



CARBON NANOTUBES: SUPER-FABRIC TINY MATERIALS AS LUNG CANCER THERANOSTICS

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ABSTRACT

Lung cancer is one of the top five most death causing disease across the world. The lung cancer disease is also affecting a significant part of the economy to treat cancer patients. In last two decade, the use of modern nanomedicine has shown a possibility to treat cancer disease. The efficiency of any cancer treatment successively depends on its detection at primary stages on time to provide efficient and potential delivery of cancer targeting treatments with minimum side effects. Carbon nanotubes (CNT) have emerged as a promising tool for biomedical purpose due to their unique properties which have led to a future prospect in clinical applications. Currently, various researchers and their group are working on nanomaterials based drug delivery systems toward the therapeutic and diagnostic (e.g. theranostic) applications to lung cancer treatments. The combination of theranostic approach to CNT based drug delivery, can enhance its drug targeting ability, therapeutic and diagnostic ability during lung cancer therapy. Also, CNT are self-theranostic nanomaterials, can be highly adequate to diagnose and deliver loaded therapeutic drugs and efficiently target the lung cancer cells with fewer side effects for the complete lung cancer therapy. This review is an attempt to assimilate all the drug targeting, diagnostic and therapeutic studies that have been carried out using CNT, especially as lung cancer theranostics.

KEYWORDS: Surface Modifications, Lung Cancer, Pharmaceutical Nanotechnology, Targeting Ligands, Theranostics

INTRODUCTION

Since the discovery of carbon nanotubes (CNT), it has been considered as novel nanomaterials for a variety of applications including electromagnetic, nanotechnology and nanoscience applications. The CNT have several extraordinary features like high

specific surface area, capacity to cross biological barriers, optical, electrical, mechanical properties, high tensile strength, flexibility, adsorptivity, durability, lightweight, biocompatible with absorbed and conjugated to macro or micro bioactive molecule, diagnostic agents, and polymer to make them theranostic nanomaterials itself



(Kirkpatrick et al., 2012). Such features make them excellent nanomaterials to use in biomedicine. In the last two decade, CNT have introduced in biomedical application, especially for lung cancer treatments. CNT have been proved as better cancer cell targeting nanomaterials for lung cancer treatments.

CNT are broadly classified into two categories, single walled carbon nanotubes (SWCNT) and multi walled carbon nanotubes (MWCNT). The types of CNT depends on the rolling of graphene sheet into a seamless cylinder that can be open ended or capped, having a high aspect ratio with diameters as small as 1 nm and a length of several micrometers (μm) (Jeyamohan et al., 2013). These both types of CNT are frequently used for the cancer treatments. From last decade, CNT is frequently used for the purpose of drug delivery, therapeutic and diagnosis application in cancer treatments (De Volder et al., 2013; Muthu et al., 2013). Researchers have observed that CNT have most cargo efficacy to target the drug molecule at cancer cells (Datir et al., 2013; Wu et al., 2013).

In 1991, a most potential novel drug delivery system was introduced in the field of medical research, which is based on carbon derivatives such as CNT drug delivery system. Their own unique electrical, physicochemical, and structural properties have to make a very useful nanomaterial to many fields, including the emerging one of biological nanotechnology in both in-vitro and in-vivo models. CNT have high capabilities for medical

applications which include in-vitro and in-vivo drug/peptide delivery, targeted drug delivery, gene silencing, gene therapy, cancer imaging and cancer chemotherapy. It is most convenient to absorb and bind to macro or micro bioactive molecule, diagnostic agents, and polymer to make them theranostic nanomaterials itself (Iijima 1991 and Elhissi et al., 2012; Ronzani et al., 2012; Kirkpatrick et al., 2012; Muthu et al., 2013).

CNT are widely used as a great potential to bring benefits to targeted cancer treatments. potential to bring benefits to targeted cancer treatments (Figure 1). CNT have been identified as a novel and targeted drug carrier in lung cancer disease treatments (Sobhani et al., 2011; Mahmood et al., 2013; Chen et al., 2013). In cancer targeting, CNT has offered highly targeted drug effects of many anticancer drugs such as doxorubicin (Liu et al., 2007), methotrexate (Iverson et al., 2008; Samorì et al., 2010), paclitaxel (Liu et al., 2008; Tian et al., 2011; Sobhani et al., 2011), cisplatin (Harper et al., 2010; Li et al., 2014), gemcitabine (Arsawang et al., 2011), quercetin (Dolatabadi et al., 2011; Li et al., 2011; Lu et al., 2012; Jeyamohan et al., 2013), and oxaliplatin. (Wu et al., 2013), where an enhanced drug targeting and theranostic effects on experimental models were observed. The biodistribution study of CNT has suggested that CNT was highly capable to (Datir et al., 2012). CNT used in cancer therapy, are also showing more effectiveness and decrease in the chance of adverse effects (Omidi2011; Deveza et al., 2012).

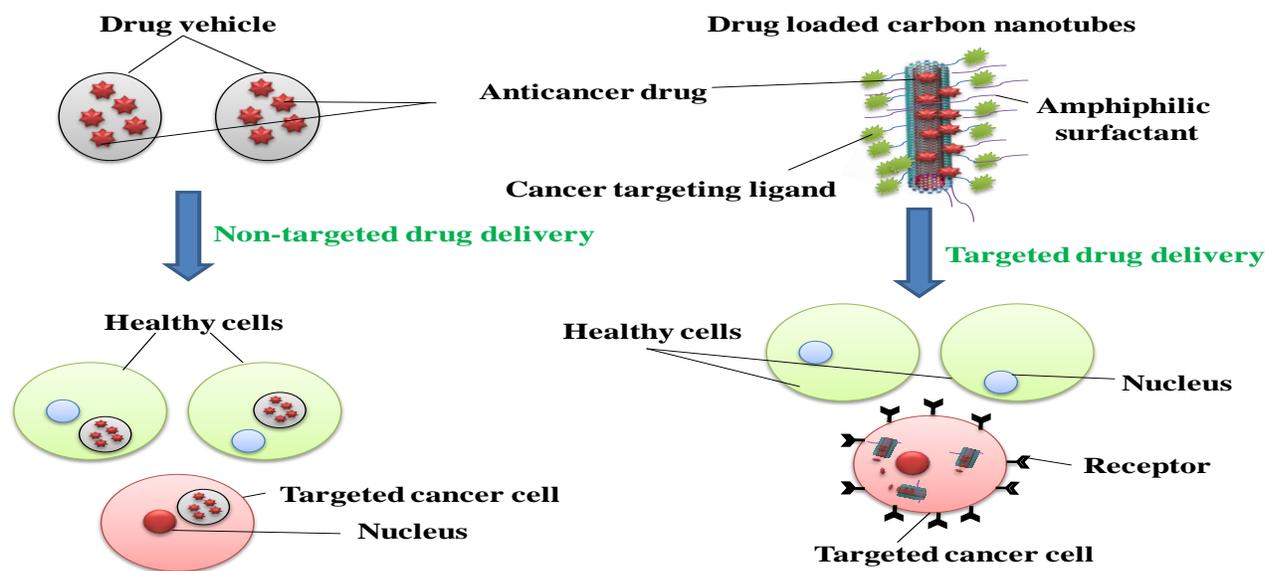


Figure 1. Targeted and non-targeted drug delivery

The word “theranostic” refers to the simultaneous integration of diagnosis and therapy, which can be very useful to optimize efficacy and safety of therapeutic agents. The role of theranostic CNT (*t*-CNT) in nanomedicine is gaining importance in biology and medicine (Janib 2010; Tan et al., 2011; Thakor and Gambhir, 2013). It takes advantage of the high capacity of nanoplateforms to transfer payload and load onto them both imaging and therapeutic functions (Xie et al., 2010). The word “theranostic nanomedicine” can be explained as, colloidal nanoparticles ranging in sizes from 10 to 1000 nm (<1 μ m). Theranostic nanomaterials, in which both imaging and therapeutic capabilities are integrated into a single platform, have shown potential for targeted drug delivery, image-guided surgery and minimally invasive interventions (Li et al., 2014). CNT can be used as highly efficient carriers for therapeutic and theranostic purpose after the attachments of various drugs, ligands, and macromolecule to its surface for the targeting of lung cancer treatments. The CNT can then be further conjugated with molecular probe or ligands, which can bind

to receptors that are overexpressed on the membrane of the cells to be targeted as advanced nanomedicine. The CNT can be circulated for prolonged periods in the lungs, lymph, blood, evading host defenses, and release drug and diagnostic agent together in the diseased cells and simultaneously facilitate in vivo imaging and therapy (Muthu et al., 2014).

