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Neural interfaces at the nanoscale

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Abstract

Bioelectrical neural interfaces provide a means of recording the activity from the nervous system and delivering therapeutic stimulation to restore neurological function lost during disease or injury. Although neural interfaces have reached clinical utility, reducing the size of the bioelectrical interface to minimize damage to neural tissue and maximize selectivity has proven problematic. Nanotechnology may offer a means of interfacing with the nervous system with unprecedented specificity. Emergent applications of nanotechnology to neuroscience include molecular imaging, drug delivery across the BBB, scaffolds for neural regeneration and bioelectrical interfaces. In particular, carbon nanotubes offer the promises of material stability and low electrical impedance at physical dimensions that could have a significant impact on the future on neural interfaces. The purpose of this review is to present recent advances in carbon nanotube-based bioelectrical interfaces for the nervous system and discuss research challenges and opportunities.

Keywords

charge density; deep-brain stimulation; iridium oxide; microelectrode array; nanofiber; neuron; recording; stimulation

Nanotechnology has had a substantial impact on neuroscience, the study of the brain and the nervous system. Nanotechnology is of particular interest to neuroscience because molecular and signal processing occurs at the micron scale of neurons, which have distinct nanoscale compartments, including synapses, axons and dendrites. Novel applications of nanotechnology to neuroscience have led to improved molecular imaging using quantum dots [1], new strategies for drug/biomolecule delivery across the BBB [2] and control of neural regeneration [3–5] and differentiation [6,7]. These topics have been addressed in previous comprehensive reviews [8,9]. Recently, there has been significant progress in the use of nanotechnology to form bioelectrical contact with cells within the nervous system. These findings have significant implications for decreasing the size and improving the selectivity of neural interfaces, which are devices that enable communication between computers or other devices and the nervous system. The purpose of this article is to review the implications of these recent findings and raise future research directions for the development of nanoscale neural interfaces.

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Neural interfaces for stimulation & recording

Neural interfaces that rely on electrical transduction consist of arrays of electrodes that are in intimate contact with neurobiological substrate. These devices have proved useful in basic science research to elucidate how the nervous system encodes information and have had a significant impact on reducing the burden of neurological disease and injury in afflicted individuals. Examples of clinically useful neural interfaces include the cochlear prosthesis [10], deep-brain stimulation (DBS; FIGURE 1A) [11,12] and neuro-motor prosthesis [13], each of which rely on implanted electrodes delivering electrical stimulation. Arrays of microelectrodes (FIGURE 1B) have been used to monitor microvolt-amplitude extra-cellular potentials from neurons *in vitro* for pharmacological-assay and environmental-biosensing applications [14–16] and *in vivo* to elucidate neural networks involved in behavior [17,18]. Arrays of microelectrodes (FIGURE 1C) have been implanted in the cortex for recording from brain regions associated with movement control or planning [19–21]. In addition, penetrating cortical-electrode arrays capable of stimulation are being pursued for restoration of vision [22]. Despite these advances, reducing the size of the bioelectrical interface to minimize damage to neural tissue and maximize selectivity has proven problematic.

Implantable neural interfaces: size & electrical characteristics

As shown in FIGURE 1, the sizes of the electrodes range from tens of microns to millimeters. For DBS, the surface area of each electrode contact is approximately 6 mm², a size that limits the specificity of stimulation and may contribute to the well-known side effects associated with DBS for movement disorders, such as difficulty with speech [23]. In the case of intracortical microelectrodes, the areas are typically much smaller, less than 2×10^{-3} mm² [24]. Reducing the size of conventional metal electrodes raises the impedance, thereby increasing the thermal or Johnson noise and compromising the ability to transfer electrical charge between the electrode and the tissue [25]. The thermal noise content at an electrode—electrolyte interface is proportional to the square root of the resistive component of the electrode impedance. Large impedance electrodes make it difficult to resolve small extracellular potentials from baseline noise. For electrical stimulation, it is important to avoid faradaic reactions that may result in nonreversible, toxic interactions with the surrounding tissue [26]. Both charge density and charge per phase interact to determine the threshold for neural-tissue damage [27]. To evoke a neural response, a certain magnitude of charge must be delivered in a pulse paradigm that is balanced. However, the amount of charge per electrode surface area should not exceed the maximum charge injection density, a parameter that is a function of electrode material. Surpassing the maximum charge-injection density for a polarizable electrode material may result in excessive faradaic currents owing to electrolytic decomposition of aqueous-phase constituents. The exploration of deposited films, such as activated iridium oxide [28] and conductive polymers [29], to decrease microelectrode impedance and boost charge-injection capacity is an active area of research and development, although significant concerns about the stability of some of these materials exist [30,31]. It is important to note that, in the absence of changes in size, simply a reduction in electrode impedance could decrease the power requirements from DBS implantable pulse generators to improve the operational lifetime of device batteries.

