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Review Article

Carbon Nanoparticles: A Complete Review on Origin and Medical Application

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Abstract

Carbon nanotubes were introduced in the 21st century into biomedicine for the delivery of therapeutic, diagnostic and imaging agents. CNTs are unique one-dimensional structures having fascinating physical, mechanical and chemical properties and also drug loading properties. Ease in cellular uptake allows them to be explored for a wide range of applications in biology and medicine. This current review is an informative compiling of the origin of CNTs, and how CNTs are used in different aspects of biomedicine including drug delivery and cancer treatment, bio-sensing, biomedical imaging, and tissue engineering. The current review also focuses on the pharmacokinetics, toxicity, and metabolism of CNTs and discusses the potential advantages and obstacles associated with biomedical applications.

Keywords: Diagnostic, Cancer, Toxicity, Bio-medical, CNT, Bio-sensing.

1. INTRODUCTION

Development in Nano drug delivery systems has increased exponentially in recent years, due to their quantum behavior, prolonged action, dose reduction, target ability, toxicity & minimization(Ferrai, 2005). These systems can be prepared by controlling their composition, shape, size, morphology (Cui *et al*., 2010). Use of this drug delivery to a particular drug increases its sensitivity and selectivity. Research on CNTs as drug delivery & drug carrier has increased over past 3 decades(Huang, Zhang, Xu, Bao, & Li, 2010). Earlier it has been used as additives in electronics optics plastics. However, because of their ultra-small size and large surface area, conjugation with a variety of molecules can be done so that the use of CNTs has been extended into a pharmacy (Bao, Tian, & Estrada, 2010). Drug delivery through CNTs has appeared promising delivery in short interfering RNA, DNA plasmids, proteins, particularly in cancer therapy(D. Chen *et al*.).

CNTs belong to the fullerene family of carbon allotrope with a cylindrical shape. It is one of the most magnificent elements that revolutionized material science due to its strong fibers, porous gas absorptivity with the best solid lubricant. CNTs have an essentially non-crystalline impermeable material, which are the hardest substance and most occupying space(Thess *et al*., 1996). These CNTs have a specialized mechanism called 'penetration mechanism' that enters into a cell by endocytosis independent and this allows the direct cytoplasmic delivery of drug molecules (Donaldson *et al*., 2006; Peigney, Laurent, Flahaut, Bacsa, & Rousset, 2001; Ye *et al*., 1999). CNTs can be functionalized and those moieties are used in genetic engineering and Nano-medicine. Functionalized moieties are hydrophilic and easily enter into cancer cells (Ivanova, Lamprecht, Loureiro, Huzil, & Foldvari, 2012; Z. Yang *et al*., 2010).

2. Structure Morphology and Types of CNTs

Allotropy of carbon forms various shapes and configurations. These forms include graphite, Graphene, diamond, coal, bucky balls (Jia *et al.*, 2007; Lucente-Schultz *et al*., 2009; Zhu, Yudasaka, Zhang, & Iijima, 2004). The typical carbon nanotube measures about 20-150 angstroms in diameter and 1000-2000 angstroms in length. (Georgakilas, Tzitzios, Gournis, & Petridis, 2005; O'connell *et al*., 2002; Rao *et al*., 1997). Due to similarity with graphite, CNTs are arranged like rolling up the graphene sheets. Based on the rolling of

graphene sheets CNTs are classified as single-walled carbon nanotubes, multi-walled carbon nanotubes as shown in figure 1, and carbon Nano fibers. Single-walled carbon nanotubes are folded with a single graphene sheet and are mono cylindrical carbon layers with a diameter range of 0.6-2.4nm. They can be organized with chiral, armchair, zigzag arrangements. The multi-walled carbon nanotubes are folded by multiple graphene sheets with an average diameter of 1-3nm for central cylindrical tubes and 2-100nm for an external tube.

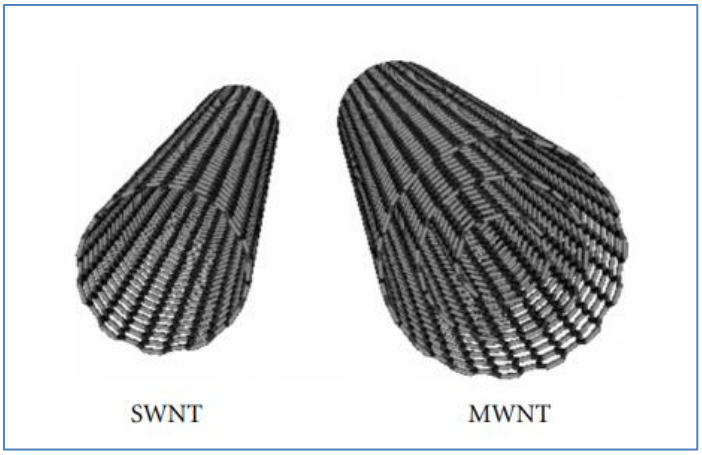

Fig-1: Structure of single walled carbon nanotube and multi-walled carbon nanotubes (Elhissi, Ahmed, Hassan, Dhanak, & D'Emanuele, 2012)

The special property of MWCNTs was it exhibits a striking telescoping property& has various textures. It includes herringbone texture in which graphene layers are at an angle concerning to nanotube axis and the other is bamboo texture in which graphene sheets are oriented perpendicular to the nanotube axis. The MWCNTs have more strength than SWCNTs. Due to its folding SWCNTs can be easily twisted and more accountable than MWCNTs. Reports suggest that CNTs are the strongest, stiffest, materials discovered to date in terms of tensile strength, elasticity, with intrinsic superconductivity (28) as shown in figure 2.

