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Emergence in the functionalized carbon nanotubes as smart nanocarriers for drug delivery applications

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4.1 INTRODUCTION

Carbon nanotubes (CNTs) are nanomaterials with one-dimensional structure, considered second generation allotropes of carbon, next to diamond and graphite. Chemically, CNTs constitute pure carbon, where inherent carbon atoms present in a repetitive manner in hexagonal pattern for cylindrical tubes (Bianco et al., 2005). The size of CNTs varies in terms of their length-to-diameter ratio. They are also considered as members of the fullerene family, they differ from each other by the number of carbon atoms (i.e., C₂₀, C₃₀, C₃₆, and C₇₀) and their arrangement. The individual fullerenes are called graphenes. Many describe CNTs as the folded form of graphenes, which are also considered allotropes of carbon. Graphenes are single-layered graphitic sheets with properties identical to nanotubes. CNTs were first discovered accidentally by Ijima (1991) as an allotropic form of carbon by electric arc discharge (EAD) of graphitic materials at high temperature. Led from his discovery, CNTs have been explored for their numerous applications in the area of physical chemistry due to their quasi-one-dimensional property, incredible strength, and fascinating electromechanical properties.

Important properties of CNTs which make them possible as a novel nanomaterials eligible for drug delivery applications are: their specific atomic architecture coupled with high aspect ratio, characteristic configuration, excellent functionalization ability, and dynamic surface modification properties (Beg et al., 2010). Furthermore, high inner volume and ability for immobilization helps in making them biocompatible, accordingly CNTs are most sought after nanomaterials in drug delivery due to their versatile applications in controlled and targeted delivery, cancer treatment, bioavailability enhancement, transdermal delivery, theranostic applications, drug discovery and delivery of therapeutic molecules such as proteins, peptides, RNA, DNA, siRNA, etc. (Bianco, 2004; Bianco et al., 2005; Pantarotto et al., 2004). The unique physicochemical, conformational and biocompatible properties of CNTs with easy surface modification have led to a surge in the number of publications in this interesting field.

4.2 CLASSIFICATION OF CARBON NANOTUBES

Depending on the structural conformation and shape, CNTs are classified into four major categories: single-walled carbon nanotubes (SWCNTs), double-walled carbon nanotubes (DWCNTs), multiwalled carbon nanotubes (MWCNTs), and functionalized carbon nanotubes (f-CNTs) (Beg et al., 2010). SWCNTs are tube-shaped structures formed from folding graphene sheets, and exist in open or close structures at both the ends. DWCNTs have a double-layer structure containing two sheets of graphene folded upon each other. MWCNTs are formed by folding of 2–10 graphenes sheets on each other or by rolling a single graphene sheet to produce a complex multiwalled structure (Mehra et al., 2015). DWCNTs and

MWCNTs differ from SWCNTs by an open-end structure on both sides, as depicted in Fig. 4.1. SWCNTs have an internal diameter of approximately 1 nm, whereas MWCNTs are having an internal diameter of 5–20 nm. Similarly, SWCNTs are insoluble in water and form aggregates soon after sonication, whereas MWCNTs are partially water soluble in nature and form slightly translucent dispersions. The native CNTs or pristine CNTs obtained from synthetic routes are generally water insoluble in nature. Functionalization is a synthetic process whereby requisite functional groups or therapeutic molecules can be tagged on the surface of CNTs. Functionalization imparts high solubility, enhances biocompatibility, and reduces toxicity of CNTs. Accordingly, f-CNTs have higher importance in the area of drug delivery and many more.

Besides pristine nanotubes, several structural variants of CNTs are observed, such as nanohorns, nanotorus, and nanobuds, which are found to have significant applications in drug delivery. Nanohorns are modified forms of SWCNTs, having a single layer cone shape or horn-like structure, closed at one end by a single hexagonal ring of carbon. These are useful in drug delivery due to their unique feature of increase in diameter with increase in length. Accordingly, they are widely employed in targeting drug molecules to malignant cancer cells. Nanobuds are materials with modified structure having a bud-like surface outgrowth, which acts as a reservoir for encapsulation of drug molecules for effective delivery. Nanotori are the ring-shaped structures formed by the folding of single layer sheet of graphene (Purohit et al., 2014). These are theoretically described CNTs bent into a torus or doughnut shape structure. However, the nanotorus has limited importance in drug delivery. Another, structural variant of CNT, coiled nanotubes, are regular or irregularly helical structures formed by the peculiar arrangement of heptagonal and pentagonal rings in the hexagonal backbone structure of nanotubes (Hanus and Harris, 2010). The present review gives an exhaustive account on various types of functionalization of CNTs and applications of functionalized CNTs in drug delivery.

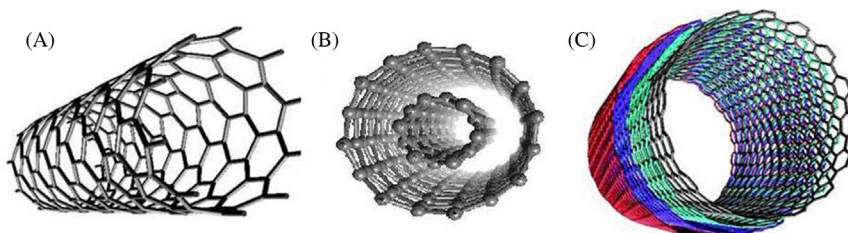


FIGURE 4.1

Pictorial depiction of various types of CNTs; (A) SWCNTs, (B) DWCNTs, (C) MWCNTs.

Source: Modified with permission from Beg, S., Rizwan, M., Sheikh, A.M., Hasnain, M.S., Anwer, K., Kohli, K., 2010. Advancement in carbon nanotubes: basics, biomedical applications and toxicity. *J. Pharm.*

Pharmacol. 63, 141–163.

4.3 METHOD OF PREPARATION OF CARBON NANOTUBES

The conventional methods of producing CNTs involve high heating of carbon black and graphites at specified temperature conditions. However, CNTs produced by this method possess uneven physical properties such as irregular shape and size, mechanical strength, quality and purity, ostensibly owing to the uncontrollable natural environment (Beg et al., 2010).

