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The carbon nanotube paradox

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ABSTRACT

Carbon Nanotubes are the latest generation of nanomaterials that have been a subject of intense study during the last two decades. These mosaics of carbon atoms form sheets that roll themselves into seamless tubes spontaneously, imparting them with incredible physical, chemical, electrical, mechanical and thermal properties. They are widely employed in several fields like biomedical diagnostics, cancer therapy, biosensor and imaging technology, molecular electronics, DNA technology, environmental technology, etc. In several of these applications, CNT is used in pristine as well as in functionalized forms. Its hybrid composites have also found usage in specialized industries. The movement of CNT from the lab setting to the factory, production and product settings has raised concerns regarding their toxicity to the ecosystem and also to biological organs, tissues and cells at various levels. Several studies have implicated its high aspect ratio for causing pulmonary and other organ toxicity in animals and humans. Such effects are now thought to be mediated by oxidative and inflammatory stresses and by immunological reactions to CNT in the system. However, it is a common consensus today that more detailed studies encompassing several experimental parameters and structural types of CNT needs to be carried out to assess its hazard potential unambiguously. Till then, this promising material needs to be cautiously employed for applications in fields of research and product development.

KEYWORDS: Nanotechnology, Carbon nanotubes, Biomedical applications, Cancer therapeutics, Nanotoxicology

1. INTRODUCTION

Nanotechnology is evolving as a versatile field that is slated to provide exemplary solutions to complex problems in several thrust areas of science, technology and particularly medicine. Since the period of Richard Feynman's landmark speech at Caltech University in 1959, where he stated that "there is plenty of room at the bottom" (Feynman, 1959) considerable time and resources have been devoted to the development of several nanomaterials involving manipulation of atoms and molecules at the nanometer scales. This alteration leads to an enormous increase in the surface area to volume ratio of the concerned material as compared to the bulk form of the same composition. In this scenario, gravity becomes less consequential and Vanderwals forces as well as surface tension predominate. Quantum effects come forth to play a major role in altering the properties of such materials with respect to electrical and thermal conduction, optical and magnetic features and also mechanical and tensile strength among other properties.

Currently, nanomaterials are being increasingly and vehemently applied in several important arenas of industry and research (Kostoff, 2008). But recently, apprehensions about their toxicity and adverse effects are also starting to be observed in animal models and in humans inadvertently exposed to them. It is very relevant to note here that the very properties that are responsible for their versatile applications may be required to be implicated for their undesirable effects on health and environment (Shvedova, 2009). In this review, the above paradox of balancing the favorable aspects with the hazard concerns is reviewed, discussed and analyzed with respect to today's most popular and application oriented nanomaterial, *viz.*, the carbon nanotubes (CNT's). An attempt has been made to unravel its complex nature based on its structural features, functional aspects and assessment of toxicity potential. The precautions that need to be undertaken for its efficient and safe exploitation are also highlighted.

Carbon nanotube - A versatile nanomaterial: A major nanomaterial that has been extensively explored, researched, synthesized, studied and applied in diverse fields in the Carbon Nano Tube (CNT). As the name suggests, it is an addition to the list of existing carbon allotropes, and seen as a sheet of graphene rolled into a cylindrical form with the hexagonal carbon lattice giving it tremendous strength and stability. It rarely occurs free in nature and can be synthesized by several artificial methods. In fact, Ijima observed CNT in 1991 as an insoluble material, in arc burned graphite rods. Much before that, in 1952, its 50nm clear image was published, albeit in a Russian journal, and hence its importance went largely unnoticed (Radushkevich, 1952). It was finally Ijima's report that enlightened the scientific community about CNT (Ijima, 1991).

