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CHAPTER 18

Interfacing neurons with carbon nanotubes: (re)engineering neuronal signaling

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Abstract: Carbon nanotubes (CNTs) are cylindrically shaped nanostructures made by sheets of graphene rolled up to form hollow tubes. Owing to their unique range of thermal, electronic, and structural properties, CNTs have been rapidly developing as a technology platform for biological and medical applications, including those designed to develop novel neuro-implantable devices. Depending on their structure, CNTs combine an incredible strength with an extreme flexibility. Further, these materials exhibit physical and chemical properties which allow them to efficiently conduit electrical current in electrochemical interfaces. CNTs can be organized in scaffolds made up of small fibers or tubes with diameters similar to those of neural processes such as axons and dendrites. Recently, CNT scaffolds have been found to promote growth, differentiation, and survival of neurons and to modify their electrophysiological properties. These features make CNTs an attractive material for the design of nano–bio hybrid systems able to govern cell-specific behaviors in cultured neuronal networks.

The leading scope of this short review is to highlight how nanotube scaffolds can impact on neuronal signaling ability. In particular, we will focus on the direct and specific interactions between this synthetic nanomaterial and biological cell membranes, and on the ability of CNTs to improve interfaces developed to record or to stimulate neuronal activity.

CNTs hold the potential for the development of innovative nanomaterial-based neurological implants. Therefore, it is particularly relevant to improve our knowledge on the impact on neuronal performance of interfacing nerve cells with CNTs.

Keywords: carbon nanotubes; neuronal network; spontaneous activity; hippocampal culture; afterdepolarization; neural interfaces.

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Introduction

Among the emerging applications of nanotechnology to neuroscience, the development of artificial nanomaterials, such as carbon nanotubes (CNTs) or nanofibers, raises particular interest for their potential exploitation in next-generation scaffolds for nerve tissue engineering (Gilmore et al., 2008), or in long-term implants such as neural interfaces (Keefer et al., 2008; Kotov et al., 2009). The unique physical–chemical features of CNTs allow the development of a variety of miniaturized devices with remarkable properties (Krishnan et al., 1998). CNT soluble preparations have been also proposed for the fabrication of novel molecular sensing, diagnostic, or drug delivery devices (Pantarotto et al., 2004).

More recently, CNTs have shown the ability to govern several neuronal behaviors when used as platforms to support the growth in cultured cortical circuits (Cellot et al., 2009; Galvan-Garcia et al., 2007; Hu et al., 2004; Lovat et al., 2005; Mattson et al., 2000; Ni et al., 2005).

CNTs are cylindrical nanostructures, composed of a single graphene sheet rolled up and closed at each end by hemispherical fullerene caps. They show a radius of a few nanometers and are characterized by a high aspect ratio and a variable length, usually below 1 μm. In the past decade, two different types of CNTs have been extensively used in basic neuroscience research: (i) single-walled carbon nanotubes (SWCNTs) and (ii) multi-walled carbon nanotubes (MWCNTs), made up of multiple concentric graphite cylinders. SWCNTs' and MWCNTs' shape and size mimic the morphology of small neuronal processes (Gilmore et al., 2008) and their high electrical conductivity combined to the large surface area may increase charge injection capacity of CNT-based microelectrodes (Kotov et al., 2009). These features make CNTs intriguing candidates for nervous system applications.

CNT-based devices are expected to fulfill the requirements of safety of use, biocompatibility, and mechanical and electrochemical stability in a biological environment, in order to be used for the development, implementation, and improvement of neuronal interfaces and electrodes (Keefer et al., 2008; Kotov et al., 2009; Lu et al., 2010; Nguyen-Vu et al., 2006). In this framework, it is highly relevant to improve our knowledge on the direct effects that nanotubes might have on neuronal signaling ability, by means of CNTs/cell membrane "direct" interactions, or, alternatively, on neurite formation ability, by instructing dendrites' and axons' growth and/or branching. SWCNTs- and MWCNTs-induced modulations may thus, in principle, alter neural signal integration at multiple levels.

This review will highlight the impact of CNTs on nerve cell functions (e.g., electrical regenerative properties and synaptic activity) and on nerve cell morphological features (e.g., adhesion, growing, and neurite/dendrite extension abilities) in cultured neuronal networks. We will also address the more recent issue regarding the application of CNTs as neural interfaces for recording and/or stimulating neuronal activity.

