Chapter

Cytotoxicity Evaluation of Carbon Nanotubes for Biomedical and Tissue Engineering Applications

Narsimha Mamidi

Abstract

Carbon nanotubes (CNTs) are one of the most studied allotropes of carbon nanomaterials. The exceptional chemical and physical properties of CNTs make them potential candidates for several applications such as electrical, gene therapy, biosensors, and drug delivery applications. However, the toxicity of CNTs has been a major concern for their use in tissue engineering and biomedical applications. In this chapter, we present an overview of carbon nanotubes in biomedical and tissue engineering applications. We discussed various factors including impurities, length, agglomeration, and size of CNTs that cause toxicity of CNTs. Further, other toxic methods are also examined, and possible ways to overcome these challenges have been discussed.

Keywords: carbon nanotubes (CNTs), biomedical and tissue engineering, cytotoxicity, agglomeration, size, length

1. Introduction

Amalgamation of nanotechnology with biomedical and tissue engineering offers an admirable opportunity for developing great nanomaterials that would significantly improve treatment and diagnosis of diseases [1]. It is also anticipated that the development and use of nanomaterials at industrial scale would be the driving forces for the emerging industries and economies. Carbon nanotubes are novel carbon nanomaterials, and they have attracted a wide range of applications due to their inimitable properties. Particularly, CNTs have the potential to modernize biomedical and tissue engineering because of their impeccable chemical, electrical, thermal, structural, and mechanical properties, which have made them as an area of great research interest [1]. CNTs exhibit semiconducting, metallic, and superconducting electron transport properties, and they also display high elastic modulus compared to all other nanomaterials. Numerous research studies have been conducted on the applications of CNTs in the biomedical and tissue engineering fields. Most specifically, CNTs have been used in a variety of applications such as diagnostic tools, biosensors, nanofluidic systems, radiation oncology, quantum dots, drug delivery, nanorobots, and nanosensors [2–4]. However, low dispersibility, toxicity, and solubility of unfunctionalized multi-walled carbon nanotubes (MWCNTs) have been the main concern for their potential use in biomedical and tissue engineering applications. Therefore, the interaction of CNTs with biological systems is very complex and unpredictable. Biological properties, performance, and behavior of CNTs have

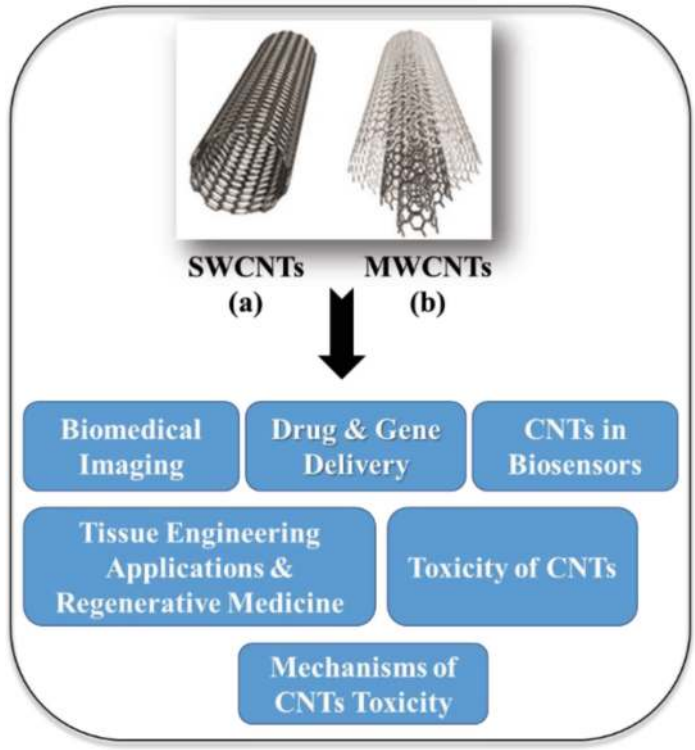

Figure 1.
(a and b) Structures of carbon nanotubes and schematic representation of the issues addressed in this chapter.

to be thoroughly understood. It is reported that CNTs exhibit different levels of toxicity based on their manufacturing method, shape, surface-area-to-volume ratio, concentration, size, composition, functional groups, applied dosages, and extent of oxidation [5–8]. In addition, CNTs have the ability to damage the cell membrane and DNA due to their high hydrophobicity. CNTs can also extend their toxicity through protein synthesis, oxidative stress, mitochondrial activities, modifications, apoptosis, necrosis, as well as intramolecular metabolic paths [8].

This chapter discusses the critical roles of CNTs in biomedical and tissue engineering applications. It explains synthetic methods and recent advances in the application of CNTs in bioimaging, drug delivery, biosensing, and tissue engineering applications. In the end, this chapter also encapsulates the surface chemistry, shape, size, and the route of synthesis of CNTs which can affect their toxicity levels (**Figure 1**). At the end the mechanism responsible for CNS toxicity and potential remedies to overcome their drawbacks has also been discussed.

