems associated with CNTs is their hydrophobic surface, which causes insolubility in aqueous systems that in turn restricts their use in biological systems. There are 2 approaches discussed in the literature regarding the improvement of solubility.[60] The first is surface modification by adsorption, electrostatic interactions, or covalent bonding of different molecules that make them more compatible with water. This approach also reduces the Van der Waals forces that cause bundling or aggregation of CNTs.[15] Noncovalent functionalization includes the use of surfactants and polymers. Surfactants increase the stability of CNT dispersions. By using 10 mg/ml of sodium dodecyl benzene sulfonate (SDBS), dispersion was obtained and was stable for more than a month. The chemistry behind this is π–π interactions of aromatic moieties of CNTs and SDBS, and stability was increased because of SDBS long lipid chains. SDBS/CNT dispersions showed uniformly coated individual tubes on atomic force microscopy (AFM) and transmission electronic microscopy studies.[61] Polymers were wrapped around CNTs and attached to the nanotube's surface due to steric repulsion of the polymer, which results in dispersion stability.[62] Star et al[63] suspended SWNTs in organic solvents by using substituted poly(metaphenylenevinylene). The second approach includes chemical modification of CNTs by attaching functional/organic groups to the sidewalls or tips of CNTs or by additional reactions.[64] Despite enhancing solubility, this technique could be used for attaching the active agents that will facilitate the delivery of drugs, antigens, and genes. Dyke and Tour[65] explored the enhanced solubility of CNTs by sidewall functionalization using aniline (Fig. 6). The main achievement was covalent functionalization of individual nanotubes, which facilitates greater surface area for the attachment of actives with enhanced solubility.[65] Jain et al[66] explored improved water dispersibility of galactosylated MWNTs. Functionalization was carried out through sequential steps of chemical reactions as carboxylation, acylation, amine modification, and galactose conjugation.[66] Carboxyl-based coupling is also a mode that provides the maximum number of attachment sites by the introduction of a carboxyl group, which can easily

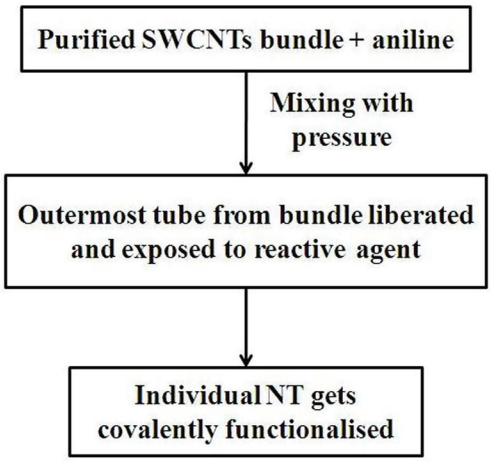

Figure 6. Flowchart eliciting sidewall functionalization of carbon nanotubes (NTs) using aniline. SWCNTs, single-walled carbon nanotubes.

make amides and esters.[67] The main steps are highlighted in Fig. 7. Yoon et al[68] carried out functionalization by inductively coupling commercially available carboxyl-functionalized SWNTs with N_2/H_2 plasma to form amine-functionalized SWNTs. The results showed that amine-functionalized SWNTs were well dispersed in the nanocomposites, improving adhesion of SWNTs to the surrounding polymer matrix. It can be concluded that upon functionalization, many features of CNTs are improved.[68] Another potent application of functionalization is to target diseases. Cheng et al[69] worked on PEGylation of MWNTs to overcome multidrug resistance, which is a significant problem in chemotherapy. Upon PEGylation, MWNTs showed effective penetration into mammalian cells without destroying the plasma membrane. PEGylated MWNTs were found to accumulate in multidrug resistant cells as they penetrate nonsensitive and sensitive cancer cells, as observed in HepG2-DR cells and sensitive HepG2 cells, respectively.[69] Apart from this, PEGylation also enhances the blood circulation time.