Carbon nanotubes as a bioelectrical interface

There has been noteworthy interest in the use of carbon nanotubes (CNTs) for a range of biomedical applications. CNTs fall into several classes: single-walled, double-walled and multi-walled tube structures. Single-walled CNTs are cylindrically shaped and have a wall thickness of a single atom, and are considered comparatively difficult to fabricate. Double- and multiwalled structures have wall thicknesses of two or more carbon atoms, in which the

simplest structural analogy for double- and multiwalled nanotubes is a rolled-sheet of parchment. Single-walled CNTs appear to offer more precise functionalization strategies that may ultimately improve the robustness of the tissue—device interface [32,33]. In general, CNTs exhibit high aspect-ratio structure and can be treated to yield reasonable electron-transfer kinetics for electrochemical applications [34]. For bioelectrical interfaces, a particularly attractive feature of CNT-coated electrodes is that they can exhibit high specific capacitance and, in fact, are well suited for ‘super-capacitance’ applications [35] showing reduced impedance. Moreover, the maximum charge density for CNT-coated electrodes has been reported to be more than twice that of similarly sized iridium oxide electrodes [36].

Biocompatibility of CNTs

The foremost requirement of any useful neural interface technology is biocompatibility. To date, the majority of studies exploring the biocompatibility of CNTs has focused on comparisons with glass or plastic as a culture substrate in which cell adhesion, neurite extension and synapse formation have been considered surrogate measures of material biocompatibility. Several groups have shown that multiwalled CNTs deposited as intertwined mats are permissive for the growth of rodent primary hippocampal, dorsal root ganglion, cortical and cerebellar neurons, especially after functionalization of the CNTs [37–40]. Similar results have been demonstrated with functionalized single-walled CNTs that form hair-like fibers and deposit on substrates as mats using neuroblastoma-glioma cells, dorsal root ganglion neurons and pheochromocytoma cells [33,41]. There is evidence that these CNT mats can enhance aspects of neuronal growth and function, while also having the capacity to decrease astrocytic function [42]. Based on observations that cultured hippocampal neurons, 8–10 days *in vitro*, exhibited elevated spontaneous synaptic currents on multiwalled CNT mats, Lovat and colleagues suggested that the nanotubes may be providing a pathway for electrotonic-current transfer to reinforce electrical coupling between neurons [38]. It is important to note that the expression of functional synapses in primary neuronal networks *in vitro* is time dependent and subject to significant changes at the beginning of the second week in culture [43]. An alternative explanation may be that the CNT substrates simply accelerate the development of the cultures *in vitro*. Consistent with that notion, growth-cone dynamics in cultures of primary neurons appear to be augmented significantly on CNT substrates [40].

Despite the promising *in vitro* work with CNT substrates, there are a number of studies that demonstrate activation of oxidative-stress pathways in cultured cells. Although these studies have been performed with cells that are not of neural origin, inflammation and reactive-oxygen intermediates are implicated in the performance degradation of chronically implanted neural probes [44,45]. Cell culture studies with keratinocytes [46], fibroblasts [47] and lymphocytes [48] have revealed that high concentrations of CNTs induce cytotoxicity, possibly through oxidative stress [49]. In macrophages, CNTs trigger overproduction of TNF- α , a cytokine implicated in inflammation [50]. Aggregates or bundles of CNTs may be even more problematic. *In vitro* cytotoxicity of agglomerated CNTs was demonstrated in both murine lung macrophage [51] and human lung [52] cell lines. The effective local concentrations of CNTs agglomerated at microelectrode sites may be sufficiently large that local cytotoxic effects may emerge and contribute to the loss of recording sites *in vivo* during chronic recording. Interpretations from the present literature are complicated by the observations that CNT bio-compatibility may be different for single- versus multiwalled CNTs and may be influenced by purity and functionalization [53,54].