2. Synthesis of carbon nanotubes

CNTs exist approximately 1 nm in diameter and 1–100 μm in length and formed by cylinder-shaped graphite layers [8]. CNTs are mainly divided into two types: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). SWCNTs are formed by a single layer of graphene, and MWCNTs are formed by multiple layers of graphene (**Figure 1a** and **b**). In addition, a variety of nanomaterials are reported including fullerenes, nanohorns, carbon nanobuds, carbon nanoporous, carbon nanopeapods, and carbon nano-onions [2, 8, 9]. Mainly three different techniques are used to fabricate CNTs such as the arc discharge technique, the chemical vapor deposition (CVD), and the laser ablation technique [2].

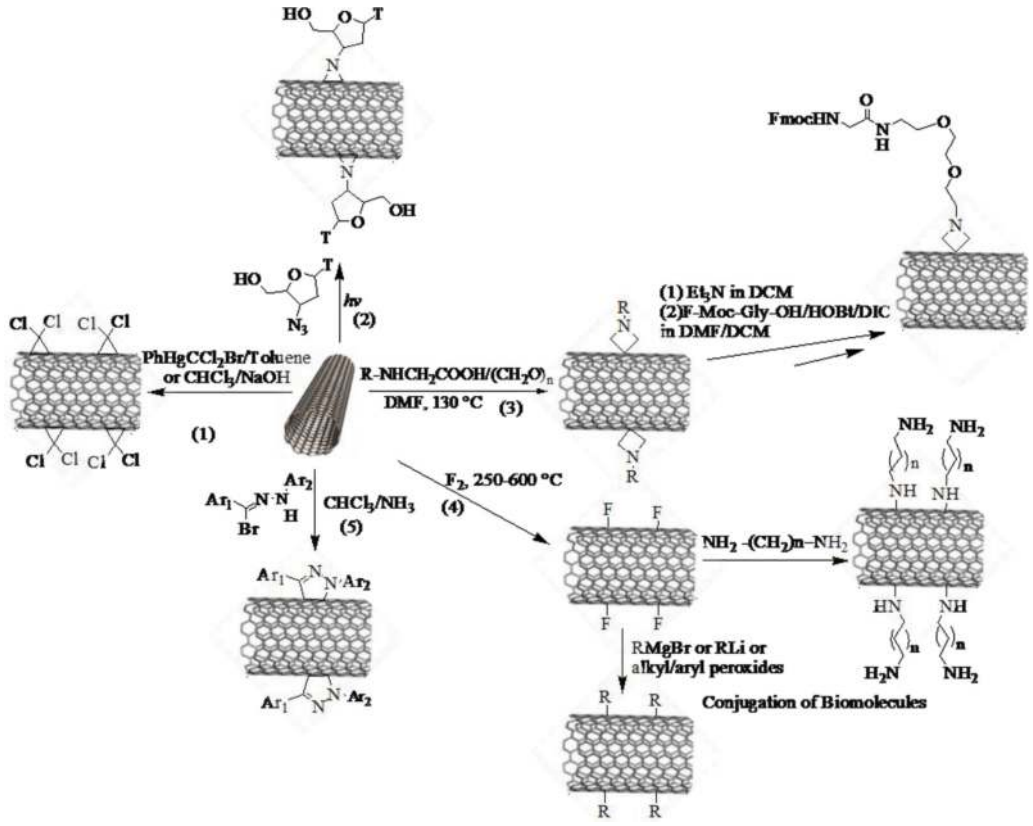

Figure 2.
Different covalent functionalization strategies of CNTs: (1) cycloaddition with dichlorocarbene, (2) photoinduced production of reactive nitrenes, (3) 1,3 dipolar cycloaddition of azomethine ylides, (4) fluorination of nanotubes and defunctionalization followed by derivatization reactions, and (5) 1,3 dipolar cycloaddition of nitrile amines.

First time in 1991, Ijima used the arc discharge technique to fabricate MWCNTs; later, metal catalyst was used in the same technique to provide the first SWCNTs [10, 11]. After that, the laser ablation technique was used by Thess to produce aligned SWCNTs [12]. Yacamán developed the catalytic growth of MWCNTs using CVD technique [12]. Further, cobalt (Co) catalyzed low-pressure chemical vapor deposition which was used on silicon oxide as a silicon substrate to produce Co-MWCNTs films and used as sensors to detect carbon dioxide (CO_2) [13].

Pristine or unfunctionalized CNTs are highly hydrophobic and insoluble in an aqueous solution and organic solvents that cause great limitation for their tissue engineering and biomedical applications. Therefore, it is important to functionalize CNTs to make them hydrophilic and amalgamate into numerous organic solvents and biological systems [4]. Three main methods have been reported to modify CNT structures, namely, (a) the covalent functionalization (**Figure 2**), (b) the noncovalent adsorption of many biomolecules, and (c) the endohedral filling of their inner empty cavity [14].