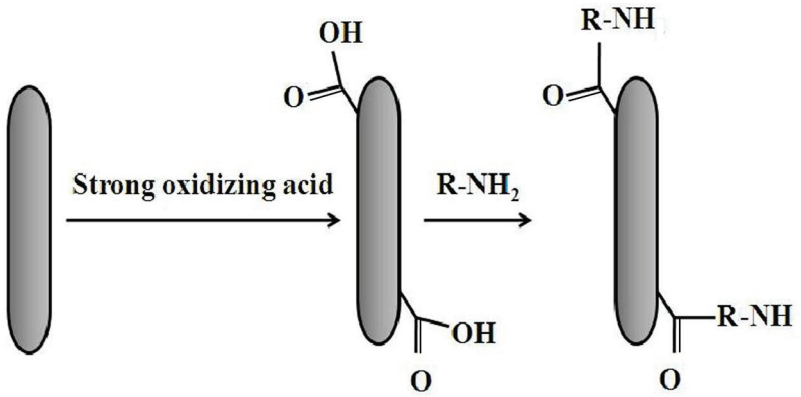

Figure 7. Carboxyl-based coupling of carbon nanotubes using a strong oxidizing agent.

A problem associated with CNTs is their intrinsic toxicity, which is mainly associated with their insolubility. For instance, results of in vitro studies conducted on functionalized SWNTs (covalent bonding with phenyl-SO_3H or phenyl-$(COOH)^2$) and aqueous dispersions of pristine (stabilized in surfactant pluronic-F108) showed that functionalized SWNTs produce less cytotoxicity compared with aqueous dispersions of pristine.[70] Functionalization also helps in drug targeting, as in the case of bone regeneration. SWNTs when functionalized with polyaminobenzene sulfonic acid through a series of reactions results in deposition of a hydroxyapatite layer, which is the main inorganic component of bone[71] (Fig. 8). Specifically for colon cancer, carcinoembryonic antigen (CEA) is a tumor marker for the identification of metastatic diseases. The antigen–antibody reaction approach could be utilized for the identification of CEA-bearing cancerous cells. Heister et al[72] carried out functionalization of doxorubicin-loaded SWNTs using monoclonal antibody and bovine serum albumin fluorescent marker at noncompeting binding sites. In vivo studies on WiDr human colon cancer cell lines showed that the drug was delivered successfully to the nucleus while nanotubes remained in the cytoplasm.[72] Similarly, McDevitt et al[73] functionalized CNTs by covalently attaching tumor-specific monoclonal antibodies, radiometal-ion chelates, and fluorescent probes. In in vivo and in vitro studies, nanotubes were found to be targeting human cancer cells specifically.[73] These techniques of functionalization make CNTs more facile, which in turn gives an advantage of applying them in the pharmaceutical/medical field. Dumortier et al[74] displayed the in vitro effectiveness of functionalized CNTs; quick uptake by B and T lymphocytes as well as macrophages was seen.

V. APPLICATIONS OF CARBON NANOTUBES IN THE BIOMEDICAL AND PHARMACEUTICAL FIELDS

Despite the issues with toxicity reported earlier, inorganic nanomaterials such as CNTs have importance in the field of biology. As discussed, functionalization alters the proper-