In this review, the recent development on CNT for lung targeted cancer therapy, lung cancer theranostic, biodistribution and clearance of CNT including various strategies for reducing toxicity of CNT are discussed.

LUNG CANCER

As reported by the American Cancer Society 2015, lung cancer is one of the top five most life threatening cancer than any other cancer in both men and women in across the world level. The lung cancer is caused by many factors i.e., chemical modification, cigarettes smoking and genetic disorder. The extreme lethality of lung cancer is ascribed to the lack of early diagnostic strategies as in almost 50% of the cases the disease is



confirmed in stage IV, leaving low chance of survival. Lung cancer is classified as small cell lung cancer (SCLC) (13%) or non-small cell (NSCLC) (83%) for the purposes of treatments. Based on type and stage of cancer, as well as specific molecular characteristics of cancer cells, treatments include surgery, radiation therapy, chemotherapy, and/or targeted therapies (Sukumar et al., 2013). For early stage NSCLC, surgery is usually the treatment of choice; chemotherapy (sometimes in combination with radiation therapy) may be given as well. Advanced-stage NSCLC patients are usually treated with chemotherapy, targeted drugs, or some combination of the two (Ferlay et al., 2010; American Cancer Society 2014; American Cancer Society 2015).

From beginning of chemotherapy, some highly potent anticancer drugs are used to destroy cancer cells for maximum treatment efficacy, but these drugs also produce severe side effects on the normal cells during the therapy and post the therapy due to reduced solubility, poor non-selective biodistribution, and restriction by dose-limiting toxicity. Unfortunately, the transport of these anticancer drugs from plasma membrane to nucleus is not very well-characterized. In fact, transports of drugs to nuclei have been found to be rather difficult, and even if it could happen, it is considered to be nonspecific and passive. Last two decade a lot of targeted drug delivery systems have been developed by using different mechanism with the conjugation or attachment of several macro or micro molecules to the drug molecules (Das et al., 2013; Sonali et al., 2016a).

CANCER IMAGE DETECTION TECHNIQUES

Now-a-days some important techniques are used for early lung cancer detection purpose

like magnetic resonance imaging (MRI), spiral computed tomography (CT), positron emission tomography (PET), and single photon computed tomography, optical imaging, ultrasound (US) and X-ray techniques. Among all of them, spiral CT technique is most reliable to detect lung cancer as compared to other techniques (Sharma et al., 2006; Janib et al., 2010). Thus, detecting cancer in its early stage in combination with controlled and targeted therapeutics may provide a more efficient and less harmful solution to the limitations of conventional techniques (Jeyamohan et al., 2013; Muthu et al., 2015; Sonali et al., 2016b). Presently, several scientists are using CNT based drug delivery for targeted lung cancer treatment and theranostic purpose and they have observed better results as compared to existing drug delivery systems in the market or pure drugs (Sobhani et al., 2011; Das 2013; Muthu et al., 2016). In this review article various applications of CNT in aspects to lung cancer therapy targeted CNT for lung cancer treatments, imaging application, theranostic application, biodistribution, elimination and toxicology of CNT, after exposure with cell lines or the body have been discussed.

CARBON NANOTUBES FOR TARGETED LUNG CANCER THERAPY

Based on the carbon nanotubes bonding structure, nanocarbons are classified into sp^2 -carbon nanomaterials, such as 0D fullerene, 1D CNT, 2D graphene and carbon dots (i.e., nanoclusters of amorphous carbon less than 10 nm in size). Indeed, fullerenes and CNT have a high aspect ratio with a hollow structure (Muthu et al., 2013). Additionally, CNT have several advantages for biomedical drug delivery including i) size in the range of 10-40 nm, ii) ability to provide a rod-like scaffold, iii) increased



capacity to carry drugs, iv) ability to deliver drugs to the nucleus and v) inert and nontoxic in nature (after the appropriate surface modification). Therefore, CNT are most popular in delivering of anticancer drug, they have capability of specific targeting to cancer cells, and also improve imaging/sensing methods, they provide biocompatible long circulating bio shuttles for simultaneous delivery of targeting moiety and imaging agent (Omidi 2011; Deveza et al., 2012). Among these nanocarbons, CNT have shown their preclinical utilities for the diagnosis and treatment of highly challenging diseases (Liu et al., 2013; Fubini et al., 2010). Since the development of CNT, several scientists are working toward the therapeutically application of CNT (Sobhani et al., 2011; Chen et al., 2012). Many of them have worked on lung cancer targeting CNT. CNT have large surface area, high drug encapsulation efficiency, nano scale size, ability to encapsulate therapeutic/diagnostic agent and suitability for surface modifications of CNT itself made them suitable for theranostic applications (Muthu et al., 2014). Several molecules can be attached to the surface of CNT including therapeutic agents, drug, genetic materials, protein, peptides/aptamers, targeting moiety and diagnostic agents such as fluorescent dyes, gold nanoparticles, quantum dots and iron oxide. CNT is able to perform multi-modality effects due to the attachment of several molecules (Figure 2).

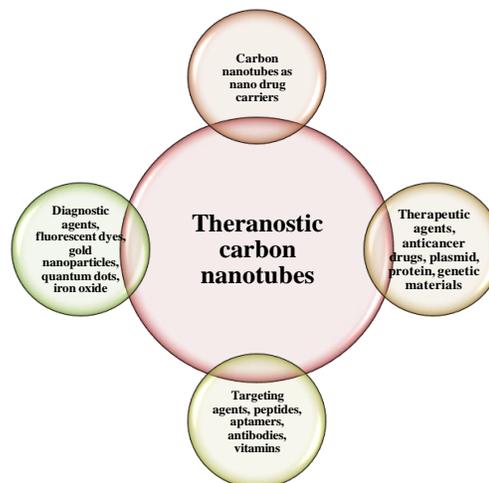


Figure 2. Theranostic applications of carbon nanotubes for biomedical purpose

In one study, Podesta et al., 2009 have demonstrated therapeutic efficacy of MWCNT in the lung cancer treatments. In this study, they have prepared two different types of drug delivery systems including liposomes and amino-functionalized MWCNT for the comparatively toxicity study of both systems on xenograft-bearing animals. In comparative study, both types of drug delivery systems indicated that only amino-functionalized MWCNT gave better cytotoxic effects than liposomal formulation and it also delayed tumor growth and increased the survival of xenograft-bearing animals. siTOX delivery via the amino-functionalized MWCNT were biologically activated in vivo by triggering an apoptotic cascade, leading to extensive necrosis of the human tumor mass. The study suggested that MWCNT mediated delivery of siRNA by intratumoral administration leads to successful and statistically significant suppression of tumor volume, followed by a concomitant prolongation of survival of human lung tumor-bearing animals. The direct comparison between CNT and liposomes demonstrated the potential advantages offered by CNT for the intracellular delivery of therapeutic agents in vivo. This work may act as the impetus for



further studies to explore the therapeutic capacity of chemically functionalized CNT to deliver siRNA directly into the cytoplasm of target cells and achieve effective therapeutic silencing in various disease indications where local delivery is feasible or desirable (Podesta et al., 2009).