Mesh-deposited CNTs as bioelectrical interfaces

Demonstrations that CNTs can be used for recording and stimulation of neural tissue have been reported recently, most of which have been accomplished using meshes of deposited CNTs on a substrate or electrode contact (FIGURE 2A). Liopo *et al.* showed that whole-cell currents elicited by cathodic stimulation through single-walled CNT-based extracellular electrodes were indistinguishable from those currents triggered through whole-cell voltage clamping with step potentials in both neuroblastoma—glioma and rat dorsal root ganglion neurons [33]. Although these initial results suggest simple resistive coupling with the extracellular region surrounding the cell depolarizes the membrane effectively, a more complex coupling between the single-walled CNT substrate and cultured neurons has been proposed. Based on simultaneous patch measurements and modeling of hippocampal neurons on single-walled CNTs, Mazzatenta *et al.* raised the possibility of more intimate and direct resistive coupling into the interior of the cell via the CNT substrate [55], although a more definitive characterization of the CNT—cell-membrane junction is still required. Beyond substrate coatings, there have been recent efforts to produce CNT-coated microelectrodes for neural recording and stimulation. Gabay *et al.* fabricated conducting tracks and recording sites of conductive titanium nitride on p-type silicon substrates using lithography [56]. After deposition of a Ni catalyst layer on recording sites, CNTs were synthesized by chemical-vapor deposition at 900°C. They reported that dense and intertwined meshes of CNTs grown over microelectrode contacts results in a large drop in impedance over bandwidths appropriate for resolving extracellular potentials. In fact, proof-of-concept recording from rat cortical neurons shows well-resolved spikes with exceptional signal-to-noise characteristics. The manufacturing process, however, is a significant limitation. The use of extremely high temperatures and Ni as a catalyst may limit the types of electrode materials and raise concern for Ni leaching. Most recently, Keefer *et al.* has shown directly that multiwalled CNTs deposited as a mesh on microelectrode sites enable improved neuronal recordings *in vitro* and *in vivo* [57]. *In vitro* studies with embryonic mouse cortical neurons were conducted on planar microelectrode arrays in which the microelectrode sites consisted of patterned indium-tin oxide coated with CNTs using electrodeposition. CNT-coated microelectrode sites showed significantly lower impedance and noise levels, as well as enhanced charge capacity for stimulation, compared with gold-coated microelectrode sites. *In vivo* studies in the rat motor cortex and the monkey visual cortex were performed both using gold-coated tungsten sharpened wire electrodes. CNTs were either covalently attached to amine-functionalized gold surface of the electrodes or combined with the conductive polymer polypyrrole and electropolymerized to the electrodes. Both strategies yielded *in vivo* measurements that showed reduced impedance and noise, enabling simultaneous measurements of local field potentials and spike activity from the same electrode site. It is important to note that coating procedures, which included electrochemical deposition, covalent modification and electropolymerization of conductive polymers, could be conducted at room temperature with metallic substrates typically used in neurophysiological recording.

Vertically aligned CNTs as bioelectrical interfaces

Most of the previously described work involves meshes of CNTs on electrodes, however, alignment of CNTs may offer added advantages to interfacing with cells and tissues by providing a 3D character to the electrode (FIGURE 2B). Yu and coworkers demonstrated a vertically aligned carbon-fiber electrode array in which the electrodes comprised conical CNT fibers, grown 10 µm in height, at sites lithographically defined through chemical-vapor deposition [58]. Although the impedance of the spire-shaped electrodes was not reported, the noise levels and charge injection capacity were consistent with other types of similarly sized electrode contacts and the extracellular recording/stimulation data from organotypic

hippocampal slices were presented. With respect to dense packing of aligned CNTs, there has been progress in the development of vertically aligned CNTs that can tolerate aqueous conditions necessary for *in vitro* and *in vivo* applications. Nguyen-Vu *et al.* showed that a thin layer of polypyrrole provided the necessary mechanical strength for a carbon-nanofiber array, consisting of multiwalled CNTs, to maintain its architecture in aqueous environment [59]. The resulting array was permissive for the cultivation of a model neural cell type, PC12, such that neurites grew interwoven among the nanofibers [60]. Importantly, electrodes with aligned CNTs still exhibited significantly reduced impedances compared with a standard metallic interface, iridium oxide, of similar surface areas, which suggests that sizes for stimulation and recording electrodes may be minimized readily without performance decrements [59]. Reports of extracellular recording from bioelectrically active cells using these densely packed CNT-coated electrodes are likely to emerge in the near future.

Conclusion & future perspective

Progress with CNT-based electrodes has thus far been promising for improving the quality of the bioelectrical interface with the nervous system. Beyond enhanced electrical stimulation and recording capabilities, CNTs offer the possibility of voltammetric detection of oxidizable neurotransmitters, such as dopamine, which could be used in an implantable device as part of a feedback-control system [61]. Nevertheless, there are several opportunities for research and development to more fully understand these nanoscale interfaces and translate these findings from the bench to the clinic.