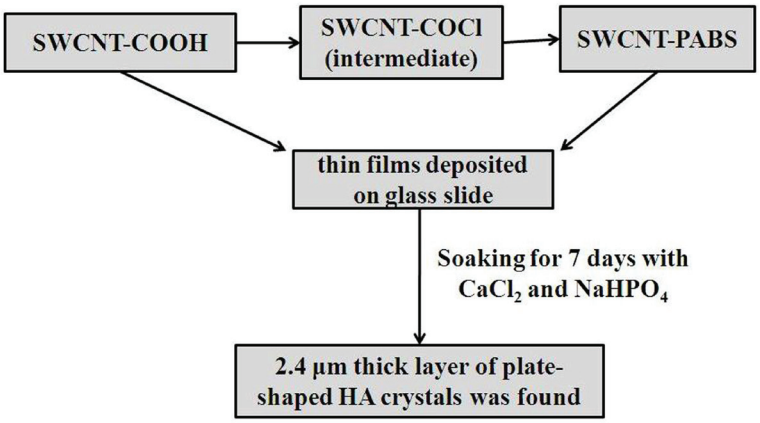

Figure 8. Carbon nanotube functionalization for bone regeneration via a series of reactions. Hydroxyapatite (HA) ; PABS, polyaminobenzene sulfonic acid; SWCNTs, single-walled carbon nanotubes.

ties of CNTs; that is, it makes them better able to be applied in a therapeutic system by increasing solubility and reducing/eliminating toxicity.[75]

V.A. Cancer Therapy

One of the deadliest diseases in the world is cancer, and it is becoming the worst because of the low specificity and low efficacy of conventional chemotherapy.[76] Previously, the way to overcome low specificity was to increase the dose that could reach the target, but this can result in severe systemic toxicity and increased drug resistance, which results in low efficacy of drug.[77] So, the focus came on the 2 main aims, which are the specificity and efficacy exhibited by CNTs. Liu et al[78] observed higher therapeutic efficacy of paclitaxel conjugated with PEGylated SWNTs in suppressing tumour growth in a murine 4T1 breast cancer model, with fewer side effects due to prolonged circulation time, slower release, and higher uptake. Tripisciano et al[79] developed a cis-platin (cis-diamminedichloroplatinum)–loaded SWNT and analyzed the system in vitro using prostate cancer cells (PC3 and DU145). Results showed a reduced number of living cells at the highest concentration of cis-platin–loaded SWNTs, which proves the effectiveness of developed nanocarriers. Taghdisi et al[80] investigated selective targeting of daunorubicin when conjugated with SGC8C aptamer and SWCNTs. The daunorubicin -aptamer-SWNT complex selectively targeted acute lymphoblastic leukemia T-cells (Molt-4 cells) with a pH-dependent release from the complex, as shown in Fig. 9.[80] Zhang et al[81] formulated a pH-triggered SWNT drug delivery system. Two polysaccharides were employed for the coating of the system: sodium alginate (ALG) and chitosan (CHI). Folic acid (FA) and doxorubicin (DOX; binds at pH 7.4 but releases at a lower pH, such as that of a tumor) were conjugated as actives with nanotubes. At a lower pH, DOX was released effectively to induce death by entering the cell nucleus. Upon incubation with human cervical cancer cells (HeLa

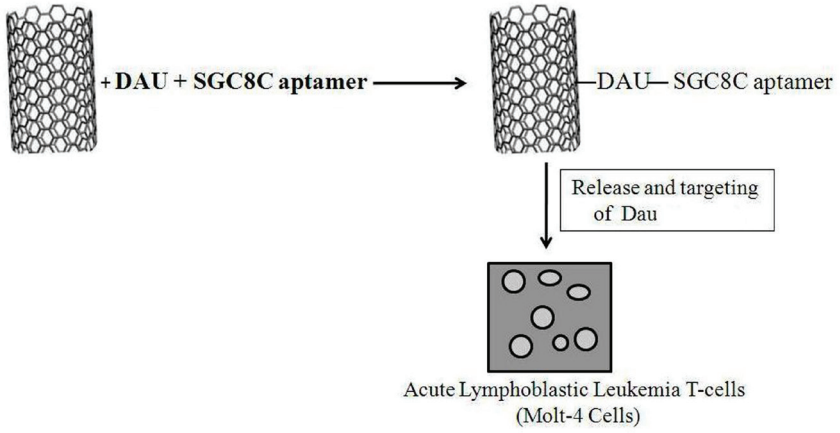

Figure 9. Representation of selective targeting of daunorubicin (DAU) using an aptamer–multiwalled carbon nanotube complex.