The advanced methods for CNTs have now been reported in literature, viz. chemical vapor deposition (CVD), EAD, and laser ablation (LA) methods. Such techniques rely on high frequency electric and laser sources, with fine control on their reactivity with carbon material. Among these, the CVD technique is used as the most sought after technique for the laboratory scale and bulk industrial production of diverse types of CNTs. It produces CNTs by catalysis of carbonaceous materials at very high temperature (700°C) in a hot furnace (Kumar and Ando, 2010). This technique has an advantage of being economical in nature for bulk production of CNTs. Fig. 4.2 depicts the process of synthesis of CNTs using CVD technique. Recent literature instances have shown the utility of several advanced CVD techniques viz. plasma-enhanced CVD, thermal CVD, laser-assisted CVD,

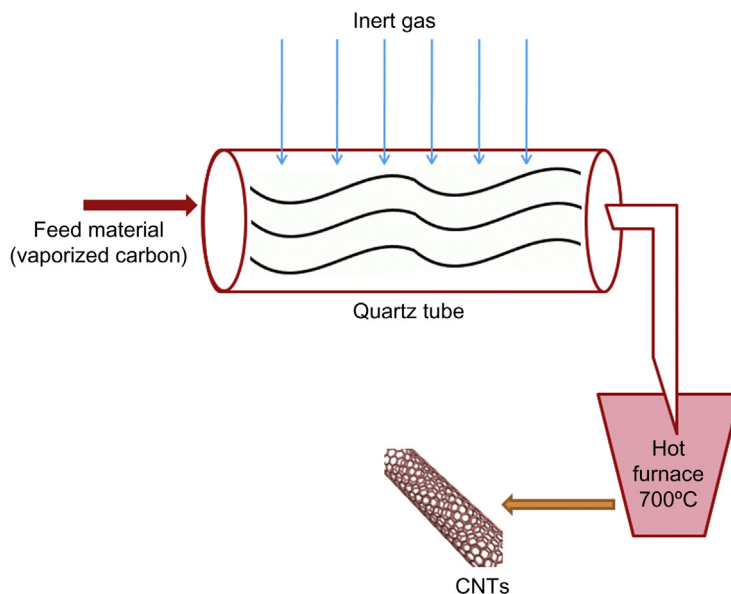


FIGURE 4.2

Flow layout of carbon vapor deposition method of preparation of CNTs.

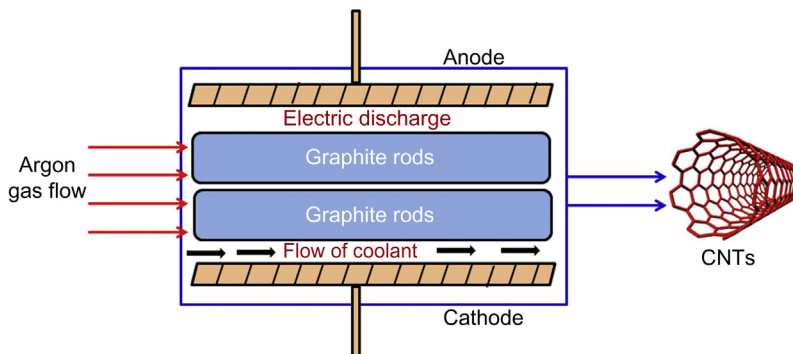


FIGURE 4.3

Flow layout of electric arc discharge method of preparation of CNTs.

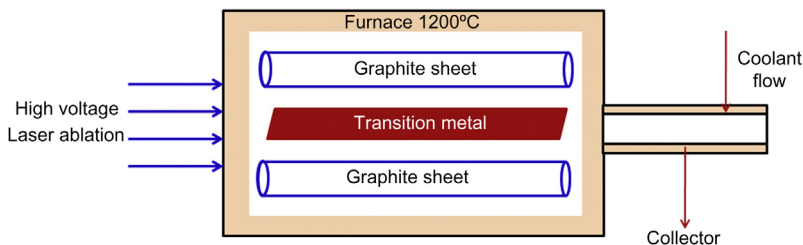


FIGURE 4.4

Flow layout of laser ablation method of preparation of CNTs.

and high pressure CVD for effective manufacturing of CNTs (Nessim, 2010). In the EAD technique, the CNTs are prepared by electric discharge of a high voltage beam (100 A) on graphite, used as feed material, as shown pictographically in Fig. 4.3. This method has the advantage of producing CNTs of superior quality with very high tensile strength (Sharma et al., 2015). However, in the LA technique the CNTs are produced by hitting a graphitic object with a high-speed laser beam, as shown in Fig. 4.4. It produces CNTs of very narrow diameter and suitable for laboratory production of both SWCNTs and MWCNTs (Hornbostel et al., 2006). Lately, other important techniques are being used for industrial and bulk scale production and manufacturing of CNTs, including cobalt-molybdenum catalytic (CoMoCat) process, high pressure carbon monoxide disproportionation technique, fluidized-bed synthesis (Dasgupta et al., 2011; Harris et al., 2008), flame synthesis (Wal et al., 2000), and solar energy-based techniques (Dasgupta et al., 2011).

4.4 FUNCTIONALIZATION OF CARBON NANOTUBES

Functionalization involves modifying the architecture of native pristine CNTs by the attachment of possible functional groups, drug molecules, ligands, polymers, proteins and peptides, nucleic acids, dyes, or radiocontrast agents for therapeutic applications (Meng et al., 2009; Vardharajula et al., 2012). The native pristine CNTs prepared by chemical synthesis are water insoluble, highly toxic in nature, and accumulated in organs. The process of functionalization imparts high solubility with enhanced biocompatibility to the CNTs. Accordingly, functionalized CNTs are highly suitable for encapsulation of therapeutic molecules for multimodal targeted delivery (Perry et al., 2011; Kumar et al., 2017). Functionalization is broadly classified into two major categories: noncovalent functionalization and covalent functionalization. Various approaches for functionalization of CNTs are elaborated below:

4.4.1 NONCOVALENT FUNCTIONALIZATION

It involves the attachment of therapeutic drug molecules onto the surface of CNTs by adsorption mechanism. CNTs act as nanoreservoirs to adsorb the drug molecules by host-guest interaction. The hydrophobic and π - π stacking interaction force between the chains of adsorbed molecule and surface of CNTs helps in the functionalization on the surface (Tang et al., 2012; Sharma et al., 2016). Noncovalent functionalization is greatly important for enhanced biocompatibility and biomedical applications of CNTs (Tasis et al., 2006; Kumar et al., 2017). By this functionalization process, surfactants, polymers, and biomolecules such as DNA, siRNA, proteins, and peptides can be successfully loaded onto the surface of CNTs. Furthermore, lipophilic drug molecules can also be grafted on CNTs by hydrophobic forces for targeted drug delivery application. The presence of charge on the nanotube surface helps in adsorption of drug molecules (Liu et al., 2010; Chen et al., 2002). Such functionalization can be achieved simply by exposing the CNTs to the solution containing drug molecules to be loaded onto its surface. Noncovalent functionalization of CNTs is particularly attractive, as it offers the possibility of attaching chemical modifications without affecting the electronic network of the tubes. Examples of various types of noncovalent functionalization of CNTs are described below.

