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Pharmaceutical and biomedical applications of surface engineered carbon nanotubes

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Surface engineered carbon nanotubes (CNTs) are attracting recent attention of scientists owing to their vivid biomedical and pharmaceutical applications. The focus of this review is to highlight the important role of surface engineered CNTs in the highly challenging but rewarding area of nanotechnology. The major strength of this review lies in highlighting the exciting applications of CNTs to boost the research efforts, which unfortunately are otherwise scattered in the literature making the reading non-coherent and non-homogeneous.

Introduction

In the current scenario, CNTs are one of the forefronts in a diverse field of medicine, which are continuously being explored by researchers, scientists and academicians for the development of new, safe and effective nanomedicine. The CNTs are promising quasi one-dimensional nanomaterial with well ordered flat networks of fused benzene rings or hollow graphitic cylindrical tubular structure consisting of hexagonal arrangement with sp^2 hybridized carbon atoms [1–4]. CNTs are mainly classified into four types, based upon the structure and diameters including single-, double-, triple-, and multi-walled carbon nanotubes. The length of the tubes can be extended depending on the type of production method [5–8]. CNTs are one of the attractive tools in nanotechnology with surface engineered carbon nanotubes (*f*-CNTs), emerging as a new family of carbon nanovectors that enabled numerous biomedical applications. Functionalization plays a pivotal role in the design of CNTs-based nanomaterials by rendering them more biocompatible and less toxic with increased dispersibility in comparison to pristine CNTs. The methodology of functionalization of CNTs can be classified broadly into two categories: (a) covalent attachment of active biochemical moieties through chemical reactions onto CNTs skeleton, and (b) non-covalent adsorption or wrapping of different functional

molecules [7,9]. The various types of nanocomposites have been summarized in Fig. 1.

Cellular trafficking mechanism of carbon nanotubes

The study of cellular uptake mechanism of the surface engineered CNTs has been intensely addressed in the last two decades [10,11]. Lacerda *et al.* have demonstrated that the surface engineered CNTs have propensity to enter the cells *via* an energy-dependent, endosomally-mediated cellular internalization approach and direct cytoplasmic translocation through insertion or passive diffusion in a non-invasive manner (tiny nanoneedle mechanism) [11–13].

The *f*-CNTs have been easily cross the blood brain barriers (BBB) without requirement of any external transporter device owing to nano-dimension and tiny nano-needle tubular structure morphology (Fig. 2A). The main pathways speculated by scientists for cellular uptakes of *f*-CNTs are: (i) phagocytosis, (ii) CNTs piercing into membrane by passive diffusion, (iii) caveolae-mediated endocytosis, (iv) clathrin-mediated endocytosis, and (v) caveolae-clathrin mediated endocytosis. The intracellular uptake mechanisms of CNTs are crucial for the controlled uptake and devising new strategies in the field of novel drug delivery [12,13].

Reports have shown that the nanotubes may enter cells rapidly with free trafficking into the cytoplasm in the first hour of internalization. This penetration of surface engineered CNTs into phagocytic and non-phagocytic cells is mediated by three alternative pathways: i) *via* membrane wrapping as individual tubes; ii) *via* direct membrane translocation of individual nanotubes;

