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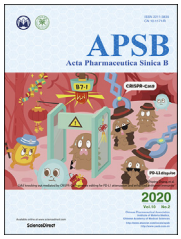

REVIEW

Biomimetic carbon nanotubes for neurological disease therapeutics as inherent medication

Chenyang Xiang[a], Yuxuan Zhang[b], Weisheng Guo[a,*], Xing-Jie Liang[a,b,*]

[a]*Translational Medicine Center, Key Laboratory of Molecular Target & Clinical Pharmacology and the State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & the Second Affiliated Hospital, Guangzhou Medical University, Guangzhou 510260, China*
[b]*CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Beijing 100190, China*

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KEY WORDS

Carbon nanotubes;
Nervous system diseases;
Drug delivery;
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Toxicity

Abstract Nowadays, nanotechnology is revolutionizing the approaches to different fields from manufacture to health. Carbon nanotubes (CNTs) as promising candidates in nanomedicine have great potentials in developing novel entities for central nervous system pathologies, due to their excellent physicochemical properties and ability to interface with neurons and neuronal circuits. However, most of the studies mainly focused on the drug delivery and bioimaging applications of CNTs, while neglect their application prospects as therapeutic drugs themselves. At present, the relevant reviews are not available yet. Herein we summarized the latest advances on the biomedical and therapeutic applications of CNTs *in vitro* and *in vivo* for neurological diseases treatments as inherent therapeutic drugs. The biological mechanisms of CNTs-mediated bio-medical effects and potential toxicity of CNTs were also intensely discussed. It is expected that CNTs will exploit further neurological applications on disease therapy in the near future.

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; aSWCNTs, aggregated SWCNTs; BBB, blood–brain barrier; CNS, central nervous system; CNT-N, nitrogen-doped carbon nanotubes; CNTs, carbon nanotubes; CpG, oligodeoxynucleotides; DTPA, diethylentriaminepentaacetic; EBs, embryoid bodies; EDC·HCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; *f*-CNTs, functionalized carbon nanotubes; GO, graphene oxide; HD, Huntington's disease; hNSCs, human neural stem cells; MCAO, middle cerebral artery occlusion; METH, methamphetamine; MPO, myeloperoxidase; MWCNTs, multi-walled carbon nanotubes; MWCNTTs, multi-walled nanotube towers; ND, nanodiamond; NHS, *N*-hydroxysuccinimide; NR, nanorod; NSCs, neural stem cells; PBEC, porcine brain endothelial cells; PCL, polycaprolactone; PD, Parkinson's disease; PEG, polyethylene-glycol; PET, position emission tomography; PMo11V, tetrabutylammonium salt of phosphovanadomolybdate; POCs, polycyclic organic compounds; PPy/SWCNT, polypyrrole/single-walled carbon nanotube; RES, reticuloendothelial system; siRNA, small interfering RNA; SWCNTP, single-walled nanotube paper; SWCNTs, single-walled carbon nanotubes; TLR9, the toll-like receptor-9; TMZ, temozolomide.

*Corresponding authors.
E-mail addresses: tjguoweisheng@126.com (Weisheng Guo), liangxj@nanoctr.cn (Xing-Jie Liang).

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1. Introduction

Nowadays, nanotechnology is revolutionizing the approaches in different fields from manufacture to health. Nanomaterials have been intensely attractive with the speedy progress in nanotechnology, due to their unique physico-chemical properties, such as high reactivity, quantum effects, small size effect, and large surface area. Nanomaterials with distinct biological activities have been increasingly applied in various biomedical areas, while the nanoscience is rapidly developing as one of the three pillars of the world science. Nanotechnology has made material progress in interdisciplinary settings, particularly in those combining nanomaterials in biological and biomedical applications. For example, with numerous efforts on investigating cutting-edge brain-specific therapies, new research fields have been established to tackle the complex central nervous system (CNS) diseases as well as to perform neuroengineering.

Carbon nanotubes (CNTs), first described by Iijima[1], are a kind of emerging nanomaterials that has attracted great scientific interests in the last decade due to their intrinsic mechanical, electronic, and physico-chemical properties[2]. CNTs get great attention all over the world in recent years[3−5]. CNTs can be classified as multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs) depending on the number of inner layers viewed as rolled up graphene sheets[1]. The diameter of CNTs can range from 0.4 to 2 nm for SWCNTs while 1.4−100 nm for MWCNTs. However, the length of CNTs varies and can reach several micrometers[6]. The high aspect ratio results in a large surface area, allowing a high degree of multiple functionalization with biocompatible functional groups[7,8]. The biological applications of CNTs are restricted with their initial hydrophobic surface that prone to severe aggregations upon exposure to aqueous solutions. Functionalization of CNTs is thus urgently required to harness this problem in biomedical applications. Chemical functionalization is one of the main approaches used to increase dispersibility, reduce toxicity, and impart desired characteristics on CNTs in biological environments[9]. Upon surface modification of CNTs with targeting ligands and drug molecules, the obtained functionalized CNTs (*f*-CNTs) are less toxic[10] and immunogenic[11,12] and show great superiorities as drug delivery systems capable of high drug payload and specific delivery into 3 living systems[13]. With their distinct physical-chemical properties, CNTs have been widely employed across many scientific fields, including composite materials[14,15], nanoelectronics[16], biosensing[17], bioimaging[8,18,19], and drug delivery[20,21]. Benefiting from their relatively low weight and large surface area, CNTs can be developed as carriers of drugs, cellular or intracellular component targeting moieties, as well as contrast agents. Due to the unique physiochemical properties and novel tubular structures, CNTs thus have emerged as promising options particularly for tracking, CNS diseases treatment, and neuroengineering[22]. The therapeutic and diagnostic applications of CNTs are widely studied all over the world and considerable progresses have been achieved to date.