4.4.1.1 *Types of noncovalent functionalization*

4.4.1.1.1 Polymer functionalization

Various polymers, such as polyethylene, nylon, and poly(ethylene glycol) (PEG) have been utilized for the functionalization of CNTs. Such functionalization processes usually involve van der Waal forces and electrostatic interactions. One of the functionalization techniques, i.e., PEGylation, has recently gained immense

importance, owing to its enhanced biocompatibility, solubility, and reduced toxicity (Bottini et al., 2011). PEG-functionalized CNTs are most widely used in drug delivery due to their nontoxicity and improved solubility. The increased solubility can be attributed to the hydrophilic segments orientated toward the aqueous phase (Ravelli et al., 2013; Hadidi et al., 2013). Fig. 4.6 depicts the methodology for PEGylation of CNTs. Noncovalent functionalization of pristine CNTs with amphiphilic polymers, such as phospholipid-poly(ethylene glycol), produces CNTs with high aqueous solubility, stability, and enhanced biocompatibility are suitable for drug targeting to cancer cells (Zeineldin et al., 2009).

Importantly, biopolymers, such as chitosan and alginate, have been employed to wrap up the CNTs for effective drug delivery of drugs such as dexamethasone and doxorubicin (Naficy et al., 2009; Zhang et al., 2009). Recent literature had shown that functionalization of MWCNTs with chitosan in the presence of sodium tripolyphosphate causes cross-linking of chitosan chains onto nanotube surfaces to produce nanohybrids for effective delivery of bovine serum albumin into cells (Li et al., 2011a,b). Functionalization of MWCNTs block copolymer, Pluronic F127, increased the cellular uptake of the CNTs and enhanced cytotoxicity (Ali-Boucetta et al., 2011). Similarly, noncovalent functionalization of SWCNTs employing amphiphilic diblock copolymer (polyoxyethylene-polycaprolactone) showed enhanced solubility of the CNTs in aqueous media (Park et al., 2007). Functionalization of MWCNTs with an hydrophilic polymer, such as carboxymethyl guar gum, showed higher solubility, enhanced biocompatibility, and superior drug loading ability (Giri et al., 2011).

4.4.1.1.2 Dye functionalization

Organic dyes can be grafted onto the surface of CNTs through noncovalent sidewall functionalization involving π - π stacking and ionic interactions. Important dyes successfully functionalized on CNTs include: congo red, trypan blue, prussian blue, fluorescein, rhodamine B, anthracene, and alizarin, etc. for various applications (Chen et al., 2011a). Drug-loaded CNTs functionalized with fluorescent dyes such as fluorescein isothiocyanate have recently been utilized for tracking CNTs into the living cells (Kang et al., 2012).

4.4.1.1.3 Nucleic acid functionalization

Nucleic acids, such as DNA, RNA and siRNA, can be noncovalently attached on the surface of CNTs for enhanced cellular delivery and multimodal targeting. CNTs have a tendency to easily penetrate into the cells by crossing the cell membrane and release the attached biomolecules in the nucleus for treatment of gene defects and related disorders (Pantarotto et al., 2004). Polyamidoamine functionalization of MWCNTs can effectively deliver the target gene through cellular trafficking to effectively produce transfection (Liu et al., 2011). Functionalized CNTs show higher affinity toward nucleic acids viz. single-stranded DNA and RNA, and form complexes by electrostatic attraction forces. Surface functionalization of

SWCNTs/MWCNTs with ammonium functional groups can easily penetrate into human and murine cells, and helps in the effective delivery of plasmid DNA, which leads to the expression of marker genes. Apart from ammonium functionalization, SWCNTs functionalized with lysine groups can easily penetrate through cellular and nuclear membrane, due to cationic charge present for enhanced delivery of plasmid DNA-nanotube complex (Singh et al., 2005). Noncovalent functionalization of CNTs with single- and double-stranded DNA by nonspecific binding interaction provides enhanced dispersion of hydrophobic CNTs in aqueous media, compared to the pristine CNTs, and better cellular penetration of the CNTs to deliver the DNA (Dieckmann et al., 2003). The enhanced aqueous dispersion of CNTs is due to the interaction of hydrophobic region of the peptide with aromatic rings of the CNTs, while the hydrophilic face promoted self-assembly through peptide-peptide interactions. Functionalization of siRNA molecules on PEG-SWCNTs allows faster cellular entry of siRNA-PEG-SWCNTs complex, owing to higher binding and association with cells by hydrophobic interactions with the cell membrane (Zhang et al., 2006).

4.4.1.1.4 Proteins and peptide functionalization

The application of functionalized CNTs in drug delivery has been explored significantly by many researchers. Of late, CNTs have also proved their worth in delivery of biological macromolecules such as proteins, peptides, and oligosaccharides (Baker et al., 2002). Noncovalent functionalization of bio-macromolecules onto the surface of CNTs provides an advantage of enhanced molecular recognition of the bound biomolecules (Bale et al., 2007). Importantly, macromolecular proteins such as fibrinogen, γ -globulin, hemoglobin, and fibronectin have been successfully functionalized with MWCNTs to achieve enhanced biorecognition (Wei et al., 2010). Recently, it has been reported that multifactorial noncovalent functionalization of MWCNTs with arginine-glycine-aspartic acid-serine peptides can produce cell capture, attenuate glycosylation on carcinoma cell surface, and play a significant role in preventing oncogenic transformation, cell differentiation, and cancer metastasis (Xue et al., 2011). Similarly, a functionalization strategy has been described by Liu et al. (2010) for functionalization of arginine-glycine-aspartic acid peptide residues for enhanced delivery of doxorubicin and anti-CXCR4 siRNA to cancer cells (Liu et al., 2009). SWCNTs noncovalently functionalized with peptide 427 undergo specific binding to major histocompatibility complex (MHC) molecules of antigen presenting cells and induces human CD4 T-cell responses for treatment of Wilm's tumor (Villa et al., 2011).