3. CNTs in biomedical applications

CNTs show numerous unique properties that make them promising carbon nanomaterials for a wide range of biomedical applications. For this, the surface of CNTs can be functionalized with suitably biocompatible moieties. These moieties can interact with cell membrane receptors that can guide their cell internalization. These receptor-mediated methods can support in drug loading, inflammation, and minimizing toxicity [15].

3.1 Drug and gene delivery

CNTs have been used in several drug delivery systems for the treatment of many diseases. Anticancer drug-loaded CNTs have attracted much attention mainly in two strategies such as selective targeting and controlled release of drugs [15, 16]. It is reported that CNT-based anticancer drug was developed that could control the multidrug-resistant cancer cells without affecting cell cycles and proliferation [17]. Further, an anticancer drug, tamoxifen-loaded peptide-modified SWCNT, was developed that showed a high level of antitumor effect and proficient tumor targeting. SWCNTs successfully delivered acetylcholine (Ach) into the mice brain to control Alzheimer's disease [18]. Anticancer drug-appended MWCNTs have been used for effective breast cancer treatment and intranuclear drug delivery [19].

In addition, functionalized CNTs have shown other advantages through covalent conjugation methods. For instance, functionalized SWCNTs with ester and amide enabled to sustain drug delivery and improved the solubility in aqueous and organic solvents [20]. Liposomes were covalently attached to MWCNTs that facilitated the delivery of large doses of the drug [21]. Peptide-based MWCNTs were also developed to deliver therapeutics into the target mitochondria to treat genetic disorders [22]. Some other functionalized MWCNTs have been used in the central nerve system (CNS) through neural tissue cell interactions [23]. On the other hand, noncovalent functionalized CNTs have been used in a wide range of medical applications that release the drugs in tumor environments at low pH and kill the cancer cells [24]. Polyethyleneimines bearing noncovalent functionalized SWCNTs were developed for effective gene delivery [25]. In addition, biomolecules including genes, DNA and siRNA, also can be loaded into CNTs. CNTs were functionalized with poly(lactic-co-glycolic) (PLGA) to deliver proapoptotic protein caspase-3 (CP3) into osteocarcinoma cells [26]. CNTs have also been used in in vivo gene silencing without any toxicity and induction of immune response. Polyethylenimine-functionalized CNTs successfully delivered siRNA into the HeLa-S3 cells [27].

3.2 Biomedical imaging

Biomedical imaging is a powerful tool that can provide high-resolution imaging of cells, organs, tissues, and even the complete body of animals or humans. Due to unique physicochemical properties, CNTs have been used in different biomedical imaging technologies [28]. There are three major methods such as fluorescence emission, photoacoustic imaging, and magnetic resonance imaging that can be executed on CNTs to detect the nanotubes in live cells [29]. It is reported that CNTs can be directed using external magnetic source toward a specific organ [30]. Surface modification, as well as the addition of elements to CNT structure, can provide a new perspective of the analysis of their performance. For example, by assembling different nanoparticles including quantum dots, gold nanoparticles, upconversion nanoparticles, iron oxide nanoparticles, PET imaging nanoprobes into CNTs enhancing their properties and wider of their applications [31]. Thus, biomedical imaging is a potential and easy platform to accomplish imaging of living cells in tissue engineering and biomedical engineering.

3.3 CNT-based biosensor

CNTs are highly effective sensing elements for biosensors due to their excellent electrical, tensile, and electrochemical properties; high surface area; and high exposure sensitivity to various biomolecules [32]. Specifically, their high surface-area-to-volume ratio has made them a potent tool to acquire fast biological species detection and

