

# Biocompatibility of carbon nanotubes with stem cells to treat CNS injuries

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**Abstract:** Cases reporting traumatic injuries to the brain and spinal cord are extended range of disorders that affect a large percentage of the world's population. But, there are only few effective treatments available for central nervous system (CNS) injuries because the CNS is refractory to axonal regeneration and relatively inaccessible to many pharmacological treatments. The use of stem cell therapy in regenerative medicine has been extensively examined to replace lost cells during CNS injuries. But, given the complexity of CNS injuries oxidative stress, toxic byproducts, which prevails in the microenvironment during the diseased condition, may limit the survival of the transplanted stem cells affecting tissue regeneration and even longevity. Carbon nanotubes (CNT) are a new class of nanomaterials, which have been shown to be promising in different areas of nanomedicine for the prevention, diagnosis and therapy of certain diseases, including CNS diseases. In particular, the use of CNTs as substrates/scaffolds for supporting the stem cell differentiation has been an area of active research. Single-walled and multi-walled CNT's have been increasingly used as scaffolds for neuronal growth and more recently for neural stem cell growth and differentiation. This review summarizes recent research on the application of CNT-based materials to direct the differentiation of progenitor and stem cells toward specific neurons and to enhance axon regeneration and synaptogenesis for the effective treatment of CNS injuries. Nonetheless, accumulating data support the use of CNTs as a biocompatible and permissive substrate/scaffold for neural cells and such application holds great potential in neurological research.

**Key words:** Carbon nano tubes, Biocompatibility, Stem cells, Stem cell differentiation, Central nervous system injuries

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## Introduction

Advances in nanomedicine are expected to have a major impact in neurological research, contributing to our further understanding of the central nervous system (CNS) and the development of novel therapeutic strategies for neurological intervention [1, 2]. CNS has limited regenerative potential

when inflicted with lesions resulting from trauma, stroke, or neuropathological conditions. Repair from CNS injuries is complicated because of the activation of inhibitors for nerve regeneration, notably neurite outgrowth inhibitors and myelin-associated glycoproteins [3]. Recently, stem cell research has provided hope for replacing lost neuronal cells for several neurodegenerative diseases and CNS injuries. Functional recovery following brain and spinal cord injuries likely require the transplantation of exogenous stem cells either neural stem cells (NSCs)/human mesenchymal stem cells (hMSCs), since the mammalian CNS has little capacity for self-repair [4-7].

Stem cells are defined as self-renewing, primordial cells possessing the capacity to give rise to a differentiated

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progeny with all neural lineages, and are posited to exist in embryonic and fetal germinal zones and participate in CNS organogenesis [8, 9]. The subventricular zone (SVZ) is a dynamic place serving as the most predominant source of adult neurogenesis. But axon regeneration and functional recovery do not occur in the CNS due to a post-injury increase in inhibitors of axon extension, leading to permanent disability [3]. Normal axon growth and guidance is regulated by extracellular cues that promote or inhibit advance of the tip of an extending axon (the growth cone) [3]. It is known that cells with stem cell-like characteristics can be isolated from the mammalian CNS at all ages, propagated in culture, and reimplanted into injured CNS regions to compensate the damaged neurons within the injury site [3, 8, 10-13].

But the transplantation of stem cells has limitations, because of low survival rate at the injury sites [6]. Several methodologies were developed to enhance the efficacy of the stem cells to withstand the pathological situation around the injury site during transplantation. Among the various methodologies used, one of the promising approaches is building biocompatible substitutes for efficient culture of stem cells to repair or replace the function of damaged nerve tissues [14-16]. Among the various biomaterials available, exploitation of carbon nanotubes (CNTs) is one of the most attractive candidates for neurological applications. During the last decade, CNTs have shown evidence of their electrical conductive capacity, strong mechanical properties and morphological similarity to neurites [17] leading to the advancements for tissue engineering and regenerative medicine using CNT based stem cells for tissue repair. CNTs have unique advantages in controlling stem cell function and in tissue regeneration [18] due to their biomimetic characteristics and special biological and mechanical properties [19]. In the past, stem cell therapy seemed like it may present a cure for all medical ailments, but problems such as immune system clearance, control of differentiation in the body, etc have hindered progress. However, with the synergy of carbon nano-dimensional materials, researchers have been able to overcome these tissue engineering and regenerative medicine obstacles and have begun developing treatments for strokes, bone failure, cardiovascular disease, and many other ailments. Application of nanomaterials including CNTs in the biomedical field provides a provision of an appropriate nano-biointerface for the control of cellular behavior, and, therefore, optimizes tissue regeneration [20, 21].