4.4.1.1.5 Endohedral functionalization

This is a type of noncovalent functionalization used for trapping drug molecules inside the hollow cavity of CNTs (Tasis et al., 2006). Encapsulation of drug molecules in CNTs increases the targeting potential and provides protection from the external environment, such as degradation by action of gastric acid. Furthermore,

entrapment also increases solubility of drugs by hydrophobic interactions with the sidewalls of the CNTs (Vashist et al., 2011). Importantly, drugs such as carvedilol, amphotericin B, tocopheryl acetate, theophylline, and acetylcholine have been successfully encapsulated in various CNTs (Vizuet et al., 2012).

4.4.2 COVALENT FUNCTIONALIZATION

Covalent functionalization creates a more secure conjunction of functional groups, surfactants, polymers, drugs, or other biomacromolecules (Balasubramanian and Burghard, 2005; Vardharajula et al., 2012). In order to achieve such types of functionalization, CNTs are subjected to treatment with chemical and high temperature reflux conditions. 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, carboxyl groups, arynes, or carbenes. Moreover, such reactions often require extreme conditions for covalent bonding. Furthermore, characterization of such functionalized nanotubes to determine the precise functionalization location and mode of addition are also very difficult. In drug delivery perspectives, the direct covalent functionalization of drug molecules to CNTs surface have been less reported in literature. However, for therapeutic biomacromolecules, such as proteins, peptides, DNA, and siRNA, covalent functionalization has been widely practiced (Chen et al., 2011a).

4.4.2.1 End-defect functionalization

This is a special type of covalent functionalization, where oxidation of native pristine CNTs is carried out using strong acids, such as H_2SO_4 or HNO_3 . This causes reduction in the length of CNTs, followed by ring opening at both the ends. Furthermore, it generates carboxylic groups at the “end,” on the surface of tips, after the ring opening due to 1,3-dipolar cycloaddition reaction. Consequently, the process is also called carboxyl functionalization, which is used for increasing the dispersibility of CNTs in aqueous solutions (Jain et al., 2011). In the case of SWCNTs, strong acid treatment with reflux causes opening of side caps to produce a hollow cylindrical structure and generation of carboxyl groups at the tips, whereas in MWCNTs, the acid treatment only causes generation of carboxyl group at the end. Long-term treatment of CNTs with strong acid solution causes severe disruption of π -networks of the CNTs, and results in possible loss of flexibility and electrical and mechanical properties of pristine CNTs (Peng and Cho, 2000). Consequently, alternative approaches such as addition reactions of hydrophilic groups to the CNTs’ external walls and tips can also make them soluble in water. Such types of functionalization are widely employed in drug delivery for therapeutic molecules, such as methotrexate, paclitaxel, doxorubicin, for treatment of diverse types of carcinomas (Karchemski et al., 2012; Pastorin et al., 2006; Lay et al., 2011).

4.4.2.2 Side-wall functionalization

Such functionalization is primarily used for the dispersion of CNTs in aqueous solutions, which can be assisted by covalent binding of surfactants, proteins, and peptides on the surface of CNTs. Furthermore, sidewall functionalization can also be achieved by directly reacting CNTs with organic species such as nitrenes, carbenes, and other radicals to generate respective functional moieties. In this regard, SWCNTs are more susceptible toward sidewall functionalization than MWCNTs (Saini et al., 2003).

4.5 MULTIFUNCTIONAL APPLICATIONS OF CARBON NANOTUBES

CNTs have recently emerged as efficient carriers in the arena of drug delivery. An explicit account of diverse drug delivery applications of CNTs is detailed further. Fig. 4.5 portrays a typical instance of delivering drugs functionalized on the surface of CNTs to the cells.

4.5.1 CONTROLLED RELEASE DRUG DELIVERY APPLICATION

The applications of CNTs in drug delivery have been explored primarily owing to their ability to deliver drug molecules in controlled release manner (Luo et al.,

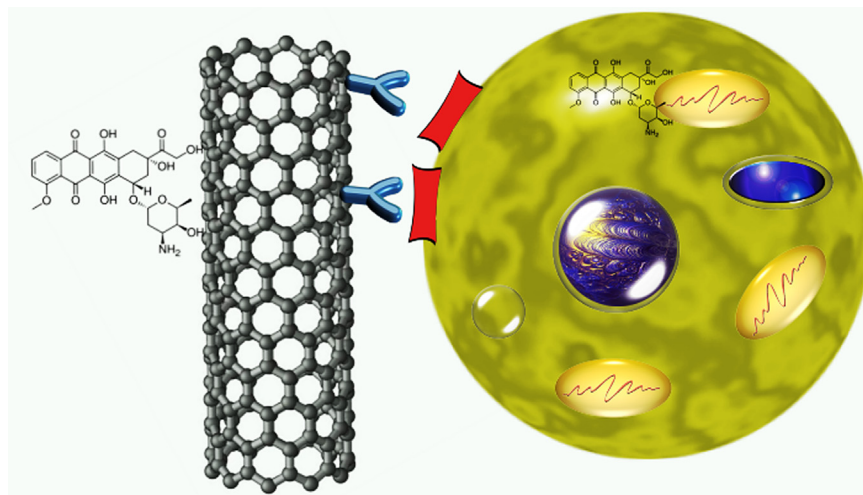


FIGURE 4.5

Drug-anchored functionalized SWCNT for drug delivery to cells.

2011). Accordingly, they are employed for controlled delivery of drugs as well as of genetic material such as nucleic acids and biomolecules. For instance, the use of mesoporous silica surface functionalized with amine groups (NH_2 -MSNTs) and blue fluorescent CdS quantum dots have been found able to deliver antiinflammatory drugs in a controlled manner (Yang et al., 2009b). Furthermore, nanohybrid hydrogels of poly(methacrylic acid)-functionalized carboxylated MWCNTs (MWCNT-COOH) have also been employed as controlled drug delivery vehicles. With an increase in MWCNT-COOH content, such hydrogels exhibit low micropore densities and large mesh sizes, which undergoes swelling upon contact with water due to pH-responsive properties, providing controlled release delivery of drug.

Advancement in CNTs has produced “smart bio-nanotubes” as next-generation nanomaterials with supramolecular properties over conventional nanotubes. These trilayered structures are made up with the help of microtubular protein, tubulin, coated with a lipid bilayer. The important formulation variables regulating the rate of drug release from such bio-nanotubes are the thicknesses of protein lipid and protein coats. Lately, several pharmaceutical formulations of CNTs have been developed for exploration in diverse controlled drug delivery applications. Fig. 4.6 illustrates various formulation systems prepared from CNTs for multiple drug delivery applications.