The biocompatibility of CNT-based substrates and the impact of CNTs on nerve cell signaling

The first report on CNT biocompatibility dates back to 2000, when Mattson et al. (2000) showed that dissociated hippocampal neurons attached and grew on glass coverslips coated with as-prepared MWCNTs or with 4-hydroxynonenal functionalized MWCNTs.

Several studies assessed and confirmed later, in cultured neurons, the biocompatibility of CNT growth platforms (Gheith et al., 2005; Hu et al., 2004; Lovat et al., 2005; Malarkey et al., 2009), but the properties of MWCNT/neurons hybrid networks from the electrophysiological and functional point of view were investigated for the first time only in 2005 by Lovat and collaborators. These authors studied how neurons reconstructed a functional network when integrated to non-functionalized MWCNTs (Lovat et al., 2005).

In this work, a homogeneous and purified meshwork of MWCNTs on glass (Fig. 1a) was obtained by first functionalizing CNTs by means of the 1,3-dipolar cycloaddition reaction, then dissolving them in dimethylformamide to allow purification, deposited on glass substrate and heated to eliminate residual functionalization (Fig. 1b). The authors cultured dissociated hippocampal neurons on MWCNT-covered glass substrates and recorded neuronal activity using traditional, single-cell electrophysiological techniques. They confirmed that cultured hippocampal circuits grew well on a conductive MWCNTs meshwork and, remarkably, they report that the frequency of spontaneous synaptic activity (measured as postsynaptic currents) was always significantly enhanced when compared to that of control neurons grown on pure glass coverslips. This boost in spontaneous network activity was accompanied by an increase in the frequency of action potential firing (Fig. 1c). The subsequent work by Mazzatenta et al. (2007) demonstrated that SWCNTs were able to improve synaptic activity in cultured networks in a similar fashion. The hypothesis that the increased network activity found in neurons grown on CNT scaffolds

