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Carbon Nanotubes: A Brief Review on Its Use for Biomedical Imaging Purpose

Abstract *Carbon nanotubes (CNTs) belong to the "fullerene family", also known as "graphene". These graphenes are similar to the graphite sheets and when these are turn up in the cylindrical form they are known as carbon nanotubes. Currently, the most common methods used for CNTs preparation are: Electric-arc-discharge methods, Chemical-vapor-deposition method and Laser-ablation method. In order to cross the cell membrane, functionalization of the pristine CNTs is performed. Because of the sp2 hybridization and closely packed hexagons in their structure, functionalization of the pristine CNTs can be done easily with either therapeutic agent or the imaging agent. They have wide applications in the field of bio-imaging because of their intrinsic optical, mechanical and electrical properties. They can be used as efficient contrast agents and the biosensors as well as efficient carriers for the delivery of therapeutic or imaging agents*

Key Words: Carbon-Nano-Tubes, Biomedical, Imaging, Functionalization, Drug Delivery

Introduction

Carbon nanotubes (CNTs) belong to the 2nd generation allotropic form of carbon atom just like graphite and diamond. They have structures in nano-range with 1-D. They are made up of solely carbon atoms which are arranged in such a way to form the hexagons in the long cylinder like tubes (Beg et al., 2018). The carbon nanotubes have different size depending on the ratio of their length and diameters. Moreover, carbon nanotubes vary depending on the number of carbon atoms they are made off. The number of carbon atoms in CNTs ranges from 20-70 C-atoms. CNTs belong to the "fullerene family", also known as "graphene". These graphenes are similar to the graphite sheets and when these are turn up in the cylindrical form they are known as carbon nanotubes (Beg et al., 2018) **(Figure 1)**.

CNTs were first discovered by Sumio Iijima in 1991. These are basically the new constructional form of already discovered allotropes of carbon fullerene. They have cylinder like construction that's why these were entitled as carbon nanotubes (CNTs). The basic constitution of CNTs is not other than graphene sheets but these sheets are turned up to form a tube shape structure having ends either open or closed. Their diameter can be of nanoscale i.e, 1 nm while length can be of many μm (Rastogi et al., 2014).

CNTs can be composed of only one graphene sheet or upto many graphene sheets. Thus, on the basis of number of graphene sheets, CNTs are divided in to two main types known as: single walled carbon nanotubes (SWCNTs) and multi walled carbon nanotubes (MWCNTs) (Liu, Tabakman, Welsher, & Dai, 2009). In single walled carbon nanotubes only

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one graphene sheet is present that is turned up in to a tube shape and this shape is detained by the weak Vander Waals forces. Due to these weak forces SWCNTs have elastic and flexibility properties. The diameter of SWCNTS ranges from 0.4-3.0 nm while their length ranges from 20-1000nm (Rastogi et al., 2014). While, in multi walled carbon nanotubes, multiple single-walled graphene sheets are coaxially arranged over one another. The range of their "outer diameter" is (2-100 nm) and "inner diameter" is (1-3 nm). While their length varies upto several μm (Dresselhaus, Dresselhaus, & Eklund, 1996).

There has been extensive research on carbon nanotubes during the last few decades because of their distinctive natural physicochemical characteristics which make them feasible for the use in various biomedical and biological fields. After the introduction of functionalization technique for CNTs the sensitivity and specificity of carbon nanotubes can be enhanced. Thus, CNTs can be used for the detection of various biological molecules. Moreover, CNTs can also be used for the targeted delivery of different pharmaceuticals or the diagnostic agents. On the other hand, because of the in-build optical properties CNTs especially SWCNTs have been used in different imaging techniques i.e, Raman spectroscopy, Photoacoustic imaging and photoluminescence etc. Further research is needed in different fields in order to find applications in the biomedical field (Liu et al., 2009).