Methods of Production: Since then several methods of CNT production have been developed and they are constantly being improvised to provide nanotubes of better quality and suited to specific applications (Prasek, 2011). Among these plasma arcing, developed in the 1960's, is the most simple method that involves passage of current in a helium atmosphere between two graphite electrodes in the presence of metal catalysts. Optimization of the current and voltage parameters produces good quality crystalline CNT with minimal impurities (Sharma, 2015). Laser ablation is an alternate method wherein a laser beam abrades off and vaporizes nano carbon, again from graphite electrodes, for subsequent condensation and deposition. Both the methods produce high quality nanotubes albeit in small quantities, with the latter technique being more expensive but highly controlled. Ball milling involves grinding

of carbon physically to reduce its size and initiate nucleation for subsequent annealing and hence involves a top down followed by a bottom up approach. Contamination issues however need to be contended with here. Today, chemical vapor deposition (CVD) is the most popular production method wherein decomposition of carbon monoxide (or any other suitable hydrocarbon) gas occurs to form CNT in the presence of nanopowdered metal catalysts, like iron nanoparticles. This process can be graded up for large scale production with minimum contamination from residual byproducts. Controlling the temperature, flow rate and the kinetics of carbon supply like in an innovative mist flow technique may have implications on the diameter of the end product (Kumar, 2010; Sun, 2012). Subsequent to synthesis, the nanotubes can be separated according to their aspect ratio, that is, the ratio of their length to diameter, by ultracentrifugation and ultimately powdered for further use. The aspect ratio in case of CNT is known to reach upto or more than 1,000,00 and this qualifies it to be classified as a High Aspect Ratio Nanoparticle or HARN. Aligned, rather than tangled CNT are preferred for different high end applications (Arya, 2015).

Types, configurations, characterization and properties: The intrinsic structural feature of CNT is important to determine its applications as well as its toxicity standards and hence understanding its structural types is of utmost importance. In this context, it would be relevant to state here that CNT can be either single walled (SWCNT) or multiwalled (MWCNT) depending upon the number of layers that go into its formation. It has been observed that each of these types has its own levels of hazard and usage potential which highlights the influence and importance of the diameter and subsequently the aspect ratio, as the MWCNT obviously would have a higher diameter and width when compared to SW ones (Jie, 2010; Maruyama, 2015). In the former case, the interlayer distance (of approximately 330 picometer) is slated to play a major role in validating its versatility. In case of SWCNT, the configurations/geometries can be chiral, armchair or zig zag depending on the alignment of the carbon lattice in the hexagonal rings. The MWCNT projects two models, *viz.*, parchment model, wherein the same graphene sheet is rolled onto itself several times (the confirmations would obviously be the same here) and the Russian doll model wherein several independently rolled graphene sheets, maybe with different configurations, are inserted one into the other. In any case, narrower the dia, the more its intrinsic properties depend upon its specific type. Double walled CNT's are the simplest archetypical manifestation of the latter type with unique scientific novelty and application potential (Pfeiffer, 2008).

The properties of any type of CNT are very versatile and are hence responsible for its varied applications in several areas of health care and other related technologies. In terms of elastic modulus and tensile strength, it is the strongest and stiffest material known on earth. However, their hollow structure facilitates buckling when subjected to bending or compressive stress. Electrical conductivity is almost 1000 times that of copper and thermal conduction is also proportionately high. Molecular nanotechnology is seen in action when the tubes slide one inside the other with almost zero friction to create an atomically linear perfect structure. Extensive development in the field of CNT metrology techniques has facilitated the measurement and analysis of such intricate properties. This has involves establishment of parameters and protocols as well as development of electron microscopies and optical spectroscopic techniques for physical characterization of the nano world making nanometrology an exciting and thrust area of research on its own (Jorio, 2008; Belin, 2005). The chemical reactivity of CNT is controlled by the curvature of the tubes and ones with smaller dia are in general more reactive. In general, it is not especially reactive, but can be made to react under strong chemical conditions (e.g., by incorporating hydroxyl or carboxyl groups onto the side walls) Wang, 2005.

Its solubilisation was initially a matter of considerable concern as CNTs are sparingly soluble only in a very limited number of solvents like dimethylformamide, dimethylacetamide, and dimethylpyrrolidone Kim, 2006. Simple dispersion in solvent blends like that of polydimethyl siloxanes and dibutene has served well for specific applications Hong, 2011. But their covalent functionalization with different groups has facilitated dissolution in several solvents, both polar and non-polar and this has proved beneficial for different biomedical applications like drug delivery (Georgakilas, 2002; Battigelli, 2013; Pastorin, 2009). But such procedures may lead to modification of the physico chemical properties of native CNT. Hence, their dispersion in biocompatible surfactants like Pluronic F108 and hydroxyl propyl methyl cellulose have also been attempted for carrying out various *in vivo* and *in vitro* toxicological studies (Piret, 2010). Procedures involving the doping of CNT to fine tune and control the physicochemical properties have been tried out successfully using wet chemistry. Exohedral, endohedral and substitutional doping are identified as favorable techniques and preliminary experiments have revealed that doped CNT's are pathologically less harmful to living cells as compared to undoped ones (Terrones, 2008).