cells), DOX-FA-CHI/ALG SWNTs showed brighter red fluorescence than DOX-CHI/ALG SWNTs and DOX (used as a control), which indicates that FA-conjugated CNTs are taken up more effectively into cells.[81] Liu et al.[22] attached DOX to prefunctionalized CNTs for in vivo chemotherapy. In vitro cytotoxicity studies on MCF7 human breast cancer cells showed an increase in cytotoxicity of the CNT-DOX complex compared with DOX alone. Use of prefunctionalized CNTs surpasses the problem of destruction of nontargeted cells.[82] The unique optical properties of CNTs make them a nanovector of choice for chemotherapy via biophysical techniques (hyperthermic treatment using an infrared beam).[83] Kam et al[84] functionalized SWNTs with folic acid and investigated internalization of nanotubes by cancer cells (in vitro), which have FA receptors on their surface. Treatment with 700- to 1100-nm near-infrared light results in damage of cancer cells without effecting receptor-free normal cells.[84] In a similar study, Moon and his co-workers[85] treated nude mice bearing human epidermoid mouth carcinoma KB tumor cells on their backs using SWNTs and near infrared radiation (NIR). PEG-conjugated SWNT solution was injected directly into the tumor and the tumor region was exposed with NIR (power density of 76 W/cm^3) for 3 minutes. After 20 days of treatment, the tumor was completely destructed with complete clearance of SWNTs via the biliary/urinary route in about 2 months.[85] Tumor cells are rich in folate, epidermal growth factor (EGF), and biotin receptors. This feature was exploited for targeting anticancer drugs. Dhar et al[86] prepared SWNT-loaded platinum (IV) complexes comprising a cell receptor targeting moiety (i.e., FA). Observation of the internalization of CNTs showed that SWNT-platinum complexes were specifically highly bonded to folate receptors, resulting in enhanced cell killing properties.[86] Another group carried out in vivo cancer cell viability studies on squamous cancer cells, which overexpress EGF receptors. The results provide an evidence of selective and effective targeting of oxidized SWNTs bioconjugated with cisplatin and specific receptor ligand EGF.[87] Similar studies were carried out by Chen et al,[88] who observed the internalization of a biotin-SWNT-taxoid fluorescein conjugate inside the cancer cells (L1210FR leukemia cell line) by means of confocal fluorescence microscopy. They concluded that the conjugate specifically targets cancer cells, and there could be potential further research on the development of such delivery systems.[88] Apart from chemotherapy, CNTs are also used as contrast agents in noninvasive cancer imaging techniques such as photoacoustic imaging. This technique offers deep tissue imaging in living subjects and provides high spatial resolution. Gambhir et al[89] conjugated SWNTs with cyclic arginine–glycine–aspartic peptides for the photoacoustic imaging of tumors. In vivo studies in mice bearing tumors showed 8 times greater photoacoustic signal in the tumor compared with nonconjugated CNTs.[89] In a similar study, Pramanik et al[90] demonstrated SWNT-enhanced photoacoustic imaging of the sentinel lymph node in a rat model. The sentinel lymph node is most likely to have metastatic breast cancer, which can only be removed via biopsy. SWNT provides noninvasive imaging and shows a high contrast to noise ratio with good resolution.[90] In addition to various research, there are also many patents regarding the delivery via CNTs of chemotherapeutic agents,[91–95] which are shown in Table 5.