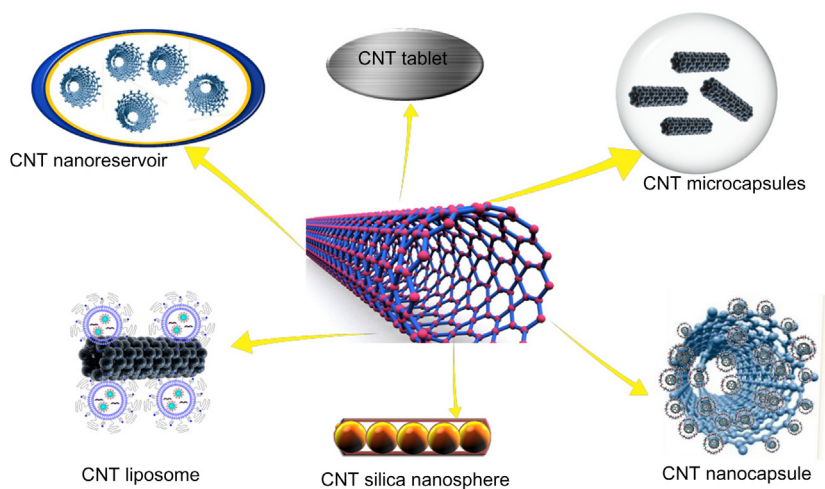


FIGURE 4.6

Pictorial depiction of various drug formulations of CNTs.

4.5.1.1 Carbon nanotube-microcapsules

Encapsulation of CNTs in synthetic and natural polymer composite material produces microcapsules. Such formulations have piqued great interest in controlled drug delivery and biomedical applications. Importantly, alginate is a widely used biopolymer for preparing the microspheres loaded with CNTs for drug delivery (Kawaguchi et al., 2006). These are prepared by dispersing the drug-loaded CNTs into the polymer composites, followed by cross-linking in presence of Ca^{2+} ions, which causes gelation of the polymer to form microcapsules. Jiang et al. (2006) prepared the CNT-bound alginate biocomposites for cellular immobilization of bovine serum albumin. Similarly, Zhang et al. (2010) prepared the alginate microspheres filled with CNTs for controlled delivery of theophylline. It has been observed that incorporation of CNTs in alginate microcapsules decreases the leakage problem, increases the drug loading, prevents drug degradation in gastric acidic pH conditions, and provides sustained therapeutic action.

4.5.1.2 Carbon nanotube-nanoreservoirs

CNTs have been recently used as nanocarriers for controlled drug delivery of therapeutic molecules, due to their electric property and hollow tubular structure. Likewise, selective functionalization of CNTs with polymers having electrical conductivity provides controlled drug delivery upon electrical stimulation. Recently, Luo et al. (2011) prepared the nanoreservoirs of MWCNTs by functionalization, employing polypyrrole films for controlled delivery of dexamethasone. The open-ends in MWCNTs were sealed with polypyrrole film to prevent the leakage of drug and controlled release profile of drug delivery.

4.5.1.3 Carbon nanotube nanospheres and nanocapsules

Functionalization of CNTs with pH-sensitive polymeric materials and inert silica-containing core particles produce nanospheres. Such systems provide controlled delivery of loaded drug molecules at the desired site of action. Chen et al. prepared the nanospheres of CNTs for controlled release delivery of fluorescein. The carboxyl-functionalized CNTs were closed at both the open-ends, employing silicon dioxide by formation of disulfide bonds (Chen et al., 2011b). Such systems are also called stimuli-responsive drug delivery systems, as they provide controlled release profiles of drug release by slowly opening silicon dioxide moieties by cleavage of disulfide bonds. Similarly, attachment of doxorubicin onto SWCNTs functionalized with hydrazinobenzoic acid provides pH-responsive, site-specific controlled drug delivery at the tumor microenvironment (Gu et al., 2011). Similarly, Guven et al. demonstrated that the application of ultra-short SWCNTs as nanocapsules for the delivery of cisplatin can provide enhanced chemotherapeutic activity. The drug molecules are entrapped in the ultra-short SWCNTs with end-wall defects by noncovalent interaction mechanism (Güven et al., 2011).

4.5.1.4 Carbon nanotube-chitosan nanocomposites

Recently, surface engineering of CNT functionalization with chitosan in the form of a film producing a variety of nanocomposites has been widely described in literature for controlled drug delivery and biomedical applications (Carson et al., 2009). Such systems provide initial burst release, followed by controlled drug delivery application, preventing the leakage of drug and protecting it from the external environment. These are prepared by electrostatic interaction between the positively charged chitosan with negatively charged surface of CNTs (Baekb et al., 2008). Zhao et al. prepared the CNT-chitosan nanocomposite films for pH-responsive controlled delivery application of polyoxometalate at the tumor site. Furthermore, such a nanocomposite provides high drug loading and higher stability to the drug in the tumor microenvironment (Zhao et al., 2009). Similarly, Naficy et al. (2009) prepared and evaluated the chitosan-conjugated CNT films for modified release drug delivery of dexamethasone.

4.5.1.5 Carbon nanotube tablets

The applications of CNTs have lately been transformed for their use in controlled drug delivery through the oral route. For example, CNTs are now used in tablet coating, along with hydrogels such as ethyl cellulose, cellulose acetate, and hydroxypropyl methylcellulose (HPMC), for achieving the desired sustained release profile of drug delivery. The CNT causes cross-linking of cellulose chains of hydrogels to provide the sustained action, following zero-order kinetics. Madaenia et al. (2012) prepared the membrane-coated drug delivery tablets, employing carboxyl functionalized MWCNTs for sustained therapeutic action of indomethacin.

4.5.2 TARGETED DRUG DELIVERY APPLICATION

Functionalized CNTs are of remarkable attention in the field of nanomedicine for targeted delivery of drugs, nucleic acids, proteins, peptides, antibodies, and other biomolecules to the desired target site (Kumar et al., 2014; Mehra and Jain, 2016). CNTs can easily deliver peptides, proteins, and nucleic acids into cells, owing to their cell membrane penetration property. Use of CNTs for targeted delivery has produced useful results in the treatment of diverse disorders such as choriocarcinoma, Burkitt's lymphoma, cervical, breast, and testicular tumors (Thakare et al., 2010; Zhang et al., 2011).