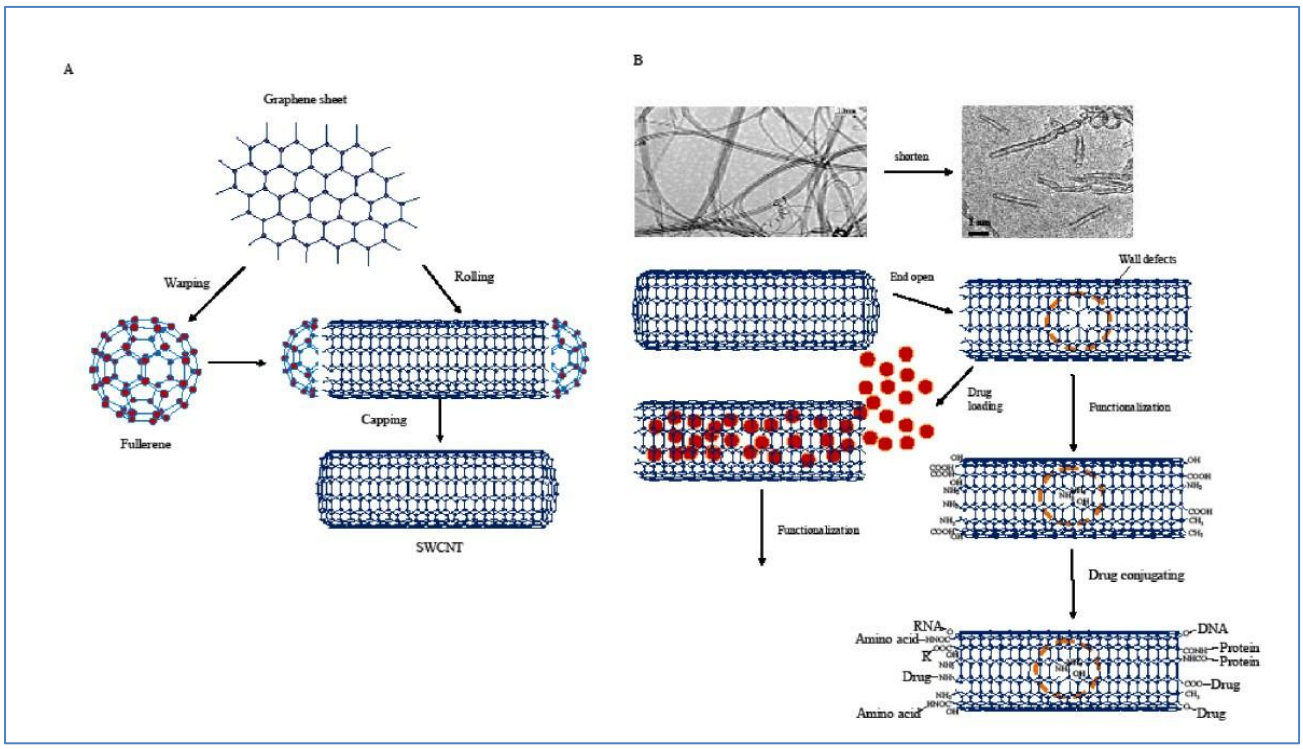

Fig-2: The formation of SWCNT and its physical and chemical treatment for use as drug carriers. (A) The schematic illustration of the structure formation of SWCNTs with the two ends closed. (B) The schematic illustration of the strategy for the preparation of the CNT-based drug delivery systems(Zhang, Zhang, & Zhang, 2011).

3. Fabrication of CNTs

Various methods for preparation of carbon nanotubes include Arc discharge method, Laser ablation method, and chemical vapor deposition method.

3.1 arc Discharge Method

This method is widely used and the simplest way for the production of nanotubes. The arc discharge method uses high temperatures for production of nanotubes, which mainly depends on the atmospheric conditions and catalyst used(Bystrzejewski *et al.*, 2008; Krätschmer, Lamb, Fostiropoulos, & Huffman, 1990). The arc discharge method involves igniting the arc between two electrodes under gaseous background like argon, hydrogen(Bystrzejewski *et al.*, 2008). This arcing evaporates the carbon; hence it cools and condenses leaving some product as filamentous forms at the cathode(Arepalli, 2004; Journet *et al*., 1997). Thus, optimizing the metals around the anode and use of catalyst results in the growth of single-walled carbon nanotubes. For the production of MWCNTS catalyst is not required as shown in figure 3.

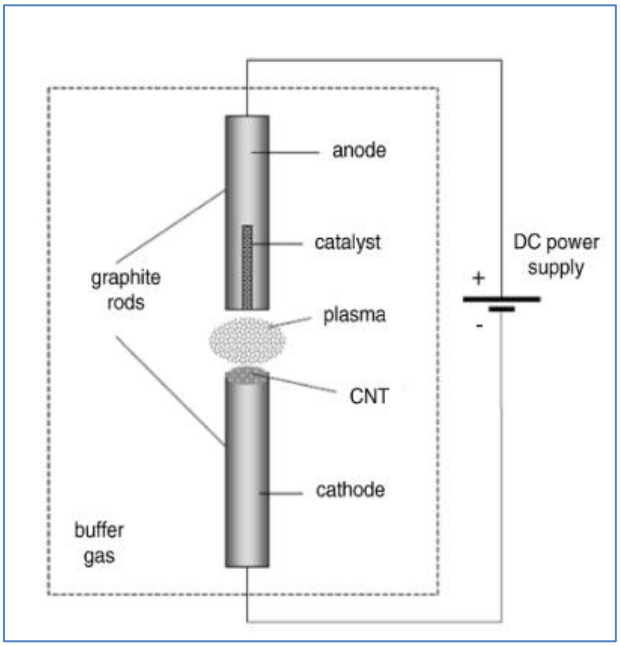

Fig-3: Preparation of carbon nanotubes by arc discharge method (Rümmeli, Ayala, & Pichler, 2010)

3.2 Laser Ablation Method

In order to prepare CNTs through laser ablation method it requires quartz tube, inert gas, graphite target with the catalyst placing furnace and maintaining high temperatures. This process involves the striking of laser pulses at graphite target at temperature of 1200℃ that makes carbon evaporates from graphite. Inert gases like helium move the carbon towards cooler surfaces of reactor that eventually condenses the carbon to nanotubes. Nanotubes were collected at cooler surfaces. This method is more expensive with 70% yield and high quality SWCNTs with controlled diameter. This can be examined by reaction temperature (Kantamneni & Gollakota, 2013; Yakobson & Smalley, 1997) as shown in figure 4.

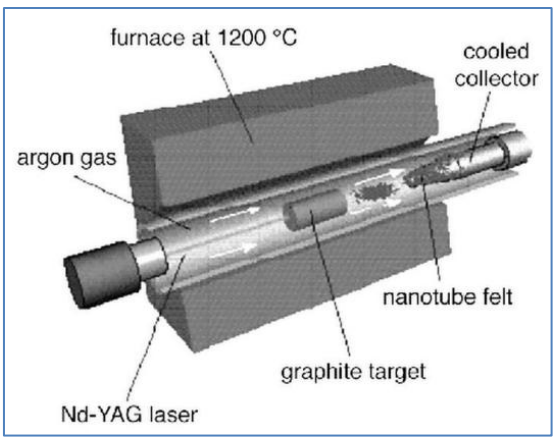

Fig-4: Preparation of carbon nanotube by laser ablation method(Walker Jr, Rakszawski, & Imperial, 1959).

3.3 Chemical Vapour Deposition Method

Chemical vapor deposition method is simple, low cost, and oldest method for preparing carbon nanotubes. CNT growth through this method mainly depends on temperature of the reaction, duration of the treatment, the composition and flow rate of carrier gas, catalyst form and its size, substrate material and surface morphology. Of all these, type of hydrocarbon and catalyst used are most important one. This method involves the passing of carrier gases like nitrogen, hydrogen, and argon with hydrocarbon gases like acetylene, methane to the chamber previously filled with catalyst and heated up to 720℃ (Beg *et al*., 2011). Hydrocarbon releases carbon and hydrogen by catalyst, hence nanotubes can be produced freely. The carbon nanotubes are formed at solid phase catalyst by two mechanisms: basing the substrate and catalyst used.

1) In base growth model, catalyst is close to surface of support hence nanotubes grow above the catalyst. 2) In top growth model catalyst, support has weak adhesion hence nanotubes are produced below the catalyst. 90% productions of CNTs were yielded by this method. This method is well accepted in industrial scale (Shifrina, 2011).

4. Functionilization

Process of chemical synthesis with introduction of desired functional groups on walls of CNTs for various applications called functionalization(Kantamneni & Gollakota, 2013). This enhances the biocompatibility, encapsulation tendency, solubility, multimodal drug delivery in cancer treatment(Y. Wang, Iqbal, & Malhotra, 2005). These modifications involve: covalent bonding, non-covalent bonding(Kirikova, Ivanov, Savilov, & Lunin, 2008).

4.1 Covalent Functionilization

Functionalization through covalent bonding provides strength to carbon nanotubes. This involves oxidation, carboxyl-based couplings. CNTs functionalized through covalent bonding are stable in bio-environment because it provides 'Robust Attachment'. This method involves the opening of tube cap and creating the holes on sidewalls by oxidation with nitric acid. Thus, it enhances solubility of CNTs in aqueous solutions. Covalent coupling can be done through amide and ester bond from carboxylic groups. Due to presence of carboxylic groups on side walls reduces Vander-walls interactions between tubes and thus it enables separation of nanotube bundles into individual and separate tubes(Kirikova *et al.*, 2008).