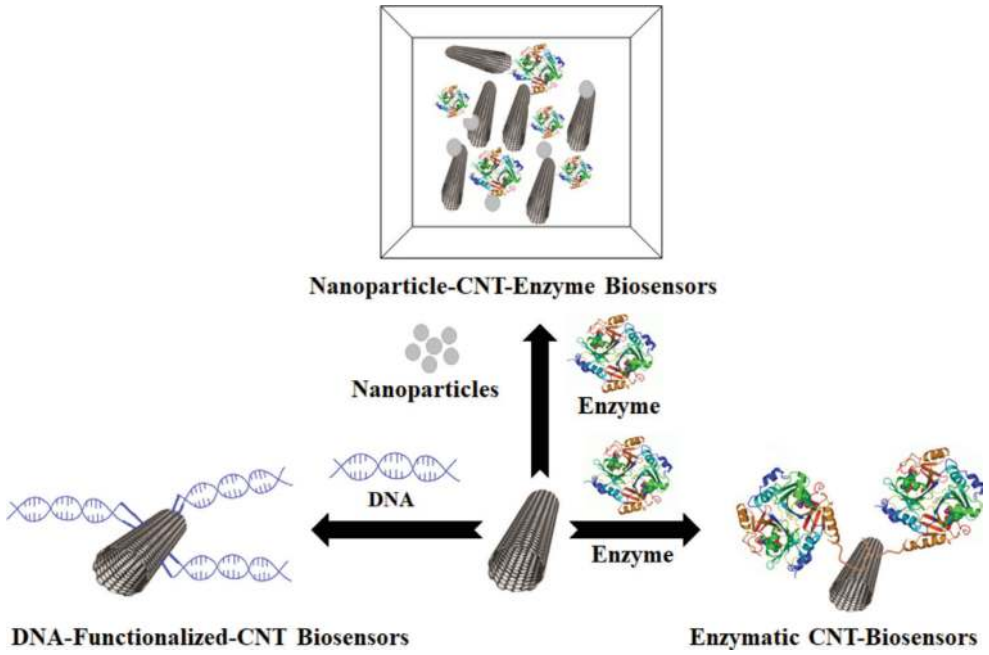

Figure 3.
Schematic illustration of CNT-based biosensors.

CNT-based biosensors which are frequently used in ultrasensitive biosensing applications [32]. In addition, enzyme biosensors are also one of the most important and commonly used biosensors (**Figure 3**). For example, durable and stable tyrosinase biosensor was developed from functionalized MWCNTs, 1-butyl-3-methylimidazolium chloride (IL), and tyrosinase (Tyr) within a dihexadecyl phosphate (DHP) film, and improved response signal was observed [33]. 3α-Hydroxysteroid dehydrogenase was incorporated into CNTs/IL/NAD+ composite electrode to develop a biosensor that detected androsterone. Further, CNT-based enzyme biosensors were also used in the detection of glucose in the blood, and it is one of the potential applications of enzyme biosensors [34]. DNA biosensors are other famous CNT-based biosensors, and they have been used in medical diagnostics, forensic science, and several other applications. Single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) is the major sensing element of DNA biosensors (**Figure 3**). It is reported that ssDNA is highly adsorptive to CNTs rather than dsDNA. For example, SWCNT-FET-based electronic DNA biosensor was developed and used in chip-on system applications [35]. Ultrasensitive DNA biosensor was developed to control DNA methyltransferase (DNA MTase) from MWCNT signal amplification and fluorescence polarization detection [36]. In addition, a glassy carbon-based sensitive DNA biosensor was fabricated to detect DNA sequencing using polydopamine (PDA), MWCNTs, and gold nanoparticles [36]. Thus, the inimitable properties of CNTs have made them a powerful tool for biosensing applications.

4. Tissue engineering applications

Tissue engineering is a new approach to fabricate artificial tissues for graft replacement and tissue models for in vitro diseases and drug discovery [37]. Maintaining proper electrical, mechanical, and biological properties of CNT-based biomaterials is a challenge in tissue engineering [38]. For example, CNTs have been used in a variety of tissue engineering applications such as enhancing electrical and mechanical properties of scaffolds, tracking of cells, sensing the cell

microenvironments, and delivery of appropriate chemical and biological agents [39]. Development of CNTs bearing scaffolds for in vitro nerve generation opened a new route for neural tissue engineering. In fact, CNT-based scaffolds have been used to improve cardiac tissue growth, bone, and neural growth [40]. In cardiac and nerve growth, SWCNTs greatly improved the electrical properties of scaffolds, whereas in bone growth, SWCNTs enhanced the attraction of calcium cations [41]. Some scaffolds were developed and used for bone formation via functionalization of MWCNTs with fibroblast growth factors [42]. In addition, MWCNT-gelatin nanofiber scaffolds were used for myoblast (C_2C_{12}) growth, and MWCNTs have improved tensile properties of the fiber scaffolds [43]. Thus, CNTs are used as a potential component for many biomaterials in tissue engineering applications.

5. Toxicity of CNTs

Many toxicological investigations of CNTs have been done both in vivo and in vitro; however, they are inconsistent to each other due to the variabilities in the type of functionalization, the synthetic method, and the dose of CNTs. In addition, a different type of cell viable indicator dye also contributed different cytotoxicity results. Some of the common indicator dyes are alamarBlue, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Coomassie Brilliant Blue, neutral blue, and water-soluble tetrazolium salt (WST-1) [44]. Several factors that contribute to the toxicity of CNTs include metal impurities, diameter, length, type of carbon nanotubes, surface functionalization, and existence of dispersant [36].

Toxicological effect of CNTs has been studied; particularly, commercial and acid-purified MWCNTs and SWCNTs have shown a significant effect on toxicity levels. This study explained that commercial CNTs showed increased reactive oxygen species (ROS) that enhanced oxidative stress and decrease mitochondrial membrane potential. In contrast, acid-purified SWCNTs with less metal impurity showed less toxicity effect [45]. However, MWCNTs exhibited some toxicity at high concentration due to metal impurities [46]. Further, iron-contaminated MWCNTs presented improved CD8+ levels and CD4+/CD8+ ratio of peripheral T cell in mice models as well as increased ROS [46].

