Carbon Nanotubes: A Versatile Technique for Drug Delivery

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Abstract
Carbon nanotubes (CNT’s) have recently gained the interest in the area of novel drug delivery system. This novel carrier can effectively administer the drugs for achieving safe and effective therapeutic regimen. CNT’s can be surface engineered (i.e functionalized) with certain groups in order to alter their physicochemical and biological properties to enhance the solubility of many drugs for efficient tumor targeting as well as drug delivery. Basically carbon nano tubes have larger surface area, due to this unique property CNT’s are capable to penetrate into the cells and deliver the therapeutic molecules at targeted site. Apart from targeting cancer cells, CNT’s can serve as a potential carrier for peptides, proteins, nucleic acids and wide range of therapeutics. In addition, CNT’s have capacity to capture the pathogenic bacteria as CNT itself posses anti microbial properties. Nearly a decade CNT’s exploring the phenomenal growth in the research activity in various fields. At the same time, it is believed that the constant development and applications of CNT’s can serve as a versatile tool for drug delivery as well. Therefore, the present review described about application of carbon nanotubes in drug delivery system.

Key words: carbon nano tubes, drug delivery, production, applications, toxicity.

1 Introduction
In recent year’s many attempts have been carried out to deliver the drugs and therapeutics through carbon tubes due to its unique structure and properties. First carbon tube is synthesized by Iijima in 1991, carbon tubes are cylindrical molecules formed by rolling single layer or multiple layer of graphene sheets into cylinder [1]. Carbon nano tubes can be synthesized by using following techniques via, electric discharge, laser ablation, thermal or plasma enhanced chemical vapor disposition (CVD) [2]. Based on their structure they can be classified into single walled (0.4-3nm), double walled (1-3nm) and multi walled (2-100nm) carbon nano tubes. Among these, single walled CNTs can readily penetrate into the cell and this property makes them a suitable carrier for drugs to be delivered in to the cells [3].

Apart from these properties CNTs have high mechanical strength and high aspect ratio and more bio compatible. Due to its high surface area they are capable of conjugating and adsorbing with wide variety of therapeutics and bio molecules. CNTs help these molecules to penetrate into the target cells and to treat disease like cancer [4-6]. The main disadvantage of CNT in drug delivery is their poor soluble in nature. However, the solubility of the CNTs can be enhanced by adding surfactants in order to functionalize their organic group and make them soluble and suitable carrier for drug delivery [7]. The main aim for using CNT as carrier for drug targeting is to release the drug in controlled manner to the targeted site. Targeting drug delivery system improves the therapeutic efficacy and reduces the systemic toxicity. Hence, the present review described about application of carbon nanotubes in drug delivery system.

2 Types of carbon nanotubes
2.1 Single wall carbon nano tubes
Single wall carbon nano tubes (SWCNTs) [8] are discovered in 1993 and which is having diameter close to 1 nm, with a tube length that may be many thousand times larger and up to orders of centimeters [9]. The structure of a SWCNT can be formed by wrapping a one-atom-thick layer of graphite (or graphene) into a seamless cylinder.

2.2 Double wall carbon nano tubes
Double-wall nanotubes (DWNT) are an important sub-segment of MWCNT. These materials have same morphology and other properties of SWCNT, while significantly improving their resistance to chemicals. This property is especially important when functionality is required to add new properties to the nanotube. Since DWNT are a synthetic mixture of both SWNT and MWNT, they have good electrical, thermal stability and flexibility when compared to both SWCNT and MWCNT. SWNT that have been functionalized are more susceptible to
3.1 Arc discharge method
Zujin shi, et al. reported the production of SWCNTs by arc discharge method [10], by applying a current of 40-100 A in helium with pressure of 100-700 torr at the electrode, the arc is generated. Due to the constant distance of electrode, electric discharge is produced which is collected in the inner wall of the chamber. Product is extracted by Cesium oxide and washed with 1:1 con HCL and dried at 100°C to remove the impurities, fullerenes and catalyst.