As stated above, over the past few decades there have

been significant advances in stem cell therapy and tissue engineering for the repair and replacement of damaged tissue and organs. In parallel, nanotechnology and nanomaterials, in particular CNTs have emerged showing a pronounced potential for creating the next generation of biomaterials.

## Characteristics of CNTs to Support Neuronal Differentiation

CNTs structural features and dimensions are very much similar to many elements of the neuronal network (ion channels, signaling proteins and elements of the neuronal cytoskeleton) enabling the use of CNTs at the molecular level and consequently better control over physiological activity and neuronal information processing [22]. The CNTs structural backbone is exclusively composed of carbon atoms and exhibits exceptional properties, such as high electronic and thermal conductivity as well as great strength. Two main types of CNTs have been explored in biomedicine: 1) single-walled nanotubes (SWNT), consisting of a single sheet of carbon benzene rings rolled up into a tubular structure; and 2) multi-walled nanotubes (MWNT) that consist of multiple concentric layers of carbon sheets. CNTs (pristine carbon nanotubes) are insoluble in most aqueous solvents and the development of functionalization chemistries of the nanotube surface led to a notable enhancement in aqueous dispersibility that has allowed their application in physiological environments including the CNS [23]. Two main strategies have been described to enable the application of CNTs under physiological conditions, namely non-covalent and covalent functionalization [24]. Non-covalent functionalization involves the coating of nanotubes with hydrophilic macromolecules and the introduction of repulsive forces [25]. This has been achieved by coating or wrapping the CNT with surfactants [26] such as, polymers [26], peptides [27], or single stranded DNA [28].

Covalent modifications of CNTs, could increase the retention of an attached compound/group and play important role in brain signaling. A study report suggested that, nerve growth factor and brain-derived neurotrophic factor, and co-polymer covalently bound to SWNTs/MWNTs, could be used to regulate the growth of neurons [29] indicating that biologically active molecules like neurotrophins attached to CNTs can retain their activity and interact with cells to promote their function [30].