Similarly, Sobhani et al., 2011 have designed paclitaxel (PTX) loaded poly citric acid (PCA) conjugated MWCNT nanoformulations (PTX-PCA-MWCNT) for the estimation of cytotoxicity of PTX on A549 cells and ovary cancer cells (SKOV3). PTX have hydrophobic nature and here it has very low solubility in aqueous medium. In this study, after the PTX conjugation to PCA-MWCNT they have observed significant cytotoxic effects on both cancer cells in comparison to free PTX. Additionally, they concluded that PCA-MWCNT based drug carrier produced higher drug penetration effects into cell nucleus. At very short incubation time of MWCNT with cancer cells, this drug delivery offered higher cytotoxic effects on both cancer cells. PTX-PCA-MWCNT have not shown any effects on cells viability which suggested that the effect was due to the PTX conjugated MWCNT (Sobhani et al., 2011). In another study, Chen et al., 2012 have investigated cytotoxic effects of etoposide (ETO) conjugated to unmodified SWCNT, SWCNT-COOH, modified SWCNT (m-SWCNT) and EGF (epidermal growth factor)-functionalized SWCNT (f-SWCNT) as the targeted carrier on A549 cells. In this study, when ETO was attached to chitosan (CHI) conjugated SWCNT-COOH a higher solubility and stable dispersion in aqueous solution was observed. The m-SWCNT and f-SWCNT with drug loading demonstrated minimal cytotoxicity. ETO was released from EGF/Chitosan/SWCNT-COOHs/ETO at low pH and taken up by tumor cells via adenosine triphosphate (ATP)-dependent

endocytosis. The cell death induced by EGF/Chitosan/SWCNT-COOHs/ETO was as much as 2.7 times than that of ETO alone. The results demonstrated that SWCNT based drug delivery had a greater anticancer effect than free ETO in vitro. These results demonstrated that their targeted drug delivery system had high anticancer efficacy due to active targeting by EGF to epidermal growth factor receptor (EGFR) and through the passive targeting of the acidic tumor pH sensitivity of CHI in-vitro (Chen et al., 2012). In another investigation Das et al., 2013 have investigated the drug targeting and cytotoxicity of gemcitabine hydrochloride loaded functionalized MWCNT (GEM-HCl-f-MWCNT) on A549 cells. In this research work MWCNT were functionalized by using 1, 2-distearoylphosphatidyl ethanolamine-methyl polyethylene glycol at different ratio. In vitro drug release study, MWCNT were most efficient in drug targeting in to acidic medium than the basic medium. In this study GEM-HCl-f-MWCNT demonstrated higher cytotoxicity on A549 cells in comparison to free GEM. Here, MWCNT played important role in of targeting drug on cancerous cells (Das et al., 2013). Similarly Arya et al., 2013 have investigated cytotoxicity of PTX loaded dual targeted drug carriers such as SWCNT and graphene oxide (GO) on lung cancer. The results showed higher cell death by using combination treatment of SWCNT/GO and PTX (Arya et al., 2013).

CARBON NANOTUBES FOR LUNG THERANOSTIC APPLICATIONS

CNT have been explored in almost every single cancer treatment modality, including drug delivery, lymphatic targeted chemotherapy, thermal therapy, photodynamic therapy, and gene therapy (Figure 2) (Ji et al., 2010). In a study, Minati et al., 2012, have demonstrated theranostic



application of DOX loaded CNT/Gold hybrids into A549 cells. DOX were adsorbed in high quantity on both inner and outer surfaces of oxidized CNT by π - π stacking interactions between DOX aromatic groups and CNT backbone. CNT/gold hybrids display a broad absorption band in the red and near-infrared regions and give higher cytotoxic effect on A549 cells, which allowed their use for imaging applications. The in vitro cellular uptake study showed that the CNT can efficiently transport and deliver DOX inside the cells. Transmission electron microscopy analysis confirmed the formation of gold nanostructures around the CNT. The visible absorption spectroscopic analysis puts in evidence that the presence of a broad absorption band in the near infrared region attributed to the plasmon resonance of the branched gold nanoparticles on the CNT. Finally, laser scanning confocal microscopy (LSCM) analysis on cell culture demonstrated that CNT-Au can be exploited as drug delivery vehicles and image contrast agent in in-vitro cell experiments (Figure 3) (Minati et al., 2012). In another study, Datir et al., 2012 have assessed in-vitro and in-vivo cytotoxicity of DOX loaded and hyaluronic acid (HR) conjugated MWCNT for theranostic platform. Near-infrared fluorescent dye, Alexa-Fluor-647 (AF-647), and radiotracer Technetium-99m (^{99m}Tc) were used to track its whereabouts both in-vitro and in-vivo via optical and scintigraphic imaging techniques respectively. In this research work hyaluronic acid (targeting ligand) was used to promote HR mediated endocytosis. Covalent functionalization of MWCNT with HA facilitated their internalization into A549 cells via hyaluronan receptors (HR) mediated endocytosis. Internalized CNT showed lysosomal trafficking, followed by low pH-triggered DOX release under endolysosomal conditions. Consequently,

DOX-loaded HA-MWCNT exhibited 3.2 times higher cytotoxicity and increased apoptotic activity than free DOX in equivalent concentrations. Organ distribution studies in Ehrlich ascites tumor (EAT) bearing mice model indicated that tumor specific localization of ^{99m}Tc -MWCNT-HA-DOX is significantly higher than both free drug and non-targeted MWCNT. In addition, pharmacodynamic studies in chemically breast-cancer-induced rats showed that the tumor-growth inhibitory effect of HAMWCNT-DOX was 5 times higher than free DOX in equivalent concentration. DOX delivered through HA-MWCNT was devoid of any detectable cardiotoxicity, hepatotoxicity, or nephrotoxicity. All these promising attributes make HA-MWCNT a “smart” platform for tumor-targeted delivery of anticancer agents (Datir et al., 2012).

Similarly Kumar et al., 2012 have performed an important study towards the investigation of fluorescence behaviour in pristine carbon nanoparticles (p-CNT), which were prepared from lamp soot, and their application in imaging of normal and cancer cells. They have executed three study viz., cell imaging, cellular uptake and cytotoxicity assay on the HL-60 (acute promyelocytic leukemia) and K-562 (chronic myelogenous leukemia) cancer cell lines. In the experiments, the nanoparticles preparation exhibited considerable fluorescence with average emission life-time of 3.54 ns and the photoluminescence behaviour of these particles was excitation dependent and gave off blue, green and red fluorescence under UV, blue and green excitation, respectively. Cellular uptake of these NCNP yielded excellent results for cell imaging of human embryonic kidney, lung carcinoma and breast adenocarcinoma cells. The cell imaging was further correlated with cytotoxicity in the above mentioned cell



lines and also leukaemia cell lines. Dose dependant cytotoxicity was observed after 24 h up to 48 h of incubation of nanoparticles. Fluorescence microscopy of nanoparticle-cell interaction clearly indicated aggregation of the particles. It was clearly observed that these nanoparticles were entered inside cells though there was no surface functionalization and some of these were seen adsorbed to cell

boundary. Bio-imaging application of these particles has been explicitly shown for three types of cells with excellent results (Kumar et al., 2012). In another study, Das et al., 2013 have prepared fluorochrome (Alexa-fluor, AF488/647), radionuclide (Technitium-99m) decorated folic acid (FA) conjugated methotrexate (MTX) loaded theranostic MWCNT nanoformulations.