However, in contrast to the popular applications of CNTs in drug delivery and bio-imaging, their potential applications as therapeutic drugs to the central nervous system have been overlooked. Benefitting from the distinct properties, CNTs can effectively regulate the behavior of the nervous system. CNTs have recently made great contributions to therapeutic applications against different neuropathological disorders *in vitro* and *in vivo* (Fig. 1). In this review, we summarized the state of art in the use of CNTs for neurophysiology and neuropathology applications both *in vitro* and *in vivo*, and highlighted the challenges and forecast of future applications of CNTs in clinical setting.

2. *In vitro* application of CNTs and biological mechanisms of CNTs-mediated bio-medical consequence

2.1. Neuronal differentiation

Some of nervous system diseases need to be eased through repairing neurons structure. In the process of development, the flexible structural characteristics at the growth cone play critical roles in mediating the neurite growth and the synapse formation. Studies on the mechanisms on regulating neurite outgrowth have been widely conducted through cultivating of dissociated neurons from brains or spinal cords of embryonic rodents. In cultures, the neurons inoculated in dishes played as seeds, and the petri dish surface coated with cell adhesion molecules could promote neurite outgrowth. Thanks to the persistent explorations of the researchers, more molecules have been identified as neurotrophic factors that can promote or inhibit neurite outgrowth. However, it is still unclear how to regulate the neurite outgrowth existence due to the lack of methods to control the growth environment at the nanometer scale. Alternatively, CNTs showed great potential applications in neuroscience research, benefitting from their distinct properties, including good strength, flexibility, electroconductivity, and additional functions with different molecules. CNTs have been widely demonstrated as good electrical conductors and scaffold, which can influence neurons growth and differentiation through changing the conductivity and spatial structure. Malarkey et al.[23] showed that the specific conductivity level of CNTs was of great importance to mediate neuronal growth and differentiation. In their study, cells growing on the substrates (0.43−0.9 S/cm) behaved the strongest reaction on cellular adherence, cellular migration, and protein expression[23]. In consideration of physiological environment (pH = 7.35), Hu et al.[24] produced three different MWCNTs with different surface charges. The results showed that the different surface charge had little effects on the number of neurites every neuron. However, the positively charged and zwitterionic MWCNTs contributed to the longer and more branching of neuritis. The CNTs offer not only morphological structure but also durableness, inertness, and easy modification. Therefore, the prosthesis using MWCNTs has an advantage over other growth substrates[24]. Taking advantage of the super adsorption, CNTs can be coated with other molecules to maintain the physiological status of neuronal cells. Mattson

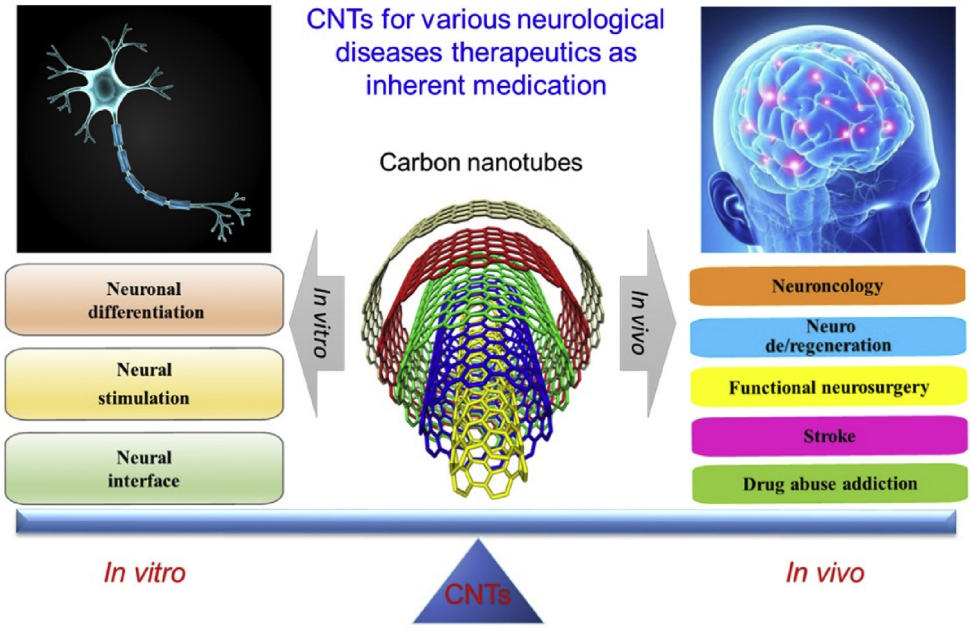

Figure 1 Schematic illustration on the applications of CNTs as inherent medication for various neurological diseases therapeutics *in vitro* and *in vivo*.

et al.[25] have successfully coated nanotubes with the bioactive molecule 4-hydroxynonenal to make the neurons more extensive branching. It is the first paradigm to develop a method to grow embryonic rat brain neurons on MWCNTs. Not only the chemical molecules, the nerve growth factor can also be connected on CNTs. Matsumoto et al.[26] reported MWCNTs modified with neurotrophin *via* EDC/NHS [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/*N*-hydroxysuccinimide] conjugation, where the neurotrophin could regulate the differentiation and survival of neurons, and thus promoted the growth of the neurons to a certain extent. For artificial nerve regeneration conduit, CNTs have been used for imitating the construction and subassembly of autologous nerves. However, there are still some problems of CNTs in many aspects, including mechanical properties, electrical conductivity and sustained release. In the study, Yu et al.[27] combined the MWCNTs with a collagen/PCL (polycaprolactone) fibrous scaffold. The results showed that the carboxyl MWCNTs could greatly alter the composite scaffold to enhance the cell adhesion and growth *in vitro*.