TABLE 5. List of Some Important Patents Regarding the Use of Carbon Nanotubes (CNTs) in Therapeutics and Diagnosis

Patent Number	Patent Description	Reference
US20100209479	Composition and methods comprising MWNTs linked with chemotherapeutic agents such as oxiplatin or mitomycin C	91
US20100055705	Composition and methods of diagnosing and treating cancer using a protein tristetraprolin	92
US20090062785	SWNTs were linked to proteins (annexins), which specifically bind to tumor and cancerous cells. Irradiating these CNTs with a specific wavelength results in the death of targeted cells; apart from this, an immunostimulant is also administered to the patient to enhance his or her immune response to the antigens released from the cancerous cells	93
US20090136987	Comprised of CNTs as imaging agents, internally loaded with a contrast agent. The method involves introducing the imaging agent into a cell and then imaging the cell to detect the presence of the imaging agent	128
US20080227687	Protein (annexins) linking was carried out with SWNTs, resulting in targeting cancerous cells, especially tumor vasculature endothelial cells rather than healthy ones. Specific wavelength radiation was used to detect and destroy bound SWNTs.	94
US20080193490	CNTs were loaded with an anticancer agent. CNTs were modified with a functional group from biotin, biotin conjugating moieties, antigen binding moieties, and tissue recognition moieties.	95

MWNTs, multiwalled nanotubes; SWNTs, single-walled nanotubes.

V.B. Drug and Gene Delivery

A drug delivery system should enhance the pharmacologic and therapeutic profile of a drug. It can be achieved by specific targeting of the drug via nanocarriers, which improves the efficacy of the system.[96,97] A group studied the delivery of amphotericin-B, the most effective antifungal drug, which was conjugated with CNT. Rapid internalization inside mammalian cells with reduced toxicity was observed compared with free drug.[98] Another group explored the binding and release of dexamethasone loaded on single-walled carbon nanohorns. Single-walled carbon nanohorns are spherical aggregates of nano-sized graphitic tube. Dexamethasone was absorbed successfully in the oxidized nanohorns and it maintained its therapeutic efficacy after release, which was confirmed by activation of glucocorticoid response in bone marrow cells of mice.[99] Yang et al[100] studied successful delivery of SWNTs embedded with acetylcholine into the brain for the therapy of experimentally induced Alzheimer disease. Targeting was done preferentially on lysosomes by precisely controlling the doses within the safety range without affecting mitochondria.[100] Earlier, viruses were used for gene delivery because

a virus can penetrate cells easily and have high gene expression; however, because of its immunogenic nature, viral gene delivery was discontinued. Nonviral gene delivery includes microinjection (DNA injected with a microneedle), but this also destroys skin cells.[101] CNTs overcome this problem because it involves minimal cell membrane disruption and it can deliver a large number of DNA cells at one time. Delivery of DNA to cells was achieved by functionalization of CNT by electrostatic interactions. Compared with DNA alone, CNTs showed 10 times higher gene expression levels.[102] Likewise, a green fluorescence protein gene was delivered in cultured human umbilical vein endothelial cells by conjugation of MWNTs with plasmid DNA.[103] Hormones are difficult to administer via the oral route because of their degradation in the acidic environment of the gastrointestinal tract. CNTs overcome this problem by delivering erythropoietin to the intestine. Venkatesan et al[104] formulated CNTs loaded with erythropoietin, casein (an intestinal enzyme inhibitor), and labrasol (an absorption enhancer). CNTs were used as an adsorbent. In vivo studies in rats via small intestine administration showed that CNTs can be used for the oral delivery of protein-based drugs because it reveals stability of the drug after it reaches the intestine.[104]