3.2 Catalyst chemical vaporization method
CNTs produced by catalytic chemical vaporization method could be very low cost in large scale. Flahaut et al. reported the synthesis of mixture of SWCNT and MWCNT by decomposition of H\textsubscript{2} /methane on nano composite powder of Fe alumina [11]. Colomer et al. reported the SWCNTs can be prepared by addition of magnesium oxide with ethanol solution of metal salts with respective concentration mixture is sonicated for 1 hr. Ethanol is removed by rotary evaporator and material is dried at 130°C for 12-15 hr. The obtained coarse material is grind into fine powder. Final portion of each catalyst is hydrogenated in H\textsubscript{2}/N\textsubscript{2}. SWCNT synthesis is carried out in fixed bed reactor at 1000°C with a typical reaction time for 10 mins for every synthesis; 1gm of catalyst is placed in center of reactor. Hydrogen and methane are used as catalyst. Carrier gas allows the methane and hydrogen to flow through fixed bet reactor. Once reaction completes catalyst are removed by using con. HCL. 1gm of sample and 50ml of HCl is placed in to Sonicator for 15 mins and then filtered, washed and dried at 130°C [12].

3.3 Laser ablation technique
Continuous laser is used to vaporize a graphite target in an oven at 1200 °C. The oven is filled with helium or argon gas in order to keep the pressure at 500 torr. This method is very expensive so it is mainly used for SWNTs. Laser vaporization results in higher yield of SWNTs with narrower size distribution than those produced in arc discharge process. Catalyst used for SWNTs is Nickel: Yttrium (4:2). Carbon and catalyst are evaporated at 1200°C by using high power laser in these procedure are carbon condenses into SWCNT with the help of catalyst [13].

3.4 Electric arc technique
C. Journet, W. K. Masser and their co workers reported the laser production of SWCNT by electric arc technique [14]. Arc is produced between two electrodes under helium atmosphere. Both the cathode and anode are drilled and filled with graphite powder and metallic catalyst by applying a voltage of 100A. A voltage drop of 30V is produced between two electrode during this condition, rubbery products condensed on chamber wall and it forms web like structure between cathode and the reactor walls, at cathode end deposits black, very light porous material is formed around the cathode.

Figure 1: Conceptual diagram of SWCNT, DWNT & MWCNT
breakage. Creating any structural imperfections can modify their mechanical and electrical properties. However, with DWNT, only the outer wall is modified, thereby preserving the internal properties. DWNT can be applied to gas sensors and dielectrics, and to technically-demanding applications like field-emission displays, nanocomposite materials, and nano sensors.

2.3 Multiwall carbon nano tubes
MWCNTs consist of multiple layers of graphite rolled on themselves to form a tube shape with an interlayer spacing of 3.4 Å. The outer diameter of MWCNTs may range from 1 to 50 nm while the inner diameter is usually of several nanometers. Two models are used to describe the structures of MWCNTs such as the Russian Doll model are the sheets of graphite are arranged in concentric cylinders. The Parchment model consist a single sheet of graphite is rolled in around itself, resembling like a rolled up newspaper.

3. Production of carbon nano tubes
3.1 Arc discharge method
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Figure 2: Single walled carbon nanotubes by arc discharge method
4 Mechanism of cellular uptake
CNTs are capable to penetrate into cellular membrane and active cellular constituents without causing damage to the cells; this is achieved due to their needle shape. Water soluble CNT are able to enter the cells and cellular uptake is based upon size and surface chemistry. CNT functionalized by oxidation, coated with surfactants or polymers are engulfed by cells via endocytosis path way. Due to their needle shape CNTs are capable to penetrate into the cellular membrane and pass into the cellular components without causing cell damage. Chen and coworker designed the nano injector using atomic force microscopy. In that, tip of functionalized MWCNTs were attached to the model carrier compound through disulfide linker and it was successfully transported into the cell where disulfide bond breaks that results in the release of the compound into the cell [15]. Vertical positioning of CNTs to the cell membrane shows that uptake of CNTs was similar to nano needle which diffuses into the cell by without causing cellular damage. Fluorescinated protein was attached to the SWCNT-biotin was detected inside the endosomes, that showed the uptake of CNTs by endocytosis. On other hand, absence of CNTs manifest that no protein was detected in the cell reported by Kam and
coworkers [16]. CNTs labeled with fluorescent compound shown to be penetrated via cell into cytoplasm or the nucleus [17]. In another study it was reported that uptake mechanism of MWCNTs is fully depend on their size and length of nano tubes which are shorter than 1µm were easily penetrate into cell this uptake mechanism is not through endocytosis [5].