Applications in Biology and Medicine: CNT has now been established as a very important nano tool to achieve amazing results in several niche areas of health care technology. Its utilization in biomedical and therapeutic arenas has been extensively researched, reported and critically reviewed in the last decade (Soleyman, 2015). CNT as well as its hybrids have been utilized very successfully as tissue scaffolds, cell growth substrates, intracellular transporters, biological imaging agents, biosensors, etc. This material has thus provided natural game changing solutions to unaddressed problems in the biological milieu.

A very important property of CNT is its ability to provide a simulated cellular environment by virtue of its porous meshwork with variable pore diameters (Asanithi, 2009). That could serve as a one, two or three dimensional network. Different types of cell phenotypes preferentially adhere and proliferate on it and on its functionalized versions. The meshwork also acts as an interface for specialized cells like neurons, osteoblasts and more importantly cultured human embryonic stem cells. Hence exploring the role of CNT in nerve tissue engineering, serving as a bone prosthetic substitute to titanium and in stem cell technology is an area of intense research that has also been extensively reviewed (Fabbro, 2013; Heister, 2013). The role of CNT in serving as a vaccine scaffold has also shown considerable promise as they lack intrinsic immunogenicity and have the ability to be attached to multiple copies of antigens. Additionally, their particulate nature also facilitates quick and favorable internalization into antigen presenting dendritic cells as well as macrophages within 5 minutes. Such mechanisms may prove very useful in the treatment of infectious diseases (Scheinberg, 2013). A similar role can be envisaged for CNT as a gene therapy vector due to several benefits offered over conventional delivery systems. These features include fine tuning of the length to diameter ratio which is a critical factor determining its complexation with nucleic acids and consequently its transfection efficiency. Surface modifications also play an important role in facilitating improved delivery of favorable genetic cargo that include plasmid DNA, si and mRNA, aptamers and oligonucleotides. *In vitro* and *in vivo* studies for treatment of cancer, leukemia, ischemic strokes, vascular diseases, restenosis, HIV and insulin resistance have shown excellent outcomes with good geno pharmacological profiles. However, the clinical efficacy of such these routes and strategies needs to be more firmly established (Bates, 2013).

Targeted delivery of drugs liked doxorubicin and paclitaxel, have been successfully attempted with CNT. For loading them both covalent and non-covalent functionalization (PEGylated phospholipids) have proved to be tremendously effective. The CNT-Doxo complex shows enhanced toxicity towards breast cancer cell line (MCF-7) as compared to the drug alone or the drug Pluronic complexes thus validating the important role played by CNT in ensuring the delivery of doxo to the cells and also facilitating its enhanced cellular uptake. Further, confocal microscopy studies have revealed that after the uptake, doxo is released from CNT and translocated to the nucleus leaving the CNT in the cytoplasm (Ali-Boucetta, 2008; Heistera, 2009). CNT assisted drug delivery has also proved to be an alternative route to the delivery of several active pharmaceutical ingredients by viral vectors that are preferred due to their inherent capability for transfecting cells efficiently. The latter however carries the risk of secondary infection. Other safer delivery vehicles like dendrimers, liposomes and even metal nanoparticles have poor transfecting capacity. CNT combines the safety aspects along with favorable structural features that facilitate their internalization into cells thus transporting the drugs too simultaneously. Also, their higher surface area to volume ratio proves to be beneficial for increasing the number of functional groups at their surface that can be exploited for binding more number of drug molecules. Alternatively, in case a drug needs to be encapsulated into it without any chemical interaction, it could also be facilitated to a greater extent through their void volume.