Functionalized nanotubes have been vastly employed for targeted delivery of nucleic acids, proteins, antibodies, drugs, and other therapeutics agents to their respective sites of action. The use of CNTs for targeted delivery is primarily accepted in treating various malignant disorders, which includes choriocarcinoma, carcinoma of cervix, breast cancer, prostate cancer, brain gliomas, and testicular tumors (Dineshkumar et al., 2015). As drug-loaded functionalized CNTs

encounter problems in the release of their drug contents, these have been encapsulated in novel membrane microcapsules, constituting a copolymer containing alginate–L-lysine-alginate units in the form of its membrane (Degim et al., 2010). Excellent drug release profile and ability for protection from the external environment have been used in targeted delivery. In addition, functionalization of chitosan on the surface enhances cell attachment to the sidewalls of the nanotubes, resulting in the desired targeted release to the cells, and with improved drug absorption (Zhang et al., 2011; Son et al., 2016). Such systems have significant potential for the delivery of drugs, peptides, and nucleic acids.

4.5.2.1 Cancer-targeting application

It is common in cancer chemotherapy that it tends to destroy the cancer cells along with healthy normal cells, accordingly causing severe side effects. CNTs, in this regard, are helpful in treating cancer cells safely without affecting the collateral tissues. CNTs loaded with chemotherapeutic agents can effectively deliver them to the malignant cells, due to high cellular uptake. The CNTs allow effective delivery of drugs into the tumors cells, owing to their enhanced permeability and retention (EPR) effect (Fang et al., 2011). Fig. 4.7 depicts the approach of

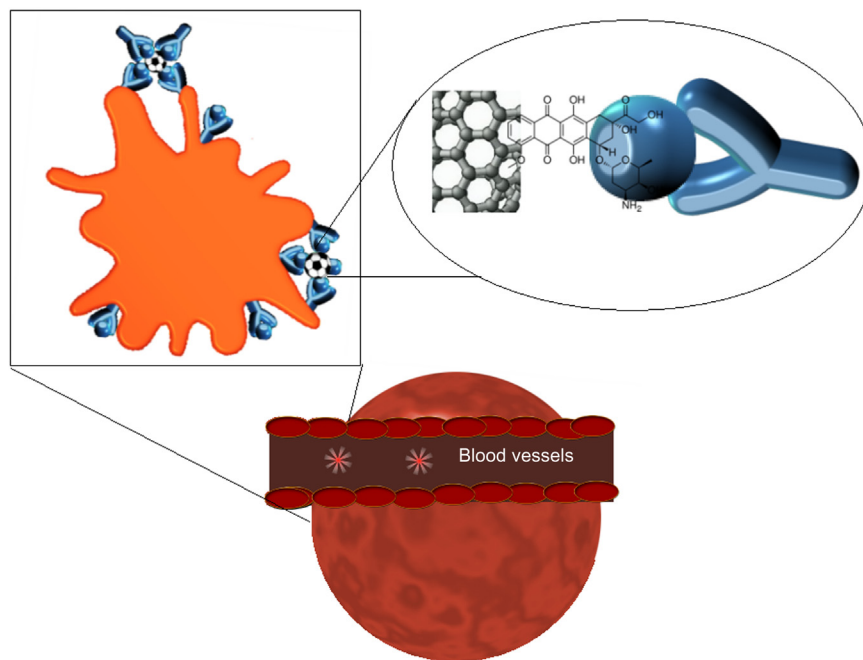


FIGURE 4.7

Enhanced permeation retention effect of the antibody guided CNTs used for tumor targeting.

EPR effect used by CNTs for traversing through the impregnable blood-tumor barrier. These easily enter into the cell by crossing the cell membrane through disorganized and leaky endothelial junctions on the surface of solid tumors, owing to their small size, which helps in easy retention in the tumor mass (Madani et al., 2011; Rahman et al., 2012). Consequently, nanotubes reduce the dose of drug by localizing its distribution at the tumor site only. This can be further fortified by functionalizing the drug-containing CNTs with an antibody molecule and targeting it to the antigen of cancer cells. Functionalization of SWCNTs with monoclonal antibodies has been employed for targeting drug molecules and diagnostic agents to provide theranostic benefits, i.e., both therapeutic effect and diagnostic imaging (Liang et al., 2016). SWCNTs functionalized with PEG and conjugated with the monoclonal antibodies are able to selectively target the CD20 cell surface receptor present on B-cells, with little nonspecific binding to negative T-cells. This approach has proved to be an alternative way in targeting drug molecules to the cancer cells (Fig. 4.7). Furthermore, the CNTs functionalized with antibodies are also capable of multimodal drug delivery, i.e., delivering more than one type of anticancer drugs to the tumor site. This approach has the advantage of delivering both hydrophilic and lipophilic drugs to the target site. Importantly, the drug molecules and antibodies to be conjugated on the surface of nanotubes must be compatible with each other. The antigenicity of antibodies must also remain unaltered after their attachment with the surface of CNTs (Liang et al., 2016).

Owing to several beneficial aspects of f-CNTs, cancer chemotherapy using such nanomaterials is found to be superior over the conventional chemotherapy to date. Numerous reports have already suggested the effectiveness of CNTs in delivery of chemotherapeutics such as doxorubicin, methotrexate, taxanes, platinum analogues, camptothecin, etc (Fabbro et al., 2012). CNTs act as the macromolecular agents which extravasate in tumor tissues over time to elevate the concentration of the drug at the tumor tissue vis-à-vis in blood plasma (Prato et al., 2008). As reported, paclitaxal conjugated with SWCNTs shows higher suppression of tumor growth, compared to unconjugated drug in vivo (Sobhani et al., 2011). These effects are due to the sustained release of the drug from the nanotubes, leading to prolonged blood circulation and higher uptake by the tumor cells. There is also reported literature for another anticancer drug, cisplatin; when conjugated with SWCNTs, it shows increased concentration in cancer cells (Bhirde et al., 2009).

CNTs are also highly effective in cancer chemotherapy in reducing the multi-drug resistance property of the malignant cells, due to increased efflux of the drug molecules by overexpression of P-glycoproteins (P-gp) on the cell surface. It has been observed that covalent functionalization of CNTs with anti-P-gp antibody can effectively deliver doxorubicin for treatment of human leukemia by reducing P-gp efflux (Li et al., 2010).