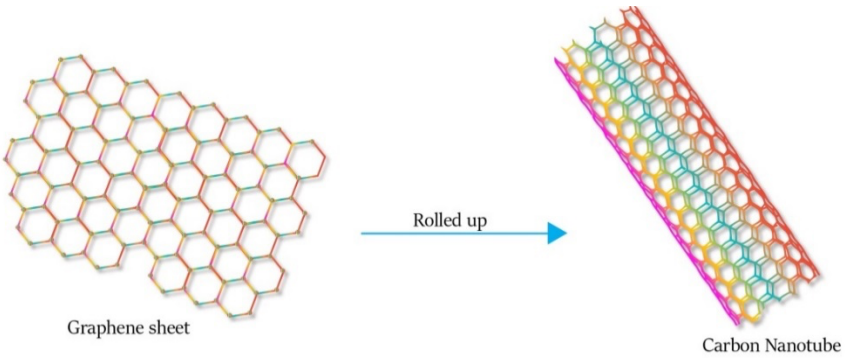

Figure 1: Carbon-Nano-Tube (CNT)

Methods of Preparation of CNTS

Generally the preparation of carbon nanotubes involves the conversion of carbon source into the cylindrical nanotubes by using the high temperature and low pressure condition. The nature of the CNT thus formed depends on these preparation conditions. The carbon nanotubes formed initially contain so many impure materials either metallic particles or the carbonaceous materials. Thus, in order to obtain the pure CNTs, the initially formed impure CNTs are subjected to the purification step (P.-X. Hou, Liu, & Cheng, 2008). Various methods for the purification of CNTs are utilized i.e, chemical methods, especially oxidation (gas phase (Zimmerman, Bradley, Huffman, Hauge, & Margrave, 2000), liquid phase (Hu, Zhao, Itkis, & Haddon, 2003)), physical methods (e.g. centrifugation technique (Yu et al., 2006), microfiltration technique (Bandow et al., 1997) and high vacuum & high temperature annealing (Huang, Wang, Luo, & Wei, 2003) etc) and/or collective or repetitive purification (P. Hou, Bai, Yang, Liu, & Cheng, 2002).

Currently, the most common methods used for CNTs preparation are: Electric-arc-discharge methods, Chemical-vapor-deposition method and Laser-ablation method (Rastogi et al., 2014).

Electric-Arc-Discharge Methods

This is the earliest method known for the preparation of CNTs and in 1991, it was first used by Sumio Iijima when he first time prepared the CNTs (Iijima, 1991). In this approach two carbon electrodes are placed in a vacuum chamber having inert gas (He or Ar). After applying a direct current of 200A-20V across the c-electrodes, the positively charged electrode is driven closer to the negative electrode in order to strike the arc. When arc strikes the electrodes, these become red hot and thus increased temperature causes the conversion of inert gas in to plasma state. This plasma then generates the very high temperature which results in the vaporization of carbon from carbon electrodes. After the stabilization of arc, the electrodes are placed about 1 mm apart. The CNTs thus formed are deposited on the negatively charged

electrode. The electric arc generated and the inert gas utilized in the chamber effects the whole performance of this method (M DeRosa, Greco, Rajamani, & Sitharaman, 2010; Sinha & Yeow, 2005). **(Figure 2)**

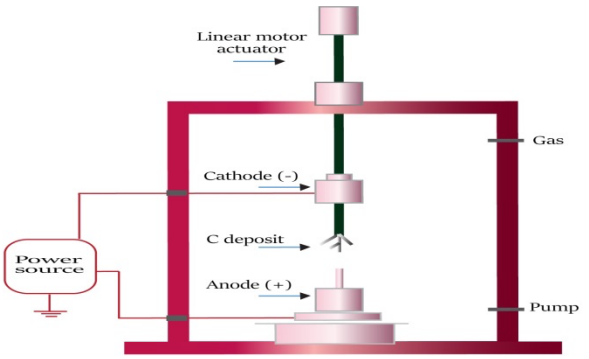

Figure 2: Electric-Arc-Discharge Method

Chemical-Vapor-Deposition Method

Although electric arc discharge method can produce immense amount of CNTs but these initially produced CNTs are impure and thus purification step is required. While in case of chemical vapor deposition method more control environment is utilized and the CNTs thus produced are pure and of desired characteristics (Sinnott & Andrews, 2001). This method is more suitable method than others as CNTs prepared in this method have caped tips. In this approach, two types of gases are utilized i.e. hydrocarbon gas (ethylene, methane etc) and process gas (nitrogen, hydrogen etc). These gases are reacted in a chamber having a metal substrate and a very high temperature of 700◦C to 900◦C. The free carbon atoms are formed and turn into nanoparticles. These nanoparticles are then deposited on the surface of metal substrate in the form of nanotubes. The temperature used and the composition of hydrocarbon gas and substrate effects the nature of CNTs formed in this method (Rafique & Iqbal, 2011). By changing the conditions of the reaction and the catalyst employed, one can produce either single-walled or multi-walled CNTs by this method (Herrera & Resasco, 2003). **(Figure 3)**