Besides the repair on injured cell, neural stem cells transplantation has attracted intense attentions as an emerging treatment strategy. Nowadays, neuroprosthetic devices are still facing several challenges that deserve further proper address. Metal electrodes detachment from implanted substrates occurs because of delamination and degradation. Then, the performance of electrodes at the cell−electrode interface would be highly compromised once the electrode size was cut down to adapt to implantation. Electrodes would also activate immune response after long-term contact with neural tissues followed with neuron depletion and replacement of reactive astrocytes. The signal transduction will be blocked. Semiconductor devices seem to be promising candidates to resolve part of above issues. However, mechanical compliance of semiconductor devices with the neural tissues cannot be maintained for desired time period. Neural stem cells (NSCs) as a life-long source of neural cells have the capacity of self-renewal and multi-directional differentiation. CNTs have been considered as the ideal scaffold, which is able to regulate NSCs growth. Kam et al.[28] combined SWCNTs with laminin to form a structure of layer-by-layer. Laminin−SWCNT thin films were found to be conducive to NSC differentiation and suitable for their successful excitation[28]. Shao et al.[29] reported that CNT nanocomposites *via* layer-by-layer assembly of negatively charged MWCNTs and positively charged poly (dimethyldiallyammonium chloride) provided potent regulatory signals over NSCs, including differentiating, neurite outgrowth and electrophysiological maturation of NSC-derived neurons (Fig. 2B). CNT-based scaffolds were considered to possess the ability to enhance growth factor sequestration of human neural stem cells (hNSCs) after surface elemental analysis. The nanofibrillar morphology of CNT also provided much larger surface area and facilitated attachment of embryoid bodies (EBs). Growth factors and attachment are two key factors to regulate the differentiation of stem cells. Therefore, the differentiation yields of neurons derived from EBs were compared when attached to two kinds of surfaces: pure polymer surface and polymer grafted CNT-based substrate. EBs on the latter one yielded more differentiated neurons, which demonstrated that CNT can provide ideal microenvironment to promote hNSC differentiation at neuron direction. Park et al.[30] also developed a CNT-based method to control the growth and differentiation of hNSCs into neurons (Fig. 2A). A CNT network was created to provide nanotopography surface and optional laminin adsorption, by which growth and adhesion of hNSCs were successfully achieved. Compared with classical cell-culture substrates, CNT network-coated structure can induce better and stronger growth and adhesion of hNSCs. Based on above findings, architecture-related neuronal differentiation was realized on CNT networks. This demonstrates that CNT network pattern provides an easy and efficient way to manipulate polarization and growth of hNSCs on multiple surfaces[30].