4.2 Non-covalent Functionilization

Functionalization through non-covalent bonding protects the conjugated system of CNTs sidewalls and therefore final structural properties of material are not affected. This type of functionalization is mainly done for the drug delivery. Non-covalent functionalization is done through aromatic compounds, surfactants, polymers, employing pi-pi stacking or hydrophobic interactions. This is mainly done to conserve their properties and improve their solubility. Surfactants have dual characters like hydrophilic region, hydrophobic region that forms the amphiphilic molecules(Strano *et al*., 2003). These amphiphilic molecules adsorb at interface between two immiscible liquids and reduce surface tension. Polymers like amphiphilic polymers, soluble polymers are used to enhance the solubility of CNTs(O'Connell *et al*., 2001). Polymers are mostly used than surfactants because they reduce the entropic penalty of micelle formation and high-energy interaction of conjugated polymers with nanotubes. Pi-Pi bonding is obtained by assembling of pyrene molecules on surface of CNT and it is mainly applied to single strand of DNA by virtue of aromatic DNA base units(Bianco, Kostarelos, & Prato, 2005; Jia *et al*., 2007) as shown in figure 5.

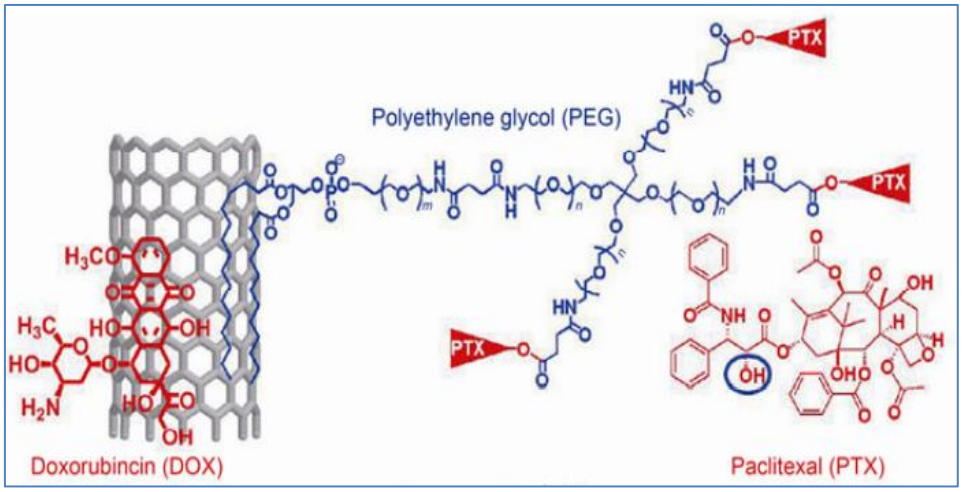

Fig-5: Conjugation of PEGylated SWCNTs with non-covalent supramolecular with $\pi - \pi$ stacking of doxorubicin and covalent conjugation of paclitaxel. (X. Wang & Liu, 2012).

5. In vivo fate of CNTs

5.1 Absorption

To show the drug activity, it must be absorbed from site of administration to target site. It can be administered through oral or parenteral like intravenous, subcutaneous, abdominal injections. These are transported via blood and lymphatic circulation. CNTs are absorbed through various mechanisms and research in absorption mechanism is still updating. The study of Yukako *et al.* determines the absorption of erythropoietin. It involves loading of carbon nanotubes with erythropoietin and addition of surfactant for absorption enhancing. From this, effect of fiber length i.e., long and short fiber length is studied. ELISA results stated that CNTs have capability of absorbing themselves and short fiber length CNTs deliver more erythropoietin. Transmission Electron microscope confirms that physically shortened CNTs when administered orally they are absorbed through columnar cells of intestinal mucous membrane. When CNTs administered subcutaneously or abdominally some part of CNTs are absorbed into lymph and some resides in local tissue. This is due to fenestra in the endothelial cells of blood for about 30nm-50nm but in the lymph it is larger than 100nm in diameter. Hence lymph absorption is faster easier than blood (D. Yang *et al*., 2009; Z. Yang *et al.*, 2010). These CNTs are used for clinical delivery of anti-cancer drugs to kill metastatic cancer cells. Gemcitabine was successfully delivered to lymph nodes by basing magnetic MWCNTs through lymphatic delivery. Administration of CNTs through veins enters directly into blood and distributed to all over the body. Intravenous injection of CNTs demonstrated that blood clearance mainly depends on the surface modification. PEGylation i.e., polyethylene glycolytion is the most accepted strategy for extending the circulation time of CNTs in blood due to surface coverage and PEG reduces the immunogenicity and avert their nonspecific phagocytosis by reticulo endothelial system (Cheng *et al*., 2008; Ji *et al*., 2010; Li *et al*., 2010; McDevitt *et al*., 2007; Schipper *et al*., 2008; Singh *et al*., 2006) as shown in figure 6.

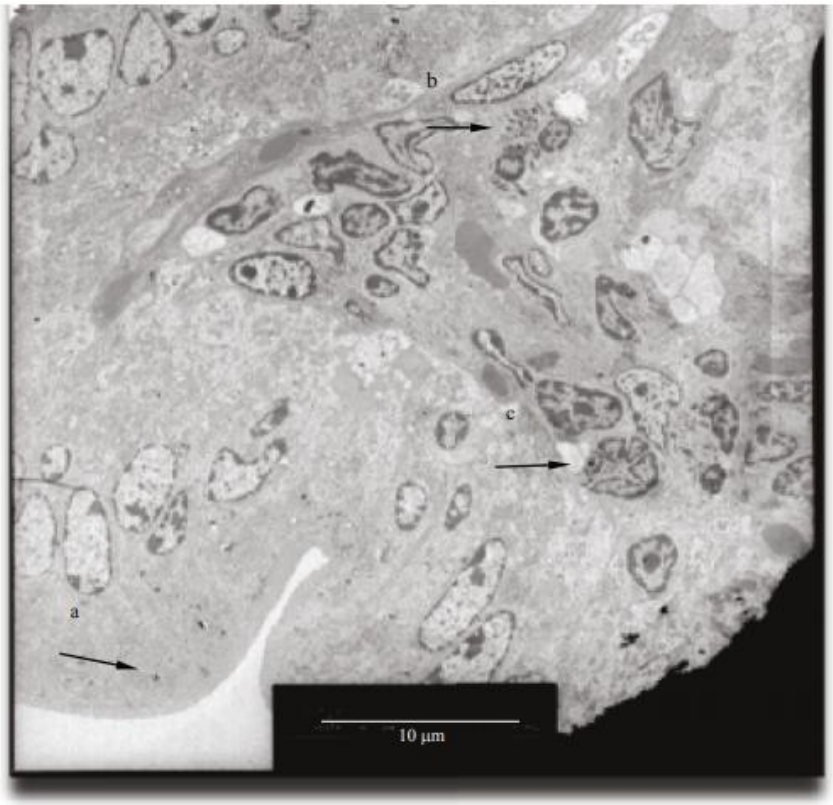

Fig-6: Absorption of carbon nanotubes.(Zhang *et al*., 2011)

5.2. Distribution

Distribution is a reversible transfer of drug from one location to another within the body. Various experiments had done to determine the *In vivo*, ex vivo bio distributions along with tumor targeting ability for SWCNTs. This includes diameter, length, non-covalently functionalized with phospholipids-PEG. Bio distribution and circulation of CNTs can also be determined by PEG chain lengths i.e., PEG-5400-modified SWCNTS has t½=2h and PEG-2000-modified has 0.5h. Combining of PEG with SWCNTs reduces the RES uptake. Further functionalization of SWCNTs with PEG branches increases the blood circulation time through intravenous injection(Hong *et al*., 2010; Kolosnjaj-Tabi *et al*., 2010; Liu *et al*., 2007; Liu, Davis, *et al*., 2008; Liu, Tabakman, Welsher, & Dai, 2009; Prencipe *et al*., 2009).