The length of CNTs also showed a great impact on the toxicity of CNTs, and smaller CNTs are less toxic than long CNTs. Smaller CNTs can easily penetrate into cell membrane as well as the success of their cellular internalization, whereas long CNTs cause biopersistence or retaining [47]. Another study demonstrated that short CNTs were cleared from the pleural cavity, whereas long CNTs were retained [48]. Similarly, the retention of long CNTs (825 nm) has exhibited induced inflammation than small CNTs (220 nm) [48]. In addition, long MWCNTs (5–15 μm of length and 20–60 nm of diameter) showed more genotoxicity in alveolar carcinoma epithelial cells (A549) than smaller MWCNTs (1–2 μm of length and 60–100 nm of diameter) [49].

Based on the structural characteristic, carbon nanotubes are divided into SWCNTs and MWCNTs. These CNTs differ in length, structure, and chemical surface. SWCNTs have a smaller diameter (0.6–2.4 nm), whereas MWCNTs have a larger diameter (2.5–100 nm) [6]. SWCNTs have a higher surface area that helps to form a bundle of CNTs. MWCNTs have a lower tendency to form a bundle due to lower surface area and their side walls [44]. Similarly, smaller size and length of SWCNTs form less aggregation of 5–30 μm that can easily phagocytosed, whereas longer MWCNTs generate larger aggregation around 300 μm, which cannot be phagocytosed [50]. The fibrous surface of MWCNTs and SWCNTs showed different mechanisms in the plasma membrane. MWCNTs caused toxicity due to the plasma membrane damage and aberrant phagocytosis, while SWCNTs initiated oxidative damage to cells [51]. As

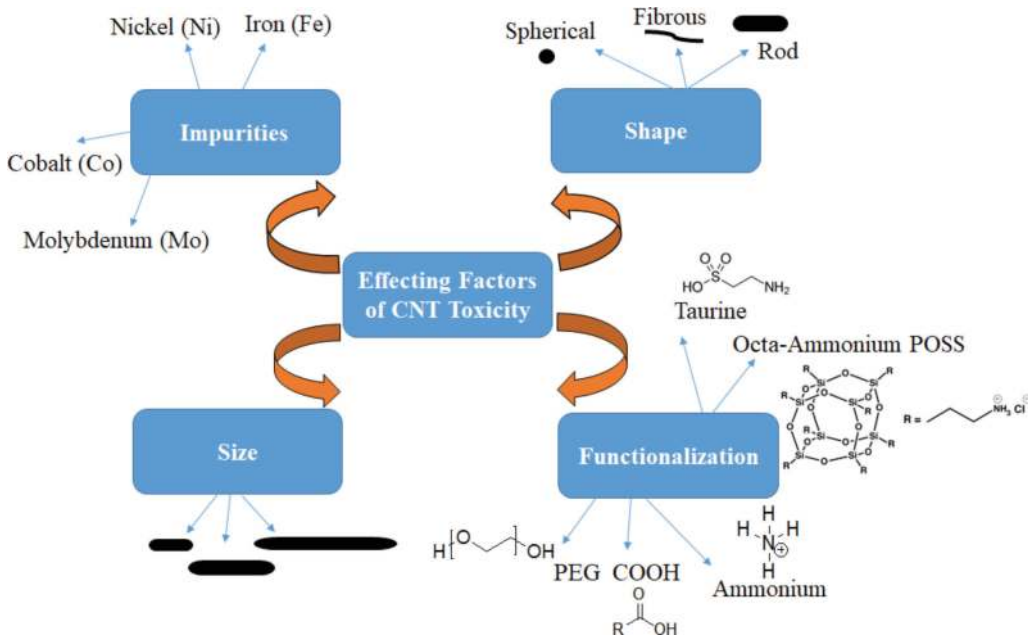

Figure 4.
Schematic representation of toxic factors of CNTs. It shows size, impurities, shape, and functionalization which are the major factors to cause CNT toxicity.

a result, based on the various characteristics (size, aggregation, and surface state) of SWCNTs and MWCNTs, they exhibit a different level of toxicity.

The surface structure of CNTs can be modified by functionalization by introducing several functional groups. The functionalization enhances solubility, biocompatibility, dispersibility, and agglomeration of CNTs. The functionalization may be either covalent bonding or noncovalent binding [52]. Functionalization enables conjugation of various groups with CNTs that help in cell receptor binding as well as cellular processing and elimination. The use of biomolecules (proteins and antibodies) in conjugation strengthens the specific binding of CNTs to targeted biomolecules. In fact, functionalized MWCNTs with 220 kDa lectin protein exhibited apoptosis and reduced toxicity in J774A macrophage [53]. On the other hand, functionalized SWCNTs-COOH showed higher toxicity in the HUVEC cell line than pristine SWCNTs [54].

The degree of toxicity of CNTs has a great influence on the type of physicochemical properties, size, shape, impurities, and functionalization of CNTs (**Figure 4**) [55]. Further, CNT-based polymer nanocomposites also developed and cytotoxicity was assessed with human cells. For instance, unfunctionalized MWCNTs were reinforced with ultrahigh molecular weight polyethylene (UMWPE), and biocompatibility was evaluated with human fibroblasts [5]. The results revealed that MWCNTs exhibited a positive influence on fibroblast cells. Therefore, several studies reported that cell membrane injury, oxidative stress, and genotoxicity are the possible mechanisms of CNT toxicity [36].