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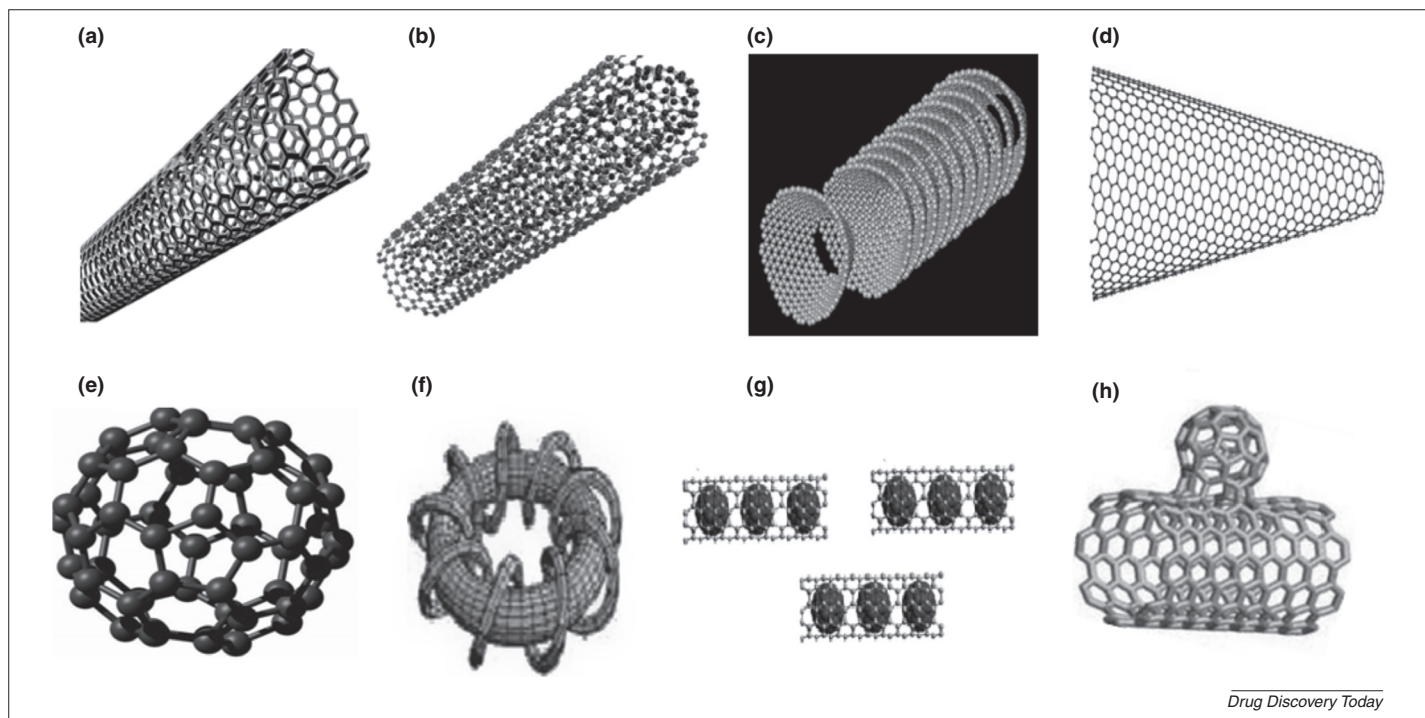


FIG. 1

Various nanocomposites: (a) SWCNTs, (b) MWCNTs, (c) Cup-stacked CNTs, (d) Carbon nanohorns, (e) Fullerenes, (f) Carbon nanotorus, (g) Carbon nanopeapods, and (h) Carbon nanobuds (Adopted from www.google.com; accessed date: 21st September, 2014).

and iii) in bundles within vesicular compartments (Fig. 2B) [13]. These exciting facts about intracellular trafficking of CNTs have opened a new door for the development of a promising delivery system for therapeutic and diagnostic agents [11].

Pharmaceutical and biomedical applications of surface engineered carbon nanotubes

The potential of surface engineered CNTs and possible biomedical applications are discussed below and various CNTs conjugates employed in bioactive delivery shown in Fig. 3.

Transdermal drug delivery

Transdermal drug delivery system (TDDS) represents a convincing as well as promising alternative to oral or injectable delivery of drugs. Recently, CNTs have been investigated as TDDS due to their tiny nano-needle shape structure and superior adsorption capacity. Im and co-workers prepared an electro-sensitive TDDS of CNTs using electrospinning method to control drug release with polyethylene oxide and pentaerythritol triacrylate polymers. In this TDDS, CNTs facilitated the electro-sensitive-transdermal drug delivery [14]. The advantages of CNTs in TDDS include high loading efficiency and enhanced transdermal penetration of drugs by application of a small electrical bias to create a programmable drug delivery system. The future explorations of *f*-CNTs in TDDS present a feasible solution to limitation of current smoking cessation and opioid withdrawal symptom treatments [15,16].

Ophthalmic drug delivery

Several approaches have been exploited in the development of safe, biocompatible and comfortable nanovehicle for controlled ophthalmic drug delivery with enhanced patient compliance and

reduced toxicity. Ophthalmic drugs have a short residence time (<5 min) and only 1–5% of applied therapy may penetrate the cornea and enter into the intraocular tissues. The CNTs-based ocular drug delivery has been found to enhance the bioavailability of few ophthalmic medications overcoming the limitation of traditional ophthalmic drug delivery methods [17]. CNTs could also be used for ocular targeting of different therapeutic agents to the different ocular sites. Unfortunately only very few reports are available in this area [18]. Application of 'Kajal' (also known as *kohl* or *surma*) is a common practice in Indian families. Kajal has been claimed for prevention and treatment of various eye diseases (blepharitis, cataract and conjunctivitis *etc.*) and also said to ward off an 'evil eye'. Cosmetic application of CNTs is well documented in various literatures [19,20,21].