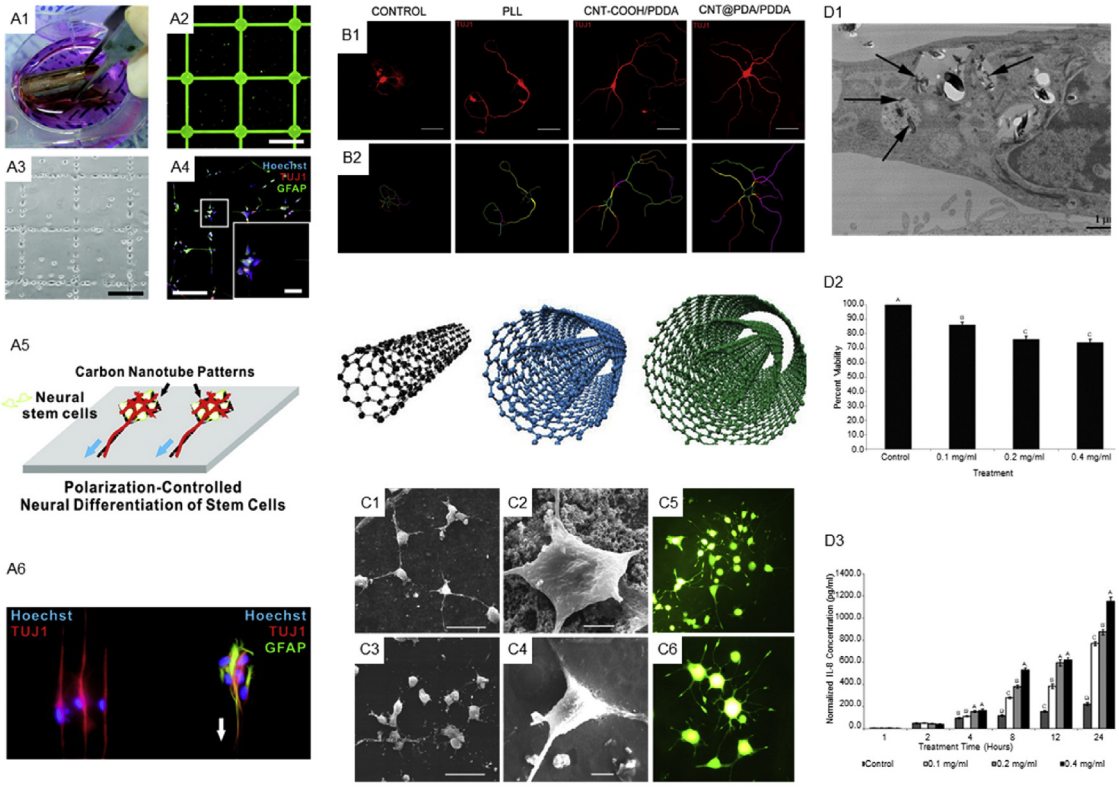

Figure 2 The application and biological mechanisms of CNTs *in vitro*. (A1)−(A6) Control of human neural stem cells (hNSC) growth and differentiation on biocompatible and flexible polyimide substrate. Adapted with permission from Ref. 30 © 2011 American Chemical Society. (B1) 2D reconstruction of NSC-derived neuron cultivated on different substrates. (B2) The contours of 2D structures of the corresponding neurons presented in (B1). Adapted with permission from Ref. 29 © 2018 Elsevier. SEM microphotos of PC12 cells cultured on PPy/SWCNT deposited ITO substrate (C1 and C2) and ITO substrate (C3 and C4) in the presence of 50 μg/mL NGF at day 7. (C5 and C6) Calcein fluorescent images showing living PC12 cells cultured on PPy/SWCNT deposited ITO substrate in the presence of 50 μg/mL NGF at day 7. Adapted with permission from Ref. 36 © 2010 Elsevier. (D1) Keratinocyte monolayer grown on a Permanox surface. (D2) Mean viability of the HEKs 24 h following exposure to MWCNT. (D3) Mean IL-8 increase with time and concentration of MWCNT. Adapted with permission from Ref. 37 © 2005 Elsevier.

2.2. *Neural stimulation*

In the clinical research, many nervous system disease treatments need electrical nerve stimulation to repair damage, for example, the deep brain stimulators and cochlear implantation. Compared with the traditional electrode, CNTs have been demonstrated to possess many distinct advantages in recent years. The CNT composites have unique molecular structures with larger specific surface area and better electrical conductivity. Li et al.[31] demonstrated that the highly porous 3D nature is the most distinguishing feature of CNTs. They compared single-walled nanotube paper (SWCNTP), multi-walled nanotube towers (MWCNTTs), and heat-treated multi-walled nanotube towers. The result showed that SWCNTP had the largest effective capacitance due to its higher density and the film remains permeability of the electrolytes[31]. Wang et al.[32] used MWCNTs to form vertically aligned pillars. Iron-modified hydrophilic CNT microelectrodes have a high charge injection limit (1−1.6 mC/cm^2) without faradaic reactions[32]. It's the first report about repetitive CNT electrodes stimulation of hippocampal neurons *in vitro*. These results suggested that compared with previous metal electrode approaches, CNTs can provide safer and more effective solutions for neural prostheses.

In addition to the porous 3D nature, the CNT can also make the electrodes thin, lightweight, and flexible. Meng et al.[33] created an interesting paper-like nanotube/polyaniline composite with CNT network as the template. The whole device was as thin as 0.45 mm and could operate well under full mechanical flexibility. For some time past, it has been reported that the chemical doping of CNTs with heteroatoms (*e.g.*, nitrogen and/or boron) can dramatically change their properties on the aspect of chemistry and electricity. Fernandes et al.[34] prepared a new nanocomposite by immobilization of the tetrabutylammonium salt of phospho vanadomolybdate $[PMo_{11}VO_{40}]^{5-}$ ($PMo_{11}V$) into nitrogen-doped carbon nanotubes (CNT-N), and designated as $PMo_{11}V$@N-CNT. For the $PMo_{11}V$@N-CNT-modified electrode, the oxidation peak current of acetaminophen increases ∼127% when compared with that obtained with a bare glass-carbon electrode and the peak potential shifts only 0.031 V. These variations suggested that the $PMo_{11}V$@N-CNT composite material has a stronger interaction with other species[34]. Jan et al.[35] carried out a new approach to modify CNTs, named activated electrochemically deposited iridium oxide (IrOx) and poly(3,4-ethylenedioxythiophene). Their findings provided the significant evidence about the potential of carbon nanotube composite material serving as excellent new material for neural interfacing. In the report from Lu et al.[36], a

research of electrochemically co-deposited polypyrrole/SWCNT (PPy/SWCNT) films for offering the electrode-neural interface was reported. The PPy/SWCNT microelectrodes showed a particularly high safe charge injection limit of ~7.5 mC/cm^2 and low electrode impedance at 1 kHz, as well as good stability (Fig. 2C).

2.3. *Neural interface*

It is of importance for nanomaterials as neural interface to fay surfaces with nervous system and reliability. Although CNTs have been are widely used in the interaction with cells as promising candidates for neural interface, they are still troubled with the certain host response and the resulted biocompatibility issue reported by several groups. The potential cytotoxicity of CNTs deserves more careful investigations and assessments prior to their extended biomedical applications, especially in human healthcare. Some researchers have shown that aggregated bundles of CNTs presented higher toxicity than well-dispersed CNTs did. Riviere's group[37] revealed that even micrometer-sized well-dispersed CNTs were less toxic than the aggregated ones. The assumption that aggregated CNTs may adhere to cells through their extracellular proteins and change the cell membrane's ability to pass nutrients and waste, which thus caused severe cytotoxicity (Fig. 2D). Alternatively, Wang et al.[38] reported an effective route to improve the dispersion of SWCNTs. They built a system to bind SWCNTs with polycyclic organic compounds (POCs), such as ethidium bromide, rhodamine 123, 1-pyrene butyric acid and fluorescein isothiocyanate. In the presence of the $\pi-\pi$ stacking, electrostatic interactions and coating surfactants, the SWCNT−POC conjugates showed better dispersity in aqueous phase and improved the transmembrane ability[38]. In agreement with the results in other studies, SWCNTs could alter the cell function through interaction with subcellular organelles[39,40]. Furthermore, the CNT dimension was considered to have great impacts on the toxicity of CNTs. Although the underlying exact mechanism is not clear yet, a hypothesized theory was put forward that the CNTs dimension is similar to the dimension is close to the dimension of a virus and thus CNTs may simulate viruses and induce an immune response.

