R E V I E W

The Applications of Carbon Nanotubes in the Diagnosis and Treatment of Lung Cancer: A Critical Review

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Abstract: The importance of timely diagnosis and the complete treatment of lung cancer for many people with this deadly disease daily increases due to its high mortality. Diagnosis and treatment with helping the nanoparticles are useful, although they have reasonable harms. This article points out that the side effects of using carbon nanotube (CNT) in this disease treatment process such as inflammation, fibrosis, and carcinogenesis are very problematic. Toxicity can reduce to some extent using the techniques such as functionalizing to proper dimensions as a longer length, more width, and greater curvature. The targeted CNT sensors can be connected to various modified vapors. In this regard, with helping this method, screening makes non-invasive diagnosis possible. Researchers have also found that nano-particles such as CNTs could be used as carriers to direct drug delivery, especially with chemotherapy drugs. Most of these carriers were multi-wall carbon nanotubes (MWCNT) used for cancerous cell targeting. The results of laboratory and animal researches in the field of diagnosis and treatment became very desirable and hopeful. The collection of researches summarized has highlighted the requirement for a detailed assessment which includes CNT dose, duration, method of induction, etc., to achieve the most controlled conditions for animal and human studies. In the discussion section, 4 contradictory issues are discussed which are invited researchers to do more research to get clearer results.

Keywords: carbon nanotubes, toxicity, lung cancer detection, lung cancer treatment, drug delivery

Introduction

Lung cancer was the most significant reason for the mortality in consequence of cancers in which the women were more suffered.[1] Moreover, a recent review article noted that lung cancer is the second most common cancer in humans. Due to the growth in silence, this cancer is often associated to the death. Lung cancer is in two basic forms with the names of small cell and non-small cell lung cancer.[2] Lung cancer attempted to treat with methods as chemotherapy, radiation therapy, and various medications.[3] These methods were not highly effective due to the non-targeted and damaging healthy tissues such as hair follicles. Based on these approaches, damage in the cell cycle, break in the double strands of DNA, inflammatory responses, tissue fibrosis, etc., will have occurred. On the other hand, there were a series of treatment obstacles; low stability, solid solubility in water, and cell resistance to treatment in chemotherapy method.[4,5] Finally, in 2017, a review article collected the new research achievements which is called nano-drug delivery as

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a new cancer treatment strategy.[6] By advent of the nano-particles, a new approach to treatment emerged to discuss purposefulness and effectiveness in therapy.[5] However, tumor-targeting has many difficulties even with using the specific antibodies to bind to cancer cells; therefore, the activity of specialists is required in this field. Nevertheless, pulmonary tumors are among the invincible types of cancer, on which the researchers work to solve this issue.[7]

Several nanocarriers were used for drug delivery and improved treatment like Liposome, dendrimer, polymeric micelle, carbon nanotube (CNT), gold nanoparticle, magnetic nanoparticle, solid lipid nanocapsules, and inhalable nanocomposites. Besides, quantum dots, gold nanoparticles, CNTs, and magnetic nanoparticles were a series of nanomaterials that could be utilized to detect lung cancer.[1,2,8] Researchers have recently applied CNTs to diagnose and treat cancers such as lung, breast, prostate, liver, colon, etc.[9] Indeed, CNT structure explained in the carbon atoms were placed next to each other in a honeycomb structure, creating a tube with high physico-chemical strength.[10] In other words, CNTs are known as tubes made of carbon which has nanometers dimensions. The dimensions of CNTs expressed in various ratios with 10–15 nm diameters. Moreover, two long length ranges of CNTs in 545 ± 230, and 10,451 ± 8422 nm and short length with lower toxicity with 192 nm lengths are synthesized. Also, CNTs are divided into two groups of single-walled (SW) and multi-walled (MW) nanotubes, and they have different dimensions (Figure 1).[11] CNTs are widely used in many fields due to their thermal conductivity, electrical properties, strength, and excellent hardness.[12] Nowadays, the CNTs are utilized in drug delivery carriers, biomedical purposes, genetic engineering, artificial implants, imaging, cancer treatment, antioxidant activity, bio-sensing, etc.[13,14] In fact, CNTs are made in five ways, which are described as arc discharge, laser ablation, chemical vapor deposition, flame synthesis, and silane solution methods. Also, CNTs are purified in three ways as air oxidation, sonication, and acid refluxing.[13] On the other hand, the CNTs are a fascinating substance that can be employed to bind proteins, peptides, nucleic acids, and various drugs.[15] Furthermore, CNTs have a high potential for drug delivery due to their tubular and fiber-like structure.[16] The usable techniques to evaluate the CNTs and drugs with each other were collected as transmission electron microscopy (TEM), scanning electron microscopy (SEM), Raman spectroscopy, Fourier transforms infrared spectroscopy (FTIR), and X-ray diffraction (XRD), etc.[17]
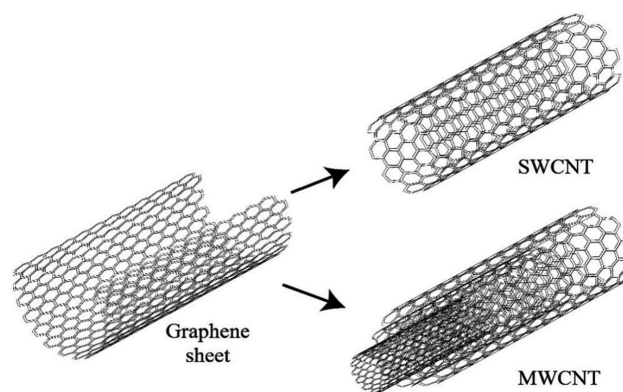