Central nervous system disorders

Surface engineered CNTs based delivery systems have been explored as a new platform to repair the damaged CNS tissue/cells in the field of neuroscience. The CNTs have been claimed to be able to cross the blood brain barrier (BBB) by different targeting mechanisms and act as safe and effective targeted drug delivery system [22,23]. Since the last one-decade researchers have continuously explored and assessed the CNTs as substrate for neuronal tissue growth, regeneration and its application in neuroscience research. CNTs have numerous salient features with a simple and inert molecular tubular nanoneedle structure and porosity beneficial to neural interface for trafficking of cells, sensing of microenvironments, delivery of transfection agents, and scaffolding for incorporating within host body, to provide the support for adhesion, proliferation and migration of cells. Ren and co-workers developed a dual targeted delivery system based on the oxidized PEGylated

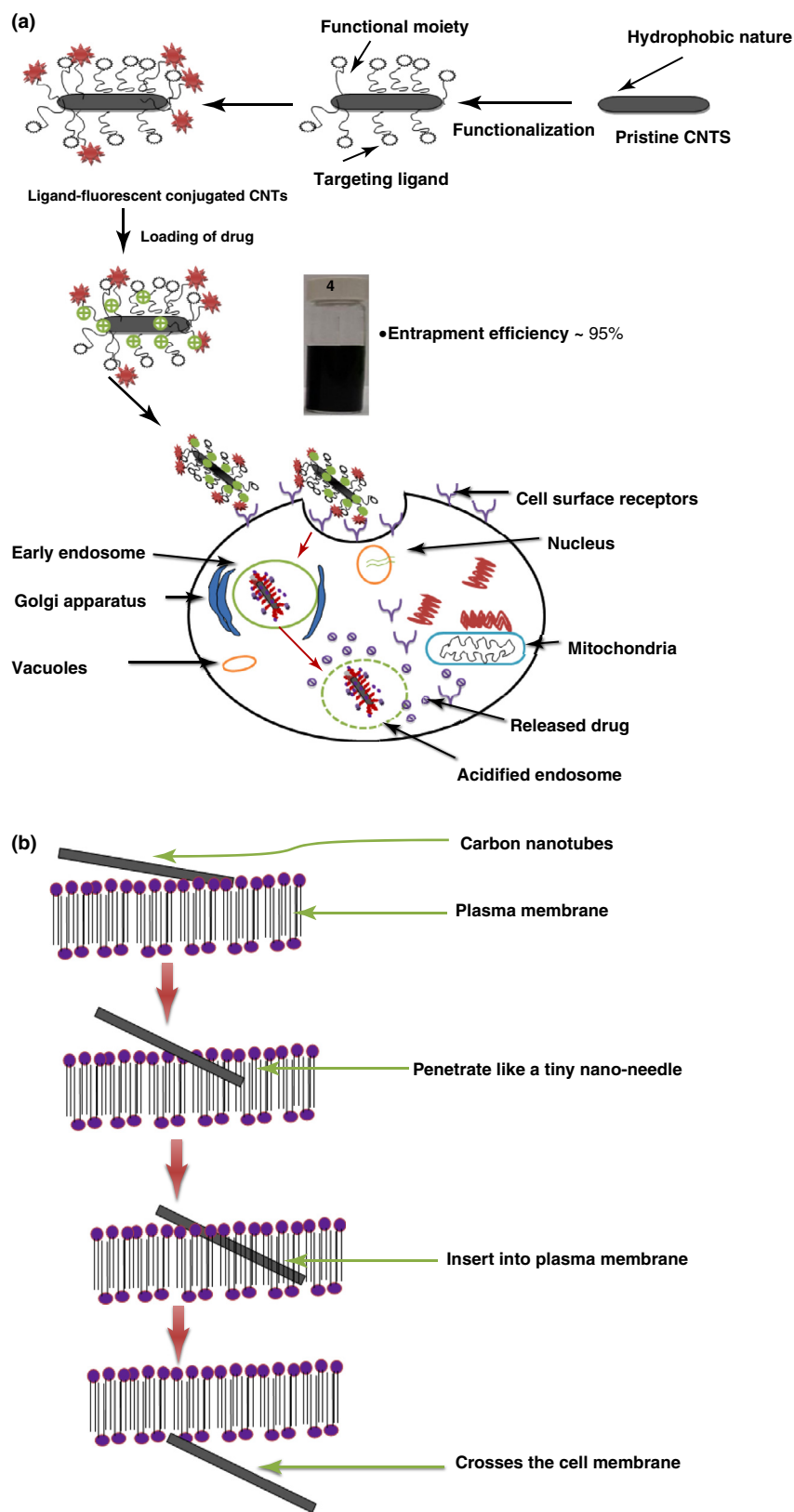


FIG. 2 (A) Receptor mediated endocytosis targeted drug delivery via parenteral administration of CNTs formulation. (B) CNTs penetrate and cross the plasma cell membrane due to their tiny needle dimension.

