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Toxicity Issues Related To the Applications Of Carbon Nano Tubes: A Review

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***Abstract*— Different particles of different size are present in the environment, out of which some are produced intentionally and beneficial and some are undesired and produced unintentionally. Out of these intentionally produced particles, some nano size particles are produced by engineering methods. These particles are Nano Particles like CNTs, Fullerenes, Nanohorns etc. The nano-particles engineered from the Carbon are called Carbon Nanotubes. These are SWCNTs (Single-Walled carbon Nanotubes) and MWCNTs (Multi-Walled Carbon Nanotubes). Carbon Nanotubes produced from different Synthesis, Purification and Post processing methods exhibiting several unique Electrical, Mechanical, Chemical and Physical properties have recently emerged as a potential material for the possible use in many technologic applications Biomedical Engineering Applications, Electronics and Computer Related Application, Gene Therapy, Tissue Engineering, Targeted Drug Delivery, Nano-medicine, Cancer Treatment, Neuroengineering and Biosensors technology for diseases diagnosis. Widespread applications and unique properties of CNTs make it important to understand their harmful effects. Determining the toxicity is hot issue in Nanotechnology. The toxicity of CNTs due to different factors like size, shape, functionalization, method of preparation is still not completely known. Due to inconsistent data availability on the toxicity of CNTs, we are unable to use them in Nanomedicine and medically sound technologies even after exploiting the unique properties of CNTs beneficial to healthcare industries. Therefore, to understand the toxicity effects of CNTs, we are dependent on present published studies. Based on these articles, this review critically discusses potentials of CNTs in different application areas along with the key findings on the toxicological implications of CNTs at cellular, genetic & systemic level and role of different characteristics owing to toxicity of CNTs.**

***Keywords*— CNTs (Carbon Nanotubes), Toxicity, Functionalization, In-vitro Toxicity, In-vivo Toxicity**

I. INTRODUCTION

Nanotchnology is the study of nanomaterials being the materials of size ranging from 0.1 nm to 100 nm.

The length of CNTs can be from millimeters and even in centimeters [1]. Nanomaterials have been categorized into four categories by National Academies viz. Nanotubes, Quantum dotes, Metal Oxides and Nanoclays. Out of these materials, Carbon Nanotubes are of our interest. CNTs were discovered by Lijima in 1991. The unique properties of CNTs include tremendous strength, large length to diameter aspect ratio with a large surface area, excellent electrical and thermal properties, chemical and optical properties. These unique properties of CNTs make it a potential material at nanoscale for different commercial and industrial applications. The application areas of CNTs are Nanomedicine, Biomedical Engineering [2-3], As carriers in Drug Delivery [4-5], Gene therapy [6], Tissue Engineering [3, 7-9], Pharmaceuticals, Biosensors [10-16]. Nanotechnology, Engineering [17], Electronics and Computer Engineering [2], Therapeutic Applications [18], Cosmetics [19] and many others. The applications of CNTs in different areas largely depend on their toxicity in humans, animals and environment. Chemical modification to lessen the toxicity of CNTs is one of the major research areas in the field of CNTs. Our review highlights major applications and different types of toxicity associated with commercialization of these applications and ways to tackle toxicity up to some extent.

II. SYNTHESIS, TYPES AND PROPERIES OF CNTS

CNTs are long chains of Carbon material in the shape of tube having the diameter on nanometer scale. CNTs have the diameter between 0.4 and 2 nm [20-21]. It is made up of graphite layers in a way to make continuous hexagonal mess rolled into a hollow cylinder. Each Carbon atom in CNT is bonded to three neighbor Carbon atoms and thus, forming sp^2 hybridized Carbon. CNTs may have different structures depending upon thickness, length, number of layers and also on helicity.