Figure 1 Scheme of SWCNT originating from a graphene sheet, while MWCNT has several graphene sheets according to the size. Carbon atoms were placed next to each other in a honeycomb structure. Reprinted from *International Communications in Heat and Mass Transfer*, Vol 78, Yazid MNAWM, Sidik NAC, Mamat R, et al, A review of the impact of preparation on stability of carbon nanotube nanofluids, Pages No. 253–263, Copyright (2016), with permission from Elsevier.[20]

However, the side effects of pulmonary fibrosis and exacerbation have been seen in the use of this approach in rodents with a history of the previous pulmonary disease.[16] The toxicity of these carriers is fully evaluated in the "toxicity assessment of CNTs" section. On the other hand, a review paper presented that the CNTs are easily recognizable and transparent due to their intense light absorption in biological imaging.[18] As well as, other features of functional MWCNTs were their detectability by multiphoton near-infrared imaging in induced region.[19,20]

There is no study conducted on the preference of use or not to use CNTs. In this study, while mentioning the disadvantages, the effectiveness of these nanotubes is discussed. Moreover, other traditional treatments have had too destructive impacts than this method. However, it is recommended that the tissue compatibility of nanotubes should be increased to increase efficiency. Despite the collection and categorization of new methods of diagnosis and treatment of lung cancer with CNT, the feasibility of these methods in humans has been discussed. Besides, the ways to reduce the toxic effects of these CNTs are some of the achievements of this article which could be useful for developing Nanomedicine. Also, the treatment of this cancer, all drugs and substances used by experts due to their type of work, are summarized.

The Pulmonary Toxicity Assessment of Carbon Nanotubes

CNTs could quickly enter the lungs through the respiratory tract and then rapidly enter and affect the nervous, lymphatic, and circulatory systems, leading to toxic effects.[21] The main reasons of these toxic effects can be durability, the amount of

residual oxygen reactive metal, and size. By removing the residual metals and selecting appropriate dimensions, the CNTs can be safe in drug transmission.[16] The smaller sizes were with less toxicity; furthermore, the concentration of metal impurities such as iron did not contribute to toxicity.[11] On the other hand, the curvature was also among the considerable parameters in MWCNTs toxicity. Indeed, the greater the curvature, the less damage occurred to the cells.[22] This issue is illustrated in Figure 2. In animal research, CNT-long exposure could induce persistent inflammation, lung cancer, fibrosis, and gene destruction in the lung.[23,24] In general, the mechanisms of creating toxicity are divided into five sections as apoptosis, reactive oxygen species, free radical formation, the formation of granuloma, and increased inflammatory response. Due to the above mechanisms, types of toxicities in cells, skin, gene, liver, cardiovascular, pulmonary, and carcinogens substance, are created.[25]

Carbon Nanotubes and Asbestos

Due to the high structural similarity of CNTs with asbestos, DNA microarray analysis showed similar physiological effects on the human bronchial epithelial cells.[12] In humans exposed to asbestos, the malignant plaques and malignant mesothelioma are created and developed. Also, animal studies showed that the CNTs could induce lung and pleural lesions, inflammation, pleural fibrosis, lung tumors, and malignant mesothelioma when animals were inhaling.[26] A study in 2018 confirmed that stiffness, hardness, length, width, and CNT longevity were five factors that could induce harmful

effects as asbestos.[27] In addition to the mentioned factors, the review report also introduced two other effective factors in causing toxicity as exposure time and the amount of accumulation in the target tissue.[28] Forty microgram was the lowest dose of MWCNTs which could induce pulmonary fibrosis, whereas 120 μg of asbestos should be inhaled to produce the same fibrosis. Also, MWCNTs, like asbestos, could alter the expression of several genes and cell survival and proliferation.[29] Another article in 2010 stated that the SWCNT produced the least toxicity, and asbestos brought the most toxicity in mesothelial cells, in the identical dose of MWCNT, SWCNT, and asbestos.[30] Moreover, an article compared MWCNT and SWCNT in this sentence which the exposure to MWCNTs mainly causes inflammation whereas SWCNTs induce apoptosis and mitochondrial dysfunction.[31] On the other side, functional CNT showed less toxicity with higher activity toward CNT without functionalization. The safe applied dose of CNTs was not determined.[32,33] Although two agents for obtaining the non-toxic dose of MWCNTs are tetrazolium bromide salt, and lactate dehydrogenase. This dose could induce the apoptosis and create the oxidative stress in A549 cancer cells.[34] An article stated that the consequences of utilizing unsafe MWCNTs dose in the lungs include oxidative stress in healthy tissue, non-malignant lung disease, and cardiovascular disorders.[35]

Toxicity of Multi-Wall Carbon Nanotubes

The presence of MWCNTs in vivo resulted in cytokines production such as TNF-α and IL-1β from immune cells
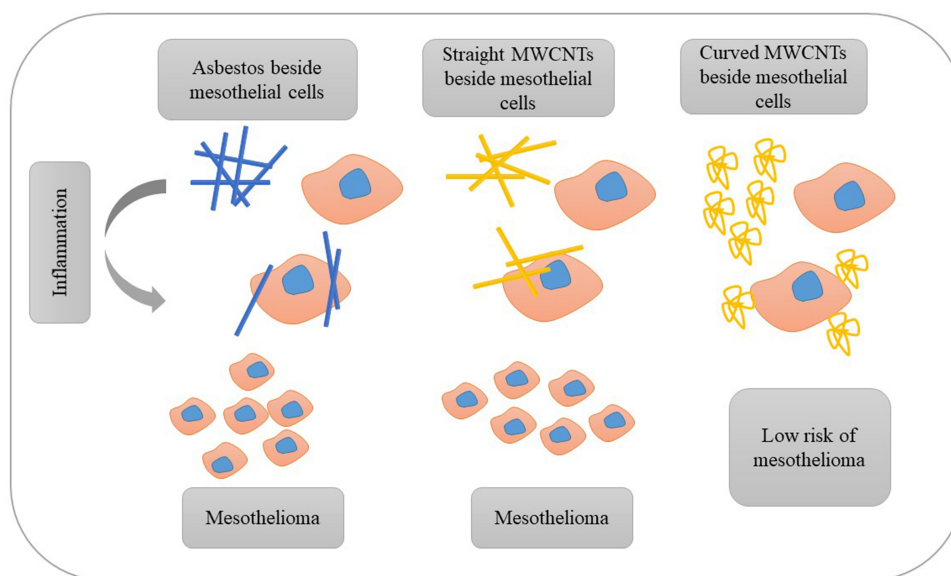