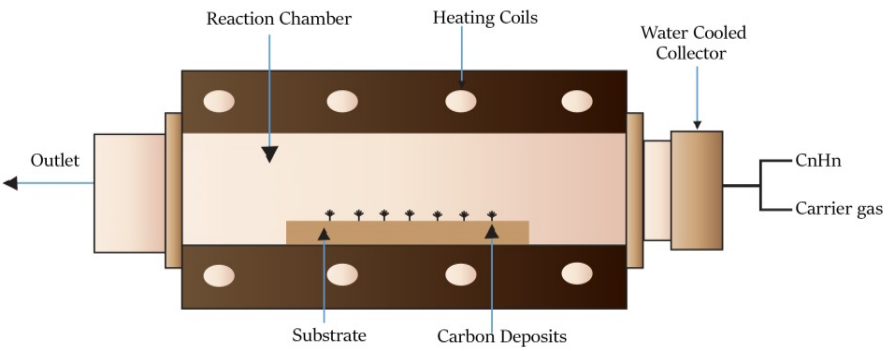

Figure 3: Chemical-Vapor-Deposition method

Laser-Ablation Method

Smalley *et al.* first developed this method, in which a direct laser beam is directed on the graphite target which is placed in the furnace having high temperature and an inert gas i.e. helium (Guo, Nikolaev, Thess, Colbert, & Smalley, 1995). When this laser beam strikes the target a high temperature is produced in the chamber that converts the graphite in to the laser plume. This laser plume is basically the mixture of vaporized carbon atoms and the metal particles which are then arranged in to the CNTs after

the condensation of carbons atoms on the cooled collector. The CNTs produced by this method are almost 90% pure but laser apparatus has very high cost which then limits the common use of this approach. Moreover only single-walled CNTs can be produced by this method while in order to produce multi-walled CNTs special conditions are required (Szabó et al., 2010). **(Figure 4)**

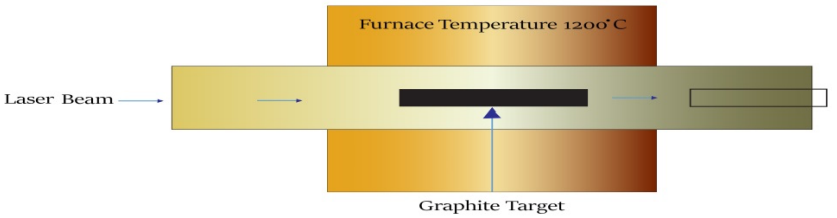

Figure 4: Laser-Ablation Method

Pathways for CNT Penetration Into Cells

The pristine CNTs are unable to cross the cell membranes, thus are not able to perform any theranostic activity. In order to cross the cell membrane, functionalization of the pristine CNTs is performed. Because of the sp^2 hybridization and closely packed hexagons in their structure, functionalization of the pristine CNTs can be done easily with either therapeutic agent or the imaging agent (Rastogi et al., 2014).

There are two main approaches for the penetrations of CNTs in the cells are (a) endocytosis and (b) endocytosis independent approaches (Lee & Geckeler, 2010). The endocytosis approach is further divided into the receptor mediated and non-receptor mediated. While in the endocytosis independent approach, CNTs can be penetrated directly by diffusion, by fusing with cell membrane or by passing directly through the pore (Fisher et al., 2012). The penetration process of the CNTs depends on its size, nature of ligands attached, length of carbon atoms, chemical groups attached to the surface and degree of hydrophobicity (Fisher et al., 2012; Lacerda et al., 2012). **(Figure 5)**

In non-receptor mediated approach of endocytosis, the f-CNTs are internalized by the cell membrane resulting in the formation of vesicle which is then transported towards the targeted site (Mu, Broughton, & Yan, 2009). The receptor mediated approach can be (a) clathrin-mediated, (b) caveolae-clathrin mediated and (c) caveolae mediated (Lacerda, Bianco, Prato, & Kostarelos, 2008; Lacerda et al., 2012). In this approach the f-CNTs are attached to the receptors which are then internalized in the form of vesicles coated with clathrin or caveolae. The ligands are detached from the receptors when the pH is dropped in endosomes, thus resulting in drug release from the CNTs after the action of lysozymes (Alberts et al., 2002).