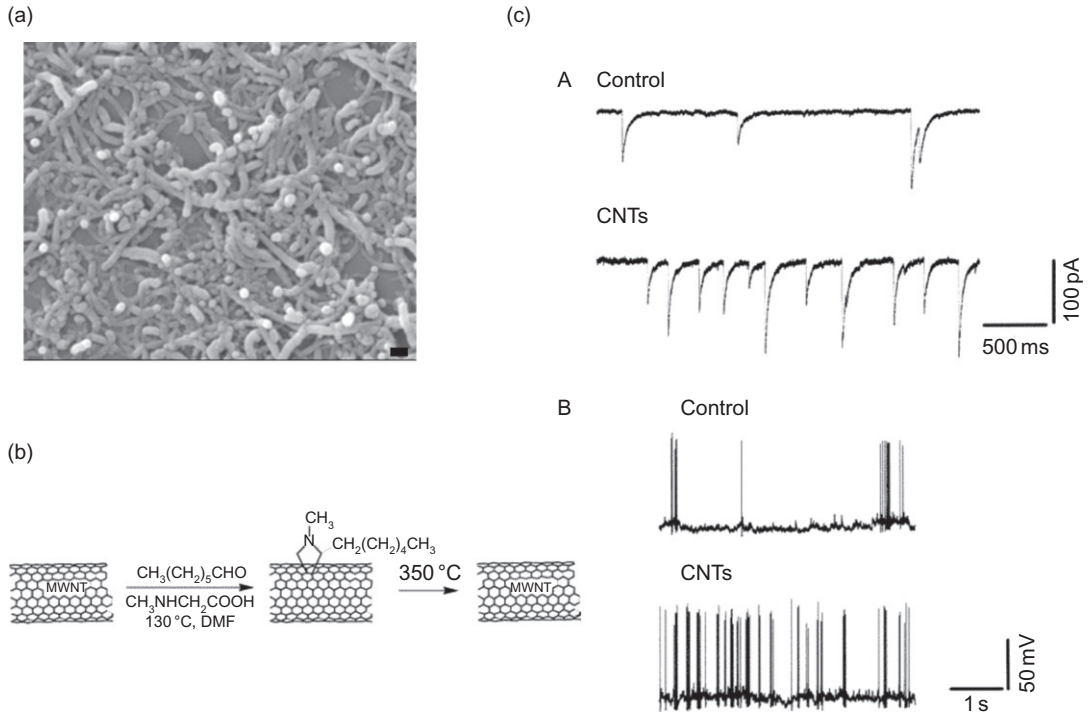

Fig. 1. Carbon nanotube impact on neuronal performance of cultured hippocampal networks. (a) SEM micrograph showing the appearance of a purified MWCNT layer, used as growing substrate for cultured hippocampal neurons. Scale bar: 200 nm. (b) Schematic representation of the protocol used for the preparation of homogeneous and purified carbon nanotubes meshwork by the 1,3-dipolar cycloaddition reaction. MWCNT is first functionalized to allow purification and then deposited on glass and heated to 350°, to eliminate the functionalization preserving the carbon nanotube network. (c(A)) Spontaneous synaptic currents recorded from hippocampal neurons in control condition (glass) and on a carbon nanotubes (CNTs) layer like the one shown in (c(A)). (c(B)) Spontaneous firing activity of cultured neurons in control and on carbon nanotubes (CNTs). Note the large increase in the frequency of both spontaneous postsynaptic currents and action potentials. Adapted with permission from Lovat et al. (2005).

could be related to an increased number of neurons adhering on such substrates was ruled out by visualization and quantification of cultured neurons via immunocitochemistry experiments, where antibodies against specific protein markers were used (Lovat et al., 2005; Mazzatenta et al., 2007). These studies demonstrated that the detected functional differences were not merely due to the construction of networks of different sizes. In particular, a direct quantification of hippocampal cells, by labeling the cultures with antibodies targeted to microtubule-associated protein 2 (MAP-2), showed that the neuronal density did not differ between control and CNT cultures and that the general morphology of neurons, monitored as the distribution of cell body diameters (Mazzatenta et al., 2007) and the number of neurites departing from the neuronal soma (Lovat et al., 2005; Mazzatenta et al., 2007), were similar in the two growing conditions (control and SWCNTs or MWCNTs). Additionally, the electrophysiological characterization of the passive membrane properties of the recorded neurons (membrane capacitance, input resistance, and resting membrane potential), generally accepted as useful indicators of the cellular dimensions and health, were fully comparable between control and neurons developed on CNT films (Cellot et al., 2009; Lovat et al., 2005; Mazzatenta et al., 2007). Overall, these results indicated that the increased spontaneous activity detected in the presence of pure, non-functionalized, MWCNT or SWCNT scaffolds were not due to differences in neuronal density or cell morphology. Thus, other phenomena, regardless of network sizes, were governing the interplay between CNTs and neurons.

In this context, the occurrence of extensive, intimate, and tight contacts between neuronal membranes and CNT growth platforms was demonstrated by scanning and transmission electron microscopy (SEM and TEM, respectively) analysis (Cellot et al., 2009; Mazzatenta et al., 2007; Fig. 2) and suggested a direct (maybe even electrical) coupling of CNTs to neuronal processes. Such interactions are potentially responsible for the effects of these scaffolds on neuronal performance, which relapse at the network level. A parallel work from Sorkin et al. (2009) supported this hypothesis, showing that process entanglement is a key mechanism by which neurons anchor to rough surfaces (such as CNTs), thus contributing to the physical interactions between neurons and nanotube substrates.

The work by Cellot et al. (2009) specifically addressed the emergence of an electrical coupling between CNTs and neuronal membranes. The authors used single-cell electrophysiology techniques to demonstrate that nanotubes are able to reengineer the integrative electrical abilities of hippocampal neurons maintained *in vitro*. In this study (Cellot et al., 2009), neurons were cultured on CNT substrates (SWCNTs or MWCNTs) or on control substrate (glass coverslips). Neurons were patched-clamped in whole cell configuration and forced to fire short trains of action potentials at variable frequencies (20–100 Hz) in order to maximize, at the single-cell level, the interactions between the regenerative properties of the proximal and distal cell compartments (Larkum et al., 1999). With these experiments, Cellot et al. (2009) showed that neurons grown onto CNT carpets were more prone to generate back-propagating action potentials, a neuronal regenerative property known to be involved in the regulation of local synaptic feedback and in the release of chemical messengers (Kuczewski et al., 2008; Waters et al., 2005; Zilberter et al., 2005). Back-propagating action potentials were unmasked due to the appearance of additional subthreshold somatic depolarization after short trains of action potentials (Fig. 3a and b; Cellot et al., 2009; Larkum et al., 1999). The authors also showed that the nanotube-induced modification of the dendritic electrogenic properties resulted in an increase in single-cell excitability: this effect may explain, at least in part, the boosting of spontaneous activity that was always observed in neurons