Figure 2 Evaluation of the similarity of straight MWCNTs and asbestos, and the effect of straight, curved MWCNTs and asbestos on mesothelial cells. Indeed, curved nanotubes are less dangerous for the cell than a straight CNTs or asbestos, and could better play their carrier role.

which involved in creating the toxicity.[36] In a 90-day study by the intravenous injection of MWCNT, no changes in mice weight were observed, while the toxic effects were observed, and also the mice survive, despite the toxicity.[37] In another study on Sprague–Dawley rats with injected intratracheally of 0.5, 2, and 5 mg MWCNTs, TNF-α discharge was increased from macrophages. After 60 days, the inflammatory and fibrotic reactions were observed. After 2 months, pulmonary lesions were formatted by the aggregation of collagen in the bronchial lumen of lungs.[38] The researches in 2012, and 2019 have shown that the amount of inflammation is directly related to MWCNTs dose volume.[39,40] Nevertheless, the MWCNT can decrease tumor metastasis.[41] In another study, due to the microarray survey results in different doses and various days, the MWCNTs led to the induction of pulmonary inflammation and fibrotic damage from miRNA and mRNA regulatory networks.[42,43] Another article has inferred that the development of inflammation, pulmonary fibrosis, and the induction of lung cancer by MWCNT were due to the elevated and decreased levels of blood mRNAs and miRNAs.[44]

On the other side, MWCNT-COOH activating TLR4/NF-κB signaling led to inhibiting lung tumor metastasis which happened due to the changes in polarized macrophages.[41] The MWCNT-induced carcinogenesis may involve low levels of DNA damage, and parallel increases or decreases in the expression of genes involved in several pro-carcinogenic pathways.[45] An adverse event which seen for lungs was adenocarcinoma, created by exposing with MWCNTs via inhalation.[46] The MWCNTs provoked hypomethylation in the promoters of genes area and CpG sites. Those were the other genetic alterations due to the MWCNT exposures to cells.[47] Suzui et al[48] in 2016 reported that the MWCNTs induce developing the pleural malignant mesothelioma and lung tumors. Nevertheless, the other examination on 344 rats exposed to inhale with MWCNTs showed no pleural mesothelioma during 104 weeks. Although there was the evidence of toxicity for the lungs, such as localized fibrosis, granulomatous change, and epithelial hyperplasia existed clearly.[49] Pacurari et al[50] presented that the MWCNTs can induce the lung tumor and mesothelioma during long-term usage due to creating changes in the expression of several cancer-causing genes. Another research work has mentioned a significant relationship between MWCNT and altered expression of cancer inducer genes, especially lung cancer.[51] In an animal model study, it was reported that the intact MWCNTs were more toxic to healthy cells than MWCNTs with an acidic agent.[52] A fascinating statement in 2020 expressed that no documented cell carcinoma effects were observed in the controlled injection of MWCNTs in their mice.[53]

Toxicity of Single-Wall Carbon Nanotubes

The following results were obtained from a series of researches performed on mice exposed to SWCNTs. SWCNTs provoke acute effects in lungs, such as inflammation, granuloma synthesis, the deposition of collagen, fibrosis, and genotoxic. Perfused SWCNTs are distributed in most organs which is mainly stored in liver, lungs, and spleen. SWCNTs are destroyed by kidney and bile ducts.[54] Sanpui et al[55] in 2014 demonstrated that using SWCNTs resulted in the suppression of some anti-inflammatory and anti-virus genes. Therefore, this type of nanotube could increase airway epithelial cells' sensitivity to viral infections such as influenza A H1N1, as a side effect. In the other research which conducted in 2017, SWCNTs association utilization and *SOX9* gene expression increase has been observed. This expression further induces tumorigenesis and metastasis through the body. Also, barricaded this gene expression led to repress tumor cells and suppresses the growth of tumor cells.[56] The long-term presence of these nanotubes can lead to the overgrowth of cells, the creation of colonies, the enhancement of cell migration, and angio-genicity.[57] Research has recorded that solo SWCNTs in adjacent epithelial cells were one of the fundamental forces of creating tumors.[58] Chen et al[59] declared that SWCNT's presence for six months led to apoptotic resistant phenotype, which could cause a tumor. Also, the changes in expressing a set of genes like increased activation of pAkt/p53/Bcl-2 signaling axis, Ras family, and Dsh-mediated Notch 1 were seen as agents of this apoptotic resistant. Besides, the decreased expression of genes regulating apoptosis such as BAX and Noxa genes occurred. On the other hand, protein caveolin-1 was known to produce cancer cells such as stem cells and the inactivation of protein P53 in epithelial cells. Research in 2014 represented that this protein could induce tumors, in the vicinity of SWCNTs for six months.[60] SWCNTs could lead to the induction of transcription factor slug enhances cell migration, invasion, and independent cell development.[61] Besides, the discussion of the toxicity of pure SWCNT compared to raw