Delivery of drugs by CNT to specific targets has achieved substantial importance in cancer therapy (Zhang, 2011). Delivery has been targeted to the lymphatic system as many types of cancers are metasized through the lymphatic canal. Radical polymerization has been used to append polyacrylic acid to CNT and increasing the hydrophilic nature to load gemcitabine with 62% efficiency. This hybrid nanoparticle when injected subcutaneously was found to localize preferentially in the local lymphatic nodes and conspicuously absent in other major organs like heart, lungs, liver kidney and spleen. It is relevant to state here that gemcitabine does not localize in the lymphatic nodes when injected as a standalone drug (Yang, 2009). Targeting anticancer drugs to tumors has remained the main focus of cancer therapy. In this context several drugs are not able to locate and enter the tumors specifically due to their short residence times in the biological milieu. For example in case of paclitaxel, its non-specific cytotoxicity and poor aqueous solubility lowers its efficacy. Conjugating it to branched PEG chains covalently linked to CNT *via* cleavable ester bonds has succeeded in increasing its residence time six fold times as compared to the clinically used Taxol in murine breast cancer model along with increased efficacy. This may be due to the enhanced hydrophilicity of the complex (Liu, 2008). An additional advantage is the reduced allergen city as compared to Taxol as the solubiliser present in the commercial formulation, *viz.*, cremophor elicits this response. Similar results with greater impact has been observed with hydroxycamptothecin attesting the superior features shown by CNT to act as a carrier for such drugs (Wu, 2009). Delivery of acetylcholine *via* CNT has been attempted to the central nervous system and it has been seen that the drug is successful in entering the brain neurons possibly by the axoplasma transformation of neuritis. The outcome is a significant improvement in the memory and learning capabilities in Alzheimer induced animal models (Yang, 2010).

CNT has also proved to be an excellent candidate for studying the bioenergetics involved in the sliding of single actin filaments over MWCNT coated muscle myosin fibers. This phenomenon, driven by ATP, has demonstrated that the biological motor activity is a temperature dependent phenomenon operating in the reversible mode from 20 - 40^0 C and tends to become irreversible beyond 40^0C. The role of CNT in deciphering similar mechanisms involving biological thermodynamics and biophysics is very promising Inoue, 2015. The one

dimensional character of CNT has been exploited for the development of optical based biosensors as this is an important character that facilitates the ultrasensitive detection of analytes. Since all its constituent atoms are present in the surface, minute changes in its environment serve to modify its relevant properties dramatically. Its diversity in terms of chirality is another obvious advantage here, but this aspect may also lower its sensitivity and selectivity. Nonetheless, such sensors have been widely used to detect several biomolecules like DNA along with its SNP's, hybridization and conformational polymorphisms, ATP, reactive oxygen and nitrogen species, glucose, proteins, nitric oxide, hydrogen sulphide, etc. because of its conducive electrochemistry (Kavan, 2008; Tasviri, 2011; Wang, 2012). It is also feasible that devices integrated with CNT will be fairly successful in collecting data from isolated cells. Hence capturing as well as profiling individual cancer cells circulating in the biological milieu may also become feasible as is being proved (Kruss, 2013). CNT mediated hyperthermia therapy for tumor ablation has been developed as an important tool to treat recurrent tumors and also those that are not responsive to conventional cancer therapies. The technology has been tuned to achieve not only the selective delivery of CNT to the tumor location but also cause its intratumoral dissemination. Further, the process also monitors its distribution in the tumor and optimizes the heat generated by exposure to near infra-red radiation for achieving enhanced efficacy. Similar ablation therapies for acting on inflammatory macrophages on animal models with induced atherosclerosis have proved to be equally beneficial Singh, 2013.