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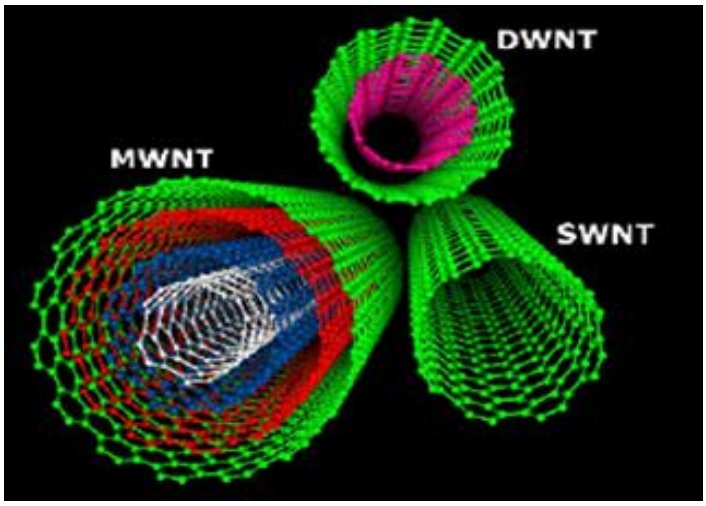

Fig 1: SWCNT, DWCNT and MWCNT [27]

CNTs may be Single Walled and Multi Walled depending upon the number of layers. Single Walled CNT has only one layer of grapheme sheet rolled into a hollow structure and Multi Walled CNT has two or more number of layers of grapheme sheets and rolled into hollow structure concentrically as shown in the figure 1.

The shape of CNT depends on the chirality or twist. Depending on the Chirality, CNTs may be divided into Arm-chair type, zigzag type and chiral type as shown in figure 2. The chirality of CNT affects its lattice structure, density and conductivity. Carbon nanotubes can be used to form different shapes like nanotorus, Nanobuds and nanohorns. Nano-torus used in the nano photonics applications are formed by bending the CNT in circular shape and its magnetic moment and other properties depend on the radius of Torus and also on the radius of CNT [22]. CNT shaped into the form of Walled tubules with a conical shape are called Nanohorns. These are used for making electrodes materials for energy storage owing to large surface area and their high purity [23].

CNTs have different types of properties like optical, chemical, thermal, electrical, mechanical and physical properties. Optical properties of chiral type CNT is due to one dimensional quasi nature and optical property disappears with increasing size of CNT. In terms of electrical conductivity, CNTs have the properties between semi conductor and metals.

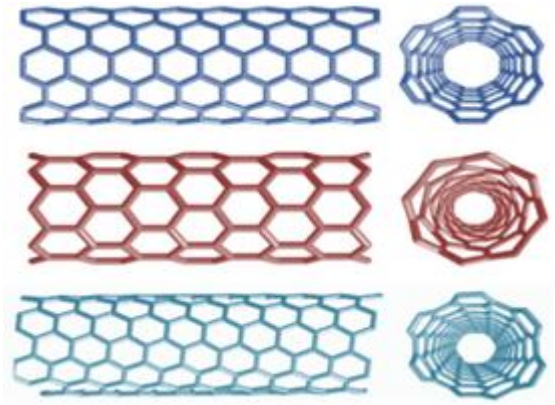

Fig. 2 Armchair, Zigzag and Chiral CNT respectively from up [24]

CNTs behave like a transmission line in response to A.C. Signal. CNTs have inductance depending on geometry and diameter of CNT and permeability of the medium [25]. CNTs can be synthesized by different methods. CNTs can be synthesized by Arc Discharge method, Laser Ablation [26], Chemical Vapor Deposition [28], Flame Synthesis, Silane Solution method [29].

The schematic diagrams are shown in figure 3. Arc Discharge method can be improved with Inert gas like Argon, Optical Plasma Control, arc torch and catalysts for the synthesis of SWCNTs.

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MWCNTs can be synthesized by ARC Discharge method with slight modifications like Liquid Nitrogen, Plasma Rotating Arc Discharge and Magnetic Field. Many types of Chemical Vapor Deposition processes for synthesis of CNT are Thermal or Plasma Enhanced (PE) Oxygen Assisted CVD [30].