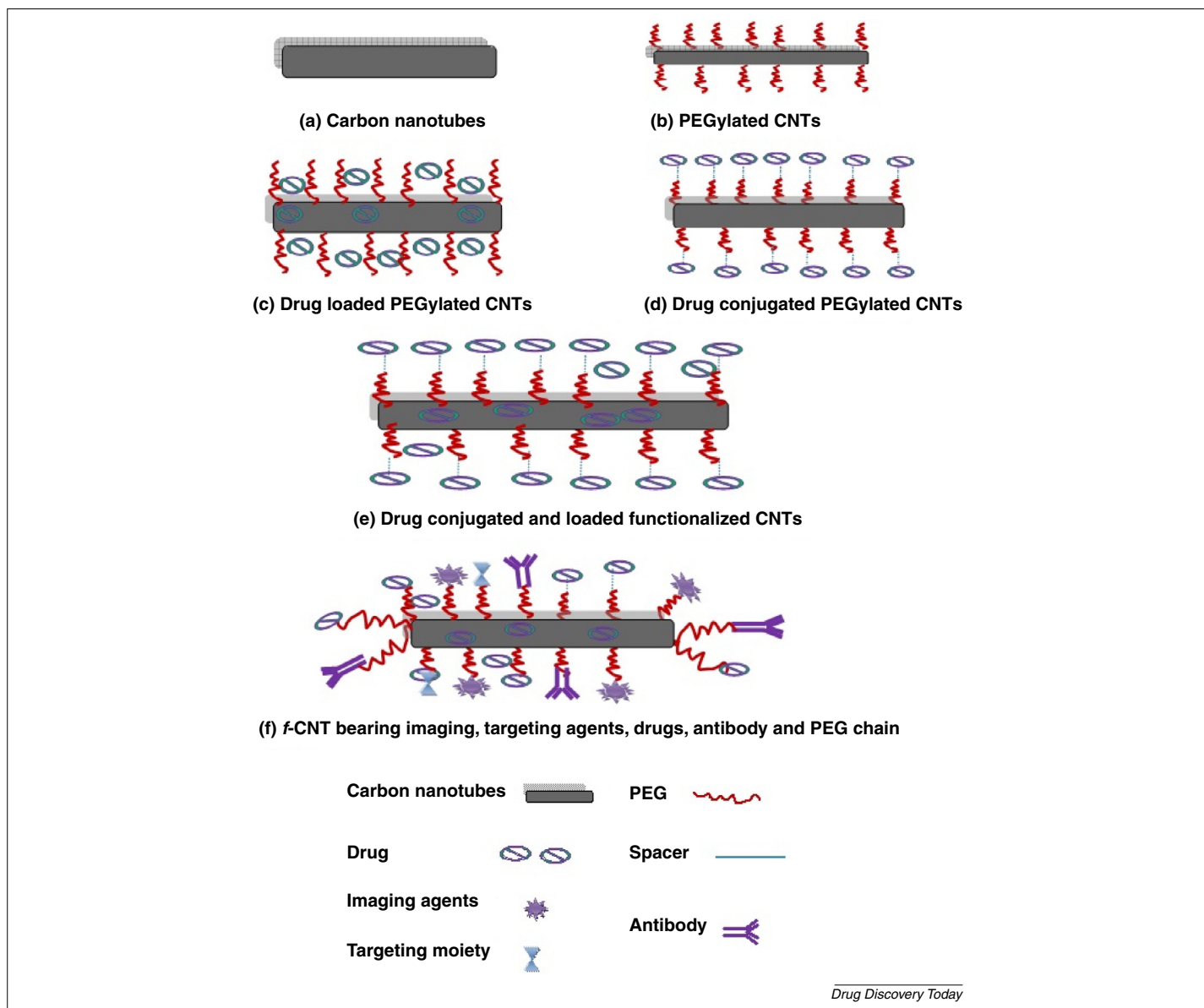


FIG. 3

CNTs conjugates employed in bioactives delivery.

MWCNTs modified with Angiopo-2 (a targeting ligand of low-density lipoprotein receptor related-protein (LRP) receptors) loaded with Doxorubicin (DOX) and systematically evaluated their *in vitro* and *in vivo* anti-glioma effect by C6 cytotoxicity and median survival time (MST) of intracranial C6 glioma bearing Balb/c mice. The combination of O-MWCNTs-PEG with angiopo-2 as targeting ligand was claimed to play a role of active dual-targeting and combination of O-MWCNTs-PEG and angiopo-2 constituted an ideal dual-targeting drug delivery to brain glioma [22].

Photodynamic therapy

Photodynamic therapy (PDT) is an effective, alternative, non-invasive, non-toxic therapy used in oncology [24]. Till date only few numbers of reports are available on the applications of surface engineered CNTs in PDT. Photosensitizer [5-aminolevulinic acid (5-ALA)] loaded PAMAM dendrimers modified MWCNTs (dMNTs) showed good biocompatibility with significant increase in the

accumulation of 5-ALA in MGC-803 tumor cells leading to a potential photodynamic anti-tumor effect [25]. Chitosan wrapped Chlorine6 (Ce6), which is a photosensitizer, loaded SWCNTs revealed high cellular uptake (high anticancer effects) and low toxicity against HeLa cancer cells than free Ce6 [26].