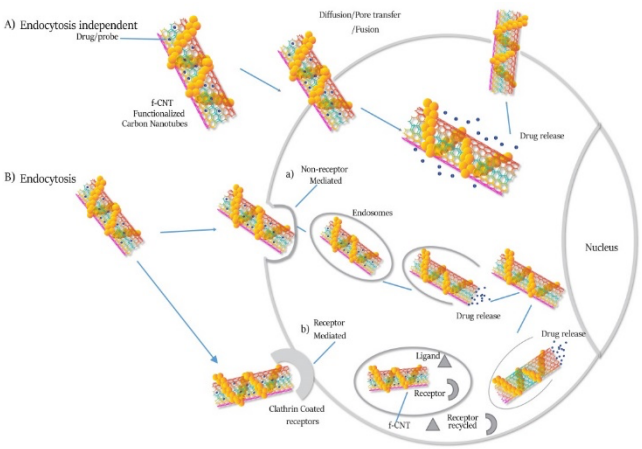

Figure 5: Penetration Pathways of CNTs into Cell

Functionalization

It is a technique in which different functional groups are attached to the CNTs in order to prevent the toxicity associated with the initially produced impure carbon nanotubes and to improve the solubility of the hydrophobic carbon skeletal in aqueous medium. By the chemical tempering several functional groups can be linked with the CNTs very precisely. This chemical tempering can be done by either simple adsorption mechanism or by different types of interactions i.e. covalent, non-covalent, electrostatic or hydrophobic etc. The most commonly used functionalization methods are PEGylation, carboxylation, esterification, purification, acylation, amidation and polymer wrapping (Ji et al., 2012; Pruthi, Mehra, & Jain, 2012; Ren et al., 2012; Singh, Mehra, Jain, & Jain, 2013; Zhang, Meng, Lu, Fei, & Dyson, 2009) etc. This functionalization can be very useful in the targeted delivery of the CNTs. The two main types of functionalization are; covalent and non-covalent methods **(Figure 6)** (Mehra, Mishra, & Jain, 2014).

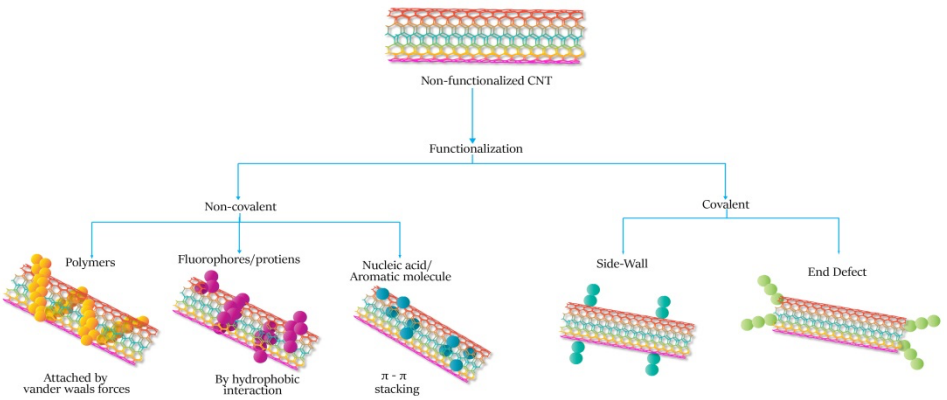

Figure 6: Classification of Functionalization

Non-Covalent Functionalization

In this type of functionalization, the functional groups are attached with the CNTs by the help of non-covalent interactions i.e. Vander-Waals interaction, hydrophobic, electrostatic or the π-π stacking interactions etc. For example lipids or any biopolymers can be attached with CNTs by Vander Waals forces, fluorophores & protein molecules by hydrophobic interactions, while nucleic acids and the aromatic molecules can be attached with the help of π-π stacking (Jain, Mehra, Nahar, & Jain, 2013; Kesharwani, Ghanghoria, & Jain, 2012).

Covalent Functionalization

In this type of functionalization covalent bonding is utilized for the attachment of different groups on the CNTs. There are two subtypes of this approach named as, "end & defect" and "side wall" functionalization. In first type, the CNTs are reacted with strong acids i.e. H_2SO_4 or HNO_3, which results in the formation of free reactive groups such as –COOH, alcoholic group, ketone or ester group at the end of CNTs. In this type not only the functional groups are produced but CNTs are also shortened into the smaller parts. While in the side wall approach the functional groups are linked covalently on the surface of the CNTs (Mehra et al., 2014).