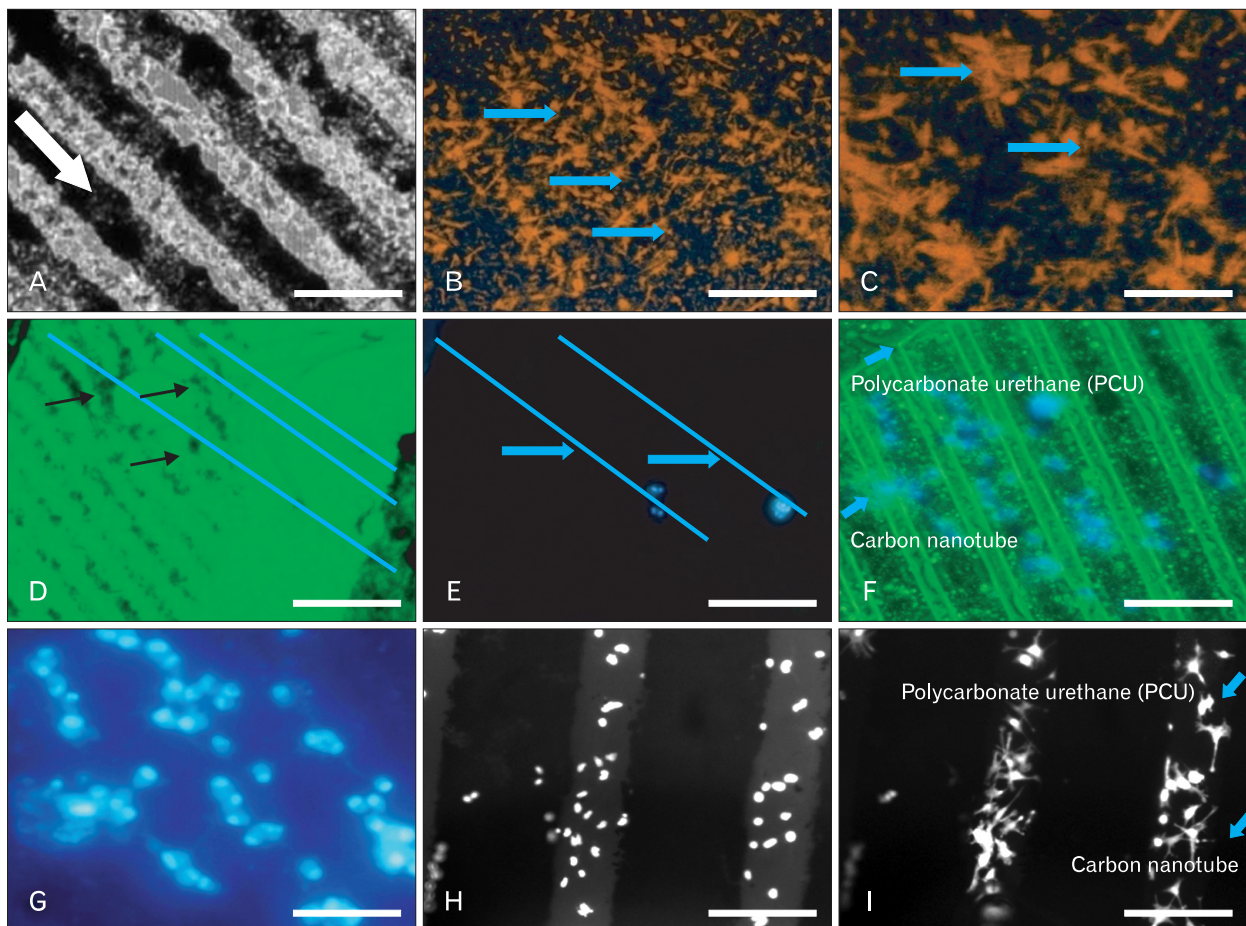
Previous studies suggest that CNT substrates can boost

neuronal electrical signaling [31], decrease astrocyte formation, macrophage density (cells which synthesize unwanted glial scar tissue in the brain) [32] and shown to increase the differentiation of NSCs to neurons [33] suggesting the promise for the use of CNTs as novel stem cell delivery vehicles for treating stroke damaged neural tissue [34, 35] *in vivo*. These attractive properties of CNTs have been attributed due to their unique conductivity and surface energetics capable of promoting the adsorption of endogenous proteins important for mediating cell adhesion (particularly the adsorption of vitronectin and fibronectin) in the transplanted region [36-38]. Importantly, CNTs have been exploited because of their “cell-friendly” nature which aids in promoting the functions of damaged neurons [32]. Specifically, researchers have developed micron patterns