Photothermal therapy

CNTs hold promise in photothermal therapy (PTT) due to extraordinary photon-to-thermal energy conversion efficiency with high absorption in the near infra-red (NIR) light [27–29]. The SWCNTs have the ability to absorb 700–1100 nm NIR radiation and convert it into heat, which kills cancer cells [29]. Moon and co-workers demonstrated the photothermal effect of PEGylated SWCNTs upon NIR irradiation on KB tumor bearing nude mice and observed that sufficiently high thermal energy was generated from optically excited PEG-SWCNTs to destroy tumor in a non-invasive manner. The mice treated with PEG-SWCNTs and NIR irradiation

TABLE 1

Various bioactives delivered through functionalized CNTs for pharmaceutical and biomedical applications

Functionalized CNTs	Cell lines used	Bioactives agents used	Physicochemical/biological parameters	Outcomes of the study	References
FITC-MWCNTs & Oxidized MWCNTs	Human Jurkat T lymphoma cells	Amphotericin B (AmB) via covalent attachment	Electron microscopy Antifungal activity	AmB covalently linked to CNTs is taken up by mammalian cells devoid any specific toxic effect. <i>f</i> -CNTs are able to enter the cell by a spontaneous mechanism.	[34]
PL-PEG-FA & PL-PEG-FITC-SWCNTs.	HeLa cells	Cy3-labeled DNA	Atomic force microscopy Confocal laser scanning microscopy	SWCNTs internalized in living cells can act as tiny NIR 'heaters' or 'antennas'.	[10]
PL-PEG-RGD-SWCNT & FITC annexin V-SWCNTs.	MCF-7 breast cancer and U87 MG cancer cell lines	Doxorubicin	Fluorescence Supramolecular loading efficiency	Enhanced uptake of DOX in case of integrin-positive U87 MG using RGD-based targeting relative to integrin-negative MCF-7 Cells.	[40]
PL-PEG-SWCNTs	Ntera-2	Cisplatin prodrug conjugate	Fluorescence microscopy	Cytotoxicity of the free platinum(IV) complex increases by >100-fold when the complex is attached to the surface of the functionalized SWNTs.	[41]
Pluronic F 127 stabilized-MWCNTs	MCF-7 human breast cancer cells	Doxorubicin through π - π stacking	Electron microscopy Confocal laser microscopy	Enhanced cytotoxicity of DOX-MWCNTs complex over free DOX.	[42]
DSPE-PEG5000-PTX-SWCNTs PEG- SWCNTs.	4T1 tumor-bearing mice (tumor size, \sim 200 mm ³).	Paclitaxel (PTX)	Pharmacokinetics and biodistribution study Antitumor efficacy Pilot toxicity study	Water-soluble SWCNTs-PTX formulation is cremophor-free. SWCNTs have shown to be safe at least in mouse models.	[43]
CNTs	Bladder cancer cells	Carboplatin	Electron energy loss spectroscopy X-ray-photoelectron spectroscopy Cell proliferation Cytotoxicity	High efficacy of carboplatin filled CNTs with reduced growth of bladder cancer cells compared to free drug CNTs.	[44]
Folic acid (FA)-chitosan (CHI)/alginate (ALG)-SWCNTs.	HeLa cell line	Doxorubicin through π - π stacking.	Fluorescence Cell viability and cell uptake studies	CNTs complex found to be more selective and effective than free DOX due to targeting based on FA and release of DOX at lysosomal pH.	[45]
mAB-BSA adsorbed-SWCNTs.	WPD _r colon cancer cell line	Doxorubicin through π - π stacking.	Electron microscopy (SEM, HRTEM) Raman spectroscopy	Uptake study revealed that delivery efficiency was 100%. All cells could take up the SWCNT complexes.	[46]
Diaminotriethylene glycol-MWCNTs.	MKN-28 cells and H22 cell lines	10-Hydroxycamptothecin (HCPT) through π - π stacking.	<i>In vitro</i> single photon emission computed tomography (SPECT). <i>ex vivo</i> gamma scintillation counting analysis	MWCNTs-HCPT conjugate enhanced superior antitumor activity both <i>in vitro</i> and <i>in vivo</i> to clinical HCPT formulation. They show relatively long blood circulation and high drug accumulation at the tumor site.	[47]
Dapsone-MWCNTs-FITC	Subvert normal macrophage (M \emptyset) and PM \emptyset	Dapsone (DDS)	Confocal laser microscopy Phagocytosis assay	Dapsone-MWCNTs did not trigger oxidative stress but caused apoptosis of PM \emptyset predominantly after prolonged cultivation (3 days).	[48]
Azomethine ylide (1.3-dipolar cycloaddition)-MWCNTs.	MCF-7 Cell line.	Methotrexate (MTX) through cleavable ester bond.	Electron microscopy Cytotoxicity study	Cytotoxic activity of CNTs conjugate was strongly dependent on the presence and type of linker.	[49]