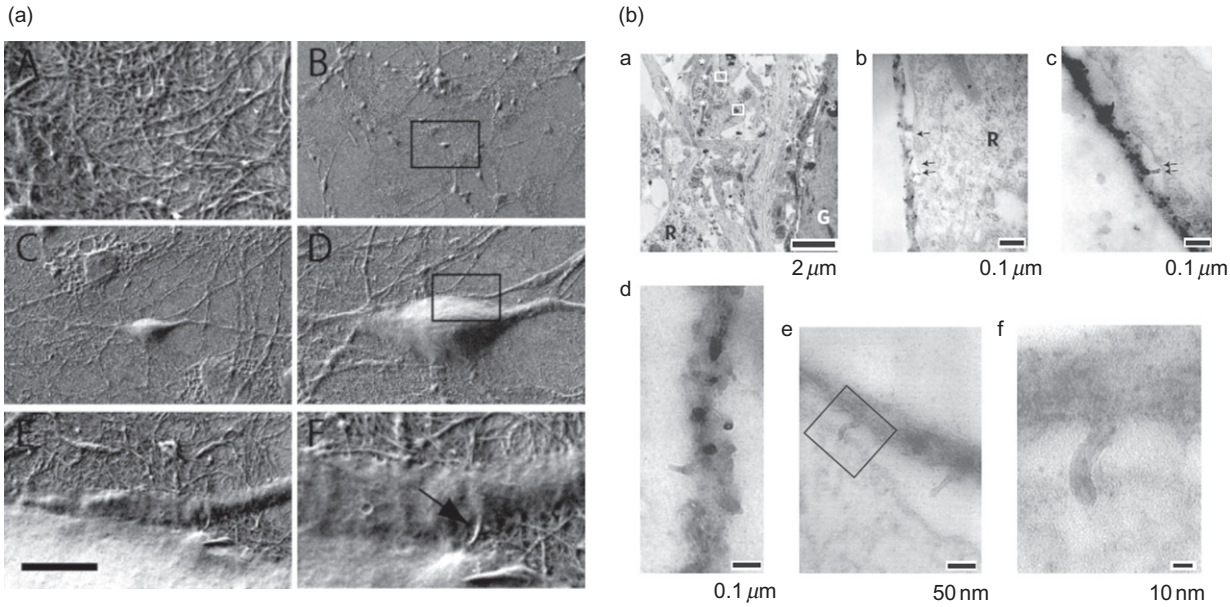

Fig. 2. Neuronal membranes of cultured hippocampal neurons make extensive and intimate contacts with the carbon nanotubes layer. (a) SEM images of hippocampal neurons cultured on carbon nanotubes. A, detail of the SWCNT layer; B–D, subsequent micrographs at higher magnifications of a hippocampal neuron grown on SWCNT. Note the extensive outgrowth of neurites on the carbon nanotube meshwork and the intimate contacts between carbon nanotube and neuronal membrane. Scale bar (in E): A, 1 μm; B, 200 μm; C, 25 μm; D, 10 μm; E, 2 μm; F, 450 nm. Reproduced with permission from Mazzatenta et al. (2007). (b) TEM images of hippocampal neurons cultured on MWCNTs. a, TEM planar section of hippocampal neurons grown on MWCNTs, showing healthy organization of neuronal networks. "R" indicates clusters of ribosomes and "G" a presumed glial cell (with the typical electron dense cytoplasm). b and c, TEM sagittal sections showing MWCNT-membrane contacts (arrows). d, MWCNT morphology. e and f, high-magnification micrographs showing MWCNT "pinching" neuronal membrane. Reproduced with permission from Cellot et al. (2009).

cultured on purified CNTs (Cellot et al., 2009; Lovat et al., 2005; Mazzatenta et al., 2007). Cellot et al. (2009) further explored which specific property of CNTs (i.e., their nanostructure, providing a characteristic nano-roughness to the scaffold surface, or their electrical conductivity) is the key one needed to successfully alter neuronal regenerative ability. To address this issue, neurons were cultured on materials different from CNTs, but presenting either comparable nano-roughness or comparable electrical conductivity. In particular, Cellot et al. (2009) used the self-assembling peptide RADA 16 substrates to mimic CNT-like three-dimensional nanostructure in the absence of electrical conductivity, and indium tin oxide (ITO) substrates to reproduce CNT-like conductivity in the absence of a three-dimensional nanostructure. In both cases, these substrates were unable to replicate CNT effects on neuronal regenerative properties and excitability. The authors therefore suggested that both SWCNTs and MWCNTs mediated their effects via the coexistence of both conductivity and nanostructure (Cellot et al., 2009).