SWCNT was observed the higher cell death, reduced antioxidant amount, and the activation of caspase cascade.[62] Furthermore, the SWCNTs can induce the resistance to apoptosis through the natural (antimycin A and CDDP) or external (FasL and TNF-a) pathways.[63] Researchers have found that the SWCNTs led to myeloid-derived suppressor cell accumulation which increased the cancer cells' induction of cancer.[64] A study in 2016 showed that the *mesothelin* gene expression reduced the bronchial part of epithelial cells, which were exposed to SWCNT.[65]

To conclude, the toxicity of CNTs for healthy tissue and a series of side effects for lung inflammation and fibrosis were reported. The set of effects generated is shown in Table 1. This issue could be controlled using the appropriate dose. Also, researchers can reduce the toxic effects by choosing the appropriate dimensions (shorter length and higher width), more curved nanotubes, and using the functionalized form. Moreover, the MWCNT was used more efficiently due to less toxicity toward SWCNT. On the other side, there were significant contrasts in using the nanotubes which induced tumor growth and tumor suppressor protein.

Using Carbon Nanotubes in Lung Cancer Detection

Generally, lung cancer's clinical manifestations include fatigue, coughing, wheezing, the pain in the chest, the brevity of breath, swallowing hardness, anxiety, and yellow fingers.[66] Also, a lung cancer diagnosis is possible with helping 4 methods with the names of radiological, non-radiological imaging such as MRI, endoscopic, and biochemical methods.[67] In usual, diagnosis, type, and degree of lung cancer are performed by CT scan imaging.[68,69] Unfortunately, the low-dose CT scans may bring false-positive answers about having lung cancer.[70] Today, the biomarkers of both protein and genetic modifications are known for lung cancer. Numerous biosensors were studied to bind to these biomarkers for non-invasive detection.[71] The detection of VOCs and tumor markers by breath analysis with helping CNT is a modern and studied method.[67]

The sensor array of CNTs has demonstrated a discrepancy between the healthy and the patient respiratory sample in the volatile organic components (VOC). Due to mass-spectroscopy and gas-chromatography, the rate of these components increased in the patients affected by lung cancer.[72] The sensors identify different biomarkers due to the solubility, polarity, and chemical associations, especially for tuberculosis disease.[73] The design of an electronic nose with CNTs to delete the VOC of lung cancer patients was an inexpensive and rapid method. Water, methanol, isopropanol, ethanol, acetone, 2-butanone, and propanol were found as polar vapors lung cancer biomarkers. Chloroform, benzene, o-xylene, n-decane, 1-hexene, toluene, styrene, n-propane, cyclohexane, 1, 2, 4-trimethyl benzene, and isoprene were discovered as nonpolar vapors.[74] SWCNTs, coated with non-polymeric organic substances, can detect VOC changes as the cancer biomarkers.[72] This method was a convenient, non-invasive, inexpensive, and rapid screening for diagnosing the biomarkers of lung cancer. In this study, the SWCNT was functionalized with tricosane, and pentadecane to have a high sensitivity for diagnosis nonpolar and VOC molecules.[75] The CNTs, doped with platinum, can detect styrene and benzene vapors which existed in the exhale of lung cancer patients. This sensitivity was very low in natural nanotubes.[76] SWCNTs decorated with Pd, Pt, Ru, or Rh elements could also be used to detect toluene gas as an indicator of lung cancer.[77] Although the sensitivity of CNT sensors toward nonpolar volatile organic compounds was low and led to the limitation of the diagnosis. The existence of derivatives Hexa-peri-hexabenzocoronene can increase this sensitivity for developing detection.[78] A research, conducted in 2018, stated that a biosensor was created by CNT which drugged with Rh, can distinguish and absorb C6H7N, and C6H6 in the exhaled air of lung cancer patients.[79] In another study, it was reported that infrared CNT could detect o-toluidine and aniline as two common gases in lung cancer.[80]

Moreover, a selective chemiresistive sensor for cancer-related VOC hexanal using molecularly imprinted polymers and multiwalled carbon nanotubes has been designed by Janfaza et al.[81] Another scheme used to enhance lung cancer detection was preparing a combination of SWCNT and chitosan.[82] Due to the differences in nicotinic acetylcholine receptors in normal and small cells of lung cancer, the nanotube-based electrode sensor for the quantitative electrophysiological monitoring of a nonadherent cell has been demonstrated.[83] Furthermore, nitrogen-filled CNT, attached to iron, could detect specific microRNAs of cancer cells which was known as a sensitive electrocatalysis biosensor.[84] Also, the changes in mRNAs and miRNAs predicted to indicate pulmonary fibrosis in individuals exposed to MWCNTs.[85]

As a result, by the advent of CNTs in the field of lung cancer detection, another way was opened in the aspect of the efficiency of these nanotubes in medicine (Figure 3).