The extensive use of CNT for imaging has been reviewed Liu, 2009. It is a fact to a certain extent that surface functionalisation is of vital importance while considering its behavior in biological systems that in turn determines their use in medicine. Their water solubility and also serum stability are resultant features of functional groups on their surface. Concurrently, these groups also determine their bio distribution as they are largely known to accumulate in the reticuloendothelial system inclusive of spleen and liver as inferred from their intravenous administration. Well functionalised CNT is excreted in the feces through the biliary pathway. If the CNT surface is rendered passive to the numerous nonspecific hydrophobic components in the biological milieu, it would facilitate the ultrasensitive detection of several clinically important species. The intrinsic optical properties of SWCNT have been exploited for imaging of isolated cells, tissues as well as whole animals. The photoluminescence associated with single walled CNT is very stable and hence facilitates longer excitation time as compared to either quantum dots or other organic fluorophores. Additionally at the absorption range between 700-1400nm, the scattering as well as autofluorescence of tissues, that are opaque, is very low. This is conducive to their detection in tissue samples and their concentration determination in blood as has been reported for CNT (Heller, 2006; Cherukuri, 2006). Their imaging after incorporation into macrophages has also been reported (Cherukuri, 2004). Photobleaching, the phenomenon observed with conventional imaging is eradicated to a large extent with CNT and this is another advantage with these materials. Radiolabelled CNT conjugated with PEG are able to circulate for a longer time in the blood and escape uptake by the reticuloendothelial system for a longer time. They are also conducive to detection by the PET scanning. Alternatively, if conjugated with arginine-glycine-aspartic acid peptide, they are helpful in targeting integrin expressing tumor cells in animal models thus attesting their potential for imaging applications in cancer Liu, 2007. Additionally, the numerous applications in energy and environment have been extensively reviewed and is beyond the scope of this review.

CNT Toxicology

Requirements and challenges: Toxicity assessment of nanomaterials, in general pose, a lot of challenges as it requires ultra-sensitive high resolution instrumental and microscopic technologies (Dhawan, 2010). The currently available ones are not cost effective or user friendly. They also need trained personnel and pose severe time constraints. Many of them are in the developmental stages for achieving better resolution, reproducibility and sensitivity.

Amidst this scenario, compared to research on the applications of CNT, systematic studies focusing exclusively on its hazard assessment are few and limited. Some CNT toxicity issues that need to be considered with regard to biological systems and nanomedicine have been extensively reviewed along with an assessment of its potential in environmental and occupational health risks (Firme, 2010; Fischer, 2012; Lam, 2006; Lamberti, 2015). Pristine CNT, in any of form has not been a much researched subject with respect to toxicity, probably because of its solubility issues, that limits its applications in several thrust areas. The functionalized ones demand the study of toxicity due to the carbon skeletons as well as the attached groups. While the effects of many such common functional groups per se may already be known, it is envisaged that variations would definitely arise when they are bonded to CNT in the diversified structural forms as each of these would have its own unique interactions with the functional groups, thereby modifying its reactivity and toxicity.

Also CNT needs to be assessed with respect to different stages of its life cycle, the first and very important one being its synthesis. Although it is considered a relatively new material, its production is expected to reach 12,000 tons shortly. This invariably increases the chances of unintentional exposure through the inhalation, ingestion and

transdermal routes by several folds (Dhawan, 2010). Intentional exposure, due to laboratory experiments is mainly by the intravenous and intratracheal routes (Buzea, 2007; Schipper, 2008).

CNT uptake, degradation and clearance: After exposure, the next stage, *ie.*, its uptake by cells, depends on the culture medium and takes place by two possible mechanisms Mutlu, 2010; Serag, 2011. One is the passive pathway that includes diffusion or pore transport by direct crossing through the membrane and in this case, it is subcelluarly localized in the mitochondria. The other more common entry route is by endocytosis that can involve either phagocytosis of long CNT's of >1um in length by macrophages, neutrophils and monocytes or pinocytosis of shorter ones. In this case, it localizes in the lysosomes and phagosomes with smaller CNT's being found in the cytoplasm (Lee & Geckeler, 2010; Zhou, 2010; Zhao, 2011). Localised CNT can also be translocated between organelles easily through carrier mediated transport and viewing CNT inside them has been made feasible by the use of Transmission Electron Microscope. It has revealed that SWCNT is localized initially within 48 hours of exposure in the lysosomes and then later after 4 days in the endosomes and finally in the nuclei after being translocated across the nuclear membrane. The process of intracellular trafficking can be fine-tuned by bonding tags onto CNT, for example attaching FITC to it facilitates its uptake into the vacuoles by carrier mediated transport. Alternatively if an inhibitor of such transport is tagged, CNT accumulates in the cytoplasm Serag, 2011. Hence, its nanotoxicity potential needs to be assessed in every organ.