V.C. Biosensing

Electrical conductance properties of CNTs serve them best as a biosensing agent because of transport of the electron propagating along the axis, which can detect small changes in current when any specific biological entity comes in contact with it.[105,106] For instance, Yang et al[107] explored and demonstrated nanotube-based biosensors that are able to detect selective proteins in a solution. Nanotubes were immobilized by polyethylene oxide chains that selectively recognize and bind the target protein. The mechanism of binding involves conjugation of specific receptors of proteins with polyethylene oxide-functionalized proteins.[107] Yang et al concluded that the work leads to the development of biosensing of a system that can be utilized for the detection of serum proteins such as disease markers, autoantibodies, and antibodies. Huang et al[108] developed a novel device for biosensing that can detect membrane proteins such as ligand-gated ion channels, antibacterial peptides, and toxins. An SWNTs net device was integrated with an artificial lipid membrane that specifically detects the presence of ionophores in their native lipid environment.[108] With the extensive development in the field of biosensing, DNA biosensors that specifically recognize nucleic acid have been designed. The design of biosensor CNTs is based on the process of electrochemical hybridization that involves immobilization of a single-strand DNA probe on a transducer surface. When duplex formation occurs, an electric signal is produced. This may help with cost-effective determination of various genetic and infectious diseases.[109]

V.D. Neural Application

Outstanding electrical and mechanical properties of CNTs can be exploited in the fabrication of electrically conductive structures such as neural tissue. The system should

have an electric current carrying capacity and should be able to support the growth of new tissue.[110] 4-Hydroxynonenal is an aldehydic bioactive molecule that increases the concentration of intracellular calcium ions and modulates signaling mechanisms (necessary for neurite growth). Mattson et al[111] exploited the property of this molecule and coated it on MWNTs to promote neural cell functions. Uncoated and 4-hydroxynonenal–coated MWNTs were cultured on embryonic rat brain neurons and the number of neurites grown was observed. Uncoated MWNTs caused growth of 1 or 2 neurites whereas coated MWCTs showed growth of 4 to 6 neurites, as shown in Fig. 10. Purification of CNTs also enhances their capacity for signal transduction.[111] As demonstrated by Lovat et al,[112] electrical signal transfer on the neuronal network was improved by the use of purified MWNTs. A group synthesized SWNTs with a polyethylenimine copolymer and compared it with a free copolymer. SWNTs showed effective lengthening and more branching of neurites.[113] Neurite outgrowth can be regulated and enhanced if it is covalently bound with neurotrophin, as explained by Matsumoto et al.[114] CNTs can impregnate stem cells on their surface, which could be used for the differentiation and increased production of neuronal cells at the site of injury in brain.[114] Following this feature, Jan and Kotov[115] cultured layer by layer on neuronal cells a SWNT/polyelectrolyte composite for 7 days and observed that the CNT composites selectively divided and developed neuronal cells while being compatible with cells. A group had used lithographically patterned CNT surfaces and kept neurons on its surface for a period of 4 days. After that time, neurons were found to be localized in CNT-rich regions with their connected axons and neurites replicated using the pattern of CNT template.[116]

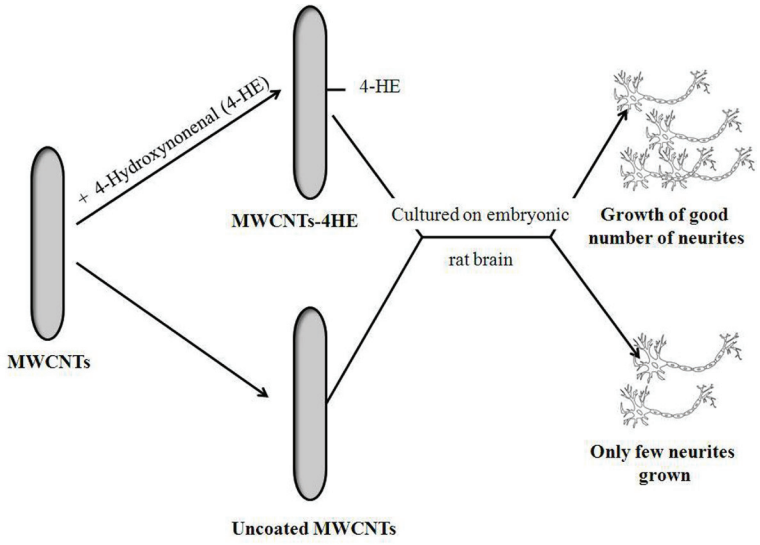

Figure 10. Comparison between the 4-hydroxynonenal (4-HE)–coated multiwalled carbon nanotube (MWCNT) and uncoated MWCNT for bone regeneration.