Table 1 Summary of Toxic Effects Obtained from Studies by Year Order

Researchers	Year	Research	Toxic Effects	Citation
Muller et al	2005	MWCNTs on lungs of Sprague–Dawley rats	Inducing inflammation, pulmonary lesions, and fibrosis	38
Srivastava et al	2011	MWCNTs on human's lung cancer cell line-A549/in vitro	Making oxidative stress and apoptosis	34
Pacurari et al	2011	MWCNTs on mice lungs	Inducing cancer, and changes in genes expression	50
Wang et al	2011	SWCNTs on mice lungs	Inducing cancer	57
Hong Liu	2012	MWCNTs on mice lungs	Producing multiple lesions	37
Morimoto et al	2012	MWCNTs on mice lungs	Inducing pulmonary inflammation, and small granulomatous lesion	40
Guo et al	2012	MWCNTs on mice lungs	Inducing cancer, and changes in genes expression	51
Yu et al	2013	MWCNTs on male C57BL/6 mice lungs	Producing tumor	52
Rittinghausen et al	2014	MWCNTs on rat lungs	Producing tumors and cancer	22
Sargent et al	2014	MWCNTs on B6C3F1 mice lung	Inducing cancer	46
Sanpui et al	2014	SWCNTs on small airway epithelial cells/in vitro	Increasing vulnerability to infections	55
Luanpitpong et al	2014	SWCNTs on mice lungs	Inducing tumor	58
Luanpitpong et al	2014	SWCNTs on human's lung epithelial cells/in vitro	Inducing tumor	60
Dymacek et al	2015	MWCNTs on mice lungs	Inducing inflammation, and fibrosis	42
Chen et al	2015	SWCNTs on lungs line cells/in vitro	Inducing cancer, and changes in genes expression	59
Virupaxi et al	2015	SWCNTs on rat's lung epithelial cells/in vitro	Decreasing antioxidant levels, and inducing cell death	62
Pongrakhananon et al	2015	SWCNTs on human's lung epithelial cells/in vitro	Inducing cancer and making resistance to apoptosis	63
Shvedova et al	2015	SWCNTs on human's lung line cells/in vitro	Inducing cancer	64
Snyder-Talkington et al	2016	MWCNTs on mice lungs	Inducing inflammation, and fibrosis	43
Snyder-Talkington et al	2016	MWCNTs on mice lungs	Inducing inflammation, fibrosis, and lung adenocarcinoma	44
Suzui et al	2016	MWCNTs on male rats lungs	Inducing malignant mesothelioma and lung tumors	48
Kasai et al	2016	MWCNTs on F344 rats lungs	Inducing lung carcinomas	49
He et al	2016	CNT on human's lung line cells/in vitro	Inducing malignancies	65
Stueckle et al	2017	MWCNTs on human's lung epithelial cells/in vitro	Producing lung tumorigenesis	32

(*Continued*)

Table 1 (Continued).

Researchers	Year	Research	Toxic Effects	Citation
Zhou et al	2017	MWCNTs on human's lung cancer cell line/in vitro	Observing genotoxic and cytotoxicity	33
Vlaanderen et al	2017	Workers in contact with MWCNTs	No functional difference in the lungs	35
Rahman et al	2017	MWCNTs on mice lungs	Inducing fibrosis, cancer, chronic inflammation, changes in genes expression, and tissue irritation	45
Voronkova et al	2017	SWCNTs on mice lungs	Inducing cancer, and overexpression of SOX9	56
Wang et al	2017	SWCNTs on human's lung epithelial cells/in vitro	Promoting tumor, and metastasis	61
Dymacek et al	2018	MWCNTs on mice lungs	Changing in gene expression, cell proliferation, survival, oxidative phosphorylation pathway, mitochondrial dysfunction, transcription, and producing fibrosis	29
Fukai et al	2018	MWCNTs on the murine lung resident cells (GDL 1) and immune cells (RAW 264.7)	Inducing mutation in GDL 1 and producing inflammatory cytokines from RAW 264.7	36
Nahle et al	2019	SWCNTs and MWCNTs on rat's alveolar macrophage cell line	Inducing inflammation mainly by MWCNTs, while SWCNTs more relying on apoptosis and destroyed mitochondrial function	31
Gaté et al	2019	MWCNTs on the lungs of Sprague–Dawley rats	Observing DNA damage	39
Wang et al	2020	CNTs on the experimental animals	Inducing pleural lesions, pleural fibrosis, inflammation, lung tumors, and virulent mesothelioma	26
Aoki et al	2020	MWCNT on the lungs of mice	No cancerous effect	53
Önera et al	2020	MWCNT on human's bronchial cells/in vitro	Inducing methylation changes in CpG sites, and promoters of genes	47

Besides, the alteration of micro RNAs and RNAs of cancerous cells was acquirable with CNTs. CNTs could assemble several biomarkers of lung cancer by the electrode and electrical current.

Using Carbon Nanotubes in Lung Cancer Treatment

Despite many efforts by the lung cancer treatment teams, many people still die for this reason annually. It is known to be among the most common cancers in the world; the nano therapy is a new method that researchers work on. CNTs themselves are effective in treating the disease such as the activation of apoptosis pathway by targeting mitochondrial organelle in cancer cells. In this regard, CNTs attached to polyethylene glycol could focus better on cancer cell repertoires which had the potential to improve the performance in Nano-drug delivery.[86] In this section, studies are divided due to the type of CNTs, drugs, and protein.

Single-Wall Carbon Nanotubes in the Presence of Effective Lung Cancer Drugs

Due to the low toxicity of SWCNT as a carrier, it was widely used in cancer therapy and drug delivery. However, the biochemical changes occurred in serum and pulmonary inflammation.[87]

