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Multi-walled carbon nanotube based multi-electrode arrays for the detection of the emergent activity in the cortical network

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ABSTRACT

We present a novel carbon nanotube growth method for the selective growth of high density arrays of multi walled carbon nanotubes (MWCNT) on multi electrode arrays systems (MEA). Platinum (Pt) has been used as the electrode material but also as the metal to catalyse the growth of the MWCNTs. These MWCNTs modified electrodes present a lower impedance compared to the original electrodes and thus, enhance the electrode sensitivity, increase its electric charge transfer and also improve the electrode-tissue contact. The system was tested for the recording of spontaneous rhythmic activity generated by ferret cortical slices before and after blockade of inhibition.

1. Introduction

In the last decades, neuroscientists around the world have dedicated their research to understand how neuronal networks work and how they malfunction in different diseases. Furthermore, the external interaction with brain networks, either for the use of brain computer interfaces or through the currently extended brain stimulation (e.g. transcranial magnetic stimulation) for the treatment of different brain disease, makes the comprehension of neuronal networks more necessary than ever. These studies have resulted in the development of different strategies to understand the ongoing neuronal activity such as the fluorescent microscopy with genetic labelling and optogenetic techniques and the recording/stimulation of tissue. In the second case, there exists an intense motivation towards systems with an increasingly larger number of electrodes to be inserted in the brain or to be utilised in cell cultures of neurons or after the preparation of slices. It is in these two last areas that the technology developed on micro-electrode arrays, commonly called multi-electrode arrays (MEAs), has grown in importance over other technologies [1–3].

A MEA can be used to perform electrophysiological experiments on tissue slices or dissociated cell cultures. With acute tissue slices, the connections between the cells within the tissue slices prior to extraction and plating are more or less preserved, while the intercellular connections in dissociated cultures are destroyed prior to plating. With dissociated neuronal cultures, the neurons form networks spontaneously.

Related to the work presented here, the analysis of brain slices instead of cell cultures is expected to result in more realistic models since the cortical architecture of the tissue is maintained. However, the use of MEAs in this area presents drawbacks such as obtaining and maintaining high intensity, low noise and time extended recordings. This is a consequence of the difficulty to assure both a good electrochemical electrode response and a good contact of the electrode with the tissue. Thus, the difficulties are related to the electrode material and to its planarity. In this frame, the use of carbon nanotube (CNT) coatings has been demonstrated extensively in the literature to improve neuronal recordings [4–6].

CNTs are high aspect ratio nanoscaled materials, they are exceptionally strong and tough, and they are good conductors and are chemically stable and enable electron exchange [7,8]. Hence, CNTs are an attractive material to form the interface with neural systems and so, they are being extensively investigated for the development of applications such as durable and robust neuroprosthetic devices [9,10]. Regarding the fabrication of these interfaces, to

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the fabrication various processes have been demonstrated for the synthesis or assembly of CNTs arrays to form electrodes. However, there is still the need to develop technologies that result in a high yield fabrication of electrodes with excellent mechanical, electrochemical and biological properties.

In our previous works we have demonstrated the modification of the surface of electrodes of MEA devices by drop casting Single Walled Carbon Nanotubes (dcSWCNTs) [11,12] and by the local synthesis of arrays of Multi Walled Carbon Nanotubes (grMWCNTs) by chemical vapour deposition (CVD) [13,14]. The drop casting of SWCNTs method was demonstrated to be an easy to perform electrode modification technique that results in a high purity CNT interfaces where the SWCNTs arrange into spaghetti like morphologies. The direct growth of MWCNTs on the metal substrate resulted in a more robust approach thanks to the scalability of the fabrication processes. However, we observed no improvement on the impedance characteristics even when the MWCNT modified electrodes were compared to bare metal electrodes. This was mainly attributed to the presence of amorphous carbon covering the MWCNTs that inhibited the electron exchange and to a non-optimised contact between the MWCNT array and the metal electrode.

Here we validate an optimisation of the technology for the MWCNT modification of Pt electrodes aimed at overcoming the issues encountered in our previous works. Again, the process is compatible with the large scale fabrication of the MEAs. The electrodes where tested in a two steps process. First, the electrode–MWCNT–electrolyte interfaces were evaluated by impedance spectroscopy and cyclic voltammetry, and then, these electrodes were compared to electrodes that were only formed of Pt (bare metal) and electrodes where SWCNTs had been drop casted. Second, MEAs formed of bare metal electrodes and electrodes modified with MWCNT arrays where compared for the recording of the spontaneous activity from slices of cerebral cortex before and after the blockade inhibition. The results demonstrate the huge potential of the MWCNTs arrays to build an interface between the neural system and the state of the art nanoelectronics.