5.3. Metabolism and excretion

The non-biodegradability in the body and non-eliminability from body gives the possibility of their successful uses in clinical practice. Animal body metabolizes the functionalized SWCNTs, carboxylation on surfaces determined their unique ability to undergo 90 day degradation of phagolysosome stimulant, and results in shortening of length and accumulation of ultra-fine solid carbonaceous debris. Non-functionalized CNTs shows no modification under similar conditions. Metabolism of CNTs includes acid carboxylation, modifiable COOH groups on CNT surface. Research says that CNTs would reside in the body up to 5 months after administration by escaping the RES. These CNTs are excreted through kidney and bile duct. Bio-degradable SWCNTs are catalyzed through hypochlorite neutrophil enzyme. Metabolism of CNT at macrophage can be seen with lesser degree. Further metabolism of CNT was revealed by molecular modeling which determines the interaction between amino acid residues on enzyme back bone and carboxyl group at catalytic site (Kagan *et al.*, 2010; Kolosnjaj-Tabi *et al.*, 2010).

6. CELLULAR UPTAKE OF CNTs:

The exact mechanism of CNTs is not known and it is still developing (Iijima, 1991). Most of researchers believe that CNTs follow two mechanisms. First one includes endocytosis dependent pathway which may be either receptor mediated or non-receptor mediated and the second includes endocytosis independent pathway which includes diffusion, membrane fusion, or direct pore transport of the extracellular material into the cell. The selection of CNTs is important because they interact with cells. Due to their shape CNTs are capable to cross the cellular membrane and pass into cellular components without causing any damage to neighbor cells. Nano injector system was developed through atomic force microscope (AFM) tip by using functionalized MWCNTs and combining cargo with di-sulphide linkage. The results have shown successful delivery of CNTs within cytosol by breaking the di-sulphide bond (Bianco *et al.*, 2005; X. Chen, Kis, Zettl, & Bertozzi, 2007). Study reports of Kham and co-workers explained that uptake of nanotubes by endocytosis. This was determined by attaching fluorinated protein to SWCNT biotin. Results were found by using epi-fluorescence, confocal microscopy with SWCNTs in endosomes & were penetrated into cell of cytoplasm & nucleus of fibroblasts (Elhissi *et al.*, 2012; Pantarotto, Briand, Prato, & Bianco, 2004). Other study determines that MWCNTs are up taken based on length of the nanotubes i.e., nanotubes having <1 micro meter are easily internalized into cells (Raffa *et al.*, 2008) as shown in figure 7.

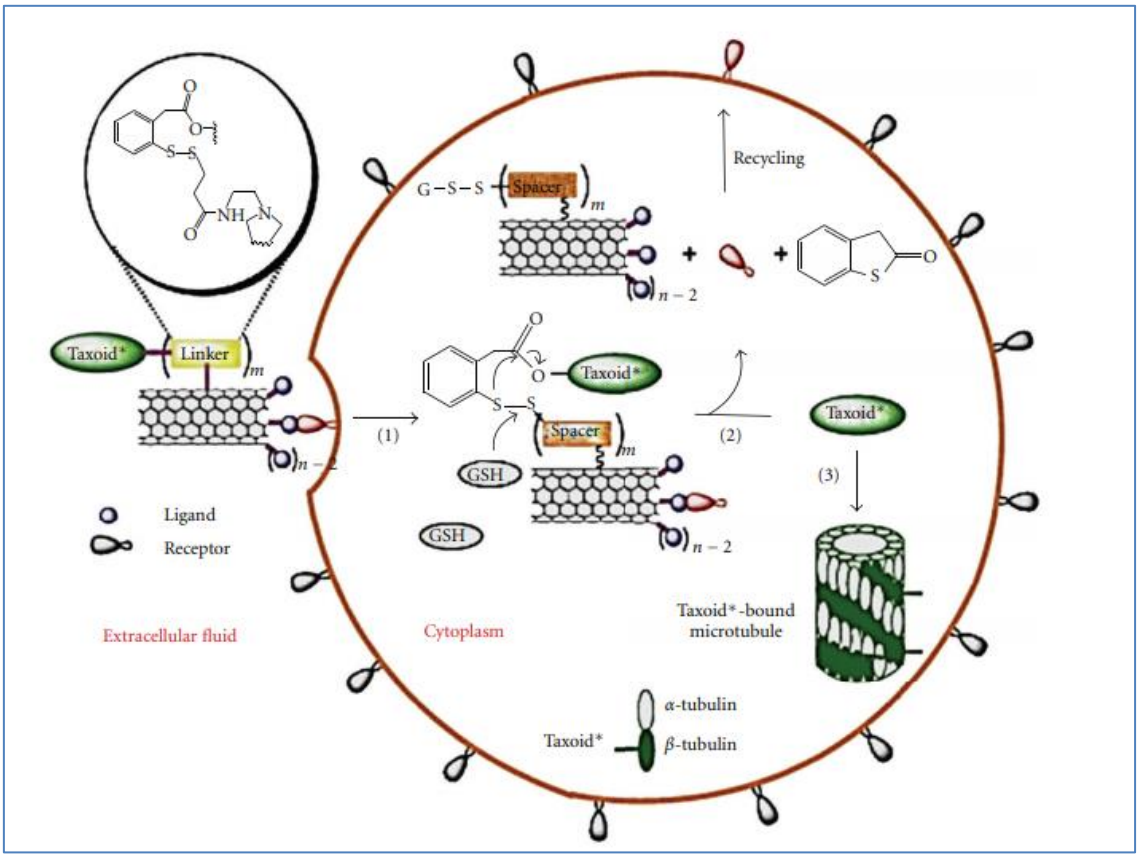

Fig-7: Cellular uptake of carbon nanotube (Kushwaha, Ghoshal, Rai, & Singh, 2013)

7. Applications of CNTs in Biomedical Imaging and Drug Delivery

CNTs have special property like biomedical imaging along with drug delivery. This involves imaging the agents with inner cavity and targeting the drug with outer cavity. Biomedical imaging of CNTs involves Computed Tomography, Magnetic Resonance Imaging (Martincic & Tobias, 2015; Pascu, Arrowsmith, Bayly, Brayshaw, & Hu, 2010).

7.1. Computed Tomography

It is equipment that diagnoses the body and visualizes it through a computer. It is mainly done to determine heart diseases, infections in the brain, internal bleeding (34, 35). A technique generates a three-dimensional representation of the sample by projecting the images and mathematical reconstruction. Various contrast agents are used in CT imaging for increasing sensitivity, visualization, and differentiation of tissues. Iodine is used as an x-ray opaque element. These x-rays visualize the body in all directions. SWCNTs filled with iodine and functionalized with serinol amide groups are dissolved in water and then visualized. The obtained images showed that iodine filled materials were more x-ray opaque than empty tubes(Ashcroft *et al.*, 2007; Mackeyev, Marks, Rosenblum, & Wilson, 2005). CT can be combined with various techniques which include single photon emission(Hong *et al.*, 2010). In this technique SWCNTs were filled with Na12I at high temperatures which results in closing the ends of SWCNTs and hence those are called 'carbon Nano capsules'(Shao, Tobias, Huh, & Green, 2006; Tobias, Ballesteros, & Green, 2010). Thermo gravimetric analysis is done to determine the amount of encapsulation in this carbon Nano capsules(Hartman, Hamlin, Wilbur, & Wilson, 2007). CNTs have also been found to have therapeutic use along with imaging ex alpha radio nucleotide.

7.2. Magnetic Resonance Imaging

It is similar to CT scan but it does not emit x-rays, hence organs are not affected by this MRI. Iron and Gadolinium are contrast agents used in MRI. Recent exploration uses nitro oxide radicals having single unpaired electron, stable free radicals, nontoxic in nature and electron paramagnetic resonance imaging. CNTs filled with gadolinium salts gives future interest on medical diagnostic purpose. *In vitro and In vivo* experiments were done by using gadolinium cation and x-ray studies have been carried out, thus indicated a high relativity than other gadolinium filled nanotubes(Law, Guven, & Wilson, 2014; Rivera *et al*., 2012). MWCNTs filled with iron is used to treat hyperthermia, were tested on epidermal growth factor that have over expression of A431 cells and suppression of receptor EAhy926 and CHO *In vitro(Mönch et al., 2007)*. Cancer cell are targeted with monoclonal antibody exohedrally and inducing the magnetic fields over hypothermia cytotoxicity resulted the selective suppression of EGFR over expressed cell line. Based on nuclear magnetic resonance copper filled MWCNTs developed the Nano scale thermometer. The sensor was developed with temperature dependent on NMR frequency and relaxation time(Rivera *et al.*, 2012) as shown in figure 8, 9.