5 Applications of carbon nanotubes in drug delivery

5.1 Cancer targeting

It is well understood that cancer cell over express folic acid receptors, from various researches it was concluded that nano carrier’s which was surface engineered to which folic acid can be attached for cancer targeting. More ever CNTs have been reported that it has high retaining capacity to accumulate in the lymph nodes for longer duration of time when compared with other nano carriers. Hence, CNTs may be used for targeting cancer cells as shown by various investigators. Such as cisplatin anticancer drug which was formulated into magnetic nano particles loaded into MWCNTS and functionalized with folic acid. With external magnet, CNTs were targeted to lymph nodes and released the drug for longer period in order to inhibit the tumor growth. In a recent study by Yang et al. revealed anticancer drug gemcitabine was loaded into magnetic MWCNT, which was studied with mice module shows high activity against lymph node metastasis when formulation was injected subcutaneously [18]. Further, N. Gsahoo, et al. reported that camphothecin poorly water soluble drug loaded into PVA functionalized MWCNT was very effective for treatment of skin and breast cancer [19].

Another platinum anticancer drug carboplatin after incorporating into CNTs showed to inhibit the proliferation of urinary bladder cancer cells in an in vitro study. Lii and co worker (2011) developed dual targeting drug nano carrier by conjugating iron nano particles and folate molecules, which was loaded with doxorubicin and it showed that there was superior deliver of drugs to HeLa cells when compared to free doxorubicin [20]. Bio adhesive polymers such as chitosan and sodium alginate were used to enhance the aqueous dispersability of the nano tubes and folic acid was use to improve targeting properties.

5.2 Lymph targeting

Lymph metastasis occur in cancer which results in frequent tumor reoccurrence even after lymph dissection. In order to overcome this issue, F. Yang et al. used magnetic MWCNT which delivered gemcitabine to lymph node under the guidance of magnetic field [18]. By using this method various chemotherapeutic agents can be delivered to lymph node.

5.3 Gene therapy

Gene therapy aims to treat disease by using genetic materials. The ability of macro molecules to cross the biological barrier and to express to the particular cell is a challenging due to its particle size and high molecular weight, with viral and non viral vectors this can be achieved. Pantratto and co worker developed functionalized SWCNT –DNA complexes and found that high DNA expression when compared to naked one [21]. Cai et al. reported the use of gene delivery formed by nickel particles enclosed on their tips and pDNA immobilized on the surface and found CNT-pDNA complex enters mammalian cell and showed gene expression in 80-100% of cell population [22]. Kam et al. reported that gene slicing can be done by siRNA-CNT conjugates [23]. The ability of the genetic material is to cross the biological membrane is poor, viral and nonviral vectors are used to carry the genes and internalize them inside the cells. M.R McDevitt et al. reported that tumor specific monoclonal antibodies, radio metal ion chelates and fluorescent probes can be attached successfully to SWCNTs [24]. J. Meng et al. reported that anti tumor response can be increased by conjugating a tumor lysate protein to MWCNTs [25]. Angeogenesis targeting antibodies were attached to SWCNTs via radio metal ion chelates which reduced the volume of tumor cells and prolonged the life time of animal models [26].