Water Assisted CVD [31-33], Microwave Plasma (MPECVD) [34], Radio Frequency CVD (RF-CVD) [35] or Hot-Filament (HFCVD) [36-37].

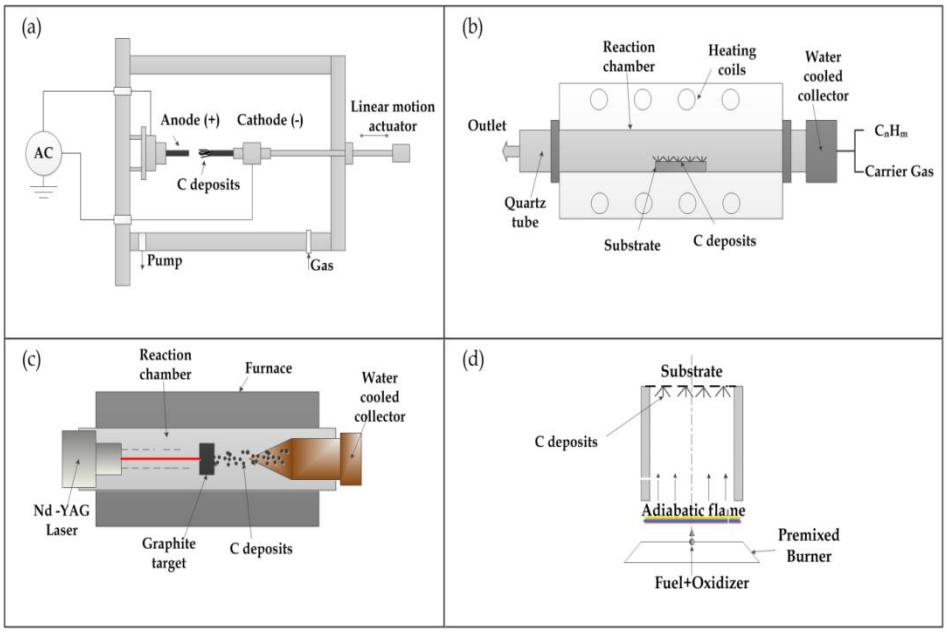

Fig 3: Schematic diagrams for synthesis of CNTs. (a) Arc Discharge Method, (b) Chemical Vapor Deposition (c) Laser Ablation Method (d) Flame Synthesis Method

Synthesis With Use Of Different Catalyst Precursors And Without Use Of Catalyst Precursors Are The Two Methods For The Synthesis Of Cnts With Electric Arc Discharge Method. Synthesis Of Mwnts Could Be Done Without Use Of Catalyst Precursors But Different Catalyst Precursors Are Used For Synthesis Of Single-Wall Nanotubes (Swnts).

III. Purification And Functionalization Of CNTs

CNTs have a large amount of impurities like carbonaceous impurities and metal catalyst particles, which should be removed before using them for drug delivery and other applications. The average percentage of impurities in CNTs is approximately 5-10%. CNTs must be purified before their use in different applications [38]. Purification methods are divided into three categories viz. Chemical, Physical and combination of both. Physical method is complicated, time consuming and less effective as compared to chemical methods. The combination of both methods exploits the merits of both Physical and Chemical methods.

CNTs can be purified by Air Oxidation [39], Acid Reflexing Surfactant added Sonication [40], Filtration and Annealing [41], Magnetic Purification [42], Chromatography [43] and Electric Field [44]. Purification of CNTs by Oxidation process includes Gas Phase Purification [34, 45-46] and Liquid Phase Purification [47-49]. Sine CNTs have the mixture of elements having semi conductiong as well as metallic properties. Several techniques for separating semi conducting and metallic mixture like Electrophoresis, Centrifugation, Solubilisation and Chromatography [50-52].

CNTs can be modified to use them into different applications. Solubility of Water insoluble CNTs can be increased by chemical modification through the use of surfactant and dispersants. Covalent bonding and Non covalent wrapping are the two methods for the functionalization of CNTs. Covalent bonding involves attachment of required molecule at the defect site or at tube ends [53]. Covalent bonding includes the oxidation process which can be performed through Wet Chemical or Dry plasma [54].