With a combination of electrophysiological techniques and theoretical modeling, Cellot et al. (2009) tried to correlate, even if speculatively, the effects detected at the single-cell level to those displayed at the network level. In particular, they succeeded in predicting by network modeling the impact of the improved dendritic regenerative properties brought about by CNT scaffolds on the

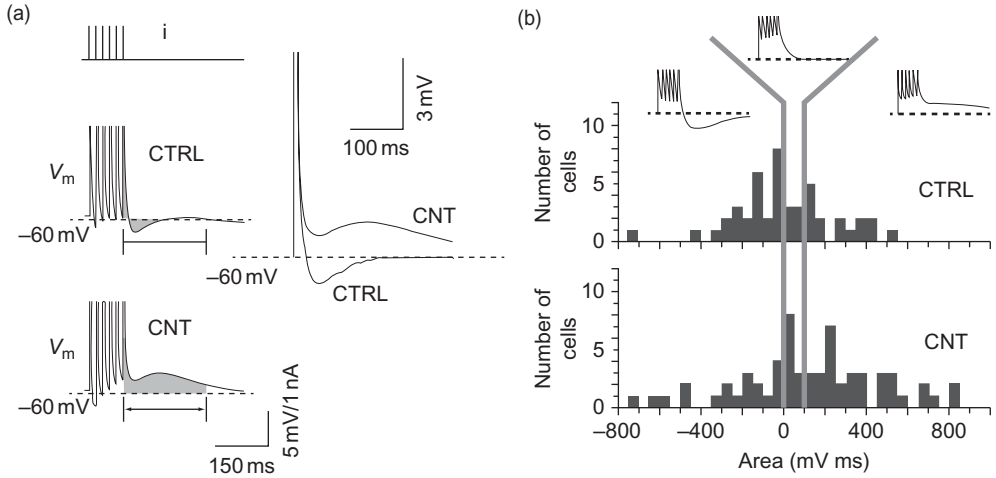

Fig. 3. Carbon nanotubes increase neuronal excitability. (a) hippocampal neurons cultured on glass (CTRL) or on a carbon nanotube (CNT) layer were forced to fire a regular train of six action potentials, to evaluate the presence of an additional hyperpolarization (afterhyperpolarization, AHP) or depolarization (afterdepolarization, ADP; shaded areas) after the last action potential of the train. Control neurons typically showed AHP; conversely, the majority of neurons grown on carbon nanotubes showed a marked ADP. (B) area distributions showing the frequency of occurrence of AHP (left), neutral response (center), or ADP (right). The distribution of carbon nanotube neurons is shifted to the right, indicating an increase in the occurrence of ADP, indicative of enhanced single-cell excitability. Reproduced with permission from Cellot et al. (2009).

emerging network behavior. In the proposed model, they enriched a circuit composed by randomly connected individual neurons with back-propagating action potentials. The resulting electrical activity generated by the modeled circuit showed the occurrence of bursting of synaptic events of prolonged duration, without a clear increase in the frequency of events. This prediction was confirmed by voltage clamp recordings performed on neuronal networks developed on SWCNT substrates (Cellot et al., 2009).

The molecular mechanisms underlying the generation of back-propagating action potentials in neurons grown on CNTs are still to be elucidated. Nonetheless, focusing on the excellent conductive properties of nanotubes and on their ability to form tight contacts with neuronal membranes, it is tempting to speculate that CNTs are able to facilitate the generation of back-propagating action potentials via promoting a direct electrical shortcut between adjacent compartments on dendrites, a provocative hypothesis supported by theoretical modeling (Cellot et al., 2009).

Regardless of the mechanisms involved in CNTs/neurons interactions, it should be considered that back-propagating action potentials have been involved to various extents in the regulation of synaptic short- and long-term plasticity (Kuczewski et al., 2008; Waters et al., 2005; Zilberter et al., 2005). This issue should probably be explored in future studies, in order to deepen our knowledge of the mechanisms underlying CNTs long-term effects on the activity of neuronal networks.

CNTs as interfaces for neuronal stimulation and recording: improving electrode performance by CNTs

The extraordinary electrical conductivity, surface area, and strength of CNTs make them excellent candidates for interfacing neural systems, in view

of the development of robust and biocompatible neuroprosthetic devices. The fundamental requirements for further miniaturization of electrodes can be significantly improved by CNT coatings, since their high surface area can drastically increase charge injection capacity and decrease the interfacial impedance with neurons (Kotov et al., 2009). The understanding of the potentials linked to CNTs exploitation in neuro-electrode engineering, or to the development of nanotubes/neural interfaces, is far from the complete realization.