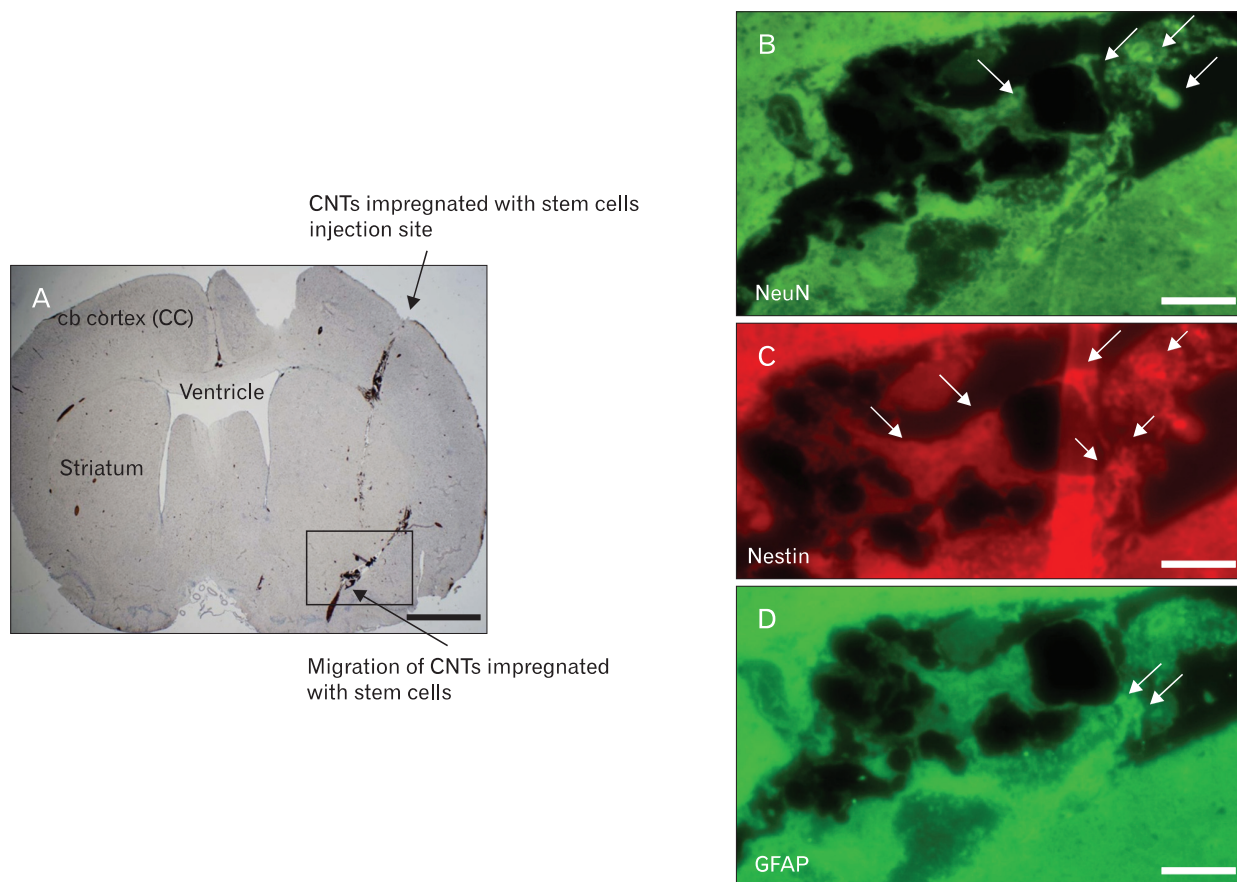
of CNTs on polymers such as polycarbonate urethane (PCU), which enable neuron cell attachment and extension of neurites along CNTs. These results highlight the ability of CNTs to direct functions of neurons to potentially heal damaged neural tissue [32, 33, 39].

### Affinity of CNTs for Supporting the Growth of Neurons, *In Vitro*

CNTs are unique formulations that provide a window of opportunity for designing more successful neural friendly materials (Figs. 1, 2). Previous studies reported the growth of astrocytes and microglia on CNT and PCU materials. The microglia preferred a PCU surrounding rather than CNT. However, many studies reported that the growth of the



**Fig. 1.** (A) Scanning electron microscope image of aligned carbon nanotube at  $\times 50$ . (B, C) Fluorescence images of the primary neural cells adhesion on aligned carbon nanotubes (CNTs) after 24 hours. (D, E) Fluorescence images of the subventricular zone neural stem cells adhesion on aligned CNTs. Cells were visualized by the use of DAPI stain. (F, G) Fluorescence images of the PC12 cells adhesion on aligned CNT rather than polycarbonate urethane (PCU) after 24 hours. Cells were visualized by the use of DAPI stain. Phase images of the selective macrophage adhesion (H) and activation (I) on aligned PCU rather than CNT after 24 hours. CNTs appear black. Scale bars=50  $\mu\text{m}$  (A, B, D, F, H), 100  $\mu\text{m}$  (C, E, G, I).