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Different methods for covalent functionalization of CNTs generate chemical bonds for Carbon atoms via chemical reactions followed by further conjugation of hydrophilic organic molecules or polymers and make the solubility of CTNs better. In non covalent wrapping, the coating of amphiphilic molecules, hydrophobic nature of CNTs is used. Covalent bonding causes less structural damage to CNTs as compared to non covalent wrapping. But non covalent wrapping is easy to perform as compared to covalent bonding. Hydrophobic portion of the surfactant is adsorbed onto the CNT surface while hydrophilic portion interacts with the polar solvent molecules [55-56]. Surfactant can be ionic [16] and non-ionic [57)]. CNTs can be effectively functionalized by cationic surfactant [58] in organic or aqueous [59-60].

CNTs are characterized by different characterization techniques like RAMAN Spectroscopy [61], Thin Film near Infrared Spectroscopy [62-63], Transmission Electron Microscopy (TEM), Scanning Electron Miroscopy (SEM), Thermogravimetric Analysis [64-65].

IV. Applications Of CNTs

The last few years have witnessed the discovery, development and large scale manufacturing of CNTs.

CNTs have remain a widespread topic of research among the researchers due to many unique properties of them which make CNTs suitable for many application in the field of Nanomedicine, Pharmaceuticals, Electronics, Drug Delivery, Therapeutic applications, Aerospace application and others as shown in figure 4. In Biosensing applications, CNT based Field Effect Transistors are used to detect low concentration analyte [66-67] and CNT based electrochemical sensors [68-70] are used for redox reaction through direct contact with the biological systems.

In Tissue Engineering Applications, CNTs are used to make scaffolds due to their mechanical strength [7], chemical stability [8] and biological inertness [71]. CNTs functionalized with polymers and sugars are used for cell adhesion and growth [72]. Applications of CNTs in the area of drug delivery include receptor functionalized CNTs as targeted vehicle, in which drugs are delivered after the insertion of CNT in cell membrane [73]. CNTs can be used to deliver drug to a single target cell [74]. CNTs encapsulated with drug and disintegration of CNT cap for delivery is another way of drug delivery with the help of CNTs [75-77]. Change in pH and temperature can be used as triggers for unloading the drugs from CNTs [78].

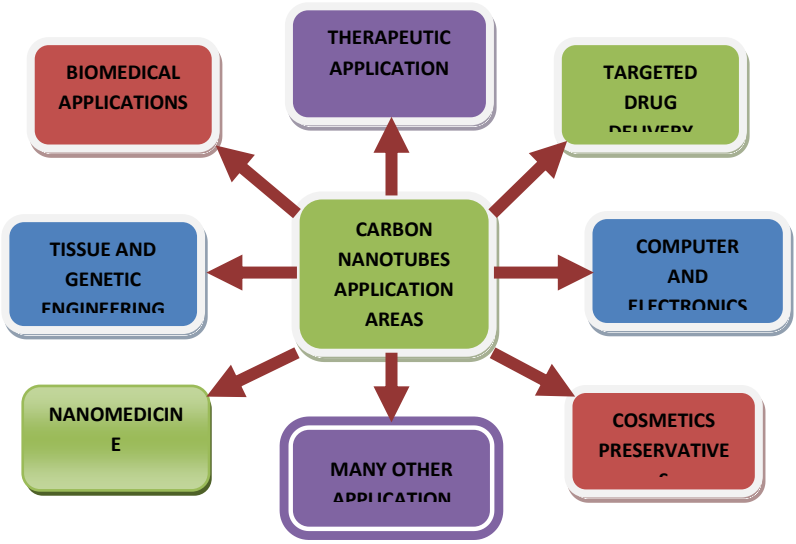

Fig. 4: Application Areas of Carbon Nanotubes

Optical stimulation is also used as trigger mechanism to release the drug in the situations where biological structures do not absorb the drugs [79]. In therapeutic applications, CNTs can be functionalized by folic acid to induce vibration in the cancer cell in order to destroy cancer cell using Infrared Radiations and acts as Cellular Bombs [18]. CNTs having Magnetic Material can be used as Hyperthermia Agents and then applied for destroying the selected Cancerous cells [80].