4.5.2.2 Cancer treatment application

Besides drug-targeting ability to malignant tumor cells, CNTs lately also have their own therapeutic properties. CNTs have been reported to exhibit cancer-curing properties, particularly when CNTs are exposed to an infrared light source, they tend to generate heat up to 70–160°C in very few seconds (<120 s) (Zhou et al., 2009). Consequently, the approach of placing such heated tubes at the tumor site can easily destroy malignant cells. This property of nanotubes makes them a tumoricidal agent. MWCNTs, especially prepared using the CoMoCat process, are of high research importance, owing to their use in cancer chemotherapy for mild tumoricidal action to reduce the size of tumor. Such nanotubes, when placed on the biological object and irradiated with near-infrared light, absorb light radiations of specific wavelength, i.e., 980 nm, and leads to programmed cell death. This approach in cancer chemotherapy is called photothermal therapy (Huang and El-Sayed, 2010). Reports also suggested that short-time exposure of MWCNTs (<2 s) to strong IR radiations (i.e., 700–1100 nm) can produce hyperthermia up to 42°C for 2 h to accelerate the programmed cell death in colorectal carcinoma cells (Huang and El-Sayed, 2010). Furthermore, hyperthermia increases the cellular uptake of anticancer drugs, plausibly due to enhanced cell membrane permeability. Similarly, MWCNTs associated with nitrogen gas have been found to induce thermal ablation causing apoptosis of cancer cells upon irradiation with infrared light source (Torti et al., 2007). The longer the CNT, the more effective would be the therapy, as less time of exposure would be required with minimal dose of radiation due to larger surface area. It can, accordingly, be concluded that the antitumor activity of CNTs might be due to heat transduction, which leads to less cellular cytotoxicity. Above all, it has been observed that functionalization of nucleic acid, i.e., DNA molecules, with MWCNTs provides enhanced tumoricidal action, which leads to death of malignant cells, plausibly owing to increased heat production and transduction. This approach has an advantage over simple radiation/heat therapy to treat human tumors because of the selective tumoricidal action of DNA-encased nanotubes (Zheng et al., 2016).

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 their efficacy in the management of cancer. CNTs loaded with chemotherapeutic agents have been shown to achieve relatively higher uptake by cancerous tissue without affecting collateral tissues (Ji et al., 2010). Consequently, nanotubes may also be beneficial in dosage reduction by localizing its 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, esp., MWCNTs, when placed at tumor site, specifically destroy malignant cells and seem to act such 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 death of cancer cells upon irradiation with an infrared light source (Zanganeh et al., 2016). It can accordingly be concluded that the antitumor activity of CNTs might be due to heat transduction, which leads to less cellular cytotoxicity.

Literature reports also suggest increased tumoricidal activity of MWCNTs, when functionalized with DNA and siRNA. SWCNT-siRNA complexes have 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 prove to be an effective solution in decreasing the prevalence of human myelogenous leukemia. Apart from these, recent investigations have proved application of CNTs in radiotherapy for killing cancer by decreasing the rate of oxygen uptake to malignant cells, thereby making them susceptible to radiotherapy. Beyond nanotubes, the application of nanohorns have now been explored in chemotherapy, where water soluble nanohorns screened for delivery of anticancer agents have shown promising results (Wang et al., 2016).

4.5.2.3 Brain-targeting application

Many disorders of CNS viz. Alzheimer's disease, dementia, parkinsonism, mood disorder, AIDS, and meningitis (both viral and bacterial) remain untreated because of restricted entry of therapeutic substances. The blood–brain barrier (BBB), an impregnable barrier is designed for protecting brain from external stimuli such as entry of foreign chemical, toxins, pollutants, drug substances to maintain the brain homeostasis. Drug molecules fail to enter into the brain by crossing the BBB, owing to poor lipophilicity. Similarly, conventional drug delivery systems lack the targeting ability to deliver the drug molecules into the brain (Alam et al., 2010). CNTs, in this regard, have been used for the delivery of drug molecules to the brain primarily because of their ability to cross the BBB (Yang et al., 2010). MWCNTs are effective in delivering neuropharmaceutical agents to brain microglial cells over the SWCNTs. Additionally, CNTs are also useful in treating neurodegenerative disorders due to their magnetic properties. Nanotubes, in combination with nerve growth factors, help in treating the neurological disorders by facilitating differentiation of brain neurons (Alam et al., 2010). As reported, both pristine CNTs and f-CNTs, have positive effects on neuronal growth and these can stimulate the growth of neurons (Spinato et al., 2016). It has been observed that MWCNTs functionalized with DNA and siRNA can internalize into brain microglia cells, i.e., macrophages derived from migratory monocytes, which are believed to be elevated in patients with malignant brain tumors.

4.5.2.4 Lymphatic targeting application

Drug targeting to lymphatics is essential for treating disorders of lymphatic systems and targeting drug molecules to the reticuloendothelial system (RES). Magnetic nanotubes are helpful in targeting drug molecules to the lymphatic system by selective functionalization on their surface (Yang et al., 2008, 2009a). The size of the CNTs is highly important for effective uptake of drug molecules into the lymphatics. CNTs, functionalized with folic acid and entrapped in magnetic nanoparticles, have shown better targeting ability to cancer cells in the lymph nodes. By placing a magnet externally, the MWCNTs can be retained in the lymph nodes for several days to facilitate the continuous release of the targeting chemotherapeutics (Ji et al., 2016).

4.5.3 OCULAR DRUG DELIVERY APPLICATION

Ocular delivery of drugs is challenging, owing to poor retention time of the dosage forms containing drug molecule because of high tear flow. CNTs, accordingly, provide the advantage of enhanced retention ability in the retinal site to release the drug molecules (Chaurasia et al., 2015). They also have the ability to cross the blood–retinal barrier for mechanizing the entry of antibiotics, anticholinergics, mydriatics, etc. for treating diverse ocular diseases (Sinha and Yeow, 2005). However, the ocular route has not been explored much in this regard. Accordingly, we are calling for further exploration of CNT application in ocular delivery (Mehra et al., 2015).

4.5.4 TRANSDERMAL DRUG DELIVERY APPLICATION

CNTs are helpful in efficient transdermal electrophoretic and iontophoretic delivery of drugs through skin. The f-CNT membranes allow fast flow of drugs through the CNT cores, owing to their dramatically high charge density and small pore size (Degim et al., 2010). Furthermore, CNT membranes are also integrated with the drug molecule to obtain switchable transdermal delivery devices. These devices offer minimal skin irritation without disruption of any skin barriers. As reported, MWCNTs, have shown enhanced transdermal delivery of nicotine with the application of electric pulses for few milliseconds (Im et al., 2010). Recent literature reports have also verified the application of indomethacin, doxorubin, and clonidine for transdermal delivery of drugs (Schwengber et al., 2015; Degim et al., 2010). Nanotube membranes have recently gained immense popularity in transdermal delivery of nicotine for cessation of smoking and opioid withdrawal symptoms (Strasinger et al., 2009; Gulati et al., 2016).