EGF-SWCNTs–cisplatin–EGF	Head and Neck Squamous Carcinoma Cells (HNSCC), HN12 cells	Cisplatin	Histological analysis Raman analysis Annexin V assay	PEG-SWCNTs, as opposed to control SWCNTs, form more highly dispersed delivery vehicles that, when loaded with both cisplatin and EGF, inhibit growth of squamous cell tumors.	[50]
Sgc8c aptamer-SWCNTs	Molt-4 (Target) and U266 non-target (B lymphocyte human myeloma) cell line	Daunorubicin (anthracycline antibiotic) through π - π stacking	Cell viability Loading efficiency ~157% Flow cytometry analysis	Dau-aptamer-SWCNTs complex was able to selectively target Molt-4 cells compared to non target cells.	[51]
CNT-PEI and CNT-pyridinium-MWCNTs	Human lung cancer cells (H1299)	siRNA	Gene silencing and cytotoxicity agarose gel electrophoresis	CNT-PEI and CNT-pyridinium did not show any added value over PEI, pDMAEMA, and Lipofectamine used as reference transfection agents for siRNA silencing activity and cytotoxicity.	[52]
Hyaluronate tethered MWCNTs	A 549 cell line	Doxorubicin (DOX)	Pharmacodynamic and organ distribution studies Tumor growth inhibition studies Apoptosis study Cytotoxicity study	The ^{99m} Tc-MWCNT-HA-DOX is significantly higher than both free drug and non-targeted MWCNTs in organ distribution studies on Ehrlich ascites tumor (EAT) bearing mice. The tumor-growth inhibitory effect of HA-MWCNTs-DOX was found to be 5 times higher than free DOX in chemically breast-cancer-induced model in pharmacodynamic studies devoid of any detectable cardiotoxicity, hepatotoxicity, or nephrotoxicity.	[53]
DOX-FA-MN-MWCNT	U87 cell lines	Doxorubicin	Electron microscopy CLSM.	Dual targeted DOX-FA-MN-MWCNTs showed the high drug loading, pH-dependent controlled release, magnetic targeting, and ligand–receptor interactions.	[37]
AmB/Mannose-MWCNTs	J774 cells	Amphotericin B (AmB)	CLSM <i>In vitro</i> & <i>in vivo</i> studies macrophage uptake study	AmBitubes showed better targeting efficiency to macrophages <i>i.e.</i> J 774 cell lines with reduced toxic effects associated with AmB.	[36]
DOX/DEX-MWCNTs	A-549 cells	Doxorubicin (DOX)	<i>In vitro</i> & <i>ex vivo</i> studies	DOX/DEX-MWCNTs found less hemolytic and more cytotoxic as compared to free DOX on A-549 lung epithelial cancer cell line.	[38]
Functionalized MWCNTs	Raw 264.7 and A-549 cell lines	–	Immunofluorescence microscopy	The chemical functionalization tailors the fate of CNTs by tilting the balance toward specific mechanisms of internalization, cellular processing and elimination/ degradation.	[13]
Angiopeptide conjugated PEGylated oxidized MWCNTs	Brain capillary endothelial cells (BCEC) and C6 glioma cells	Doxorubicin (DOX)	Anti-glioma efficacy Intracellular fluorescence distribution. Fluorescence imaging	The results showed that DOX-O-MWCNTs-PEG-ANG is a promising dual-targeting carrier to deliver DOX for the treatment of brain tumor	[22]

TABLE 1 (Continued)

Functionalized CNTs	Cell lines used	Bioactives agents used	Physicochemical/biological parameters	Outcomes of the study	References
f-SWCNTs-COS-GTX-p53	HeLa and MCF-7 cells	Glilotoxin	Cytotoxicity study Fluorescent activated cell sorter analysis	The f-SWCNTs-COS-GTX-p53 was found to be most effective delivery vehicle with a controlled release and enhanced cytotoxicity rendered through apoptosis in HeLa cells.	[54]
GL-MWCNTs-DOX	HepG2 cell	Doxorubicin	Electron microscopy Flow cytometry and phase contrast microscopy Anticancer activity	GL-MWCNTs-DOX formulation approach was found to be promising toward development of an affluent liver targeting.	[55]

MWCNTs: Multi walled carbon nanotubes; SWCNTs: Single walled carbon nanotubes; FITC: Fluorescence isothiocyanate; PEI: Polyethyleneimine; PL-PEG: Phospholipid-Polyethylene glycol; RGD: Arginine-glycine-aspartic acid; NGR: Asn-Gly-Arg; HCP: Hydroxycamptothecin; NIR: Near infrared; EGF: Epidermal growth factor; PL-PEG-FA: Phospholipid polyethylene glycol-folic acid; PEI: Polyethyleneimine; siRNA: small interfering ribonucleic acid; AmB: Amphotericin B; Amb: GTX: Gliotoxin; GL: Glycyrrhizinic acid; DOX: Doxorubicin.