2.4. *Biological mechanisms of CNTs-mediated biomedical consequence*

CNTs have been demonstrated to be able to mediate the growth situation of neural synapse, such as density, length and branch. Correspondingly, the underlying mechanism has also been widely noted by several groups. It has been proved that the electric surface charge of CNTs have a significant influence on their biological effects. When exposed to CNTs with more positive surface charge, cells would grow out more neurite branching and growth cones[24]. CNTs modified with various chemical groups can trigger more biological mechanisms. In previous studies by Malarkey et al.[41], it was found that the functionalized SWCNTs-PEG were able to increase the length of various processes of nervous activity with unclear mechanism yet. In their following further research, it's demonstrated that SWCNTs were able to prevent stimulated membrane endocytosis in neurons, which could then explain the early noted extended neurite length. Our group explored the SWCNTs-mediated biomedical consequence mechanisms on an Alzheimer's disease model. Based on a large number of biological experiments, we found that SWCNTs could upregulate autophagy and repair defects in the autophagic turnover of proteins within astroglial cells[42]. These findings herald the potential of SWCNTs in nerve disease therapy.

3. *In vivo* applications of CNTs

3.1. *Neuroncology*

Among numerous neurological diseases, neuroncology has gained intense attentions. Compared with other tumor types, brain tumor is much more challenging to be cured because of the rapid growth, invasive nature, and blood−brain barrier. Based on this kind of special case, many chemotherapeutics are not able to realize appealing efficacy in the clinical treatment. Initially, researchers utilized the strong ability to penetrate membrane of CNTs to load drug or gene. In 2007, Kateb et al.[43] demonstrated that MWCNTs showed little cytotoxicity and significant effects on proliferation of BV2 microglia or GL261 glioma cells *in vitro*. Meanwhile, the MWCNTs were able to be selectively uptaken by certain cells. Compared with tumor cells, phagocytic cells showed higher uptake efficiency on MWCNTs carrying DNA or siRNA[43]. Based on these findings, some groups turned their attentions to the combination of carbon nanotubes with oligodeoxynucleotides (CpG) that is secreted from cells and difficult to be embedded. CpG is an excellent activator can stimulate the toll-like receptor-9 (TLR9) of tumor-associated inflammatory cells and CNTs are promising candidates hold great potentials to solve the dilemma of CpG. Zhao et al.[44] conjugated SWCNTs with CpG *via* conjugation reaction. The as-prepared SWCNT/CpG conjugates were intracranially injected into tumor bearing mice at a single low dose. The experimental results indicated that 50%−60% of GL261 gliomas were eliminated by this method, where surviving animals were protected from relapse up to three months. These results were the obvious evidence that CNTs are versatile carriers for the brain delivery of immunostimulatory molecules to achieve effective immune response for tumor treatment. VanHandel et al.[45] injected MWCNTs labeled with fluorescent dye PKH26 into cranial cavity of mice. The flow cytometer showed that 75% of the cells uptaking CNTs were macrophages related to the tumor (Fig. 3A). It was also found that CNTs had a selective cellular uptake. The SWCNT/CpG has been verified as a good therapeutic based on mice experiments. Consequently, further research was inspired and carried out in the environment closer to clinical situations. K-Luc was chosen in Ouyang et al.'s study[46] on a more invasive mouse glioma model, which showed higher similarity to human glioma. Additionally, with reference to the standard of clinical application, temozolomide (TMZ) was introduced into SWCNT/CpG, which was further adapted for their antitumor efficiency assessment 4 days after injections. Compared with the control (RSS-CpG), the SWCNT/CpG in combination with subsequent TMZ treatment was much more effective than the other treatment regimens. Immunotherapy was combined with chemotherapy for improved efficacy in this study and these results provided strong evidence to the superiority of combined cancer treatments.

3.2. *Neurodegeneration and neuroregeneration*

Neurodegeneration is a heterogeneous population of either genetic or sporadic conditions all distinguished by progressive nervous system dysfunction causing the degeneration of selected neurons in the CNS. Some of the most common neurodegenerative diseases are Parkinson's disease (PD), Alzheimer's disease (AD) and