The possibility of delivering electrical stimulation to neurons through CNT layers was investigated by several groups (Liopo et al., 2006; Mazzatenta et al., 2007; Wang et al., 2006), which demonstrated that CNTs offer a suitable and efficient interface for the direct stimulation of neuronal cells seeded on the nanotubes themselves. An original approach to this issue is represented by the work of Gheith et al. (2006), who developed a layer-by-layer (LBL) technique to produce CNT films that were used as growing substrates for neurons. Using this LBL approach, the authors were able to stimulate neurons by direct applications of voltage steps to the CNT films, eliciting an extrinsic stimulation to neuronal cells. The authors hypothesized that this stimulation was due to a cation's influx inside the cell. More recently, Cho and Borgens (2010) blended MWCNTs with type IV collagen, and using this blend as substrate for PC12 cells culturing, they reported that the MWCNT/extracellular matrix molecule blend can be employed to electrically stimulate neurons.

A significant advance in the development of CNT/neural interfaces for the recording of neuronal activity was recently made by Keefer et al. (2008), who showed that CNTs coatings decreased electrode impedance and increased charge transfer, paving the way to an extensive and fruitful employment of SWCNTs and MWCNTs in the development of electrophysiological recording and stimulation techniques.

Recently, Lu et al. (2010) tested the suitability of the use of polypyrrole (PPy)/SWCNT films for nanosurface modifications of electrodes. After showing that PC12 cells grown on PPy/SWCNT-covered ITO surfaces extended longer processes and constructed more complex neurite networks, when compared to control ITO surfaces, the authors studied the consequences of the implantation of PPy/SWCNT-covered Pt devices (where SWCNTs were electrodeposited on the electrodes) in the rat brain and compared it with the noncovered Pt implants. In this experimental model, the authors demonstrated that there was less gliosis and increased neuronal survival around the site of PPy/SWCNT implants, when compared to Pt alone. Even if the authors did not dissect the effects of PPy in itself from those of SWCNTs in itself, in terms of neuronal survival and gliosis, this study paves the way for developing systematic, long-term assessments of CNT-based interfaces on neuronal tissue *in vivo*.

In the last few years, several reports focused on the use of CNTs as nanostructured electrodes for the multisite delivering of electrical stimuli or for the recording of neuronal electrical signals. One of the first works dealing with this issue was that of Gabay and colleagues (2005), who described a novel class of microelectrodes based on high-density CNT islands deposited onto titanium nitride conductor-based chips, with a lithographically defined pattern, where CNT sites served as working electrodes. CNT-based electrodes were fully biocompatible and their enhanced electrochemical properties allowed high-fidelity extracellular recordings of electrical activity of cortical neurons, directly seeded on the electrodes.

One of the first works showing that SWCNTs are able to modify neuronal performance, when employed in the fabrication of recording electrodes was that of Khraiche et al. (2009). The authors cultured rat hippocampal neurons on micro electrode arrays (MEAs) in which several drops of SWCNTs solution were deposited at the tip of gold microelectrodes. The authors observed that the electrical activity of rat hippocampal neuronal networks developed on SWCNTs-modified MEAs was detected as soon

as 4 days after seeding and continued to grow till day 7, while neurons developed on control (bare gold) electrodes showed no electrical activity till day 7. The authors suggested that the increase in surface roughness due to SWCNTs immobilized on the microelectrodes provides cells with a larger surface area to adhere leading to an increase in the activation of adhesion molecules (such as integrins), which might in turn promote a faster neuronal differentiation (Khraiche et al., 2009).

An additional work where CNTs-modified MEAs were shown to significantly improve neuron/electrode interfacing and neuronal recordings is that reported by Shein et al. (2009). The authors coated MEA electrodes with CNTs, obtaining islands with a conductive, three-dimensional, exceptionally high surface area. Dissociated cortical neurons cultured on these arrays adhered only and directly to these islands, and self-assembled in neuronal network patterned on the CNT neurochip. Once the neurons had adhered and self-organized, the CNT–MEA allowed very high fidelity, direct recording of neuronal activity, and an effective electrical stimulation of neurons, at the electrode sites.