5.4 Vaccine delivery

Vaccine delivery can be achieved by linking an antigen to CNT without losing its confirmation and by inducing antibody response with right specificity. Pantroto et al. reported the use of CNT in improving the immune response [21]. It is found that CNT protein complex enhances the immune response when attached to the antigen which strength the possibility of incorporating CNT in vaccine. Bianco et al. demonstrated that in the presence of cationic CNTs deliver synthetic oligodeoxy nucleotides, which improve the immune stimulatory properties. Synthetic oligo nucleotides have nonspecific protection against cellular pathogens and enhance antigen specific immune response.

5.5 Brain targeting

Glioma is a brain tumor which is capable to evade the host immune system, which will lead to poor beneficial effect from chemotherapy. Immunosuppressive cytokines such as prostaglandin E and TGF- beta and II-10 are secreted by glioma cells. Macrophages are capable of having high affinity towards MWCNTS when compared to glioma cells. Liu et al. developed an immunotherapy by using GL261 murine glioma cancer model; macrophages engulf CNT when compared to glioma cells. MWNMTs increase the influx of macrophages into the glioma cells [27]. This was reported that which led to increase level of IL-10expression which reported that immune modulation using CNT is a possible way to treat brain tumor.

5.6 Photo thermal therapy of cancer

Due to the unique property of CNTs, it is capable of absorbing light in the infra red region which leads to
heating of CNT; by using this method cancer cells can be destroyed. Gannon and co worker used functionalized SWCNT, which followed by incubation of hepatic tumor cells on application of radio frequency at 980nm, caused the thermal destruction of tumor cells [28]. In contrast tumor cells injected without CNTs that were viable after application of radio frequency.

5.7 Other applications

Pathogenic bacteria from liquid medium can be trapped by using surface engineered CNT. Also, CNT itself had anti microbial effect which adsorbs the micro organism into engineered surface. Nanotube induced oxidation of intercellular antioxidant glutathione, resulting in increased oxidative stress on bacterial cell and cause death [29-30]. Binaco et al. reported the antifungal activity of amphotercin B which was transported into mammalian cell through CNTs which reduced the antifungal toxicity when compared to the free drug [31]. About 40% of cells were killed by using free drug, where as no cells were killed in CNT formulation.

6 Toxicity of CNT

CNTs are considered as a potential carrier for drug delivery in the field of medicine, however some toxicity effects had been observed. Both in vitro and in vivo test had been carried out to evaluate the toxic effects of CNTs. Toxicity were raised due to presence of ferric impurities and on their length [32]. Apart from this, various factors are influence the toxic effect such as physical form, degree of functionalization and agglomeration state also leads to toxicity [33].

CNTs when administered to mouse through different routes, more adverse effects were found such as accumulation of CNTs in major organs like liver, spleen, and lungs [34]. But, negligible toxicity was found in liver and lungs after intravenous exposure to CNT of increasing concentration. However, subcutaneous administration of CNTs shows no adverse effect like drug allergy, toxicity, or ulceration. Similarly no agglomerates deposited in liver, lungs and spleen, and it was found that only small amount of CNT entered into blood, circulation, hence the toxicity is mainly depends on the route of administration. Pacurari M et al. reported that CNTs may activate many cellular pathways which will cause damage to DNA [35]. Hence, further detailed investigations are required to know the information about DNA damage.

7 Conclusion

CNTs are used as a prospective carrier in drug delivery, due to their unique properties they can be used as a carrier for drugs and bio molecule and genes. Due to their novelty and potential applications they can be used in treatment, diagnosis and to adsorb pathogenic micro organisms. The major challenge and limitation of the CNTs is the toxicity. Although many reports had suggested that well functionalized carbon tubes is safe. More pre clinical and clinical studies are needed before carbon nano-tubes based drug delivery. More specific drugs are being developed to use the specificity and potency of these drugs, new drug delivery systems must be implemented. Optimistically, carbon nano tube technology is also a one of such potential technique to deliver the most specific and potential drugs to treat acute as well as chronic diseases. Carbon nano tubes delivery are promising candidates that will enable efficient and targeted delivery of novel drug compounds.

References

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