6. Conclusions

Carbon nanotubes are a new class of carbon nanomaterials that have a potential in biomedical and tissue engineering applications including drug delivery, biosensors, biomedical imaging, and artificial tissue scaffolds. In order to decrease cytotoxicity and increase biocompatibility and physicochemical properties, CNTs can be functionalized with various biomolecules either covalently or noncovalently.

Several investigations have made to study the relationship between physical and cytotoxicity properties of short-walled or multi-walled nanotubes. In vitro and in vivo biocompatibility of MWCNTs and SWCNTs have been effectively influenced by their functionalization, diameter, and length. It is also clear that the synthesis method and metal impurities of CNTs can influence the cell viability and interaction of CNTs with cells. Although many investigations have been conducted for the toxicological characterizations of CNTs in biomedical and tissue engineering, however, a complete understanding of internalization, cellular uptake, and gene expression changes linked with CNTs has still remained elusive. This understanding would be required for the future use of CNTs in biomedical and tissue engineering applications.

Acknowledgements

The authors acknowledge the financial support from the Consejo Nacional de Ciencia y Tecnología de México (CONACyT) and Tec de Monterrey, Monterrey Campus, NL, Mexico.

Conflict of interest

The authors declare no conflict of interest.

Author details

Narsimha Mamidi
School of Engineering and Science, Tecnologico de Monterrey, Nuevo León, México

*Address all correspondence to: narsimhachem06@gmail.com

References

[1] Simon J, Flahaut E, Golzio M. Overview of carbon nanotubes for biomedical applications. Materials. 2019;**12**(4):624. ISSN 1996-1944

[2] Sinha N, Yeow JT. Carbon nanotubes for biomedical applications. IEEE Transactions on NanoBioscience. 2005;**4**:180-195

[3] Beg S, Rizwan M, Sheikh AM, Hasnain MS, Anwer K, Kohli K. Advancement in carbon nanotubes: Basics, biomedical applications, and toxicity. The Journal of Pharmacy and Pharmacology. 2011;**63**:141-163

[4] Bianco A, Kostarelos K, Partidos CD, Prato M. Biomedical applications of functionalised carbon nanotubes. Chemical Communications. 2005;**5**:571-577

[5] Mamidi N, Leija HM, Diabb JM, Lopez Romo I, Hernandez D, Castrejón JV, et al. Cytotoxicity evaluation of unfunctionalized multiwall carbon nanotubes-ultrahigh molecular weight polyethylene nanocomposites. Journal of Biomedical Materials Research. Part A. 2017;**105**:3042-3049

[6] Foldvari M, Bagonluri M. Carbon nanotubes as functional excipients for nanomedicines: II. Drug delivery and biocompatibility issues. Nanomedicine. 2008;**4**:183-200

[7] Vardharajula S, Ali SZ, Tiwari PM, Eroğlu E, Vig K, Dennis VA, et al. Functionalized carbon nanotubes: Biomedical applications. International Journal of Nanomedicine. 2012;**7**:5361-5374

[8] Mehra NK, Jain K, Jain NK. Pharmaceutical and biomedical applications of surface engineered carbon nanotubes. Drug Discovery Today. 2015;**20**:750-759

[9] Sano N, Wang H, Chhowalla M, Alexandrou I, Amaratunga GAJ. Synthesis of carbon 'onions' in water. Nature. 2001;**414**:506-507. DOI: 10.1038/35107141

[10] Iijimaa S. Helical microtubules of graphitic carbon. Nature. 1991;**354**:56-58

[11] Iijima S, Ichihashi T. Single-shell carbon nanotubes of 1-nm diameter. Nature. 1993;**363**:603-605

[12] José-Yacamán M, Miki-Yoshida M, Rendon L, Santiesteban J. Catalytic growth of carbon microtubules with fullerene structure. Applied Physics Letters. 1993;**62**:202-204

[13] Khan ZH, Ansari MS, Salah NA, Memic A, Habib S, Shahawi M. COBALT catalyzed-multi-walled carbon nanotubes film sensor for carbon mono-oxide gas. Digest Journal of Nanomaterials and Biostructures. 2011;**6**:1947-1956

[14] Tasis D, Tagmatarchis N, Bianco A, Prato M. Chemistry of carbon nanotubes. Chemical Reviews. 2006;**106**:1105-1136

[15] Chen J, Chen S, Zhao X, Kuznetsova LV, Wong SS, Ojima I. Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery. Journal of the American Chemical Society. 2008;**130**:16778-16785

[16] Zhang X, Meng L, Lu Q, Fei Z, Dyson PJ. Targeted delivery and controlled release of doxorubicin to cancer cells using modified single wall carbon nanotubes. Biomaterials. 2009;**30**:6041-6047