V.E. Peptide and Transdermal Delivery

CNTs have great potential in the field of peptide delivery. Specific peptides are attached to the surface of CNTs, which after administration induce the formation of antibodies specifically for peptides, not for CNT.[117] A study was carried out on mice that were immunized to peptide-linked CNTs and resulted in the high production of antibodies compared with free peptide. B-cell epitope of the foot and mouth disease virus was attached to the amine group of CNTs, resulting in the secondary structure of the surrounding peptides, which can be recognized by monoclonal and polyclonal antibodies. CNTs also showed a virus-neutralizing activity.[118] Another aspect of CNTs is the effective treatment of drug abuse and addiction. For example, for the cessation of cigarette smoking, a switchable transdermal drug delivery device loaded with CNT membrane has been formulated. The CNT membrane is loaded with nicotine to obtain switchable transdermal nicotine delivery rates in vitro. The device releases the drug for a long time in a programmed manner with minimal or no skin irritation.[119]

V.F. Carbon Nanotubes for Imaging

Excellent optical properties of CNTs have made them more popular for use as a novel photoluminescence agent and a Raman and photoacoustic contrast agent for the imaging of cells.[120] O'Connell et al[121] first explored NIR photoluminescence from micelle-encapsulated SWNTs, while Cherukuri et al[122] imaged the uptake of SWNTs in the phagocytic cells, which was nonspecific in nature. Xiang et al[123] functionalized SWNTs with an antibody (integrin$\alpha_v\beta_3$) for early detection of tumors with a photoacoustic imaging technique. Preliminary in vivo studies in human glioblastoma tumors demonstrated that highly efficient targeting of antibodies could be achieved with high contrast. Their experimental studies showed that the functionalized SWNTs were nontoxic. They finally concluded that photoacoustic imaging with antibody-functionalized SWNTs have the potential for effective early tumor diagnosis.[123] Likewise, Chen et al[124] have explored the potential of CNTs as probes in the field of AFM because of their well-defined geometry and robust mechanical properties. They have also exemplified the basic factors that determine the image resolution in AFM using well-characterized individual SWNT AFM probes with different diameters.[124] CNTs also have been used for in vivo imaging of animals. Leeuw et al[125] used NIR fluorescence microscopy by feeding Drosophila larvae with feed containing SWNTs. Biodistribution studies were observed by fluorescence nanotube signals.[125] Another group successfully imaged living mice bearing a tumor xenograft by injecting RGD-conjugated PEGylated SWNTs (targeted) and nontargeted SWCNTs intravenously, which was used as a Raman probe. Mice showed strong Raman signals in the tumor compared with nontargeted SWNTs.[126] CNTs also have been used for the diagnosis of myocardial infarct. One group has used CNTs with microcomputer tomography to identify areas of myocardial infarct in mice.[127] They demonstrated the ability of the system to provide cardiac information through the use of a delayed contrast enhancement technique. They concluded that CNT microcomputer tomography

provides a potentially novel tool for the study of cardiovascular biology.[127] Patents have been granted for its imaging properties,[128] as discussed in Table 5.

VI. CONCLUSION

The characteristic features of CNTs, such as structural, optical, thermal, and mechanical features, promises they will act as diagnostic and therapeutic agents in deadly diseases such as cancer and brain disorders, although studies regarding the toxicities of CNTs are underway and new schemes are being investigated to eliminate/reduce the toxicities that were raised during synthesis. Functionalization has broadened the horizons for the applications of CNTs in the biomedical and pharmaceutical fields, serving as an alternative to conventional drug delivery systems. Scientists have exploited the unique properties that make CNTs specific to the cancer treatment. With continuous research, CNTs surely will become a useful and safe tool for the positive enhancement of human health.

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