Paclitaxel

Paclitaxel utilized as an anti-cancer drug which bonded with SWCNTs beside graphene oxide. This nanostructure led to enhance the effectiveness and induce death on A549 and NCI-H460 cancer cells.[88] Another study to deliver this drug was about SWCNT modified with chitosan, which led to increase the compatibility in vivo. Also, the layer of
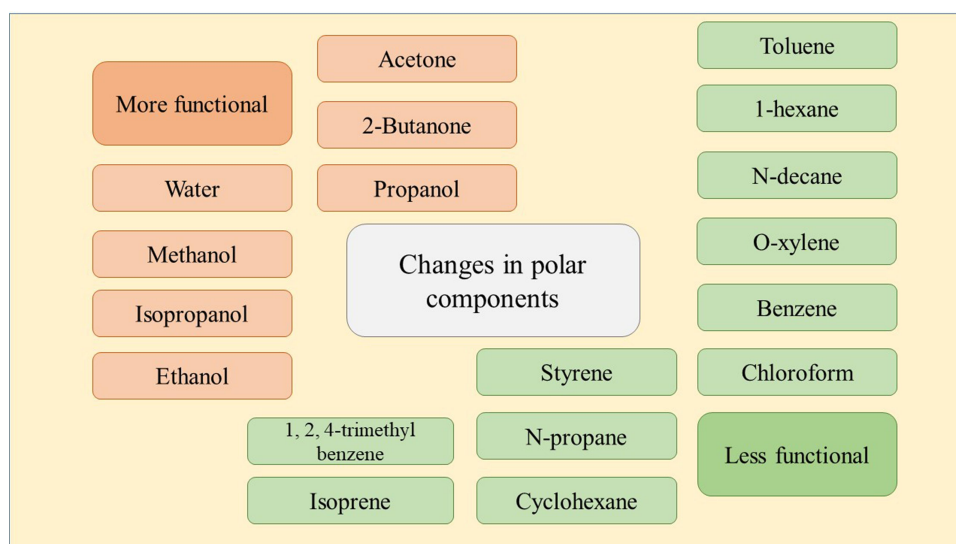
Figure 3 Summary of diagnostic cases based on the changes in polar components. Targeted CNT sensors could be connected to a variety of modified vapors. In this way, with the help of this method screening make non-invasive diagnosis possible.

chitosan has been combined with hyaluronic acid to target A549 cells.[89]

TRAIL

Apo2L or TRAIL is a protein that can bind to cancer cell receptors and induce apoptosis without a toxic effect on healthy cells. In contrast, the presence of SWCNTs, due to TRAIL due to and its high solubility in the blood led to the proper distribution of this protein and rapid pulmonary tumor eradication.[90]

Doxorubicin

Delivering doxorubicin with SWCNTs due to magnetic localization and better distribution can enhance the targeting and increased therapeutic efficiency. Indeed, this project which was performed on mice, confirmed the results of increasing the efficiency of therapy by MRI technique.[91]

Curcumin

In a study in 2018, Curcumin, a chemical produced which has therapeutic potential for A549 cancer cells, was investigated in a nano-state with an SWCNT carrier. The carrier, which was functional with chitosan and alginate polysaccharides, increased the efficacy of the drug.[92]

Survivin siRNA and Doxorubicin

A study in 2019 showed using survivin siRNA, and doxorubicin with SWCNT carrier, as two factors for increasing apoptosis and expressing less *survivin* as an inhibitor

gene apoptosis. The carrier was functionalized with poly-ethyleneimine (PEI) besides betaine.[93]

Gemcitabine

Also, Gemcitabine is among the anti-cancer drugs for non-small cell lung cancer. In a clinical trial on B6-mice, the drug was tested with an SWCNT carrier. In this study, the cell line of A549 was examined which shows interesting results repression. In fact, this research has identified SWCNTs as encouraging carriers for drug delivery due to the high loading inclination of the drug, prolonged distribution time, and notable cell membrane permeability.[94]

Multi-Wall Carbon Nanotubes in the Presence of Effective Lung Cancer Drugs

The presence of plasma-functioned MWCNT with graphene oxide decreased the expression of telomerase reverse transcriptase.[95] In another study on targeting drug-resistant cancer cells, researchers modified MWCNT with a coating ethylene glycol and antibodies against p-glyco-protein as a multidrug resistance protein. The results showed that in these cancer cells, the toxicity was significantly increased after light irradiation whereas it did not have any toxic effect on healthy cells.[96] CNTs acted as drug carriers which are categorized in this article due to the drugs used in research works.

SiRNA

CNTs are used as a highly effective vector in clinical treatment due to their high antitumor and durability

properties. MWCNT NH+3 with Si RNA can increase the viability and prevent tumor growth in the animal model.[97] Furthermore, tumor eradication via targeting the *Polo-like Kinase* gene for delivery siRNA by MWCNT-NH3+ was suggested to treat lung cancer.[98]

Doxorubicin

Doxorubicin was known as an anti-cancer growth inhibitor. Conjugated MWCNT with this drug and hyaluronic acid had more toxicity and apoptosis effect on A549 cells. This complex showed free of toxicity for kidneys, heart, and liver. Optical imaging and scintigraphic techniques could also be used to identify the location and operation of the complex.[99] The study of Lodhi et al[100] presented that Doxorubicin HCL besides MWCNTs and MWCNTs conjugated with Dexamethasone can affect the epithelial cancer cells. The complex of MWCNTs, Dexamethasone, and Doxorubicin had more cytotoxicity on these cancer cells, less hemolytic effect, and superior and quicker diffusion and dispersion.

Methotrexate

Methotrexate could conjugate with MWCNTs for lung cancer therapy to decrease drug waste and use it in the target way. Moreover, the animal tests indicated that this complex was free of toxin effects on cardiac, hepatic, and nephrotic systems.[101] In another study, chitosan-coated CNTs were used as carriers of methotrexate. In this in vitro research, less toxicity of this drug on healthy lung cells (MRC-5) and improved their anti-cancer activity in the cancerous lung cells (H1299) were admitted.[102]

Cisplatin

Li et al[103] presented that MWCNTs conjugated with cisplatin as a chemotherapeutic agent led to increase treatment and decrease the toxicity for liver and kidney as typical side effects. This efficient carrier can improve the platinum levels of accumulation in target organs such as the lungs. The study was conducted on mice showed that CNTs do not have abnormal immune and inflammatory responses.