4.5.5 SOLUBILITY ENHANCEMENT APPLICATION

CNTs, obtained through synthetic routes, are strongly hydrophobic in nature. However, treatment of such CNTs with strong acid solution (H_2SO_4 or HNO_3), causes formation of end-defects, and rendered them hydrophilic due to generation of carboxyl groups on their side-walls and tips owing to covalent functionalization (Dyke and Tour, 2004). Furthermore, secondary or double functionalization of nanotubes with surfactants, polymers, nucleic acids, also makes them hydrophilic. The poorly water soluble drugs can be further attached on the surface of CNTs to enhance their solubility too (Tagmatarchis et al., 2006). The basic mechanism by which CNTs improve the solubility is plausibly owing to “functionalized-partitioning.” As reported, carboxyl MWCNTs, are highly effective in enhancing the oral bioavailability of a poorly water soluble drug, carvedilol (Li Y et al., 2011b). The f-CNTs also have beneficial aspects of delivering several hydrophobic biomolecules (e.g., proteins, peptides, nucleic acids, enzymes) to enhance their bioavailability. SWCNTs have the advantage of formation of host–guest complexes with cyclodextrins for improving the solubility and deliverability of various guest drug molecules (Ogoshi et al., 2008).

4.5.6 VACCINE DELIVERY APPLICATION

Vaccine delivery tends to suffer from myriad problems, such as improper absorption on carriers, hypersensitivity and anaphylactic reactions by the adjuvants, and susceptibility to antigen-induced immune response (Gottardi and Douradinha, 2013). Several novel delivery strategies and carriers have been investigated for vaccine delivery, which include liposomes, microspheres, nanoparticles, etc. Lately, CNTs explored for vaccine delivery have demonstrated immense promise in improvement of effectively inducing the immune response, such as adjuvants (Kostarelos et al., 2009). Conjugation of CNTs with antigenic peptides can provide safe and effective delivery of synthetic subunit vaccines. Both SWCNTs and MWCNTs are highly useful in the delivery of vaccines, where the said nanocarriers have shown improved performance for complement activation, protein adsorption, and generating the immune response by formation of the antibodies (in het Panhuis, 2003; Salvador-Morales et al., 2006).

4.5.7 GENE DELIVERY APPLICATION

Gene delivery suffers from challenge of difficulty in transfection of DNA, mRNA, siRNA, and other nucleic acid carriers across the cell membrane or nuclear membrane. CNTs have been widely used for improving gene delivery, owing to their capability of replacement of damaged or missing genes, and transportation of DNA into cells (Bianco, 2004). Synthesis of hybrid structures constituting

nanotubes combined with dendrimers by grafting can be helpful in loading nucleic acids for correcting the gene defects, by delivering the loaded cargos into the cells (Pantarotto et al., 2004). Recently, application of CNTs in the form of matrices to support neural growth has been reported elsewhere. Ammonium-functionalized CNTs can be used as vectors for delivering nucleic acids and plasmid DNA for improving the efficacy of gene therapy, in comparison with DNA alone (Prato et al., 2008). Complexation of siRNA with SWCNTs for efficient gene delivery is a newer approach for augmenting gene silencing in genetic diseases. Short interfering RNA and SWCNT complexes can be easily taken up by splenic immune recognizing CD11c + , CD11b + and Gr-1 + CD11b + cells for faster induction of the immune response after delivering the loaded gene. It has been reported that when single-stranded DNA is tagged onto the surface of SWCNTs, it helps in the protection of DNA probes from possible enzymatic cleavage and interference from nucleic acid-binding proteins (Liu et al., 2007b). Studies have shown that a DNA probe can target a specific mRNA inside living cells when coupled with SWCNTs, which increases its self-deliverability and intracellular biostability, compared to that of the free DNA probes. Consequently, new conjugate systems provide greater potential for applications in the field of genetic engineering.

4.5.8 TRANSFECTING AGENTS

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. Recently, functionalized CNTs have shown promising outcomes in the treatment of these diseases, owing to their ability to easily conjugate drugs such as amphotericin B (Wu et al., 2005), dapsone, etc. Conjugation of AmB to these f-CNTs have shown to have reduced toxicity and enhance antimycotic efficiency. In addition, their targeted deliveries to macrophage cells have also been indicated by researchers (Ryoo et al., 2010; Vuković et al., 2010).

4.6 BIODISTRIBUTION OF CARBON NANOTUBES

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 SWCNTs and MWCNTs are excreted as intact nanotubes. Functionalizations of these SWCNTs have also shown to prolong the SWCNT blood circulation, reduced uptake in the RES, and complete clearance from major organs.

Recently, functionalized CNTs have been used for the delivery of biomolecules and drugs to the 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, their cellular internalization needs to be extensively studied. Scientists across the globe have proposed different pathways for their 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 (Liu et al., 2007a). Two most commonly understood mechanisms include direct cytoplasmic translocation and receptor-mediated endocytosis. The first pathway acts as a passive diffusion mechanism, where CNTs undergo diffusion across lipid bilayer of the cell membrane to deliver the loaded cargos into the cells (Mu et al., 2009). MWCNTs have been shown to cross cell membranes by means of an energy-dependent process, employing the usage of ATP. Some scientists have also reported that CNTs of approx 100 nm are able to fit into caveolae and clathrin vesicles, while bigger sized CNTs are taken up by means of macro-pinocytosis pathway (Mu et al., 2009).

4.7 CONCLUSIONS

CNTs have been explored as multipurpose innovative carriers for application in delivering drugs and biomolecules. Owing to this, CNTs have drawn significant attention by nanotechnologists, from industry as well as academia. In the last two decades, remarkable work has been carried out on CNTs for biomedical applications. Functionalization is a versatile area which has opened newer perspectives in the application of CNTs in advanced drug delivery. Attachment of an organic moiety to nanosized tubes has facilitated their use for diagnostic and targeting purposes, especially in cancer and treatment of infections. Despite their promising role in nanomedicines, CNTs require extensive research investigations to guarantee their safety in clinical setup. Toxicity studies, therefore, are needed for establishing evidence of safety in full scale under in vivo conditions for real time applications and commercialization marketing. Physiological, physico-chemical, and molecular processes need to be considered for understanding clinical and preclinical toxicity of CNTs. Regardless of knowledge gained in nanotoxicology, scientists have not yet been able to precisely forecast the biological behavior and biokinetics of CNTs. Furthermore, commercialization of CNTs requires strict regulations that 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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