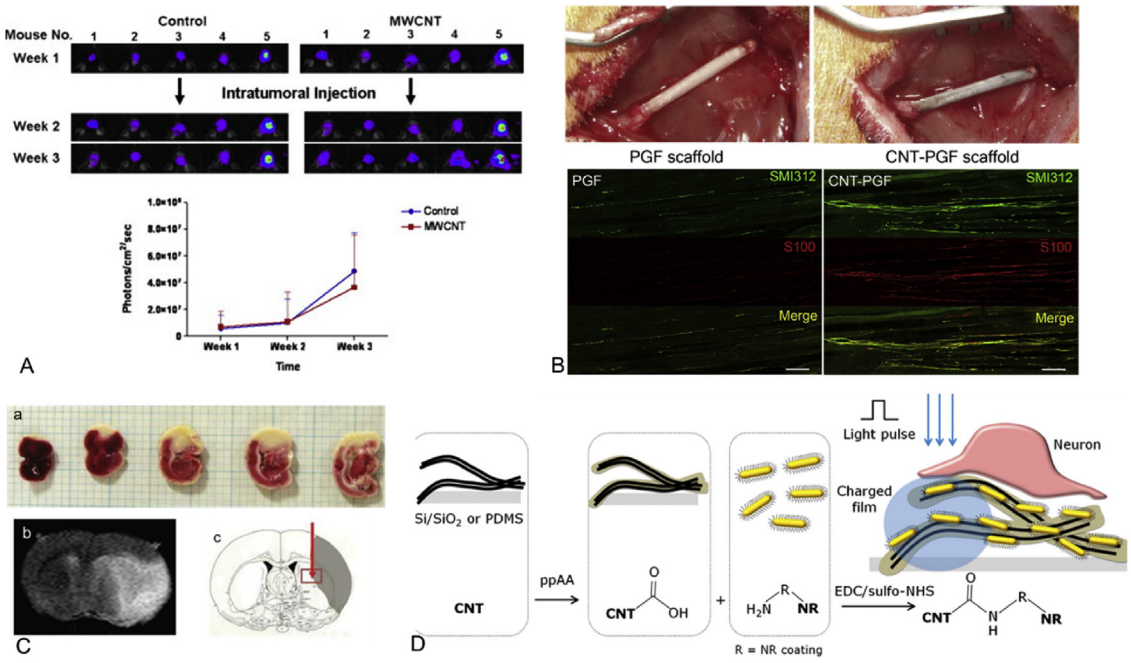

Figure 3 The application of CNTs *in vivo*. (A) CNTs applications in tumor macrophages. Adapted with permission from Ref. 45 © 2009 Elsevier. (B) CNT-interfaced nerve conduits in the regeneration of transected rat sciatic nerve. Adapted with permission from Ref. 59 © 2015 Elsevier. (C) CNTs' outstanding performance on stroke. The infarct area after middle cerebral artery occlusion injury was determined by triphenyl tetrazolium chloride staining (a) and magnetic resonance imaging (b). The subventricular zone neural progenitor cells impregnated with CNTs were transplanted into the injured brain directly by microinjection (c). Adapted with permission from Ref. 64 © 2012 National Center of Biotechnology Information. (D) CNTs are used as artificial retina in neurosurgery. Adapted with permission from Ref. 68 © 2014 American Chemical Society.

other dementias, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS)[47]. Millions of people are suffering damaged CNS worldwide every year. AD, PD and peripheral neuropathy are the most popular neurodegenerative pathologies[48,49]. There are many reasons causing neurons damage in brain and spinal cord, including aggregation and accumulation of misfolded proteins[50], oxidative stress, apoptosis, inflammation and microglia activation[51], mitochondrial dysfunction[52], impaired autophagy-lysosomal activities[53], damaged DNA and impaired DNA repair[54], epigenetic deregulation of gene expression[55], RNA processing and protein degradation pathways[56,57], as well as perturbation of vesicle trafficking and synapse dysfunction.

Over the past decades, the CNTs have been considered as a fixed system without the capability of regeneration. With keeping rapid development of related technologies, CNTs are expected to promote neuroregeneration as tissue engineering scaffolds. In recent years, CNTs were adapted to coating with nerve growth factor and neural stem cells, aimed at accelerating the growth of neural networks. Cellot et al.[58] used the CNTs as a kind of appealing electrical shortcuts to affect the neuronal regeneration behavior and the reproducible phenomenon was different from that of other substrates. Electrotonic hypothesis was proposed to explain the outstanding property of CNTs. However, the correlation study was still limited to experiments *in vitro*. Studies *in vivo* were highly urgent. Compared to as nerve scaffold, CNTs showed more appealed developments as drug carriers on the aspect of neuroregeneration. In Ahn's study[59], they reported the functions of CNT-interfaced nerve conduits in the regenerations of transected rat sciatic nerve *in vivo*. CNTs were tethered onto the surface of 9 aligned phosphate glass microfibers and placed into three-dimensional poly(L/D-lactic acid) tubes. The number of regeneration axons crossing the scaffold, the cross-sectional area of the re-innervated muscles and the electrophysiological finding were all significantly improved after implanting the engineered CNTs into the 10 mm gap of a transected rat sciatic nerve (Fig. 3B). Lee et al.[60] utilized a stereolithography 3D printer to fabricate a well-dispersed MWCNT−hydrogel composite neural scaffold with tunable porous structure. The results indicated that MWCNTs coupled with electrical stimulation have a synergistic effect on promoting neurite outgrowth for therapeutic application in nerve regeneration. Despite that great efforts have been made, few paradigms of CNTs applications *in vivo* have been reported so far. The major challenge should lie on the blood−brain barrier, which severely compromises the CNTs delivery efficiency. Some advantageous properties of CNTs, such as easy modification and conformability of cells, could be intensely exploited in the future study, which holds great potentials to overcome the blood−brain barrier.

3.3. *Stroke*

Stroke is defined as a kind of serious neurologic disorders caused by broken off the vasculature supplying the brain[61]. Disease symptoms are usually sudden, within seconds to hours, and can include one-sided weakness or numbness of the face, arms or legs, aphasia, dysarthria, visual disturbances, loss of balance and coordination, barriers to action and in severe cases, even death. The results of a stroke depend on which part of the brain has been affected and the degree of severity. The two main types of stroke are ischemic and hemorrhagic[62]. The brain−blood circulation

obstacle has a strong impact on the function of local nerve. It results in nerve functional disorder and even death.