showed the complete eradication of solid malignant tumor devoid of any toxicity or abnormal behavior 20 days post treatment. The issue concerned with residual SWCNTs accumulation inside the body after the treatment was resolved as nanotubes were excreted from mice's body in about 2 months through the biliary or urinary pathway in biodistribution study [27]. Hashida and co-workers reported the photothermal ablation activity of a novel SWCNTs-peptide composite with a designed peptide having a repeated structure of H-(-Lys-Phe-Lys-Ala-)₇-OH[(KFKA)₇] against tumor cells. The obtained results suggest the remarkable suppression of tumor growth compared with only SWCNT-(KFKA)₇ injection alone or NIR irradiation alone [28].

Gene therapy

Currently viral vector system based gene delivery is most widely used to achieve high efficiency of gene expression, which concerns the safety, as viral vector can be immunogenic, oncogenic, and may be responsible for inflammation. Further, gene therapy may suffer rapid degradation of exogenous nucleic acid. Now-a-days some effective multiple non-viral delivery systems like polymeric nanoparticles, liposomes, cell penetrating peptides, cationic lipids, dendrimers and f-CNTs are being employed to efficiently deliver nucleic acids including siRNA with reduced immunogenicity. The efficiency of gene expression mediated by these nanovectors is lower as compared with the viral vector but they provide the advantages of easy up-scaling, flexibility and reduced immunogenicity. The applications of CNTs as new vectors for delivery of nucleic acids is another area of research and development which could offer several advantages for nucleic acid, siRNA, gene and protein delivery owing to their thin and long architecture that offers a large surface area to which siRNA can be bound and delivered to target site. Further, CNTs may have protective capacity, which stabilize and lead to more efficient cellular transfection and prevent the lysosomal degradation of encapsulated nucleic acids [30]. Kawaguchi and co-workers synthesized DNA-functionalized SWCNTs to improve the dispersibility as compared to acid treated SWCNTs. The binding ratio of the acid-treated SWCNTs and DNA was calculated to be 3:1 (DNA/SWCNTs) from the phosphorous content in DNA-SWCNTs [31]. In context of the enhanced gene delivery Sanz and co-workers were first to develop and demonstrate the enhanced gene delivery using lysosomotropic anti-malarial drug chloroquine loaded polyethyleneimine (PEI)-coated DWCNTs to enhance the cell transfection efficiency [32].

Treatment of infectious diseases

Infectious diseases such as tuberculosis, leishmaniasis, severe acute respiratory syndrome (SARS), flu (swine, bird and avian) and anthrax infection indicate that infectious diseases have emerged as a critical public health issue with global concerns. Surface engineered CNTs have been used in the treatment of infectious diseases due to their ability to easily conjugate drugs like amphotericin B (AmB) and dapsone. Leishmaniasis is a microbial disease in which macrophages and other cells of reticuloendothelial system (RES) serve as host as well as replication site for the parasite [33]. Conjugation of amphotericin B (AmB) to f-CNTs can modify its properties in terms of toxicity and anti-mycotic efficiency [34,35].

Wu and co-workers explored the toxicity and antifungal activity of AmB loaded CNTs (AmB-CNTs) functionalized with fluorescein isothiocyanate (FITC) for efficient nuclear localization in human Jurkat lymphoma T cells and observed that AmB-CNTs could be easily taken up by mammalian cells precluding any specific toxic effect but preserving antifungal activity [34]. CNTs surface engineered with mannose showed promising targeted delivery of AmB to J774 macrophages cells [36].

Cancer theranostics

Cancer is one of the leading causes of death worldwide. More than 99% cases of chemotherapy destroy cancer cells along-with the normal healthy cells, with serious side effects and hence strategies based on nanotechnology are being explored as 'safe and effective' strategy to achieve selective uptake of chemotherapeutic agent by target cancer cells [7].

A significant decrease in toxicity with preferential killing of cancer cells was achieved with dual targeted nanocarrier combining MWCNTs and iron oxide magnetic nanoparticles (MNs) conjugated with FA for antineoplastic agent, DOX [37]. Jain and co-workers observed tumor targeted delivery of DOX and reduced toxicity with DOX loaded dexamethasone mesylate anchored MWCNTs to A-549 lung epithelial cancer cells [38]. Further, CNTs surface engineered with folic acid and D-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) showed tumor targeted delivery of DOX with improved cytotoxicity toward cancer cells and enhanced cellular uptake speculated to be mediated by endocytosis and tiny nano-needle mechanism [2,8]. Fig. 3 presents the different conjugates based on CNTs employed in the delivery of anticancer bioactives varying from pristine CNTs to drug loaded CNTs. From the aforesaid account it may be concluded that the surface engineered CNTs could have promising potential in cancer therapy.