[17] Cheng J, Meziani MJ, Sun YP, Cheng SH. Poly(ethylene glycol)-conjugated multi-walled carbon

nanotubes as an efficient drug carrier for overcoming multidrug resistance. Toxicology and Applied Pharmacology. 2011;**250**:184-193

[18] Yang Z, Zhang Y, Yang Y, Sun L, Han D, Li H, et al. Pharmacological and toxicological target organelles and safe use of single-walled carbon nanotubes as drug carriers in treating Alzheimer disease. Nanomedicine. 2010;**6**:427-441

[19] Das M, Singh RP, Datir SR, Jain S. Intranuclear drug delivery and effective in vivo cancer therapy via estradiol-PEG appended multiwalled carbon nanotubes. Molecular Pharmaceutics. 2013;**10**:3404-3416

[20] Khazaei A, Rad MN, Borazjani MK. Organic functionalization of single-walled carbon nanotubes (SWCNTs) with some chemotherapeutic agents as a potential method for drug delivery. International Journal of Nanomedicine. 2010;**5**:639-645

[21] Karchemski F, Zucker D, Barenholz Y, Regev O. Carbon nanotubes-liposomes conjugate as a platform for drug delivery into cells. Journal of Controlled Release. 2012;**160**:339-345

[22] Battigelli A, Russier J, Venturelli E, Fabbro C, Petronilli V, Bernardi P, et al. Peptide-based carbon nanotubes for mitochondrial targeting. Nanoscale. 2013;**5**:9110-9117

[23] Bardi G, Nunes A, Gherardini L, Bates K, Al-Jamal KT, Gaillard C, et al. Functionalized carbon nanotubes in the brain: Cellular internalization and neuroinflammatory responses. PLoS One. 2013;**8**:e80964

[24] Petrov P, Stassin F, Pagnoulle C, Jerome R. Noncovalent functionalization of multi-walled carbon nanotubes by pyrene containing polymers. Chemical Communications. 2003;**0**:2904-2905

[25] Behnam B, Shier WT, Nia AH, Abnous K, Ramezani M. Non-covalent functionalization of single-walled carbon nanotubes with modified polyethyleneimines for efficient gene delivery. International Journal of Pharmaceutics. 2013;**454**:204-215

[26] Cheng Q, Blais MO, Harris G, Jabbarzadeh E. PLGA carbon nanotube conjugates for intercellular delivery of caspase-3 into osteosarcoma cells. PLoS One. 2013;**8**:e81947

[27] Huang YP, Lin IJ, Chen CC, Hsu YC, Chang CC, Lee MJ. Delivery of small interfering RNAs in human cervical cancer cells by polyethylenimine-functionalized carbon nanotubes. Nanoscale Research Letters. 2013;**8**:267

[28] Gong H, Peng R, Liu Z. Carbon nanotubes for biomedical imaging: The recent advances. Advanced Drug Delivery Reviews. 2013;**65**:1951-1963

[29] Tong L, Liu Y, Dolash BD, Jung Y, Slipchenko MN, Bergstrom DE, et al. Label-free imaging of semiconducting and metallic carbon nanotubes in cells and mice using transient absorption microscopy. Nature Nanotechnology. 2012;**7**:56-61

[30] De La Zerda A, Zavaleta C, Keren S, Vaithilingam S, Bodapati S, Liu Z, et al. Carbon nanotubes as photoacoustic molecular imaging agents in living mice. Nature Nanotechnology. 2008;**3**:557-562

[31] Li J, Chang X, Chen X, Gu Z, Zhao F, Chai Z, et al. Toxicity of inorganic nanomaterials in biomedical imaging. Biotechnology Advances. 2014;**32**:727-743

[32] Yang N, Chen X, Ren T, Zhang P, Yang D. Carbon nanotube based biosensors. Sensors and Actuators, B: Chemical. 2015;**207**:690-715

[33] Vicentini FC, Janegitz BC, Brett CM, Fatibello-Filho O. Tyrosinase

biosensor based on a glassy carbon electrode modified with multi-walled carbon nanotubes and 1-butyl-3-methylimidazolium chloride within a dihexadecyl phosphate film. Sensors and Actuators, B: Chemical. 2013;**188**:1101-1108

[34] Pourasl AH, Ahmadi MT, Rahmani M, Chin HC, Lim CS, Ismail R, et al. Analytical modeling of glucose biosensors based on carbon nanotubes. Nanoscale Research Letters. 2014;**9**:33

[35] Tang X, Bansaruntip S, Nakayama N, Yenilmez E, Chang YL, Wang Q. Carbon nanotube DNA sensor and sensing mechanism. Nano Letters. 2006;**6**:1632-1636

[36] Alshehri R, Ilyas AM, Hasan A, Arnaout A, Ahmed F, Memic A. Carbon nanotubes in biomedical applications: Factors, mechanisms, and remedies of toxicity. Journal of Medicinal Chemistry. 2016;**59**:8149-8167