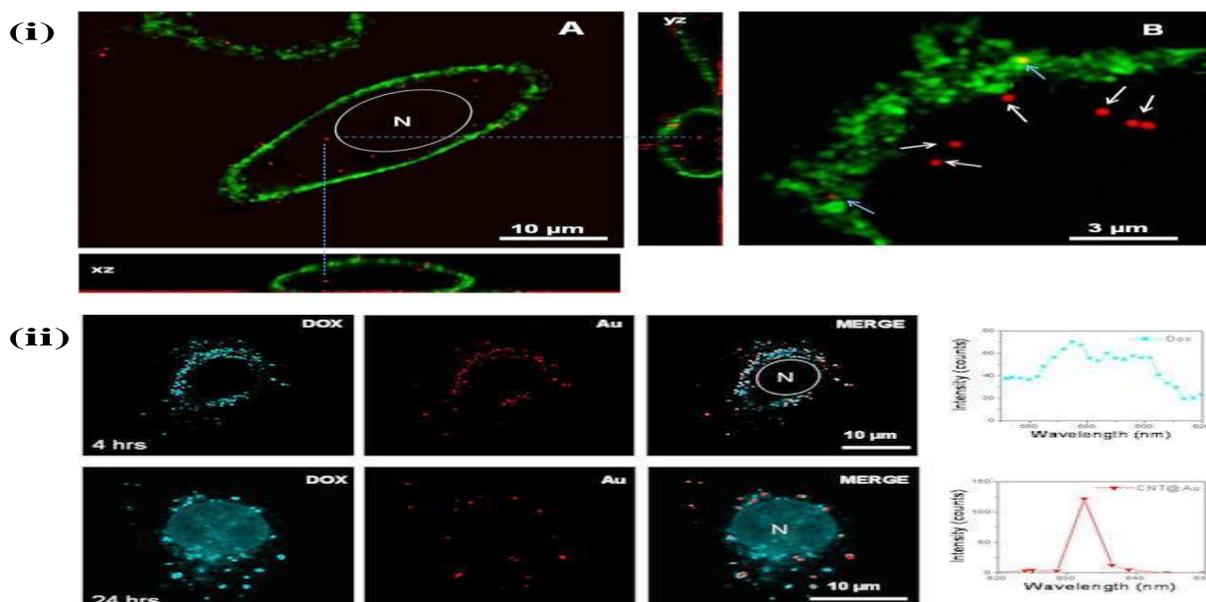


Figure 3. (i) (A and B) LSCM analysis of A549 cancer cell line incubated with CNT-Au-PEG sample for 1 h. (A) CNT-Au (red spots) and cell membrane (green). Z-series and orthogonal views (xz and yz) of A549 cancer cells incubated for 1 h. Optical images were collected every $0.7 \mu\text{m}$ beginning at the bottom of the coverslip and moving upward. Orthogonal images in the xz and yz planes indicate the CNT-Au localization inside the cell. White oval labeled by N indicates the nucleus position in the cell. (B) Detail of one cell showing the scattering of nanoparticles localized inside the cell (white arrows) and on the cell membrane (blue arrows) and (ii) LSCM analysis of A549 cancer cell line incubated with the CNT-Au-Dox sample for 4 and 24 h. Panel: DOX, fluorescence, gold scattering (Au), and merged images of A549 cancer cells incubated for 4 and 24 h. N indicates the nucleus positions. Right: $xy\lambda$ analysis (extraction of the photoluminescence line) of the two channels (cyan and red) reported in the LSCM images. Reproduced with permission from Figure 5 and 6 of ref. (Minati et al., 2012) © 2012 American Chemical Society.

In this research work, cellular uptake studies corroborated the selective internalization of AF-FA-MTX-MWCNT (AFMM) by folate

receptor (FR) positive A549 cells and breast (MCF-7) cancer cells through FR mediated endocytosis. The MWCNT showed higher



anticancer activity as compared to its non-targeted preparation that was mainly restricted to cytoplasm. In result, tumor specific accumulation of AFMM in Ehrlich Ascites Tumor (EAT) xenografted mice was almost 19 and 8.6 times higher than free MTX and FA-deprived MWCNT. During the study, MWCNT-MTX conjugates were devoid of any perceivable hepatotoxicity, cardiotoxicity, and nephrotoxicity. Overall, the delivery property of MWCNT, high tumor binding avidity of FA, optical detectability of AF fluorochromes, and radio-traceability of ^{99m}Tc could be successfully integrated and partitioned on a single CNT-platform to augment the therapeutic efficacy of MTX against FR overexpressing cancer cells while allowing a real-time monitoring of treatment response through multimodal imaging (Das et al., 2013 (a)).

Another study Das et al., 2013 have demonstrated in-vitro and in-vivo nuclear targeting capability of PEGylated-estradiol linked doxorubicin loaded MWCNT on both breast cancer cells and human lung cancer cells. The estradiol linked MWCNT (DOX-E2-PEG-MWCNT) has given better nuclear targeting cytotoxic effects through an estrogen receptor (ER)-mediated pathway in comparison to free doxorubicin. In-vitro cytotoxicity study, plain DOX-m-PEG-MWCNT and DOX-E2-PEG-MWCNT, the IC_{50} of the ER-targeted conjugate in A549 and MCF 7 cells was 1.5 –1.6 times lower than its non-targeted counterpart ($P < 0.001$) which, however, seems to be a consequence of direct, intranuclear delivery of DOX intervened by E2-PEG-MWCNT that was not operative in case of the non-targeted conjugate. In this work E2 on the surface of MWCNT played a key role in targeting DOX to its site of action (i.e., nucleus). Through this research work they concluded that CNT have higher nuclear targeting efficiency (Das et al., 2013b).

BIODISTRIBUTION AND CLEARANCE OF CNT

Since discovery, CNT are giving beneficial effects in biomedical fields, they have good tendency of penetrating cell membrane and easily target nucleus of the cancer cells (Sobhani et al., 2011; Wu et al., 2013). Elgrabli et al., 2008 have investigated biodistribution and clearance mechanism of MWCNT in rat model. In this study, they used nickel (Ni) trace element that was observed as impurity of MWCNT. MWCNT were intravenously injected in to rats and the result suggested that MWCNT can be eliminated. The MWCNT did not significantly cross the pulmonary barrier but was still present in lungs upto 6 months after a unique instillation. The MWCNT were biopersistent and clearance takes upto 6 months after respiratory administration. On the basis of tracing element, biodistribution of MWCNT were investigated. Different concentrations (0, 6.25, 12.5, 25, 50 and 100 μg) of MWCNT were instilled into rats for the biodistribution study, which revealed that no increases of Ni was detected in organs after 1 μg or 10 μg MWCNT exposures except in the lung at 10 μg of MWCNT. During the study, Ni was not detected at any time in liver, kidneys, spleen, heart, brain, thymus and testis of rats exposed to 1, 10 or 100 μg of MWCNT. A higher amount of Ni was observed in the MWCNT exposed rat's lung throughout the experiment (from 1 to 180 days) in lymph nodes at day 30. Considering that Ni represent 0.53% of MWCNT instilled, the percentage of MWCNT recovered was 63% at day 1, 78% at day 7, 97% at day 30, 38% at day 90 and 16% at day 180. Ni was not detected in BALF at any time post-exposure. About 50 % of the instilled MWCNT were located in the parenchyma of the lung after 1 month. 10 to 24% of CNT were found in the alveolar cells during this period. At 30th



day, 31% of total CNT injected was eliminated from the lung but 28% of this same nanotube was found in the lymph nodes for a transition period less than 60 days. Clearance of MWCNT was amplified at 3 and 6 months after the exposure because 63% and 84% of total MWCNT injected was respectively eliminated from the organism. For the MWCNT clearance mechanism, alveolar cells isolated from BAL were counted with Malassez cell and observed by optical microscopy. Significant increase in cell number, respectively plus 44% and plus 100%, were noted at 7 and 180 days after treatment.