Notably, Shoval et al. (2009) took advantage from an analogous MWCNTs-modified MEA and showed the impact of this device on neuronal operation, by recording the activity of whole-mount retinas isolated from the neonatal mouse (Fig. 4a). After minutes from the placement of the retinas on the electrodes, Shoval et al. (2009) could obtain electrical recordings of the spontaneous, typical, propagating retinal waves. When compared to those detected from commercially available electrodes, recordings from MWCNTs–MEAs showed a consistently higher signal-to-noise ratio (Fig. 4b). Moreover, the authors observed a prominent increase in the amplitude of the recorded spikes over a period of minutes to hours. Shoval et al. (2009) proposed that this phenomenon is probably due to an improvement in cell–electrode coupling, resulting from a dynamic interaction between MWCNTs and neurons. It should not be excluded however, that MWCNTs might have intrinsically modified neuronal network activity, because of their properties (see the section "The biocompatibility of CNT-based substrates and the impact of CNTs on nerve cell signaling"). In the same work, the authors also validated the suitability of their MWCNT electrodes for neuronal stimulation (Shoval et al., 2009).

The advantages of CNT–MEAs over metal electrodes in neuronal recordings were further confirmed by the work of Gabriel et al. (2009). These authors deposited SWCNTs directly on standard platinum electrodes to fabricate MEAs for electrophysiological recordings. In this report, the application of SWCNT-modified MEAs to record electrical activity from whole-mount rabbit retinas allowed a very low noise recording of multiunit activity in comparison with standard, platinum electrode-based MEAs (Gabriel et al., 2009).

The impact of CNTs on neuronal morphology and growth

In the perspective of employing CNT-based scaffolds to support neuronal network formation and to interface neuronal function, the ability of CNTs to promote neuronal attachment and neurite extension and/or branching is highly relevant. Several groups addressed this issue, employing either SWCNTs or MWCNTs, as-prepared or functionalized; alternatively, SWCNTs were even codeposited with (conductive) polymers (PEG, PPy, etc.; Lu et al., 2010; Malarkey et al., 2009; Ni et al., 2005).

The idea that different functionalizations may strongly affect CNT-mediated effects on neuronal morphology and survival (see e.g., Hu et al., 2004; Mattson et al., 2000; Ni et al., 2005) already emerged from these first studies. In their