Detection of glucose is one of the many indications of human health due to the relation of many diseases to blood glucose concentration. CNTs based nano-hybrids and biosensors like MWCNTs/AuNPs/Ionic Liquids [81], Polyaniline coated Fe_3O_4 MWCNTs and palladium SWCNT have been explored for glucose sensing. CNTs show significance change in the conductance due to the presence to some biomolecules and protein [82-84].

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CNT nanoelectrode assemblies have been used for selective and sensitive detection of glucose [85]. CNTs based sensors have shown superior performance as compared to conventional electrodes to detect Dopamine [86], Hydrogen Peroxide [87]. Sensors based on CNTs with β Nicotinamide Adenine Dinucleotide (NADH) have been studied [88]. SWCNTs have been used to decrease tip size of Atomic Force Microscopy, thereby increasing resolution to study surface topology at the nanoscale and have been used to study antibodies [89-90] and DNA Analysis [91]. CNTs can be used as a substrate for neuronal growth [92]. Array of CNTs combined with Information technology can be used for identification of genes and genetic materials for drug discovery [93]. CNTs filled with calcium can act as bone substitute due to their high tensile strength [94].

V. Toxicity Issues With Use Of CNTs

For effective work of a material to perform the required task after incorporation into the body, it should be biocompatible. It should perform its task and should not produce undesired effects in the host.

The toxicity of CNTs is a matter of concern before its use in many application areas. Carbon based materials like pyrolytic Carbon has been used since the long time for artificial implants. There have been different studies on the toxicity issues of CNTs for many applications. It has been found through the studies conducted in the recent years that CNTs exhibit some degree of toxicity issues. The toxicity in the unrefined CNTs may be attributed to the impurities like catalytic metals and other carbonaceous particles. Since CNTs are graphite, so they are bio-persistent [95], being nanoparticulate so more toxicity is associated with them as compared to large sized particles [96].

Toxicity is dependent on Surface characteristics of CNTs also. The results obtained after the administrations of CNTs in biological systems are still controversial. Only few studies have been published to demonstrate the bio distribution of CNTs. These are In Vitro and In Vivo studies and effects of CNTs toxicity on human, animals and environment. The toxicity issues resulted from different studies have been summarized in the tables 2 and 3.

Table 2:
In-Vitro Toxicity studies of CNTs

Type of Carbon nanotube	Animal used for study	Cell	Dosage	Duration of Exposure	Toxicity	Reference Paper/Article
Pristine and Oxidised MWCNT	Human	T Lymphocytes	40 µg/ml	5 days	Cells approximately damaged	[97]
MWCNTs	Rat	Erthyrocytes and Thrombocytes	5-25 µg/ml	1 day	No effect on cell viability. After increment in dosage to 50, cell viability decreases	[98]
MWCNTs	Human	Pneumocytes	50 µg/ml	2 days	10 % Cell death	[99]
Purified and Unpurified MWCNTs	Human	Monocytes derived macrophage cell	20 µg/ml	4 days	57% viable cell resulted	[100]
Functionalised SWCNTs to variable degree	Human	Dermal Fibroblasts	3-30000 µg/ml	2 days	Cytotoxicity becomes less with increase in Functionalisation	[101]
SWCNTs	Human	A549 cells(ATCC, CCL-185) human lung carcinoma epithelial cell line	800 µg/ml	1 day	Very low acute toxicity; Greater toxicity in the absence of serum;	[102]
SWCNTs Functionalized with aryl sulfonate groups;	Human	Human epithelial-like Hela cells	1 mg/ml	4 days	Adsorption of essential micronutrients from cell culture medium results in the toxicity (Cell viability,	[103]