In another study Yang et al., 2007 have demonstrated biodistribution mechanism of pristine SWCNT in male KM mice. In this study mice was exposed to ^{13}C -SWCNT suspension (200 $\mu\text{g}/200 \mu\text{L}$) in comparison to aqueous solution of 1 wt % Tween 80 (200 μL), SWCNT were administered via a single tail vein injection. They observed that, chemically modified/functionalized SWCNT were highly cleared from renal excretion route, whereas the pristine nanotubes could hardly be detected in urine and faeces by using ^{13}C isotope ratio measurements and TEM analysis of the samples. The chemically modified/functionalized SWCNT were highly accumulated in lungs and liver over an extended period of time (Figure 4)(Yang et al., 2007). Similarly, Li et al., 2014 have investigated theranostic effects of cisplatin loaded MWCNT (cisplatin-MWCNT) by using in-vivo biodistribution and histopathological evaluations. Cisplatin or an inert platinum (IV) complex was loaded inside functionalized-MWCNT and then followed i.v. instillation to the mice body and the effects of MWCNT on the distribution of Pt-based molecules were assessed. In the results, platinum levels in vital organs suggested that functionalized-

MWCNT did not affect cisplatin distribution, while they significantly enhanced the accumulation of Pt (IV) sample in some tissues (e.g. in the lungs, suggesting their potential in lung cancer therapy) and reduced both kidney and liver accumulation (thus decreasing eventual nephrotoxicity, a typical side effect of cisplatin). During the experiments MWCNT did not induce any intrinsic abnormal immune response or inflammation, as confirmed by normal cytokine levels and histological evaluations. Therefore, functionalized MWCNT represent an efficient nano-carrier to improve accumulation of Pt species in targeted tissues/organs. The results proved that functionalized MWCNT could significantly enhance the platinum content in almost all tissues and efficiently alter the tissue distribution in mice for the delivery of Pt (IV) compound. Therefore, functionalized CNT can be used as a promising nano-carrier to improve accumulation of drug molecules in the lungs for therapeutic treatments (Li et al., 2014).

CNT-LUNG UPTAKE MECHANISM

CNT are being investigated for a variety of biomedical applications. Despite numerous studies, the pathways by which CNT enter cells and their subsequent intracellular trafficking and distribution remain poorly determined. Al-Jamal et al., 2011 have used 3-D electron tomography techniques that offer optimum enhancement of contrast between CNT and the plasma membrane to investigate the mechanisms involved in the cellular uptake of shortened, amino functionalized MWCNT (MWCNT-NH³⁺).

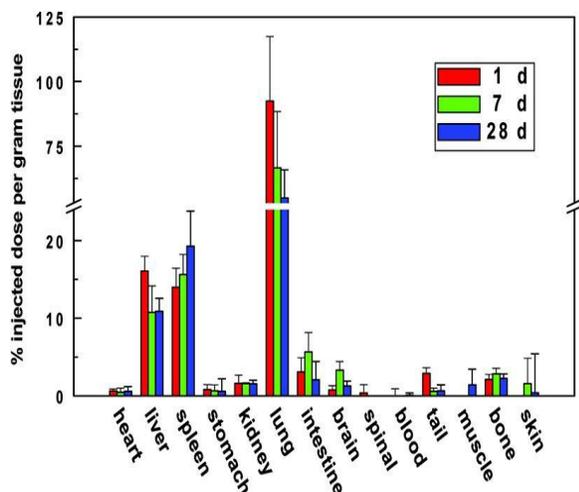


Figure 4. The biodistribution of pristine SWNTs (13C-SWCNT) in mice at different time points post exposure. Data are presented as the mean (SD (n=3)). Reproduced with permission from Figure 2 of ref. (Yang et al., 2007). American Chemical Society© 2007.

They have observed that MWCNT-NH₃⁺ were internalised in both phagocytic and non-phagocytic cells by any one of three mechanisms: (a) individually via membrane wrapping; (b) individually by direct membrane translocation; and (c) in clusters within vesicular compartments. At early time points following intracellular translocation, they have noticed accumulation of CNT within various intracellular compartments, while a long-term (14-day) study using primary human macrophages revealed that MWCNT-NH₃⁺ were able to escape vesicular (phagosome) entrapment by translocating directly into the cytoplasm (Firme and Bandaru, 2010; Al-Jamal et al., 2011). From the past literature, the exact mechanisms that lead to cell death are still unclear, but there are five methods of internalization of CNT inside the cells, phagocytosis, macro-pinocytosis, clathrin-mediated endocytosis, caveolin mediated path-ways, and clathrin/caveolin independent pathways. So far, the two

most widely accepted mechanisms of CNT internalization proposed are (i) endocytosis/phagocytosis and (ii) nanopenetration. Endocytosis represents the engulfing of an extracellular particle by the cell, for example, viruses (~100 nm in size), through the creation of a vesicle that is then integrated into the cell. Phagocytosis is similar to endocytosis but usually involves uptake of larger particles, such as bacteria (~1µm), and is characteristic to a subset of immune cells/phagocytes (eg, neutrophils, macrophages, dendritic cells) (Sonali et al., 2016c; Kaklotkar et al., 2016; Chiaretti et al., 2008; Elgrabli et al., 2008). These processes are energy dependent and are hindered at low temperature and in low ATP environments. Nanopenetration is an energy-independent passive process, where the nanotubes diffuse across the cellular membrane without causing any harmful effects on cells membrane (Jain et al., 2012). Very few studies have been performed towards investigation of cellular uptake mechanism of CNT in biological system. Antonelli et al., 2010 have proved that, the cellular uptake of SWCNT can be followed by two different mechanisms such as, phagocyte cells occurs via an endocytosis mechanism and diffusion mechanisms which are depends on its physical property and particle size. It is important to investigate the upper size limit for molecules simply inserting and diffusing through the cell plasma membrane, and the lower size limit for nanomaterials becoming too small for wrapping by a highly curved lipid bilayer to undergo endocytosis (Pantarotto et al., 2004; Antonelli et al., 2010; Lopez et al., 2004; Liu et al., 2005; Kam et al., 2006; Banco et al., 2013). In another study Lacerda et al., 2012 have focused on cellular translocation mechanism of functionalized MWCNT on RAW 264.7 cells and A549 cells. In this study, MWCNT were translocated across



cell membranes of both phagocytic and nonphagocytic cells. In initial study, at least 30-50% of f-MWCNT was taken up by cells through an energy independent mechanism. These were better indication for theranostic application for drug targeting (Lacerda et al., 2012).

CNT - LUNG TOXICITY STUDIES

Respiratory exposure of CNT can induce acute adverse effects in the pulmonary and cardiovascular systems. Additionally, CNT may produces several toxicity issues including free radical formation, reactive oxygen species (ROS) generation, granuloma formation, increased inflammatory responses, and apoptosis (Jain et al., 2012). The excess formation of free radical can be produced more toxicity than any other toxic factors. It can damage or oxidize lipids, proteins and DNA contents of cells. (Wang et al., 2011a). Oxidative stress may upregulate redox sensitive transcription factors, activator protein-1 and kinases that cause inflammatory responses. Due to the higher exposure of CNT, ROS level may increase which may further lead to harmful effects in cells such as apoptosis, DNA damage, amino acid oxidation and inactivity of enzymes (Valko et al., 2007). The higher exposure of CNT in to the body can produce chronic inflammation effects. CNT itself have asbestos based carbon molecule which may trigger the release of polymorphonuclear leukocytes and protein exudation, indicating an increased inflammatory response (Di Giorgio et al., 2011). After the exposure of CNT, the extracellular matrix protein signaling brings changes to the cell skeleton and the subsequent displacement of organelles, results in membrane deformation and finally apoptosis (Montes-Fonseca et al., 2011). The CNT mainly increase the biomarkers of inflammation, including endothelin-1 in the

bronchoalveolar lavage (BAL) fluid and plasma levels of angiotensin-1 converting enzyme, which leads to endothelial dysfunction, interstitial inflammation, peribronchial inflammation, peripheral vascular thrombosis and epithelial granuloma formation (Muthu et al., 2013).