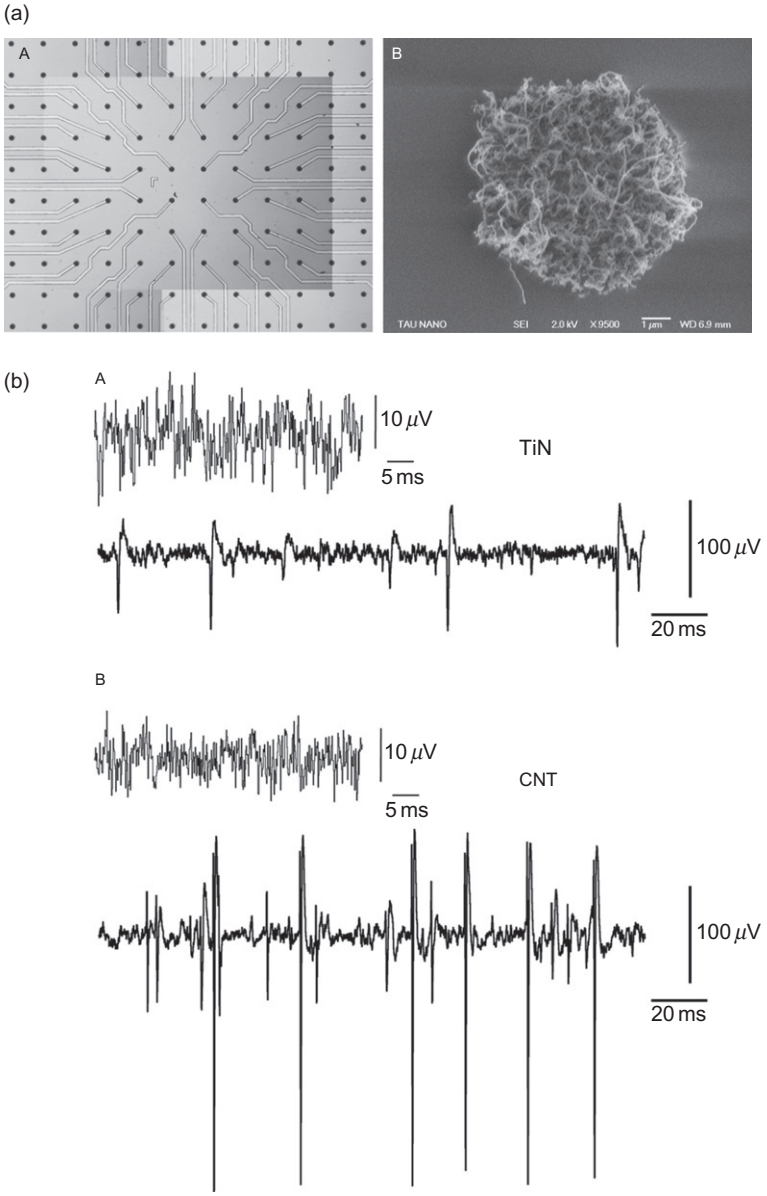

Fig. 4. Application of carbon nanotube-based MEAs for neuronal interfacing and recording. (a) Left, view of a carbon nanotube–MEA. Each of all 60 electrodes in the field ends with a carbon nanotubes island (black dots). Right, micrograph showing the three-dimensional and rough surface of an MWCNT island. (b) Application of the MWCNT–MEA to record neural activity from a whole-mount mouse retina. Top, recordings of spontaneous activity (unfiltered and filtered) with commercial titanium nitride (TiN) electrodes. Bottom, recordings (unfiltered and filtered) obtained with an MWCNT electrode. Note the striking increase in the signal-to-noise ratio. Reproduced from Shoval et al. (2009).

pioneering work, Mattson et al. (2000) showed that neurons grown on MWCNTs functionalized with 4-hydroxynonenal constructed more elaborated neuritic trees, with an increased number of processes, longer neurites, and higher branching occurrence, when compared to neurons grown on unmodified MWCNTs. In 2004, Hu and colleagues tested various functionalized MWCNTs in their ability to affect neuronal morphology. The authors tested at pH 7.35 negatively charged (−COOH), neutral (poly-*m*-aminobenzene sulfonic acid: PABS, zwitterionic), and positively charged (ethylenediamine: EN) functionalized MWCNTs, and demonstrated that the processes' average length was higher in neurons grown on positively charged MWCNT–EN and the amount of neurites' branching progressively increased from negatively (MWCNT–COOH) to neutral (MWCNT–PABS) and to positively charged (MWCNT–EN) CNTs.

The issue of the electrical charge and conductivity of SWCNTs as modulatory factors in mediating CNT effects on neuronal outgrowth was explored by Malarkey et al. (2009). These authors produced films of polyethylene glycol (PEG)-functionalized SWCNTs of increasing conductivities (from 0.3 to 42 S/cm) and cultured hippocampal neurons on these different substrates or on polyethylenimine (PEI) as control. They showed that the neurites' lengths and branches per neuron were significantly higher in neurons grown on PEG–SWCNTs with the smallest conductivity (0.3 S/cm) than those grown on PEI substrates (controls) or on PEG–SWCNTs with larger conductivity values.

It is interesting to note, however, the absence of such effects on neuronal morphology, growth, and branching, when using pure, non-functionalized CNTs. This issue was addressed in 2005 by Lovat and colleagues, who reported that the neuronal density of cultured hippocampal neurons seeded on defunctionalized MWCNTs is similar to that of neurons seeded on pure glass and that the number of neurites per neuron was again similar in both culture conditions. Similar results were obtained by a subsequent study on SWCNTs (Mazzatenta et al., 2007).

Conclusions

The present review summarizes recent advances in the study of the impact of CNTs on neuronal performance, together with the first, pioneering evidences on the interactions between CNTs and neurons. MWCNT and SWCNT substrates are biocompatible and show the intriguing ability to increase single-cell excitability and spontaneous synaptic activity *in vitro* (thanks to both their conductive and nanostructural properties), suggesting their capacity to reengineer neuronal integrative properties. In addition, CNTs are a promising material for implementing neuronal interfaces and electrodes, to deliver electrical stimuli with improved neuronal–electrode interface contact or to record electrical signals with a remarkably high signal-to-noise ratio. Thanks to these properties, CNTs hold the potential of being used as a next-generation nanomaterial for biomedical applications, particularly in the field of neural prosthesis.

Acknowledgments

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Abbreviations

ADP	afterdepolarization
AHP	afterhyperpolarization
CNT	carbon nanotube
CTRL	control
EN	ethylenediamine
ITO	indium tin oxide

LBL	layer-by-layer
MAP-2	microtubule-associated protein 2
MEA	micro electrode array
MWCNTs	multi-walled carbon nanotubes
PABS	poly-*m*-aminobenzene sulfonic acid
PC12 cells	pheochromocytoma 12 cells
PEG	polyethylene glycol
PEI	polyethylenimine
PPy	polypyrrole
Pt	platinum
SEM	scanning electron microscopy
SWCNTs	single-walled carbon nanotubes
TEM	transmission electron microscopy

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