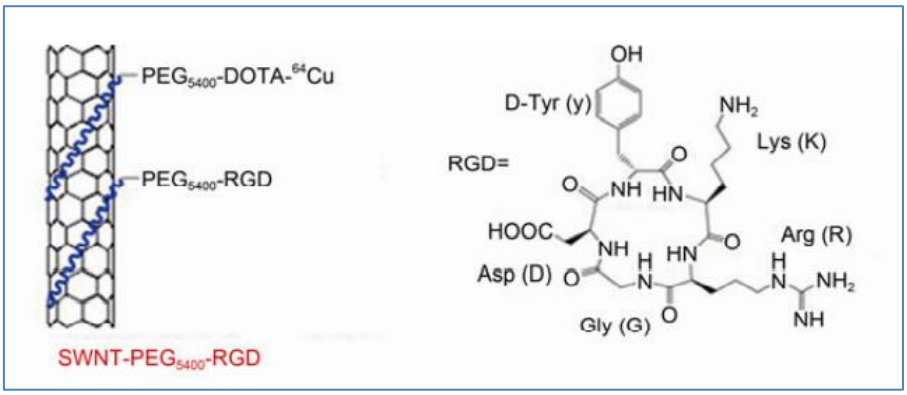

Fig-8: Biomedical imaging of carbon nanotubes (X. Wang & Liu, 2012)

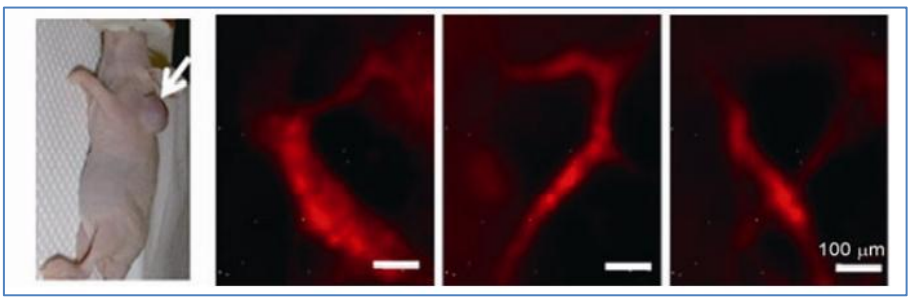

Fig-9: Biomedical imaging of carbon nanotubes (X. Wang & Liu, 2012)

8. DRUG DELIVERY WITH CNTs

8.1 Active and Passive Targeting

Delivery of drug to specific target sites or diseased cell is key challenge and it should be done without damaging healthy Cells/tissues. Various trails of antibody mediated drug has been mostly unsuccessful, due to low specificity of antibodies upon binding with drug molecules. Research suggest that nanotubes support the antibodies by maintaining their properties and targeting abilities(Kushwaha *et al*., 2013). Active and passive targeting is solution for drug targeting. These are direct result of functionalization.

8.2 Passive Targeting

It is well established targeting of drug. Under inflammation, hypoxia the endothelium of blood vessels is more permeable and engulfs existing blood vessels / develops new vessels. These vessels enhance selective permeation of macromolecules >40KDa. Retention of CNTs can be contributed by abnormal lymphatic drainage but it is not applicable to small molecules with short circulation. Hence encapsulating the small molecules with Nano sized increases their pharmacokinetics properties, selectivity, and reduces side effects. This type of targeting is called passive targeting. EPR effect is acceptable or standard design for passive targeting. Nano sized CNTs are mainly done for avoiding opsonization(Misra, Acharya, & Sahoo, 2010). Tumor specificity with EPR is increased to 20-30% in delivery. This EPR effect is mainly dependent on the 1) degree of angiogenesis, lymph angiogenesis 2) degree of perivascular tumor growth and density of stromal response 3) intra tumor pressure(Kushwaha *et al*., 2013).

8.3. Active targeting

Targeting of tumor cells with specific binding sites with functionalization called active targeting. In active targeting drug is not taken by RES. It increases the quantity of delivered drug than free or passively targeted drug. Active targeting increases the drug penetration and affinities of cancer cells and it was first proven in 1980 with liposomal surface of grafted antibodies. Brain tumors can be targeted with transferrin receptors, nicotinic acetylcholine receptors with vascular targeting. Active targeting recognizes the various antibodies *In vitro* & *In vivo* with strong ligand /receptor binding(Kushwaha *et al*., 2013; Misra *et al*., 2010) as shown in figure 10.

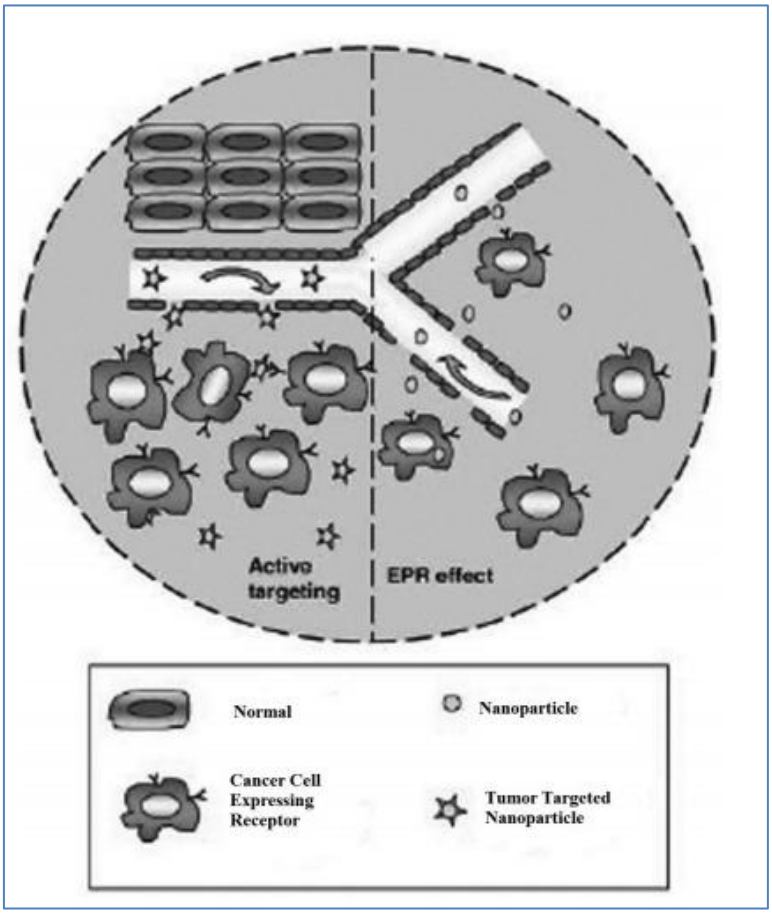

Fig-10: Active Targeting of drugs with carbon nanotubes(Kushwaha *et al*., 2013)