Macrophages targeting

Reports showed that CNTs may emerge as promising nanocarrier in targeting of macrophages owing to their exceptional physico-chemical characteristics. AmB conjugated CNTs showed improved antileishmanial activity as compared to free AmB in J774A.1 macrophage cells [39]. Later, AmB loaded mannose conjugated CNTs showed efficient macrophage targeting in J774 macrophage cell lines with significantly reduced toxicity toward RBCs and kidney cells showing considerable reduction in the nephro- and hemolytic-toxicity, which are the major constraints in clinical use of AmB [36].

A brief summary of various bioactives delivered through surface engineered CNTs is shown in Table 1 [10,13,22,34,36,40–55].

Diagnosis

CNTs alone as well as in combination with other nanocarriers like dendrimers, nanoparticles and quantum dots are emerging as promising novel drug delivery and diagnostic nano-vector tool with minimal toxicity and immunogenicity. Delogu and co-workers assessed the echogenic property of nanotubes *in vitro* and found that the ultrasound signal of *f*-MWCNTs is higher than the pristine MWCNTs, graphene oxide and *f*-SWCNTs. The authors found that the MWCNTs are highly echogenic in liver and heart as examined on pig as an experimental animal model. Thus the nanotubes

could show enormous potential as ultrasound contrast agents and theranostics moiety combining diagnostic and therapeutic modalities [56].

Biological fate of carbon nanotubes

The smart/intelligent surface engineered carbon nanotubes are still having controversy about their biodegradable/non-biodegradable nature. Previous literature suggested non-biodegradable nature of CNTs and excretion through biliary pathway, especially *via* urine and feces. However, few investigations observed that CNTs are degraded in specific environments *via* natural, enzymatic catalysis, natural horseradish peroxidase (HRP) with low concentration of H₂O₂ (approximately 40 μM) at 4 °C over 12 weeks or in the presence of human neutrophil enzyme, myeloperoxidase. Importantly biodegraded nanotubes did not produce any inflammatory response when aspirated in to the lungs of mice [57–59]. Bianco and co-workers investigated the biodegradation of SWCNTs and MWCNTs under different conditions and concluded that the oxidized MWCNTs were highly degrading in nature as compared to SWCNTs [59]. These few recent literature reports support the biodegradable nature of CNTs, otherwise previously, CNTs were considered non-biodegradable and excreted out as examined by electron microscopic studies. Additionally biocompatible nanotubes could be prepared *via* conjugation of phospholipids (PL) and polyethylene glycol (PEG) chains on to them, which may be a promising alternative candidate in biomedical applications [58–60].

Regulatory considerations

The toxicities associated with the CNTs are yet unclear. Several research studies demonstrated the toxicity of pristine CNTs, which could fortunately be minimized *via* surface engineering that renders them more biocompatible and non-immunogenic.

The specific structural properties of CNTs like needle-shape, long and thin and biopersistent nature (insoluble) may produce adverse pulmonary effect but this potential risk could be avoided by use of surface engineering of CNTs. However, till date CNTs' eco-toxicological data are limited and controversial and also lack reliable information of exposure as per Occupational Health and Safety regulations. In contrast to ecotoxic point of view, surface of CNTs can often be modified with the different chemical reactions or by 'grafting to' and 'grafting from' make CNTs more biocompatible with better stability. Additionally, there is a significant need for research and development in the field of analytical and detection methods. Strict norms and regulations are needed in the development of new drug products for being safe, effective and economic in generally regarded as safe (GRAS) prominence.

Conclusion and future perspective

CNTs' potentials as novel carriers in bioactives delivery make them promising candidate for the development of new pharmaceutical products in clinical relevance. Before the clinical use of CNTs based products; their pharmacological and toxicological parameters must be proved to be safe and effective. Surface of CNTs could be easily engineered with targeting and imaging agents, peptides, proteins, siRNA, nucleic acids, antibodies and chemotherapeutic drugs for aforementioned pharmaceutical and biomedical applications. These surface engineered CNTs

are considered promising nanomaterial for use in many biomedical applications, including biocompatible modules for the delivery of bioactives. In conclusion, before realizing the full potential of CNTs, it is very important to generate data on their safety, efficacy, and feasibility. Although the scientists are optimistic about the possible application of CNTs yet learning the lesson from past (like thalidomide tragedy), the safety and efficacy of CNTs/CNTs based products/devices *etc.* must be established beyond doubt. We have witnessed the emergence of several

materials/devices/products, which could not make their way to market but then that's the research. Let's keep our fingers crossed and wait for the **conclusive** proof regarding the possible application of CNTs, particularly in biomedical arena. No conclusion should be drawn either in haste or without conclusive evidence of safety.

Conflict of interest

The authors report no conflict of interest related to manuscript.

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