2. Materials and methods

2.1. Fabrication of the electrodes

The designed MEA chips are formed of 16 Pt electrodes that are connected to metal pads located on the sides of the chip not to interfere on the liquid based testing. We implemented different electrodes designs with circle or square shaped, and different diameter or side. However, all the electrochemical characterisations and experiments reported here were conducted with the round 40 μm electrodes.

The MEAs were produced in the cleanroom facilities at the National Centre of Microelectronics in Barcelona (Spain). The electrodes were fabricated similarly as described in [11,14] as shown in Fig. 1. The starting point of the process is a 4 inch Si wafer (Fig. 1a1). First, a 1.5 μm thick SiO_2 layer is deposited by plasma enhanced (PE) CVD (Fig. 1a2). Then, the electrodes, the contact pads and the stripes connecting them are patterned through a photolithography, the deposition of Ti/Pt (30/150 nm) and a lift-off process (Fig. 1a3). Next, the wafer is passivated by a SiO_2/Si_3N_4 (400/700 nm) that are deposited by PECVD and windows are opened at the electrodes and the connection pads by a second photolithography and a reactive ion etching process (Fig. 1a4).

The integration of the MWCNT arrays starts with the deposition of a 15 nm thick SiO_2 layer. On the one side, this layer aims to inhibit the diffusion of the catalyst material into the electrode and, on the other side, to increase the roughness of the electrode to enhance the formation of a dense array of CNTs in the subsequent steps (Fig. 1a5). Afterwards, the catalyst material for the MWCNTs to grow from is selectively patterned on the electrodes by a third photolithography, the deposition of a 4 nm thick layer of Pt and a lift-off process (Fig. 1a6). The MWCNTs are synthesised in a rapid thermal CVD system at 800 °C by H_2 and CH_4 as the main process gases in a 2 step process. The first step, where only H_2 is injected, aims at dewetting the Pt layer into a dense array of ~10 nm diameter nanoparticles. During the second step the MWCNT arrays are made to grow by injecting the carbon containing gas (CH_4 in this case) as shown in Fig. 1a7. The last step of the fabrication is the removal of the 15 nm thick SiO_2 layer by a wet HF based solution (Fig. 1a8).

The morphology of the grown CNTs was evaluated by scanning electron microscopy (SEM) and by a high resolution transmission electron microscopy (HRTEM).

Once the wafer was fabricated, the MEA devices were encapsulated prior the electrochemical characterisation. The encapsulation consisted of the dicing of the wafer by a diamond saw, the gluing of the MEA to a printed circuit board (PCB), the wire-bonding of the connection pads and their protection with an epoxy based resist, and the gluing of a ring lid to the PCB for the liquid solution to be confined during the experiments in liquid.

2.2. Electrochemical experimental

The electrochemical characteristics of the electrodes with and without CNTs were compared by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry. EIS was conducted by using a commercial impedance analysis system (SI 1260, Solartron Analytical) operated by Zplot software. Two-electrode impedance measurements were conducted to characterise the electrode–electrolyte interface impedance compared to a Pt reference electrode (Radiometer Analytical). The electrical properties of the electrodes where evaluated by comparing the impedance and phase shifts relative to the operation frequency (10 Hz to 1 MHz) in physiological saline solution (0.9 wt.% NaCl, with a nominal resistivity of 71.3 Ωcm).

The cyclic voltammetry characterisation was performed by a Model 750 Bipotentiostat (CH instruments, Inc.). The electrodes on the MEAs, a Pt electrode (Radiometer Analytical) and an Ag/AgCl electrode (Metrohom) were used as the working, the counter and the reference electrodes, respectively. The voltammograms were obtained by cycling the potential of the electrodes in a phosphate-buffered saline solution (PBS, pH = 7.4) between −0.6 V and 0.6 V with respect to the Ag/AgCl electrode at different scan rates.

2.3. In vitro experimental

Coronal slices (0.4 mm thick) from occipital cortex and containing primary and secondary visual cortical areas (areas 17, 18, and 19) were obtained from adult ferrets, as described in [15]. The MEA was inserted in a MEA1060 probe interface that pre-amplified the signal. Further amplification (1000×) was obtained with amplifiers from Multichannel Systems. The artificial cerebrospinal fluid (ACSF) where the slices were bathed contained (in mM): NaCl, 126; KCl, 3.5; $MgSO_4$, 1; NaH_2PO_4, 1.25; $CaCl_2$, 1.2; $NaHCO_3$, 26; dextrose, 10, and was aerated with 95% O_2, 5% CO_2 to a final pH of 7.4. To induce spontaneous activity a gabaergic blocker (5 μM) bicuculline methiodide (Sigma) was added at some point of the recording. The recording chamber where the slice was placed simulated an interface-style recording chamber. The chamber top was closed and the flowing air was humidified and enriched with oxygen. The bath temperature was maintained between 34.5 and 36 °C.