Lee et al.[63] reported for the first time the neuroprotective properties of CNTs in stroke models. In their study, rats were treated with aggregated SWCNTs (aSWCNTs) through lateral ventricle injection. One week later, a middle cerebral artery occlusion (MCAO) surgery was performed to cause ischemic brain injury. According to the analysis of coronal brain sections and Western blot analysis of cerebral cortex lysate, the rats treated with aSWCNTs showed fewer damages and the nervous system function recovered faster than other groups. In addition, the a-SWCNT-treated animals expressed higher level ($\sim$1.8-fold) of N-cadherin expression than the PBS-treated animals. It was turned out that the cell adhesion effect of N-cadherin plays an important role in the whole phenomenon[63]. Additionally, Moon et al.[64] showed that hydrophobic CNTs impregnated with subventricular zone neural progenitor cells had the ability to repair damaged neural tissue following stroke. The rat transplanted neural progenitor cells functionalized with CNTs exhibited improved behavior and reduced volume and area of infarct cyst compared to control groups. Most of the transplanted neural progenitor cells functionalized with CNTs broadened around the ischemic injured region and decreased microglial (Fig. 3C)[64]. Their research paved the road for further studies to develop SWCNTs-based novel protections against ischemic brain injury. Again, this report highlighted *f*-CNTs as therapeutic candidates for Stroke.

3.4. *Functional neurosurgery*

On the basis of many studies, the property of CNTs to transmit electrical signals into the nerve has been widely proven. For example, Minnikanti et al.[65] designed the implantable multi-walled carbon nanotubes, which showed almost the same charge storage capacity *in vitro* (1.008 mC/cm^2) compared to that *in vivo* (1.111 mC/cm^2). Therefore, it is hopeful to activate or replace damaged nerve cells in neurosurgery. At present, the most typical example is cochlear implantation in neurosurgery, where carbon nanotube electrodes can transform voice signal into electrical signal to brain in the surgery. By this way, the cochlear could replace the damaged cochlea effectively[66,67]. On the other hand, Bareket et al.[68] used semiconducting CNTs for retinal transplantation. The researchers found that semiconducting CNTs had excellent photosensitive properties and could transform acoustical signal into electrical signal to the neurons that are in charge of communication between the eye and brain (Fig. 3D). These successful stimulations of affected parts suggest the potential use of CNTs in functional neurosurgery.

3.5. *Drug abuse and addiction*

Addiction is defined as a chronic, relapsable brain disease that is characterized by obsessive drug seeking and abuse, along with harmful consequences. It is considered a brain disease because that drugs act on brain and change the structure and function of brains. These brain damages can continue for a lasting time and lead to many harmful and even self-destructive results. The initial decision to exposure to drug is mostly voluntary. However, a person's ability of self-control can become seriously impaired once addiction takes over. Cerebrograph imaging studies from people addicted to drugs show physical changes in areas of the brain that are fatal for decision making, judgment, memory, learning, and behavior control. Scientists believe that these changes alter the pattern of the brain works and may help to explain the enforceable and destructive behaviors of an addicted person. Fortunately, addiction is a treatable, chronic disease that can be cured successfully. Current research shows that behavioral therapy combining with medicine, where available, is the best way to ensure effective for most of the patients. Therapeutic method must be designed to address patient's drug use patterns and drug-related medical, psychiatric, and social problems. In this regard, our recent study revealed that aSWCNTs showed remarkable inhibiting effect on methamphetamine (METH) addiction. In our study, the aSWCNTs were used as active constituents of drugs to explore the outcomes and mechanism for METH confrontation. The reverse effect was outstanding and the aSWCNTs showed better efficacy than SWCNTs. Based on in-depth explorations, we attributed the mechanism to the adsorption and oxidation of dopamine, which further adjusted the dopamine reward pathway[69]. In this way, the CNTs are believed to be potential inhibitors in drug abuse and addiction, and probably will receive more attentions in the future.

4. Biocompatibility and biosafety of CNTs

Despite rapid development in CNTs and other polymeric nanomaterials, relatively less is known to date on the biological effects that exposure to these materials. Recently, potential toxicity of CNTs to humans and environment have been raised[6,70]. The biocompatibility and biosafety of CNTs and other polymeric nanomaterials has been widely addressed and is of major concern for biomedical use[71–76]. The biosafety of CNTs-based biomedical theranostics is still a moot point. The following paragraphs give detailed discussions on the toxicity analysis of CNTs *in vitro* and *in vivo*. Furthermore, the pharmacokinetics and metabolism of CNTs are summarized as well.

4.1. *Toxicity analysis of CNTs in vitro*

Cytotoxicity analysis is of fundamental significance for *in vivo* biological applications. *In vitro* assays mainly focused on cell uptake, cell viability and reactive oxidative stress. The cytotoxicity of carbon nanomaterials is intensely related with many factors, such as size, morphology, mass basis, surface property, and concentrations. Zhang et al.[77] compared cellular uptake and cytotoxicity of MWCNTs, graphene oxide (GO) and nanodiamond (ND) in HeLa cells. The cell uptake ratio of the three kinds of carbon nanomaterials was in following order: ND $>$ MWCNTs $>$ GO. However, the cytotoxicity of these materials showed no relationship with the cell uptake ratios. Although the cell uptake of MWCNTs was higher than GO, no significant differences were observed between their cytotoxicity. ND exhibited better biocompatibility than MWCNTs and GO. Some studies demonstrated that the cytotoxicity of CNTs was greatly influenced by the mass basis, and SWCNTs are believed to have higher cytotoxicity than MWCNTs. Yuan et al.[78] compared cell viability of HepG2 cells treated with graphene and SWCNTs, respectively. The results showed that SWCNTs exhibited higher cytotoxicity and might induce oxidative stress, and thereby led to apoptosis by activating P53-mediated damage checkpoint signals.