Applications of CNTS in Biomedical Imaging

The electronic distribution on the SWCNTs gives them good semiconductor or metallic properties (Monthioux et al., 2017). In case of MWCNTs, by increasing the concentric walls the metallic properties are also increased. The DWCNTs exhibit a large surface area i.e, 1000 $m^2 g^{-1}$ (Flahaut, Bacsa, Peigney, & Laurent, 2003). The SWCNTs exhibit less density (Laurent, Flahaut, & Peigney, 2010), high mechanical resistance and high flexibility, which is indirectly proportional to the number of walls. Due to excellent biocompatibility, CNTs are widely used for biomedical applications (Mohajeri, Behnam, & Sahebkar, 2019).

Drug Delivery

Due to the unique properties of the carbon nanotubes, they have potential of the optimistic agent for theranostics in the biomedical areas. Especially in case of delivery of the pharmaceutical agents, CNTs

have several advantages over the other carriers used for the drug delivery. For example by utilizing the functionalization phenomenon for CNTs, one can control the general problems associated with the conventional systems i.e. delivery of water miscible drugs, targeted delivery of drugs and more than one agents can be delivered by using CNTs as carrier. The delivery of these theranostics agent can be achieved by either linking the agents to the surface of the CNTs or by enclosing inside the cavities of CNTs. In case of enclosing the theranostic agents inside the CNTs, one can achieve more stability of the given agent, moreover there are more surfaces available for the attachment of other targeting or the dispersing agents. This approach has been utilized by the researchers for many years for the delivery of either therapeutic or the diagnostic agent (Martincic & Tobias, 2015).

The different pharmaceutical agents that have been delivered by utilizing the functionalized CNTs are; proteins, peptides (antibodies, vaccines), nucleic acids and several other therapeutic and diagnostic agents etc (Bianco, Kostarelos, & Prato, 2005).

Cancer Treatment

CNTs have several applications in cancer targeting and diagnosing. But newly the cancer curing application has been disclosed by the researchers. When they are subjected to the IR light; CNTs produce high temperature ranges from 70 to 160◦C in almost 120 seconds, which results in the cancer cell destruction, thus CNTs exhibit the cancer curing property due to this characteristic (Beg et al., 2018). The MWCNTs which are synthesized by the "CoMoCa process" have the high potential to be used as chemotherapeutic agent, because of their property to destroy the tumor cell and thus causes the reduction in size of the tumor. The mechanism of the cancerous cells killing by the use of CNTs is that; first CNTs are subjected to the near-IR radiations as a result the CNTS absorb specific wavelength e.g. 980 nm. After absorption of specific wavelength the CNTs produce high amount of heat which is then utilized for the cancerous cell killing. This whole process is known as "photothermal therapy" (Beg et al., 2018). Moreover, in a study conducted by Wang *et al.* the SWCNTs were conjugated with anti-CTLA-4 and injected intravenously, which resulted in immune system activation along with photothermal activity, resulting in complete destruction of the metastatic cancerous cells (Wang et al., 2014).

Biomedical Imaging

SWCNTs have unique natural optical characteristics which make them feasible candidate for biomedical imaging agent besides their application in drug delivery and the cancer treatment. Moreover, SWCNTs have supposedly one dimensional structure thus they show high scattering in the Raman spectroscopy, show more absorption of light and thus photoluminescence phenomenon especially in near-infrared range (Liu et al., 2009).

Photoluminescence Imaging

On the basis of the diameter and the number of carbon atoms, there exists a mini band gap of almost 1 eV in SWCNTs. Owing to this small band gap SWCNTs show photo luminescence inside the wavelength range of almost 900 to 1600 nm which is the NIR range. This phenomenon is thus utilized for the imaging purpose. As our body tissues show lucidity towards the light having wavelength between 800 to 1000 nm and also show less fluorescence in the NIR range thus SWCNTs can be effectively used for imaging of body tissues and organs. Moreover, SWCNTs exhibit a big difference betwixt the excitation (550 to 850 nm) and the emission band (900 to 1600 nm), which is useful in minimizing the background light, may develop as a result of scattering or the fluorescence phenomenon (Liu et al., 2009). This phenomenon was utilized by Welsher *et al,* in order to image the SWCNTs in the vessels and deep tissues of the mice (Welsher et al., 2009; Welsher, Sherlock, & Dai, 2011). Recently, it is being declared as an optical replacement of the positron-emission-computed-tomography (PET) especially in imaging or detection of brown fat (Yudasaka et al., 2017).