In one study, within 24 h of exposure to SWCNT, lungs displayed features of inflammation, an increase in biomarkers of inflammation, oxidative stress, cell damage and an increase in the cell count in the BAL fluid (Ge et al., 2012). CNT also induce dose-dependent lesions in the lung, which vary from necrosis to granuloma formation. The ferrous-containing CNT were present even 90 days after exposure to the lung. After 2 months post exposure to grounded CNT, lungs could be characterized by the formation of collagen-rich granuloma in the lung parenchyma. The lungs also showed features of alveolitis after exposure to agglomerated CNT. In this process, MMP-9 was involved in collagen formation and fibrogenic processes of the lung both in vivo and in vitro (Wang et al., 2011b). MWCNT showed hepatotoxicity in an in-vivo experimental study of biocompatibility in Swiss albino mice. The mice have significantly reduced glutathione and SOD activities in the liver and an increase in liver enzymes, such as serum ALP and serum ALT, in comparison with controls. During a histopathological study of the liver, it was observed that hepatocellular vacuolization, central vein necrosis and degeneration of the liver occurred in more than half of the field area (Awasthi et al., 2013). The purified carboxylated MWCNT also showed the potential to induce hepatotoxicity in Swiss Webster mice by activating oxidative stress. The administration of purified functionalized MWCNT to the mice showed a significant increase in ROS levels in the exposed group than the control group. An LDH assay in



mouse livers showed a significant increase in the level of hydrogen peroxide in the exposed group compared to the control groups. Liver enzymes, including SGOT, SGPT and ALP, were also significantly increased in the exposed groups as compared to the controls. Histopathological analysis of exposed mouse livers showed remarkable morphological alterations, including hepatocyte disruption, hepatocellular vacuolation, pyknotic changes or condensed nuclei in the hepatocytes, central vein degeneration and, subsequently, liver atrophy (Patlolla et al., 2011). The pristine CNT also showed cellular toxicity. For example, Porter et al., 2007 studied translocation of CNT inside the cell by confocal and transmission electron microscopy. During this imaging study in stained and unstained human cells, CNT entered the cytoplasm and localized in the cell nucleus. Cell mortality observed in a dose-dependent manner (Porter et al., 2007). In another study Wang et al., have demonstrated that chronic exposure to SWCNT leads to malignant transformation of human lung epithelial cells. The transformed cells induced tumorigenesis in mice and exhibited an apoptosisresistant phenotype characteristic of cancer cells. This study also provided new evidence for CNT induced carcinogenesis and indicated the potential role of p53 in CNT toxicity (Wang et al., 2011c). In another study, Coccini et al., 2010 have studied the effects of water soluble f-MWCNT by using different types of cytotoxicity methods in human astrocyte D384 and A549 cells. In this study, they indicated that cytotoxicity of MWCNT can be altered by using surface modification techniques in association with change in solubility and tendency of MWCNT for form agglomerates. They also suggested that the use of modification techniques for making CNT as drug carriers and minimize its toxicity and give better

results in drug targeting (Coccini et al., 2010).

STRATEGIES FOR REDUCING TOXICITY OF CNT

TPGSylated MWCNT

Recently, the TPGS conjugated MWCNT were developed containing docetaxel for lung cancer therapy. The IC₅₀ values demonstrated that the D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) conjugated MWCNT could be 80-fold more efficient than DocelTM after 24 h treatment with the A549 cells. The flow cytometry analysis confirmed that cancerous cells appeared significantly ($P < 0.05$) in the sub-G1 phase for TPGS conjugated MWCNT. The results of TPGS conjugated MWCNT have shown better efficacy with safety than non-coated or TPGS coated MWCNT and DocelTM (Figure 5) (Singh et al., 2016). Most recently, a transferrin receptor targeted MWCNT was designed to improve the targeted and therapeutic delivery of docetaxel for lung cancer therapy. The in-vitro cytotoxicity study revealed that the IC₅₀ values of the transferrin conjugated MWCNT was 136-fold more efficient than DocelTM, after 24 h treatment with the A549 cells. The flow cytometry analysis confirmed that cancerous cells have appeared significantly ($P < 0.05$) in the sub-G1 phase for transferrin conjugated MWCNT in comparison to DocelTM. The in-vivo safety study showed that the transferrin conjugated MWCNT achieved better safety than DocelTM in lung cancer delivery DocelTM (Figure 6) (Singh et al., 2016). In a study, Mehra et al., 2014 prepared TPGS conjugated doxorubicin loaded MWCNT and DOX loaded MWCNT (DOX-TPGS-MWCNT) formulations and estimated in-vitro cytotoxicity on human breast cancer (MCF-7); derived from pleural perfusion and in-vivo (Balb/c mice)



therapeutic potential in comparison to free doxorubicin. In in-vitro drug release study they found that doxorubicin is released in a

sustained manner at lower pH (pH 5.5), which was very efficient to cancer cells targeting.

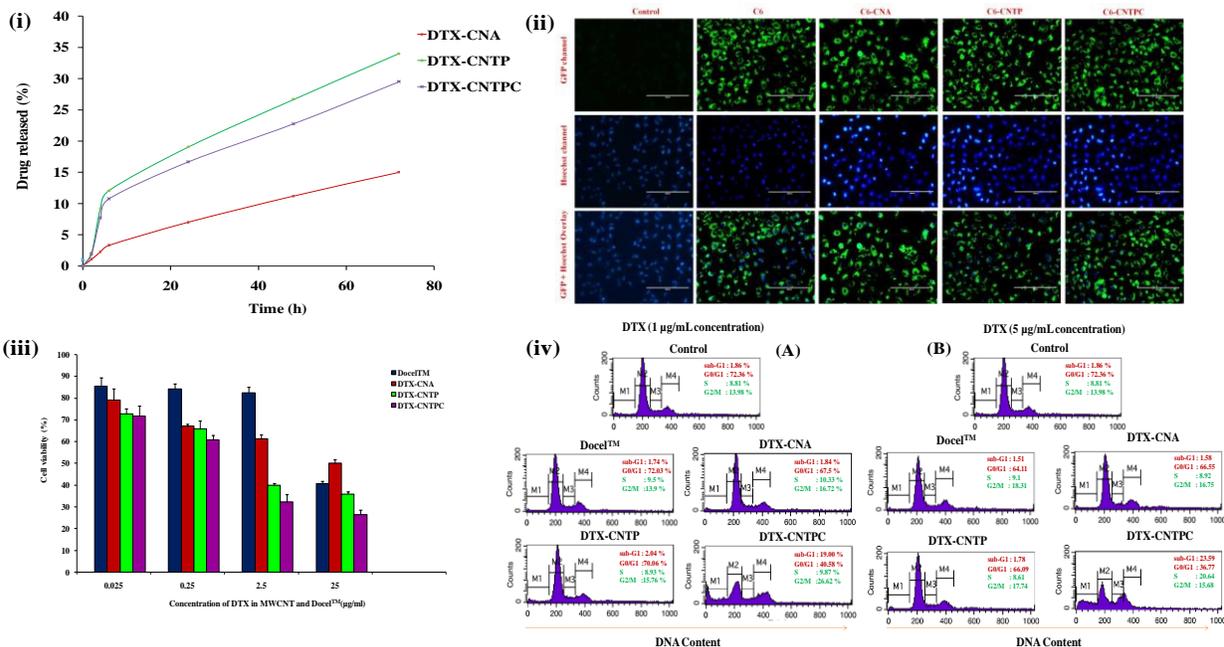


Figure 5. (i) In-vitro drug release from DTX loaded MWCNT in PBS (pH 7.4). Bar represent \pm S.D (n = 3). DTX loaded MWCNT (DTX-CNA, DTX-CNTP and DTX-CNTPC). Bars represent \pm S.D (p < 0.05, n = 3). (ii) Fluorescence microscopy images using A549 cells after 3 h incubation with the fluorescent C6 loaded MWCNT (C6-CNA, C6-CNTP and C6-CNTPC) and free C6. Green Fluorescent Protein (GFP) channel showing the green fluorescence from C6 distributed in cytoplasm in first row. DAPI channels showing the blue fluorescence from Hoechst 33342 stained nuclei in second row and overlay of GFP and DAPI in third row. Scale bar = 200 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.). (iii) Cytotoxicity and antiproliferative profile of DTX formulated as MWCNT or Docel™ for A549 cells (n = 4). Docel™, DTX-CNA, DTX-CNTP and DTX-CNTPC. Bars represent \pm S.D (p < 0.05, n = 3). (iv) (A and B) Cell cycle analysis by flow cytometry of DTX loaded MWCNT with two different concentrations as 1 and 5 g/ml on A549 cells after 3 h incubation. Control: A549 cells without drug treatment; Docel™, DTX-CNA, DTX-CNTP and DTX-CNTPC. (C) DTX loaded MWCNT induced ROS generation in A549 cells. Reproduced with permission from Figure 2, 4(A) and 5 (A and B) of ref. (Singh et al., 2016). © 2016 Elsevier Inc.