Betulinic Acid

The acid-functionalized MWCNTs loaded with betulinic acid had an anti-cancer activity which can be analyzed by ultraviolet light and thermogravimetric. In fact, lung cancer cells were more sensitive to certain concentrations of this drug.[104]

Docetaxel

In a study, conducted by Singh et al,[105] it is mentioned that docetaxel in the presence of an MWCNT carrier conjugated with the transferrin protein, 136 times more effective than the drug used alone. The drug uptake was reported to be much higher in A549 cancer cells. In another research in 2016, it was declared that this drug and MWCNT coated by d-alpha-tocopheryl polyethylene glycol 1000 succinate is more effective than non-targeted therapy.[106] Another research in 2017 demonstrated that docetaxel, loaded on MWCNTs conjugated with chitosan-folate, was 89-fold more effective in targeting A549 cells.[107]

Etoposide

Transferring this chemotherapy drug with functionalized MWCNT used in other research for the treatment of cancer. In this research, simultaneously delivery of Bcl-2 and VP-16 targeted antisense by MWCNTs for anti-cancer effectiveness in both kinds of lung cancer cells was done.[108]

IRGD Peptide and Candesartan

By placing polyethyleneimine and cystamine ligands on MWCNT, researchers modified the attachment to iRGD peptide and candesartan. These two substances boosted the efficiency of MWCNTs transfer and cell absorption. The positioning of these ligands led to the targeting of parenchymal and endothelial cancer cells, and treatment of lung cancer occurred.[109]

To conclude, in the treatment of this cancer, nanotubes can lead to recovery or serve as a carrier for several functional substances (Figure 4). In the discussion of drug delivery, nanotubes were functionalized with various substances which led to targeted binding to the tumor tissue. In general, the studies with MWCNTs were more frequent. Indeed, due to researches, it could be stated that CNTs are also useful carriers for nano-drug delivery. CNTs could induce the apoptotic pathway in targeted cells. Besides, lower efficiency may sometimes occur due to the drug resistance. Table 2 classifies the set of researches conducted under the relevant conditions and the results obtained.

Discussion

In this section, 4 challenging and contradictory issues are discussed. Also, it invites researchers to do more research to get clearer results.
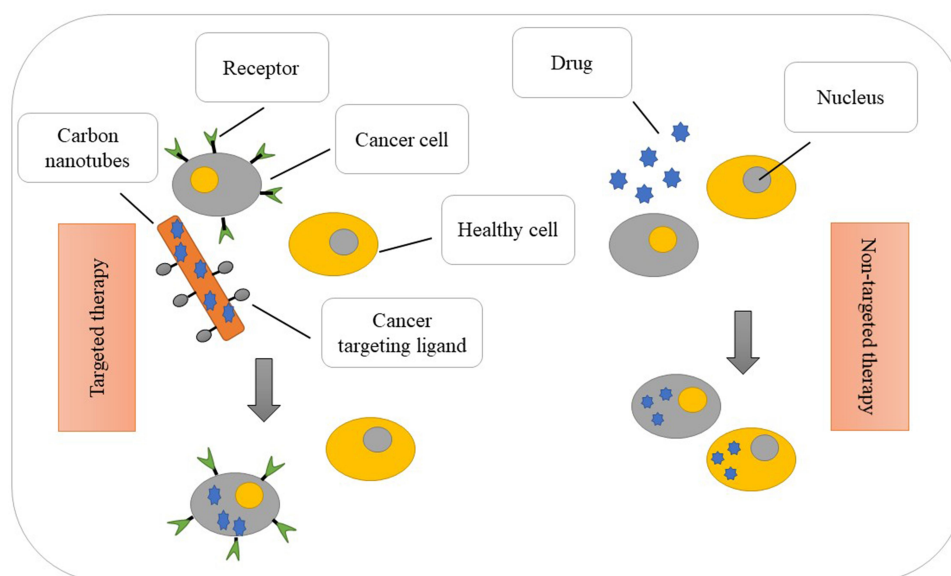
Figure 4 Targeted and non-targeted treatment differences. Indeed, the figure refers to the accumulation of drug to the target cell. The drug is not distributed freely in nano-drug delivery.

1. The efficacy and toxicity of CNTs are always an excellent opportunity for discussion.[11,16] One point of contention is that an article stated that a low mount of CNT has more potential to produce toxic effects than asbestos.[29] In contrast, Pacurari et al[30] stated that asbestos is much more toxic. As well as, the other article confirmed and mentioned that SWCNT had less toxicity in the organs or changes in immune indicators.[87]

2. On the other side, two articles stated that the amount of pulmonary inflammation was directly related to CNT dose consumption.[39,40] However, another article declared that different doses of CNTs at the same time can cause inflammation.[38] Also, increasing the length of CNTs led to heightened toxicity for healthy cells and expanded CNT, despite increased utilization resulting in tumor tissue destruction.[7,11]

3. An article stated that SWCNT led to the expression of *SOX9* created tumorigenesis and metastasis.[56] In contrast, the activation of P53 as tumor suppressor protein was seen in the presence of MWCNTs without mutagenic effectiveness.[45] Also, the insertion of nanotubes into the body resulted in increased cytokines such as TNFα and IL1β, which results in toxicity. On the other hand, enhanced cytokines IL-10, and TGF-β, restricted tumor metastasis.[36,41] Indeed, the role of tumor destruction of CNTs did not rule out. Another

study on breast cancer was performed with CNTs, which confirmed that CNTs could reduce tumor size.[110] Furthermore, an in vitro study on A569 cells also approved an increase in the anti-cancer properties of CNT decorated with naringenin.[111] A very interesting report in 2020 stated that no documented carcinogenesis had been observed in the locally controlled injection of MWCNS in their mice analyzed. In this study, the use of CNT in a controlled manner is considered safe.[53] Another study presented the other advantage of using CNT in nano-drug delivery of hepatocellular as target cells. In this article, the drug was ruthenium polypyridyl, in which cancer cells had resistance to drug and radio waves. CNT can cause to induce more apoptosis with the increased uptake and reduced toxicity.[112]

4. An investigation stated which CNTs have antioxidant activity in two groups as fight free radicals, and anti-aging activity.[14] Two other studies reported a decrease in antioxidant activity due to using CNTs.[25,62] This controversial issue needs further study in this area.