Raman Imaging

SWCNTs have supposedly one dimensional structure thus they show high scattering in the Raman spectroscopy because of their high repulsion of the electronic cloud at different states at the "van Hove singularities". This scattering can be of two type i.e, "radial breathing mode" or "tangential mode". This scattering results in the formation of highlighted peaks which can be easily seen on even the lighted background (Liu et al., 2009).

The G band present in the SWCNTs at 1580 cm^{-1} is responsible for the scattering of Raman by CNTs. In a study conducted by Liu *et al.* SWCNTs were used having different ratios of C_{13}/C_{12} isotopes and

different ligands attached to CNTs which resulted in 5-coloured Raman imaging of tumour cells (Liu et al., 2010).

Magnetic Resonance Imaging

The pristine CNTs have some metal particles as impure materials and also some metallic NPs present in them. Because of these impurities CNTs show natural magnetic properties. Thus by incorporating some contrast agents in CNTs we can use them for imaging in MRI (Martincic & Tobias, 2015). The 1,3-dipolar cyclo-addition of CNTs as compared to the oxidation of CNTs is more feasible approach because in this process the functionalization of CNTs can be done without opening their close ends which in turn avoid the unnecessary leakage of radionuclides and thus these nuclides are prevented from accumulating in stomach & thyroid gland. The conjugation of MWCNTs and gadolinium was done by using this approach which resulted in enhanced contrasting ability of gadolinium in magnetic resonance imaging (MRI) as compared to the pristine CNTs (Servant et al., 2016).

Photoacoustic Imaging

CNTs after absorbing the NIR spectrum produce the photoacoustic effect which is being used in photoacoustic imaging. In photoacoustic imaging, CNTs produce heat after exposure to NIR rays, which in result causes the expansion of tissues present around CNTs and thus produce the ultrasounds which are being measured by the photoacoustic imaging. In a study conducted by De La Zerda *et al.* SWCNTs conjugated with the Arg-Gly-Asp peptides. These conjugated CNTs showed more sensitive and targeted effects to tumour cells as well as improved imaging of tumour cells in comparison with the unconjugated SWCNTs (De La Zerda et al., 2008; Zerda et al., 2010).

SPECT

Hong et al. described another biomedical application of CNTs as a radio-probe in single-photon-emission-computed tomography (SPECT), by encapsulating the metal halides in CNTs (Hong et al., 2010).

Challenges to CNTs as Biomedical Agent

There are a number of challenges which are being faced by the CNTs in order to be used on a large scale in the biomedical field. The most important challenge is safety, i.e, use of highly pure CNT in order to prevent the release of those ions which further cause toxicity. Thus on a large scale it is not quite possible to produce ultra-pure CNTs. The other limitation is the hydrophobicity of the CNTs which make them unstable in suspension and also affects the solubility of the CNTs. This problem can be solved by the functionalization of the CNTs. In dry form CNTs cannot be easily dispersed thus in order to avoid this, drying process of the CNTs can be avoided. Another problem associated with CNTs is increase in viscosity of their dispersion by increasing their concentration (Grady, 2006), which in turn effects the dispersibility of CNTs in the nano-formulation.

Advantages over Other Carriers

Although CNTs are comparable to other carriers used for the drug delivery i.e. NPs, liposomes and dendrimers but they show some edge over others due to their tube like closed structure which is composed of hexagons or pentagons. Moreover the sp2 hybridization of these hexagons make them easy candidate for the functionalization technique. These functionalized carbon nanotubes then can easily penetrate the cell membranes of the body cells.

Conclusion

CNTs are propitious candidates in the field of biomedical imaging and drug delivery. Because of their high biocompatibility and sp^2- hybridization they can easily be functionalized with any therapeutic or diagnostic agent. They have wide applications in the field of bio-imaging because of their intrinsic optical, mechanical and electrical properties. They can be used as efficient contrast agents and the biosensors as well as efficient carriers for the delivery of therapeutic or imaging agents. There had been extensive work on the CNTs but still not enough to explore its wide potential capabilities. Despite of the number of challenges on the way of CNTs in the field of biomedical sciences, CNTs have potential to be used for cancer diagnosis as well as treatment.

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