9. Drug Delivery to Tumour

Abnormal growth of cells leads to cancer. It is second leading cause of death in the world. Cancer cells are over expressed with folic acid, hence most of projects were designed with Nano carriers & FA derivatives attachment. As discussed earlier some of CNTs are retained in lymph nodes, these CNTs are used for lymphatic cancer targeting. In this tumor targeting is done by magnetically i.e., magnetic nanoparticles were filled with cisplatin by entrapping folic acid functionalized MWCNTs. External magnetic field was used to pull the nanotubes to lymph nodes for prolonged release and specific tumor inhibitor. Campothecin, a hydrophobic drug is filled into polyvinyl alcohol functionalized MWCNTs and it has shown effective in breast and skin cancer treatment. Dhār and Co-workers developed Lang Boat delivery system. It involves the complex of cisplatin with functionalized FA by compromising the amide bonds to long boat. It is taken up by endocytosis and drug releases with subsequent interaction with nuclear DNA. Doxorubicin was targeted by FA secured SWCNTs. Aqueous dispersibility of CNTs were increased by using bio adhesive polymers like chitosan, sodium alginate for targeting. The result of TEM indicates that cargo was released to tumor cell at acidic pH of lysosome. Dual targeted drug Nano carrier was developed by Li & co-workers by binding doxorubicin MWCNTs with iron nanoparticles and folate molecules; it was determined that superior delivery to the cells than free doxorubicin. Cancer is mainly treated by using indole derived based drugs because of their high affinity of tryptophan and study of sustained delivery of small molecules by encapsulation of SWCNTs. Encapsulation of cargo is determined by RAMAN, FTIR, NMR Spectroscopy. The primary structure of SWCNT filled indole molecules is THR-ASN-TYR-LEU-PHEN-SER-PRO-ASN-GLY-PRO-ILE-ALA-ARG-ALA-TRP with fluorescent probe, Lucifer yellow on TNX peptide. Extra cys amino acid residue at N-terminal of peptide at TNX peptide was added due to affinity of receptor tumor cells. It should have long residence time in blood circulation. Anti-cancer drug paclitaxel has poor solubility in aqueous medium and non-specific cytotoxicity so that it prevents the cargo to reach tumor cells. Use of CREMOPHOR as SOLUBILIZER enhances allergic reactions. Liu *et al.* conjugated paclitaxel with branched chain and cleavable ester bond was combined for water solubility(Lamberti *et al*., 2015; Zhang *et al*., 2011) as shown in figure 11.

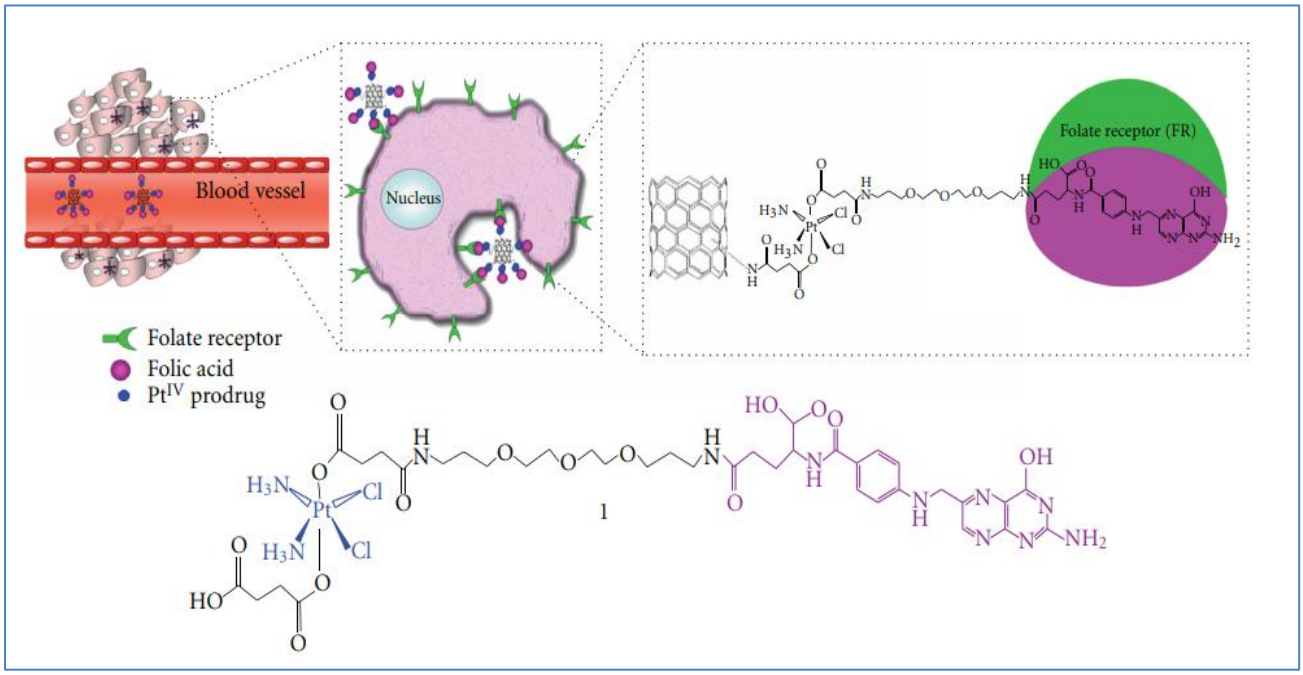

Fig-11: Delivery cancer drugs through carbon nanotubes (Elhissi *et al.*, 2012)

10. Drug Delivery to Lymphatic System

Now-a-days delivery of drug through lymphatic route was increased. This is because, to avoid the first pass metabolism and to develop prolong absorption of the drug etc. Most of cancers are targeted through lymphatic canal. Water solubility of drug can be increased by radical polymerization, poly acrylic acid. Adsorption of the PAA-CNT surface can be done by co-precipitation of FE3O4 based magnetic nanoparticles. Efficiency of EMCITABINE was up to 62%, when it is stirred with PAA-CNT and with fe3o4 magnetic nanoparticles for 24hrs. Drug delivered through lymph was absent in major organs like kidney, liver, spleen, heart after 3hrs of subcutaneous administration (Z. Yang *et al*., 2010).

11. Drug Delivery to CNS

Delivery of drug to brain is guarded by BBB. It is characterized by presence of endothelium with low permeability and cells are interlinked with tight junctions. Due to small size of Nano particles, they can easily permeate into the BBB and can cause neural toxicity. Neurotoxicity study was done by WANG *et al*. (Z. Yang *et al*., 2010). It involves the exposure of PC12 cells of SWCNTs to neurons and results were determined that these CNTs were producing the oxidative stress in neuronal cells. This results in development of cellular injuries like NEURO degenerative disorders. Study reports of Bard et al gives that oxidation of CNTs surface lead sustained inflammatory reaction in healthy brain. Alzheimer's disease is characterized by reduction of acetylcholine leading to incapability of learning, memory, and thinking. By administration of acetyl choline to brain may minimize all the above. But there is no evidence for proper delivery of acetyl choline. Results from Raman spectroscopy gives that SWCNT absorbed through axo-plasma transformation of neuritis. Even though there is no clarity on absorbing of CNTs through surfaces or in the tubes(Zhang *et al*., 2011).

12. Delivery of Small Molecules with CNTS

Small molecules can be delivered through CNTs by covalent functionalization and non-covalent functionalization. Small molecules include chitosan, albumin, and heparin conjugating to nanotubes. SWCNTs can be used for pro-drug delivery which reduces the cytotoxicity of PL (F1) compound. It was reported by Dhār *et al*. (Dhar, Liu, Thomale, Dai, & Lippard, 2008). In Liu *et al*. studies conjugation of paclitaxel with branched polyethylene glycol chains on SWCNT for *In vivo* drug cancer treatment and result found that blood circulation time was found to be more than free paclitaxel leading to increased drug retention in tumor with enhanced therapeutic efficiency that retards the tumor growth(Liu, Chen, *et al*., 2008). Nucleoside analog of gemcitabine has been using for treating cancers of pancreas, bladder, lung, breast. Incorporation of gemcitabine into SWCNTs arranges a pi-pi stacking, resulting in preventing the loss of solvation of drug molecule. Most of the time gemcitabine is used to encapsulate inside the SWCNT leading to formation pi-pi stacking at low concentration and hydrogen bonding at high concentration among the drug molecules. Selective drug targeting was done by containing epidermal growth hormone &60 chitosan monomers (Arsawang *et al*., 2011; Rungnim, Arsawang, Rungrotmongkol, & Hannongbua, 2012; Rungnim, Rungrotmongkol, Hannongbua, & Okumura, 2013). Van der Waals density was used to study different diameters of CNTs i.e., from 0.8-1.5 to 1.53-2.26nm. Suggestion of Mullikan states that the CNT, provide strong interactions with drug than when they are semi-conducted/metallic (Rezvani, Ganji, & Faghihnasiri, 2013). Molecular dynamic stimulation was used to study controlled release of drug and molecular properties. This study explains that temperature plays a key role in diffusion process with small diameter CNTs but in large diameter CNTs it is spontaneous and not so dependent on temperature (Sornmee *et al*., 2011; Stafylas & Sarafidis, 2008).