Study of its cellular clearance mechanisms in terms of its metabolism proves the exceptional stability of its basic carbon skeleton *in vivo* for a minimum period of three months that prevents its breakdown into smaller molecules which can be easily excreted in the urine or feces Yang, 2008. The focus of degradation studies hence was initially limited only to the defunctionalisation of groups attached by either non covalent or covalent bonds to the carbon structures, as invariably, solubility issues demanded the CNT to be applied only in these forms for its applications. Both these types can be metabolized into the pristine CNT's either by simple quick desorption as in case of non-covalent bonding or under more vigorous conditions involving the metabolic activities in the liver as in covalent bonding.

In the latter case, the specificity of organs in achieving this has also been reported, with the hepatic defunctionalisation being more effective than that occurring in the spleen. Since pristine CNT is known to be more harmful than functionalized ones, attaching groups by non-covalent interactions and also the accumulation of covalently bonded CNT in organs like the liver do not emerge as best possible ways of using it for *in vivo* applications. It also suggests the existence of organ specific detoxification mechanisms in biological systems. It is relevant to state here that dispersing or suspending medium like PEG-Phospholipids play an important role in not only eliminating CNT from the body *via* urine/feces but also in preventing its defunctionalization to the more toxic native forms (Yang, 2012).

An oxidative stress mechanism involving superoxide and hydroxyl radicals for the degradation of CNT in the macrophages has been established (Elgrabli, 2015). Mechanistic investigations with a peroxidase system involving horse radish peroxidase have revealed that the requirement of oxygen moieties or defective sites in the SWCNT for this phenomenon. Purely pristine CNT tends to be hydrophobic and aggregate in bundles in the solutions and hence resist degradation. Additionally, the negative charge on the COOH group of the acid functionalized CNT ensures proper orientation and proximity to the active site (heme) of the enzyme and this feature that is inherently lacking in pristine CNT prevents its degradation (Kotchey, 2013). Similarly, a neutrophil myeloperoxidase system has also been reported to significantly reduce its pulmonary toxicity Kagan, 2010.

Importance of CNT types, models and aspect ratios in determining toxicity: It is also clear that outcomes of CNT hazard assessments are critically dependent on the concentration, types, configurations, models and most importantly, on the length to diameter ratios (Lanone, 2013). The duration of exposure also seems to be of utmost importance and ascertained to be 24 hours for the initiation of certain deleterious consequences of nanomaterials in general (Lanone, 2006). Additionally, its existence in many versatile forms creates a scenario wherein every form needs to be individually assessed for hazardous effects as there are many questions that need to be answered. For example, how are single walled and multiwalled CNT comparable? Is a five (multi) walled CNT equivalent to five individual single walled CNT's with respect to toxic concentration effects? Do configurations like arm chair, zig zag and chiral have different or similar implications in case of single walled CNT? Are the Russian doll and Parchment models similar? If not, how are they dissimilar? These are only some of the important queries that need solutions after intensive and focused research that is currently lacking in this area.

Its pharmacology with respect to blood circulation, distribution, tissue accumulation, excretion and toxicokinetics has been reviewed be to very complex and variable for its multiple forms and maybe the probable reason for throwing up ambiguous results in several studies (Ali-Boucetta,2013; Dubin, 2008). Some preliminary reports have attested CNT to be harmless to certain types of cells, tissues and organs while others have revealed it to cause both chronic and acute effects in several biological systems. This is because each type of research has analysed

a different type of this base material and under a different set of variable parameters. Also, traditional toxicological assays like the MTT assay is found to be ineffective in measuring the cytotoxicity of CNT (Worle-Knirsch, 2006).