The prepared formulations of DTX-TPGS-MWCNT were giving better cytotoxicity and cellular uptake on cancerous cells via endocytosis mechanism. Additionally they have found more survival span (44 days, p < 0.001) for DTX-TPGS-MWCNT, than DOX-MWCNT (23 days), free DOX (18 days) and control group (12 days) and

demonstrated that upon increasing the concentration from 0.001 to 10 0 mM the percent viability of the cancerous cells was decreased owing to apoptosis by intercalating DOX with DNA. The blood plasma drug concentration measurement; area under the curve $AUC_{(0-\infty)}$ and area under the first moment curve (AUMC) were



calculated to be 9.33, 22.31, 46.47 and 22.00, 149.87 and 937.58 for free DOX, DOX-MWCNT and DOX-TPGS-MWCNT conjugated doxorubicin loaded MWCNT, respectively. The AUC and AUMC of DOX-TPGS-MWCNT were approximately 5.0- and 6.25-fold higher, respectively as compared to free DOX. DOX-TPGS-MWCNT formulation was found to be significant in tumor growth suppression as compared to non-targeted MWCNT and free DOX solution. In biodistribution study, they found more concentration of MWCNT in cancer infected site (Mehra et al., 2014). In another study, Wang et al., 2012 have demonstrated cytotoxicity of doxorubicin loaded SWCNT on ICR mice bearing mouse sarcoma tumor. In this work TPGS was conjugated to DOX-TPGS-SWCNT. The DOX-TPGS-DOX was subjected to intratumoral injection into the tumor bearing mice, better cytotoxicity (up to 50.2%) in comparison to free DOX (up to 40.2%) was observed. The biodistribution study reported that there was longest retention time in tumor, the highest tumor accumulation, as well as less accumulation in other solid tissues, especially in heart, when tumor bearing mice were administered with DOX-TPGS-SWCNTs (Wang et al., 2012).

PEGylated SWCNT

In another study SWCNT were functionalized with PEG and coated with folic acid (FA) by using non-covalent method. After that, DOX was conjugated to this PEG-folic acid (PEG-FA) hybrid. The PEG-FA was directly adsorbed onto the surface of SWCNT to increase water solubility, biocompatibility and cancer

targeting efficacy of SWCNT. In this work, they found higher drug encapsulation efficiency of DOX and showed excellent stability under neutral pH conditions such as in serum, but dramatic releases for DOX-PEG-SWCNT and DOX-PEG-FA-SWCNT. They have performed in-vitro cytotoxicity on human cervical cancer HeLa cells and mouse embryonic fibroblast 3T3 cancer cells and normal cell models. DOX-PEG-FA-SWCNT gave most cytotoxicity HeLa cells (about 90%) after 72h. Additionally, exposure of DOX/PEG/SWCNTs in HeLa cells, also showed significant effects. However, incubations with 50 mg/ mL free DOX did not result in appreciable cytotoxicity. These result indicated that, after PEGylation, SWCNT were capable to enter the lysosomes or endosomes by clathrin-mediated endocytosis in cancer cells and destroyed cancer cells with minimum side effects to normal cells (Niu et al., 2013). Similarly, Wu et al., 2013 have designed oxaliplatin loaded PEGylated MWCNT formulation for the purpose of in-vitro cytotoxicity of human colon adenocarcinoma HT29 cells treatment. Oxaliplatin was attached to MWCNT by nano-extraction method and MWCNT were pre-functionalized with acid treatment. In in-vitro drug release study, approximately 50 % incorporated oxaliplatin was released within the 6 hr, and remaining drug was released after 24 h, and finally 89% of drug was recovered within 5 days. In in-vitro cytotoxicity study of oxaliplatin, the cell viability of HT29 was decreased at different concentration of oxaliplatin-MWCNT and oxaliplatin-PEG-MWCNT as compared to free oxaliplatin.

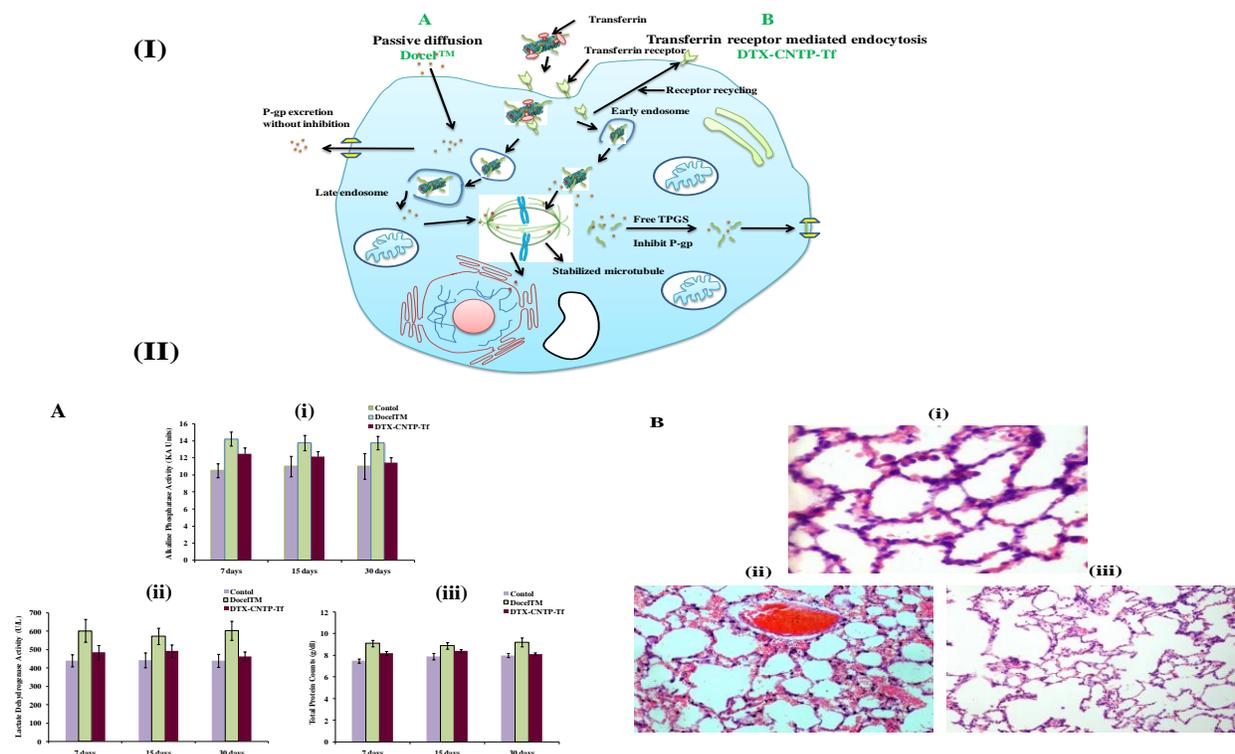


Figure 6. (I) Schematic representation of cellular uptake of DTX from formulations (A) passive diffusion for Docet™ and (B) transferrin receptor-mediated endocytosis mechanism for DTXCNTP-Tf. (II) (A) In-vivo safety study of DTX loaded MWCNT; (i) alkaline phosphatase activity (KA Units); (ii) lactate dehydrogenase activity (U/L) and (iii) total protein counts (g/dl) in BAL fluid of rats. (B) Effect of MWCNT in the lungs after i.v. administration. Animals were sacrifice after 30 days of administration and the lungs were perfused with a normal saline solution by cannulating the right ventricle. The panels present macroscopic views and hematoxylin/eosin-stained lung sections from (i) rats treated with normal saline solution (control) (ii) Docet™: DTX injection (5 mg DTX/kg) and (iii) DTXCNTP-Tf: DTX loaded transferrin conjugated MWCNT (5 mg DTX/kg). Reproduced with permission from Figure 4 and 7 of ref. (Singh et al., 2016). © 2016 Elsevier Inc.