[37] Hasan A, Memic A, Annabi N, Hossain M, Paul A, Dokmeci MR, et al. Electrospun scaffolds for tissue engineering of vascular grafts. Acta Biomaterialia. 2014;**10**:11-25

[38] Hasan A, Paul A, Vrana NE, Zhao X, Memic A, Hwang Y-S, et al. Microfluidic techniques for development of 3D vascularized tissue. Biomaterials. 2014;**35**:7308-7325

[39] Memic A, Alhadrami HA, Hussain MA, Aldhahri M, Al Nowaiser F, Al-Hazmi F, et al. Hydrogels 2.0: Improved properties with nanomaterial composites for biomedical applications. Biomedical Materials. 2016;**11**:014104

[40] Jin GZ, Kim M, Shin US, Kim HW. Neurite outgrowth of dorsal root ganglia neurons is enhanced on aligned nanofibrous biopolymer scaffold with carbon nanotube coating. Neuroscience Letters. 2011;**501**:10-14

[41] Zhao B, Hu H, Mandal SK, Haddon RC. A bone mimic based on the self-assembly of hydroxyapatite on chemically functionalized single-walled carbon nanotubes. Chemistry of Materials. 2005;**17**:3235-3241

[42] Hirata E, Menard-Moyon C, Venturelli E, Takita H, Watari F, Bianco A, et al. Carbon nanotubes functionalized with fibroblast growth factor accelerate proliferation of bone marrow-derived stromal cells and bone formation. Nanotechnology. 2013;**24**:435101

[43] Ostrovidov S, Shi X, Zhang L, Liang X, Kim SB, Fujie T, et al. Myotube formation on gelatin nanofibers-multi-walled carbon nanotubes hybrid scaffolds. Biomaterials. 2014;**35**:6268-6277

[44] Cui HF, Vashist SK, Al-Rubeaan K, Luong JH, Sheu FS. Interfacing carbon nanotubes with living mammalian cells and cytotoxicity issues. Chemical Research in Toxicology. 2010;**23**:1131-1147

[45] Vittorio O, Raffa V, Cuschieri A. Influence of purity and surface oxidation on cytotoxicity of multiwalled carbon nanotubes with human neuroblastoma cells. Nanomedicine. 2009;**5**:424-431

[46] Koyama S, Kim YA, Hayashi T, Takeuchi K, Fujii C, Kuroiwa N, et al. In vivo immunological toxicity in mice of carbon nanotubes with impurities. Carbon. 2009;**47**:1365-1372

[47] Raffa V, Ciofani G, Nitodas S, Karachalios T, D'Alessandro D, Masini M, et al. Can the properties of carbon nanotubes influence their internalization by living cells? Carbon. 2008;**46**:1600-1610

[48] Murphy FA, Poland CA, Duffin R, Al-Jamal KT, Ali-Boucetta H, Nunes A, et al. Length-dependent retention of

carbon nanotubes in the pleural space of mice initiates sustained inflammation and progressive fibrosis on the parietal pleura. The American Journal of Pathology. 2011;**178**:2587-2600

[49] Kolosnjaj-Tabi J, Hartman KB, Boudjemaa S, Ananta JS, Morgant G, Szwarc H, et al. In vivo behavior of large doses of ultrashort and full-length single-walled carbon nanotubes after oral and intraperitoneal administration to Swiss mice. ACS Nano. 2010;**4**:1481-1492

[50] Fraczek A, Menaszek E, Paluszkiewicz C, Blazewicz M. Comparative in vivo biocompatibility study of single- and multi-wall carbon nanotubes. Acta Biomaterialia. 2008;**4**:1593-1602

[51] Di Giorgio ML, Di Bucchianico S, Ragnelli AM, Aimola P, Santucci S, Poma A. Effects of single and multi-walled carbon nanotubes on macrophages: Cyto and genotoxicity and electron microscopy. Mutation Research, Genetic Toxicology and Environmental Mutagenesis. 2011;**722**:20-31

[52] Coccini T, Roda E, Sarigiannis DA, Mustarelli P, Quartarone E, Profumo A, et al. Effects of water-soluble functionalized multi-walled carbon nanotubes examined by different cytotoxicity methods in human astrocyte D384 and lung A549 cells. Toxicology. 2010;**269**:41-53

[53] Montes-Fonseca SL, Orrantia-Borunda E, Aguilar-Elguezabal A, Gonzalez Horta C, Talamas-Rohana P, Sanchez-Ramirez B. Cytotoxicity of functionalized carbon nanotubes in J774A macrophages. Nanomedicine. 2012;**8**:853-859

[54] Gutierrez-Praena D, Pichardo S, Sanchez E, Grilo A, Camean AM, Jos A. Influence of carboxylic acid functionalization on the cytotoxic effects induced by single wall carbon nanotubes on human endothelial cells (HUVEC). Toxicology In Vitro. 2011;**25**:1883-1888

[55] Madani SY, Mandel A, Seifalian AM. A concise review of carbon nanotube's toxicology. Nanotechnology Reviews. 2013;**4**:21521