Demonstration of favorable toxicological profiles for CNT: Formulations of hylauronic acid that is known for its favorable viscoelastic properties and biocompatibility is unstable thus limiting its applications. Its biocomposite with CNT stabilizes it for use in bone regeneration procedures thereby increasing its therapeutic potential Mendes, 2007. Favorably, this treatment has shown no variations in the cardiovascular parameters like blood pressure, heart rate and cardiac functions of rats after being injected into their tooth sockets as dispersion in carbopol gel and recommended for use for bone reconstruction after evaluation of certain other crucial parameters like electrical properties of the heart and the optimum concentration of injection (Joviano-Santos, 2014). Implantation of MWCNT paper for tissue engineering applications did not induce any serious toxicity except some minor inflammation (Bellucci, 2009). SWCNT did not induce any apoptosis in the organs upon its long term accumulation and the fibers were found to be compatible with mammalian cells and neurons (Yang, 2008; Dubin, 2008). Damage to the testis was caused by multiple injections of carboxyl and amine functionalized CNT in animal models, but was reversible with no obvious effects on fertility (Bai, 2010). In fact, amine functionalized CNT's protected the neurons also (Lee, 2011). Such types of CNT showed normal toxicological indicators and did not accumulate in the body. It can be inferred that their filtration is reduced by the pulmonary capillary vessels due to increased dispersion.

CNT as a potentially hazardous material for biological systems : Most of the early *in vivo* studies attesting the toxicity of CNT was based on its respiratory exposure model and monitoring the consequent lung tissue inflammation, injury and granuloma formation. Initially, although soot containing CNT was found to be safe in terms of not inducing any measurable inflammation or change in lung function of guinea pigs, later experiments in mice with SWCNT revealed it to be more toxic than carbon and quartz in terms of inducing lesions, peribronchial inflammation and necrosis extending into the alveolar septa within a period of 7 to 90 days (Huczko, 2011; Lam, 2004). Similar studies were observed in rat and rainbow trout models and with MWCNT's too (Warheit, 2004; Smith, 2007; Muller, 2005). They probably elicit effects similar to asbestos in these tissues and may lead to development of cancer in the long run Donaldson, 2013; Bhattacharya, 2013. MWCNT is also reported to cause airway fibrosis in murines with allergic asthma (Ryman-Rasmussen, 2009). Such outcomes are of major concern as CNT has been identified as a major component of particulate matter in the alveolar macrophages in a study conducted on asthmatic Parisian children. This would naturally deem to decrease the lung function in such subjects (Alloyeau, 2015). The inflammatory mechanism could possibly involve the activation of inflammasome complex in the phagocytic cells followed by the release of IL-1β, one of the key mediators of inflammation.

CNT is also reported to cause hemotoxicity, immuno toxicity, genotoxicity and carcinogenicity in biological systems (Bussy, 2013; Dumortier, 2013; Toyokuni, 2013). When introduced into the abdominal cavity, it induces inflammation and formation of granulomas Poland, 2008. MWCNT, surface bound to PVC, causes severe platelet activation *in vitro* and massive thrombosis by extracorporeal circulation in established rabbit models Gaffney, 2015. *In vitro* studies have implicated oxidative stress as the main phenomenon for its unfavorable toxicological profile. This has been specially observed in case of human HEK and keratinocyte cell lines (Cui, 2005; Monteiro-Riviere, 2005). Loss of viability in lung fibroblast cell lines has also been seen with SWCNT in a time and concentration dependent manner (Kisin, 2007). Similarly, its exposure to human lung epithelial cell lines, in high concentrations above 250ug/ml, for 72 hours leads to membrane damage and inflammatory responses (Choi, 2009). In a similar study with MWCNT, oxidative stress and NF- κB has been implicated for the cytokine production and exertion of proinflammatory effects (Ye, 2009).

2. CONCLUSION

There has been a growing interest in CNT based materials due to their tremendous potential especially in the area of biomedical theranostics including cancer therapy. Their use in these fields has become imperative and almost indispensable. Simultaneously, their hazard assessment research have thrown up contradicting and controversial results that need to be analyzed judiciously after conducting thorough toxicological experiments with its diverse forms and by incorporating important variables in the assessment protocols using an unbiased approach. Currently functionalized CNT seems to be less hazardous than pristine ones, but toxicity of the functional group incorporated into in may be another area of concern. Till the safety levels of its forms and their conditions of use are firmly established, CNT is required to be applied in the field of biological theranostics with utmost caution inspite of their remarkable potential and properties. Its utilization in other areas of energy, environment, etc also needs to be regulated as all applications tend to ultimately converge on the ecosystem.

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