Conclusion and Future Perspective

Due to the researches, it can be mentioned using nanotubes in the diagnosis of disease, has been known to be an effective method. Additionally, studies have suggested that the immune response is not unusual in the in vivo. However, researchers

Table 2 Review of Studies and Results of Nano-Drug Delivery by CNT in the Treatment of Lung Cancer (by Year Order)

Researchers	Year	Type of CNTs	Functionalized with	Type of Drugs/Protein	Targeting	Results	Citations
Podesta et al	2009	MWCNT	Cation	siRNA	A549 in vivo	Results were desirable	97
Datir et al	2012	MWCNT	Hyaluronic acid	Doxorubicin	A549	The hopeful result was observed	99
Arya et al	2013	SWCNT	Graphene oxide	paclitaxel	NCI-H460, and A549	Treatment was effective	88
Lodhi et al	2013	MWCNT	Just carboxylate	Dexamethasone	A549	Approach was more toxic for A549	100
Das et al	2013	MWCNT	Fluorochrome	Methotrexate	A549	The hopeful result was observed	101
Li et al	2014	MWCNT	....	Platinum	Mice lungs in vivo	Results confirmed that MWCNT are efficient nan-carrier	103
Tan et al	2014	MWCNT	Just carboxylate	Betulinic acid	A549	The drug was used in low concentrations and the results were positive	104
Zakaria et al	2015	SWCNT	pyrene butyric acid N-hydroxysuccinimide ester, and Polyethylene glycol	TRAIL or Apo2L	H1703	Treatment was effective	90
Guo et al	2015	MWCNT	Cation	siRNA	Calu 6	Anti-tumor effects were seen	98
Yu et al	2016	SWCNT	Chitosan	paclitaxel	A549	Cell proliferation was inhibited	89
Al Faraj et al	2016	SWCNT	Polyvinylpyrrolidone	Doxorubicin	Mice lungs in vivo	Treatment was more effective	91
Razzazan et al	2016	SWCNT	Polyethylene glycol	Gemcitabine	A549 in vivo	Anti-tumor efficacy was seen	94
Pratap Singh et al	2016	MWCNT	Alpha-tocopheryl polyethylene glycol 1000 succinate	Docetaxel/coumarin-6	A549	Results confirmed that MWCNT are efficient nan-carrier	105
Pratap Singh et al	2016	MWCNT	Alpha-tocopheryl polyethylene glycol 1000 succinate	Docetaxel/coumarin-6	A549	Efficacy with safety was seen	106
Kim et al	2017	MWCNT	Polyethylene glycol	....	Mitochondria in lung cancer cells (A549, and NHFB)	Anti-lung cancer efficacy was seen	86
Attri et al	2017	MWCNT	Graphene oxide	....	A549, and H460	The hopeful result was observed	95

(*Continued*)

Table 2 (Continued).

Researchers	Year	Type of CNTs	Functionalized with	Type of Drugs/Protein	Targeting	Results	Citations
Pratap Singh et al	2017	MWCNT	Chitosan	Docetaxel/ coumarin-6	A549	Results were desirable	107
Heger et al	2017	MWCNT	Polyethylene glycol	VP-16 and Bcl-2-targeted antisense	DMS53, and NCIH2135	Hypersensitivity to this drug was observed	108
Su et al	2017	MWCNT	Polyethyleneimine	iRGD	A549	Treatment was effective	109
Neha et al	2018	SWCNT	Chitosan	Curcumin	A549	Cell proliferation was inhibited and apoptosis was induced	92
Suo et al	2018	MWCNT	Polyethylene glycol	....	Multi-drug resistance cells	The hopeful result was observed	96
Cao et al	2019	SWCNT	Polyethylenimine with betaine	*Survivin* siRNA, and Doxorubicin	A549 (in vitro/in vivo)	Anti-tumor effects were seen	93
Giuseppe Cirillo et al	2019	MWCNT	Chitosan	Methotrexate	MRC-5, and H1299	Less toxicity for healthy cells and more anti-tumor effect were confirmed	102

Notes: A brief description of the cells mentioned: (A549, NCI-H460, H1703, Calu 6, NHFB, H460, NCIH2135, and H1299 are non-small cell lung cancer cell lines), (DMS53 is small cell lung cancer cell line), (MRC-5 is fibroblast cell line).

should seek to increase the compatibility of these carriers. The findings also showed that the treatment with CNT was much more effective and more successful than the traditional treatments for this cancer. Fortunately, this type of cancer treatment is rapidly investigated by researchers. Also, using the new methods such as functionalization, nanotubes with a longer length, more width, and greater curvature partially can be done with lower toxicity. The collection of studies summarized has highlighted the need for a detailed assessment which includes the dose of CNT, duration, method of induction, etc., to achieve the most controlled conditions for animal and human studies. Finally, other drugs have also been used to target cancer cells using other Nano methods that could be evaluated for their efficacy using CNTs.[113–115]

Disclosure

The authors declare that there are no conflicts of interest.

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