13. Delivery of Peptides

Most of the peptides were delivered through CNT for treating the immune system. Peptides easily adopt secondary structures around CNTs for the identification of specific monoclonal & polyclonal antibodies. Immunological properties of peptide CNT were determined *In vivo (Pantarotto et al., 2003)*, which involves immunization of mice with FMDV peptide. Nanotube results in high progress with conjugated peptides than free peptides. CNT for vaccine delivery was validated by combining with complement i.e. human immune system composed of series of proteins for recognizing, opsonizing, clearing, and killing pathogens/necrotic cells. Studies of Salvador moral *et al.* give that pristine CNT activate the complement by both classical and alternative through selective adsorption of proteins(Salvador-Morales, Flahaut, Sim, & Sloan, 2006).

14. Delivery of Nucleic Acids

A Molecular dynamic simulation was studied for the delivery of nucleic acids (DNA, RNA)(Kang *et al*., 2009). Predictions of GAO *et al*. unforced the single-stranded DNA encapsulation *In vivo* CNT, these double-stranded DNA molecules were encapsulated inside MWCNT with platinum under 400K&3 bar pressure(Richard *et al*., 2009). The main goal of nucleic acid delivery through CNT was to increase the gene transfer expression because plasmid DNA alone enters into cells and the nucleus. Non-encoding RNA polymers can be delivered into cells through CNT by condensing the RNA through nonspecific binding. Radioisotope labelling and confocal fluorescence were used to determine the complexes between CNT& POLY (RU) RNA polymer into MCF-7 cells resulting in reduced toxicity. Research believes that the development of the tumour is mainly due to the alteration of a gene. Hence delivery of genes through CNTs may reduce the tumour. This delivery can be done through vectors which include viral and non-viral. The Viral vector may reduce the toxicity and maintain the specificity towards gene transfer but they are unstable, cause immunogenicity, and may lead to degradation. DNA, RNA, Proteins are unable to permeate through the cell membrane and they are delivered through vehicles or non-viral vectors, which include nanoparticles, liposomes, cationic lipids, polymers. This type of transfer provides easy scale-up, pliability to nucleic acid size, and decreased immunogenicity. These non-viral vectors are formed by cationic polymers. DNA is carried by CNTs by maintaining the proper physical, chemical, biological

properties& studies of atomic force microscopy and spectroscopic determine that DNA can easily form sheaths around the CNTs. Various types of F-CNTs were developed and resulted in the effective delivery of plasmid DNA into cells. Studies of BARTHO LOMEUZ states that positively charged SWCNTs through non-covalent bonding of si RNA gives *In vivo* therapeutic silencing of hypoxia-inducible factor/alpha under animal experiment CNTs are also used to deliver through Nano spearing or Nano injection for sustainable gene delivery. CNTs were pre-functionalized with enhanced green fluorescence protein encoded with DNA plasmid and nickel is implanted on nanotubes. The External magnetic field was used to penetrate the SWCNT needle target site and release the DNA plasmid to the cell, hence increasing the expression of EGFP. Functionalization of SWCNTs with PLPEG may provide efficient transporting and releasing enzymatic cleaving by integrating the cleavable di-sulphide bonds. This functionalization not only delivers the cargo but also functionality of RNA was more potent. There is conspicuous on CNT that they can form supra molecular assembles with nucleic acids. Hence it provides a gateway for nucleic acids (DNA, RNA, GENE) for application in gene therapy, genetic vaccination, Immune potentiation enhancement (Bianco *et al*., 2005; Martincic & Tobias, 2015; X. Wang & Liu, 2012) as shown in figure 12.

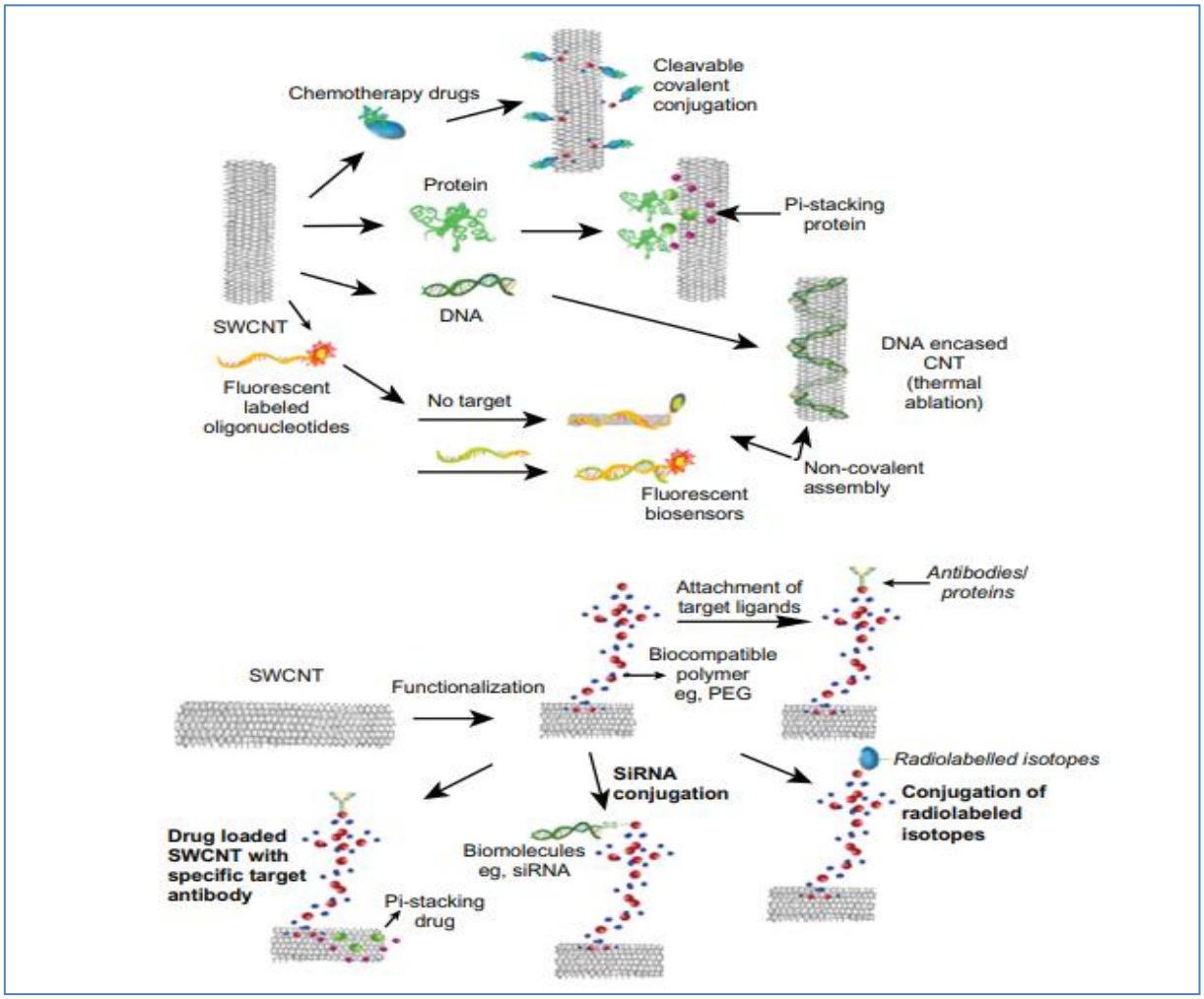

Fig-12: Delivery of nucleic acid with carbon nanotubes(Vardharajula *et al.*, 2012)