					DNA Damage and apoptosis).	
SWCNTs	Human	Human embryo kidney cells (HEK293 cells)	25 µg/ml	1-5 days	Inhibit cells growth; death of cells within 24h (250 µg/ml) only slight influence(less than 1 µg/ml SWCNTs in the medium)	[104]
Refined and Unrefined SWCNTs	Human	Human dermis Fibroblasts cells	25-100 µg/ml	1-5 days	Refined SWCNTs are more toxic than its unrefined counterpart	[105]
SWCNT in KGM basal medium	Human	Human Epidermal keratinocytes (HaCaT)	0.2-0.1 mg/ml	1-48 hours	Time dependent release of the pro-inflammatory cytokine,	[106]
SWCNTs & MWCNTs	Guniea Pig	Alveolar Macrophage	.20-250 µg/ml	6 hours	SWNTs impaired the phagocytosis at a low dose as compared to MWNts at high dose	[107]
Purified SWCNTs	Human	Macrophage	Different concentrations	6-48 hours	NO was not produced and low toxicity resulted	[108]
SWCNTs	Human	Bronchial epithelial and human fibroblasts	20-80 µg/ml	6 hours	Nitric oxide production increased and cell layers were detached and shows cytotoxic response	[109]

Table 3
In-Vivo Toxicity studies of CNTs

Type of CNT used	Animal under study	Route of Administration	Dosage	Duration of Exposure	Toxicity	Reference Paper/Article
Magnetic MWCNTs	BALB nu Male	Subcutaneously	0.94 mg	15 days	No agglomerate, no abnormalities resulted	[110]
SWCNTs	Nude mice	Intravenously	151 mg	120 days	No toxicity	[111]
SW Nano Horns	Wistar Rats	Peroraly	2g/Kg	14 days	No abnormaility	[112]
SWNT	CD ICR mice male	Intravenously	40-1000 µg	90 days	Serum Biological changes	[113]
MWCNTs	SD rats female	Intratracheally	0.5-5 mg	60 days	Granulomas formation in Bronchial Lumen and alveolitis in surrounding tissues	[114]
SWCNTs	Rat	Intratracheal	5 mg/Kg	24 hours	Lungs Granulomas formation and mortality due to blockage of large airways	[115]
SWCNTs	mice	Inhalation and pharyngeal	5-20 µg	5 hours/day for 4 days	initial inflammatory response, granulomas, fibrosis, and decreased rates of respiration, and activation of a gene that produces lung cancer	[116]

VI. Limitations Of CNTS

Despite several unique and exceptional properties, CNTs have some limitations like lack of solubility in several solvents, impurity level of CNTs, maintaining high quality production. Large scale growth of CNTs over the surface is difficult to control. Apart from these limitations, toxicity is the largest limitation in the use of CNTs for various applications.

VII. Conclusions

In this review, we made an effort to provide the overview of synthesis, properties, purification methods, applications and the most important about the toxicity issues of CNTs. Different studies related to toxicity issues of CNTs has been highlighted. CNTs have proved to be a safer and more effective alternative to drug delivery methods, therapeutic purposes for carrying drugs and vaccines, tissue engineering and many applications, but some problems in the path for the commercialization of CNTs in the medicine and biology occurs due to cyto-toxicity issues of CNTs. Although, functionalization of CNTs have solved the issue of toxicity to some extent, but further studies and researches are necessary to find out the related toxicity issues and methods to control those issues. With increasing research in the field of Nanotechnology, CNTs could prove a boon for different industries provided their toxicity issue is solved.

VIII. Future Scopes

The main obstacle in the path of commercialization of CNTs for various applications is the toxicity in human, animals and environment. The depth of toxicity of CNTs is still not known completely. Future scope of research in the field of CNTs is determining the parameter affecting the toxicity and also the adverse effects of using the CNTs in various application areas and to find the methods to counteract the toxicity issues of CNTs through different In-Vivo and In-Vitro studies. Once the toxicity of CNTs is ended by any method, CNT could prove a wonder material in many application areas.

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