**Fig. 2.** (A) Stem cell interaction with carbon nanotubes (CNTs) one week after implantation into stroke damaged rat neural tissue. Results of this *in vivo* data showed increased expression of NeuN (marker for neurons) (B), nestin (marker for stem cells) (C) and decreased the expression of the astrocytes formation evidenced by less glial fibrillary acidic protein (GFAP) positive cells surrounding the carbon nanofibers (which appears black in the histological sections) (D). Scale bars=25  $\mu$ m (B–D).

neuron preferred a CNT surrounding to a PCU one [33, 34, 39, 40]. The origin of the neuron-CNT interaction appears to be strongly affected by surface roughness. It was demonstrated that the roughness of CNTs contributes to anchoring the neuronal cells [41]. Zhang et al. [42] preferentially cultured neuronal cell line H19-7 on the MWNT patterns. Neuronal growth cones were found to make contact with the nanotube surface, and these strong interactions allowed the neurons to spread long patterns and form interactions with one another. Additional mechanism may be that long nanotubes are flexible and undergo deformation to accommodate the proliferating neurites [42].

### Biocompatibility of CNTs to Manipulate Stem Cells for Treating CNS Injuries

CNTs are often modified to improve their biocompatibility

to perform new functions by tagging various compounds to them. Lipids, DNA and various peptides can often be simply adsorbed to the CNT. If a more permanent attachment is desired, compounds may be covalently linked to CNTs by incubating CNTs with strong oxidizing agents like nitric acid, which add carboxyl groups to the ends of the CNTs. In addition other groups can then be added converting the carboxyl group to acyl chloride, which can then be reacted with the compound of interest [43-45]. CNTs have been studied as substrates for neuronal growth. Their size and shape are similar to neuronal processes and can be made conductive. These are all qualities that are advantageous for creating scaffolds for neuronal growth [46]. CNTs are not biodegradable, and as such they could be used as implants where long-term extracellular molecular cues for neurite outgrowth are necessary, such as in regeneration after spinal cord or brain injury [47]. Several studies demonstrated

the usage of CNTs as permissive substrates/scaffolds for cell adhesion and growth. The first study that explored the possibility of using CNTs as substrates for neuronal growth was done by Mattson and colleagues [46]. They grew embryonic rat hippocampal neurons on MWNTs [46] and results showed that the neurons grown on MWNTs displayed an increased number, length, and branching of neurites suggesting that MWNTs can serve as a permissive substrate for neuronal cell adhesion and growth. In a follow-up study [48] surface charge of MWNTs were systematically varied to control the outgrowth and branching pattern of neuronal processes. Hu et al. [48] examined the morphological parameters of live rat neonatal hippocampal neurons in cultures using calcein dye. Their results using live cells were consistent with the initial finding by Mattson and colleagues using fixed cells.

Liopo et al. [49] demonstrated that SWNTs were less supportive to neural cell attachment and growth than unfunctionalized nanotubes. Furthermore, Galvan-Garcia et al. [50] reported that directionally oriented CNTs promoted cell attachment, differentiation and cell growth. Additionally, when highly purified, these CNTs supported the survival of neurons to extend processes and length suggesting that interaction between neurons and CNTs may be affected by the purity of CNTs as well as by the 3-dimensional organization of the CNT substrate/scaffold. Interestingly, carbon threads made from SWNTs [51] were also compatible with neural cells as demonstrated by culturing hippocampal neurons and PC12 cells on them. After a week in culture, neurite outgrowth on the nanothread surface showed neuron specific labelling ( $\beta$ 111 tubulin staining) indicating the biocompatibility of these nanothreads for the construction of electrodes or nanowires in implantable devices. Gabay et al. [52] designed a micropatterned array of CNTs (coated with dimethyl siloxane) to study self-organization of neural networks. The neurons grown on these substrates had accumulated on the CNTs within 4 days showing neuronal processes. Similarly, Sorkin et al. [53] cultured hippocampal neurons on the polydimethylsiloxane stencil coated CNTs substrates and the results showed islands with neurites within 2 to 3 days during culture. These patterned networks could be used to study neuronal networking with CNTs as electrical connections for sensing or stimulation. Additionally, there have been some attempts to use CNTs in stem cell research. The growth and differentiation of NSCs on nanotube substrates was recently demonstrated [54]. Coverslips coated with six layers of SWNTs dispersed