15. CNTs IN DETECTION OF TOXINS

CNTs have wide variety of applications. They are used to determine the toxins that include physical, chemical and biological etc. due to their small size, large surface area, CNTs have high adsorption properties. Hence inorganic chemicals are absorbed by CNTs which act as gas sensor. SWCNTs act as gas sensor because electrical conductance of SWCNTs changes fast on exposure to gaseous molecules. NO2 gas was detected by composite film of SWCNT, mash doped with alkaline THIOL monolayer protected gold cluster. Toxic proteins can be detected by altering the electrical signals. DNA sensing is used as biological recognition of alkylating agents like nitrogen mustard, ethylene amines, alkyl SULFONATES, TRIAZENES, and NITROSOUREAS. CNTs interaction of antibodies with bond of protein is detected by SEM & electro-chemical luminescence.

15. Biosafety of SWCNT:

Discussions above clearly said that CNTs are having wide range of applications. But biosafety has becoming controversial topic in their safe & damage to *In vitro* tissues and cells even though they have attractive physicochemical properties. Swiss studies on toxicity of SWCNTs on mice taking dose, length, surface chemistry as function and observe that neither death and growth nor behavior dilemma on oral administration with fiber like structures. Spectroscopic studies of RAFEE and KAUL probed the interactions between the MWCNT & cell culture medium resulting biocompatibility of nanotubes. In order to avoid toxicity, there are various methods to follow which includes 1) Functionalization of CNTs may reduce the toxicity with safe delivery (drug carrier). 2) As CNTs are metabolized through liver, kidney, hence discussion on the persistence residence of them in bodies.3) CNTs are guarded by target organelles on pharmacological, toxicological distribution with some chemicals, hence utilization of specific target may reduce toxicity. Dose should be maintained properly to avoid toxicity of CNTs as shown in figure 13.

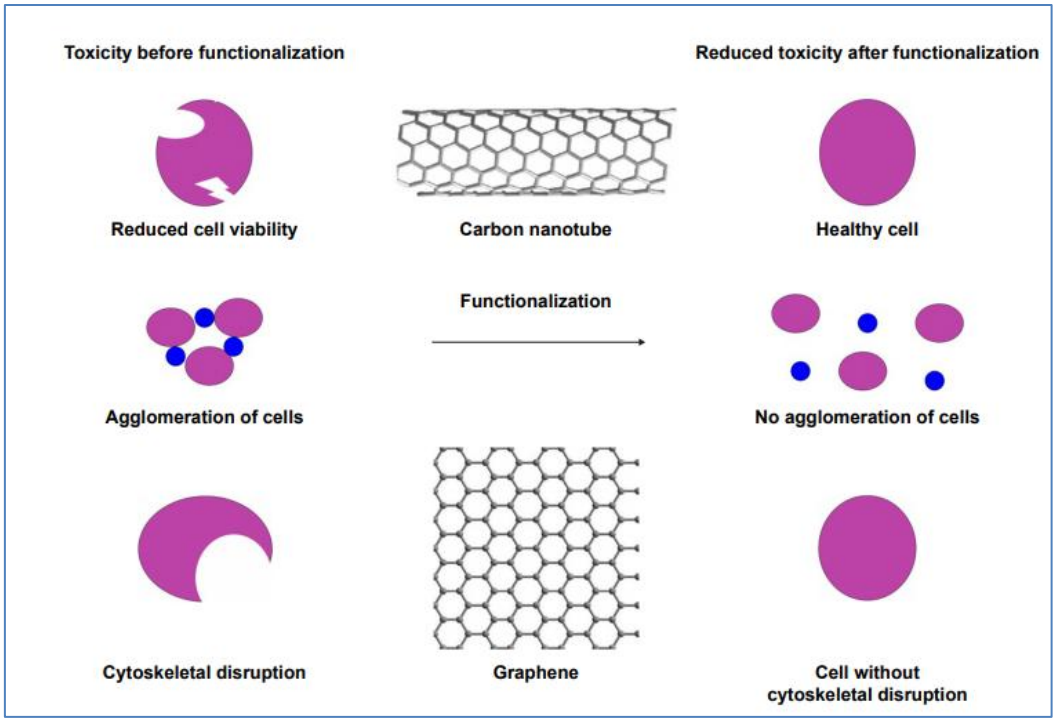

Fig-13: Bio safety of carbon nanotubes through functionalization (John *et al*., 2015).

16. TOXICITY OF CNTs:

CNTs toxicity is mainly due to their small size, large aspect ratio, length of tubes, degree of aggregation, functionalization residues of catalyst after synthesis. CNT on exposure to respiratory system leads to multi-focal granuloma, peri-bronchial inflammation, progressive interstitial fibrosis, collagen deposition. Aerodynamics was used to determine deposition of CNT in respiratory system. Most of the researchers said that administration of CNT leads to chronic inflammation and oxidative stress that induces the adverse health effects like gene toxicity & cancer. Sargent et al. Studies on mice gives that CNT, on nasal administration (inhaled) promotes pulmonary adenomas, adenocarcinomas in B6C3F1. MWCNTs on intra peritoneal administration on mice lead to abdominal mesothelium. Reports have claimed that CNTs on intravenous administration may induce platelet aggregation. Studies of Salvador Morales said that CNTs has direct interaction with plasma proteins like fibrinogen &Apo lipoprotein. CNTs are proved to have pro inflammatory action on endothelial cells, inhibition of cell growth and reduction of nitric oxide synthase. Due to small size of CNTs they are able to produce neural toxicity by easy penetration. CNTs are easily absorbed through GIT due to small size but they produce adverse effects. Animal studies of BELIAVEA showed that there is change in structure of microvilli in mice with water consumed CNT, induces the proliferation of epithelial cells and increasing unstructured villi with 2 months exposure of CNT(Firme III & Bandaru, 2010; Kam, Liu, & Dai, 2006; Pantarotto, Singh, *et al*., 2004; Szebeni *et al*., 2007).

17. RECENT PATENT ON CNTs:

1) Ammonium functionalized CNTs were invented by KHABESHEKU with increased solubility & biocompatibility for effective target delivery.
2) CNTs are used as carrier by Hirsch resulting decreased systemic side effects and reduced dose.

3) CNTs array was used for drug delivery, targeting, localization &controlled delivery of drugs by GHARIB *et al.*
4) Antibiotic delivery was done by Jennings with chitosan CNTs having high loading efficiency.
5) Dai *et al*. delivered biologically active molecules through CNT utilizing bi-functionalization linkers for complex CNT.
6) Proteins and peptides were delivered through CNT by complexing the functionalized SWCNT with chitosan and the hybrid having high transfection efficiency than free chitosan.
7) CNT conjugates were developed by Cen *et al*. by conjugating covalently with exogenous materials like protein, peptide, fatty acid, vitamin etc. at one side and drug on other side with di-sulphide link [78, 79].

CONCLUSION

As we discussed earlier that drug delivery and Nano technology has tremendous increase. Hence carbon nanoparticles are used in pharmaceutical industry for targeting and reducing side effects, toxicity. CNTs guaranteed with 85% of treating cancer. Due to their versatile properties CNTs are used in various fields. Pure forms of CNTs are highly toxic, insoluble. Hence functionalization may reduce the effects. Delivery of drug to nucleus can be done by charging CNT with biological active moieties. Research on CNT said that myeloperoxidase a particular enzyme in which white blood cells break carbon nanotube into water& carbon dioxide, thus making their elimination easy and biodegradable

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