First, there needs to be a comprehensive, quantitative characterization of neuron—CNT junctions. The characterization work to date has relied on inadequately voltage-clamped cells on relatively large substrates and coated mesh-deposited CNTs substrates, such that there are significant shunt pathways that complicate the modeling and analysis of the junction [33,55]. There may be significant differences between junctions comprising mesh-deposited CNTs versus vertically aligned, densely packed CNTs. It is possible that alignment may promote cell-electrode coupling via bridging the cell membrane in a minimally destructive manner. There are several examples in the published literature in which both single- and multiwalled CNTs have been used as transporters or ‘nanoinjectors’ to introduce bioactive molecules across membranes [62–65], suggesting that appropriately modified and oriented CNTs might promote bioelectrical access. Voltage- and current-clamp experiments of neurons in intimate contact with the CNT-coated microelectrode sites need to be performed using a range of small- and large-amplitude input signals to generate an electrical equivalent of the junction, similar to prior work with metal electrodes and field-effect transistor interfaces [66–68].

Second, robustness of the tissue-device interface needs to be fully characterized. As an initial step, the CNT-electrode durability needs to be demonstrated fully. Typically, *in vitro* soak tests in saline solutions for 6 months to 1 year are performed with an end point of measured impedance. The bathing temperature can be elevated well beyond physiological levels to accelerate life-time testing [69].

Third, long-term tests *in vivo* need to be performed to examine CNT-electrode degradation and interactions at the tissue—device interface. Degradation of the CNT electrode could be assessed by examination of tissue after implantation with ¹³C-enriched CNTs to aid in visualization [54]. *In vitro* studies would be useful to explore whether or not oxidative stress processes are activated with CNTs in neural cultures. *In vivo*, the degradation of the tissue within 50–100 μm of conventional implanted neural probes negatively impacts the recording of μV level signals [19]. Therefore, detailed histological examination in close proximity to

the device needs to be performed to characterize long-term biocompatibility. Should problems become apparent, there are options to explore. For example, CNTs can be used for drug delivery [70] and perhaps CNT-based electrodes could be loaded with anti-inflammatory compounds or other bioactive molecules to promote tissue-device integrity.

Executive summary

- Emerging applications of nanotechnology in basic and clinical neuroscience include molecular imaging, drug/gene delivery across the BBB, nanoscale materials for tissue engineering and regenerative medicine and bioelectrical interfaces.
- Carbon nanotube (CNT)-coated electrodes exhibit high specific capacitance and a high maximum-charge density, enabling the development of smaller bioelectrical interfaces with reduced impedance.
- Biocompatibility studies to date have shown that neural cells can thrive on CNT-based substrates *in vitro*.
- Recent studies have also shown that mesh-deposited CNTs improve neuronal recordings *in vitro* and *in vivo*, in which CNT-coated electrode sites showed significantly lower impedance and noise levels, as well as enhanced charge capacity for stimulation. Arrays of vertically aligned CNTs have been synthesized and recordings from neural tissue *in vitro* have been reported.
- Future efforts should include a quantitative characterization of the neuron—carbon nanotube junction, validation of CNT durability and effects of any degradation on surrounding tissue through detailed histological examination and possible incorporation of neuroprotective compounds into CNTs to promote neural tissue viability.

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Figure 1. Examples of neural interfaces

(A) Deep-brain stimulation electrode (Medtronic) used clinically to relieve the motor symptoms associated with movement disorders, including Parkinson's disease and essential tremor (generously provided by WM Grill, Duke University, NC, USA). (B) Planar microelectrode array with cultured murine neuronal network. The microelectrode array consists of a lithographically patterned matrix of indium—tin oxide conductors passivated with polydimethylsiloxane. Laser exposure to de-insulate at the end of each conductor pattern produced 64 uniformly spaced microelectrode sites (scale bar = 200 μ m). (C) Scanning electron microscope image of Utah microelectrode array consisting of 100

electrodes microfabricated from silicon with iridium oxide tips (scale bar = 1 mm; generously provided by F Solzbacher, University of Utah, UT, USA).

Figure 2. Carbon nanotube-based bioelectrical interfaces

Scanning-electron microscopy images of (A) mesh-deposited carbon nanotube-coated microelectrode site (scale bar = 2500 nm). Generously contributed by EW Keefer, University of Texas Southwestern, TX, USA. (B) vertically aligned carbon nanofibers (scale bar = 500 nm). Generously provided by J Li, Kansas State University, KS, USA).