in sodium 4-styrene-sulfonate were used to culture NSCs. Cells adhered and differentiated into neurons, astrocytes and oligodendrocytes as shown by immunoreactivity with nestin, microtubule-associated protein 2 (anti-MAP2), glial fibrillary acidic protein (anti-GFAP), and oligodendrocyte marker O4 (anti-O4). Since there was no adverse effects of growing on nanotube substrates these materials could readily be used for applications involving stem cells. Previously, it was reported that CNTs can increase the differentiation efficiency of NSCs to neurons [33] and the unique formulations of CNTs decreases the macrophage activation indicating the promise for the use of CNTs as novel stem cell delivery vehicles for treating stroke damaged neural tissue [34, 35]. Park et al. [6] used postnatal mouse hypoxic-ischemic model to combine biomaterials science and stem cell biology in the brain [9, 14]. After such a severe ischemic event, scaffolding, such as with polyglycolic acid, a biodegradable polymer, would provide the support system in which multipotent stem cells could begin to integrate with a very hostile environment and ultimately contribute to CNS tissue reconstitution [14]. Stem cells seeded with biomaterials acquire some degree of cyto-architectural organization and generate appropriate synaptic interconnectivity with the host [9]. Recent studies demonstrate that CNTs impregnated with subventricular neural progenitor cells with a structural environment to differentiate and migrate into the injured tissue and minimize the reactive microglial cells around the ischemic core region [39]. Several studies have already shown the ability of CNTs based substrates to mediate the differentiation and electrical stimulation of NSCs [54, 55]. The NSCs have been shown to be biocompatible with CNT substrates with levels of cell viability and the development of neural processes [55]. The effectiveness of carbon CNTs to deliver NSCs into CNS-injured sites, and support their differentiation into neurons, constitutes an essential requirement for the success of regeneration of damaged neural tissues (Fig. 2). Roman et al. [56] demonstrated that SWNT chemically functionalized with PEG were effective in the promotion of axonal regeneration in a rat model of SCI (at T9 vertebral level).

### Toxicity of CNTs in Medical Applications

The use of CNTs in medicine has raised interest in their potential toxicity. The effect of CNTs on living systems has become a highly active area of research over the last decade [57, 58]; however, there is still no broad consensus

on what features of nanoparticles make them more or less biocompatible. Biocompatible nanomaterials are being developed for many cell- and tissue-contacting applications, and therefore biocompatibility is necessarily an important issue. Some of these materials, including CNTs, are rapidly approaching targeted clinical use [1, 59] and it is highly likely that CNTs will find medical uses. However it has become clear that it is impossible to broadly classify CNT as 'nontoxic' or 'toxic,' since their effects on cells are highly dependent on the application. In this respect they are very similar to most other molecules and materials that are used in medicine: their efficacy and side effects depend greatly on the dose, mode of administration and type of exposure. In spite of these concerns, it is likely that CNTs will find utility as experimental tools in neuroscience and in addition will form the basis of new technologies in neuromedicine.

## Future Perspective

CNT/NSCs/MSCs composites, in which CNTs are combined with stem cells as base material, were developed to improve the neurite growth during CNS damages. Applications of CNTs both *in vivo* and *in vitro* study showed similar biocompatibility ability of CNTs impregnated with stem cells which could direct functions of neurons to potentially heal damaged neural tissue. The use of biomaterials in treatment of brain disorders and spinal cord injury may require advances in both neurobiology and scaffolds, but advances in *in vitro* uses will almost certainly underpin therapeutic applications.

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