4.2. *Toxicity analysis of CNTs in vivo*

Toxicity analysis *in vivo* is essential to evaluate the biocompatibility of CNTs following cytotoxicity analysis. Animal models

such as *Caenorhabditis elegans*, zebrafish, mice, rats and primates are widely used in biomedical research to improve the understanding of the dynamic behaviors and biocompatibility of materials *in vivo*. The toxicity analysis *in vivo* mainly focused on biochemistry and hematology analysis and histological examinations. To improve the great biocompatibility, some groups functionalized CNTs with polymers or other biosafety molecules. Liu et al.[79] determined the *in vivo* biodistribution of SWCNTs modified with polyethylene-glycol (PEG) by position emission tomography (PET). The functionalized SWCNTs exhibited long blood circulation times and low uptake by the reticuloendothelial system (RES). No obvious circumstance of toxicity was found and mice did not display negative consequence such as weight loss and fatigue. Although SWCNTs have relatively high cytotoxicity mentioned above, Xue et al.[69] reported that aggregated SWCNTs significantly attenuate the behavioral and neurochemical effects of methamphetamine in mice. Interestingly, the study did not observe significant change in behaviors such as feeding, drinking, locomotion and neuronal toxicity in the striatum or midbrain within the dosage used in the research.

4.3. *Pharmacokinetics and metabolism of CNTs*

It is also urgent to figure out the pharmacokinetics and metabolism behaviors of CNTs before their wide use in biomedical applications. Many studies have documented that different physiochemical properties such as size, shape, surface property and mass ratios can greatly influence the pharmacokinetics and metabolism. Wang et al.[80] labeled radioactive ^{125}I atoms to water-soluble hydroxylated SWCNTs to study the biodistribution in mice. The results showed that SWCNTs have little accumulation in RES and accumulated readily in the bone for a long time, stomach and kidney. The radiolabeled SWCNTs could move freely and rapidly to each proper target in a similar manner of small molecules. And the excretion percentage of SWCNTs was 94% and 6% *via* urine and feces, respectively. It suggested that SWCNTs are excreted mostly *via* kidney. Singh et al.[81] determined tissue biodistribution and blood clearance of SWCNTs functionalized with diethylentriaminepentaacetic (DTPA) and labeled with ^{111}In. It turned out that intact SWCNTs exhibited a blood circulation half-life of up to 3.5 h and rapid kidney excretion route without any toxicity side effects or mortality. Additionally, Alidori et al.[82] conducted the research in primates (*Cynomolgus macaques*), where the CNTs accumulated in liver and kidney were mostly excreted *via* urine. In particular of the brain tissue, Kafa et al.[83] found that MWCNTs located within endocytic vesicles and multi-vesicular bodies after incubation for 4−24 h, followed by a basal excretion from primary porcine brain endothelial cells (PBEC) cells after 48 h. And MWCNTs administered by tail vein injection were able to cross the BBB, displayed a maximum brain accumulation of 1.1% of injected dosage and followed by a gradually slow excretion. Nunes et al.[84] revealed the partial degradation of the chemically functionalized MWCNTs after internalization with microglia *in vivo*.

5. Conclusions and outlook

In this article, we highlight the application prospects for CNTs acting as therapeutic drugs for neurological diseases. Based on all studies above, the special characteristics and high potential of CNTs in neurophysiology and neuropathology are highlighted. Neurological diseases are a group of CNS disorders that affects the structure or function of either the spinal cord or brain. In the past, neurological diseases continued to be a significant global health problem, which negatively influenced the life quality of patients. Among all the neurological diseases, the incidence of some of them has increased in recent years, such as neuroncology, neurodegeneration, stroke, functional injury, drug abuse and addiction. The blood−brain barrier (BBB) is a major obstacle for drug delivery resulting in limited drugs reaching the market to tackle CNS disorders. CNTs, which have attractive properties, provide sorts of excellent examples for using in the CNS environment.

CNTs were typically prepared by metal-catalyzed synthesis and chemical functionalization is necessary to improve the biocompatibility and biosafety. Due to the structural and electronic properties and unique biological consequence on cells viability and cell growth, CNTs can be used as scaffolds alone or in combination with other biodegradable biomaterials to guide neuroengineering, such as protection, stimulation and regeneration, and default in neural activity improvement. Besides that, CNTs take indisputable advantages in neuroncology theranostics. In addition to the diseases mentioned above, the application of CNTs can be extended to many other neurological disease therapy because of their unique physiochemical properties in the future.

Although many efforts have been made to improve the biocompatibility of CNTs, the potential oxidative stress, free radical production, peroxidative products accumulation, DNA damage and inflammatory are still obstacles for the potentially wide applications of CNTs in neurological diseases therapy. The major concern about the use of CNTs as inherent medication for neurological disease therapeutics lies on safety, especially the brain degradation and excretion. Intense attentions are deserved on functionalization of CNTs in order to improve the brain degradation and excretion of CNTs. On the other hand, it is worthy to note that degradation and excretion may be a double-edged sword. Degraded and excreted too rapidly may influence the treatment effect, while retention for too long may cause the inherent body damage. The behaviors of degradation and excretion of CNTs can be carefully tailored based on the intended *in vivo* applications.

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Author contributions

Chenyang Xiang collected relevant studies and wrote the paper. Yuxuan Zhang helped to collected relevant information. Weisheng Guo provided constructive suggestions and modified the paper. Xing-Jie Liang conceived the paper layout and modified the paper.

Conflicts of interest

The authors declare no conflicts of interest.

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