Folic acid MWCNT

In one study, Dinam et al., 2014 have prepared DOX loaded PEGylated folic acid (FA) targeted MWCNT formulations and PEGylated non-targeted MWCNT formulations and estimated the in-vitro cytotoxicity on HeLa cells human cervical cancer cells and in-vivo on male sprague-dawley rats. The MWCNT were functionalized with PEG amphiphilic surfactant, that was attached to MWCNT and then FA was conjugated to PEGylated MWCNT. The DOX encapsulation

efficiency was higher than low-PEGylated and high-PEGylated CNT $84.3 \pm 3.1\%$ and $49.3 \pm 5.4\%$ respectively. In in-vitro cytotoxicity study, folic acid-targeted MWCNT expressed a 3.2-fold decrease in IC₅₀ value compared with non-targeted MWCNT. In this study, DOX-PEG-FA-MWCNT gave sustained drug release in comparison to free DOX. Finally, they have indicated that FA-targeted MWCNT show great potential as a targeted anticancer delivery system. Cellular uptake study of DOX showed targeted effects and much brighter fluorescence



signals for FA-PEG-MWCNT than that of non-targeted PEG-MWCNT carriers at the concentration of 50 $\mu\text{g/ml}$ of DOX, corresponding to a higher degree of cellular uptake and internalization of targeted carriers through receptor-mediated endocytosis. In in-vitro cytotoxicity, free MWCNT did not show any cytotoxicity on HeLa cells. The IC₅₀ values for free DOX, FA-PEG-MWCNT and PEG-MWCNT were 186.4 ± 20.1 , 316.8 ± 24.3 and $1020.1 \pm 162.3 \mu\text{g/mL}$ respectively. The higher IC₅₀ value of DOX-loaded MWCNT was likely to have a time-consuming DOX-release from the carrier, which is consistent with in vitro release profiles. Due to higher uptake of FA-targeted carrier, cell killing effect enhanced with a significantly lower IC₅₀ value in comparison to non-targeted MWCNT (Dinan et al., 2014). In another study Lu et al., 2012 have demonstrated the cytotoxicity of doxorubicin loaded folic acid conjugated magnetic multi walled CNT on brain cancer cells (U87 human glioblastoma cell lines). MWCNT were pre-functionalized with poly (acrylic acid) and decorated with iron oxide magnetic nanoparticles (MNs), conjugated with a targeting ligand folic acid (FA), for loading of DOX. In this research work, it was proved that DOX-MWCNT and DOX-FA-MN-MWCNT have higher cytotoxicity in comparison to free DOX on U87 human glioblastoma cells compared with free DOX, and also were capable to transport of DOX into the nucleus with the nanocarrier left in the cytoplasm. This dual targeted DOX-FA-MN-MWCNT was specific for ligand-receptor interaction with magnetic targeting. Selective killing of U87 cells at the magnetic targeting site is possible without affecting cells at the control site (Lu et al., 2012).

RGD-CNT

In another study Wang et al., 2014 have demonstrated cytotoxicity of RGD-conjugated silica-coated gold nanorods (GNs) on the surface of MWCNT for in-vivo gastric cancer cells. RGD is a short form of arginyl-glycyl-aspartic acid (RGD), which composed by tripeptide including L-arginine, glycine, and L-aspartic acid. In this work silica coated gold nanorods were covalently attached to the surface of MWCNT to the presence of carboxylic and amino groups on the surface of GNs modified with a silane coupling agent. The RGD peptide was conjugated to prefunctionalized GNs/MWCNT, resultant RGD-conjugate was used to investigate their influences on in-vitro cells viability of MGC803 and GES-1 cells and in-vivo on nude mice models with gastric cancer cells. In this study RGD-conjugated GNs and MWCNT demonstrated good water solubility and low cellular toxicity, which could target in vivo gastric cancer cells, and obtained strong photoacoustic imaging in the nude model. Additionally, they have proved good biocompatibility of GNs/MWCNT (Wang et al., 2014).

In research work, CNT have demonstrated the risk of producing some toxic effects during the therapy because it contains several manufacturing chemical impurities and strong hydrophobic environment surrounding nanotubes. However, this toxicity of CNT can be minimized by using several surface functionalization techniques including amidation, acylation and carboxylation etc. Such alternative approach involves functionalization of the surface of CNT with small coupling agents, which are able to increase water solubility of CNT and opens more complementary attachment to functional biomolecules. The most common fixation sites on CNT are COOH, NH₂ and COCH₃ but COOH is frequently used for its surface functionalization (Datir et al., 2013, Jaymohan et al., 2013). These are the main



groups which are responsible to reduce the toxicity of CNT. Wu et al., 2013 have reported that functionalized CNT (f-CNT) are most potential for cancer targeting in comparison to pristine CNT because f-CNT have some hydrophilic nature and compatible to biological cell membrane (Wu et al., 2013). Some researchers have used amphiphilic surfactant such as poly ethylene glycol (PEG), TPGS, poly methacrylic acid (PMA) and poly citric acid CNT to increase drug targeting efficiency in cancer treatment (Liu et al., 2009; Sobhani et al., 2011; Cirillo et al., 2013; Jeyamohan et al., 2013; Mehra et al., 2014). It is already approved that the insertion of small PEG-like spacer between targeting ligands and nanocarriers not only improves the hydrophilicity of the overall conjugate system but increases its accessibility toward receptor site as well (Das et al., 2012).

CONCLUSION AND FUTURE ASPECTS:

From the aforementioned various research studies, CNT have showed promising results the delivery of anticancer drugs for lung cancer treatments including theranostic application. Since the development, CNT offers attractive biomedical application towards the targeting efficacy of itself to the nucleus of the cells imaging and therapy. Several anticancer drugs (docetaxel, paclitaxel, doxorubicin, cisplatin, and etoposide etc.,) were delivered in to the pulmonary cancer cells including theranostic applications and gives highly potential actions against to the cancer. But in some cases CNT have given toxic effects in biological environments, which were performed on in-vitro or animal's models. However, the reported cytotoxic effects of CNT in mammalian cells are controversial, with some reports demonstrated their cytotoxic effect, whereas others

demonstrated their biocompatibility. Several other factors related to the structure of CNT play a role in their toxic effects, including metallic impurities, surface modification, surface area, shape, length, agglomeration and number of layers of CNT. In most of CNT toxicity effects on cells, pristine or unmodified CNT were used, which are high hydrophobic in nature, insoluble in biological fluids or remains long times in body. While functionalized-CNT are more biocompatible with physiological systems and hence reduce their toxicity compared with pristine CNT and enhances therapeutically applications. Therefore minimise this drawbacks of CNT, surface modification methods are very useful to make biocompatibilities of CNT in biological systems and also improve propensity to cross cell membranes. CNT are offers the possibility of introducing more than one function on the same tube, so that targeting molecules, contrast agents, drugs, and give theranostic effects on targeted site of action. The in-vivo theranostic studies undertaken so far indicate that the functionalized CNT can be developed as biomedical application in compare to non-functionalized and pristine CNT. From the above discussion we can estimated, CNT have an important role for the drug deliveries in to the pulmonary therapy. Overall, a detailed understanding of the pharmacological and toxicological properties of CNT and a balanced evaluation of risk/benefit ratio are required before they can be recommended for routine clinical use. CNT are extremely promising application of nanotechnology and is definitely worth as future lung cancer theranostics.

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CONFLICT OF INTEREST

The authors report no declaration of interest

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