

# Carbon nanotube functionalized neural probe platform for characterization of cell attachment and motility

J. Tyson, M. Tran, T. Patrick, G. Slaughter  
University of Maryland Baltimore County and Bioelectronics Laboratory  
Department of Computer Science and Electrical Engineering  
1000 Hilltop Circle, Baltimore, MD 21250, USA, gsslaught@umbc.edu

## ABSTRACT

We present a fabricated multichannel, flexible neural probes using standard microfabrication techniques to characterize cell adhesion and motility of PC12 cells. The cell-to-electrode interface consisted of 16 electrode sites per shank. Each electrode site is approximately  $250 \mu\text{m}^2$  and is created using gold rectangular pyramidal electrode sandwiched between two polyimide dielectric layers. The electrodes were characterized by electrochemical impedance spectroscopy (EIS) analysis in phosphate buffered saline solution (pH 7.4). The electrode test impedance values at the physiologically relevant frequency of 1 kHz was observed to be on average  $17.16 \text{ M}\Omega$ . Multi-walled carbon nanotubes (MWCNTs) were deposited on the electrode sites resulting in a significant reduction in the electrode impedance at 1 kHz to  $45.73 \text{ k}\Omega$  on average. For in vitro studies, cell adhesion and motility are modeled by electrochemical impedance spectroscopy. Results suggest that the probe induce little to no cytotoxicity in the presence of cells and the unique geometry of the electrode recording site facilitated intimate cell-to-electrode contact without restricting cell movement.

**Keywords:** Cell adhesion, motility, MWCNTs, NeuroMEMs, NeuroProbe

## 1 INTRODUCTION

The integration of neurons with neural probes in neurological studies has enabled greater understanding of the complex neurological processes. Such devices utilize electrodes to stimulate and record electrical signal from targeted neural tissues, and are particularly useful for elucidating the complex processes that contributes to the onset of neurodegenerative diseases. The application of micro- or nano-electromechanical systems (MEMs) or (NEMs) to the fabrication of neural probes, have led to the development of minimally-invasive high density probes. Although the majority of neuroMEMs probes have been used in short term implantation studies, chronic implantation has eluded the field of neuroscience and neuroengineering due to the biocompatibility challenges encountered. Tissue damage, scarring and device encapsulation eventually render these device useless. Additionally, electrode impedance is very important to the characterization of neural probes designed

for the recording and stimulation of electrogenic cells. Neural probes that exhibit high electrode impedance require a large applied voltage, which causes cell damage in addition to an increase in the electrode noise that further obscures recorded neuronal signals [1]. Therefore, it is important to employ neural probe with electrode sites that have optimal impedances and signal-to-noise ratio in order to acquire the best possible recording signals from targeted cells.

Electrochemical impedance spectroscopy (EIS) has been demonstrated to be a very useful technique for analyzing cell structure, specifically in monitoring cell adhesion to electrode surfaces [2]. Xiang et al demonstrated that gold electrodes could be made more suitable for electrochemical application through the deposition of multi-walled carbon nanotubes (MWCNTs) [3]. In addition to MWCNTs ability to lower the impedance of the electrodes, it provides a roughened high surface area catalytic material that have been shown to detect chemicals such as dopamine in the monitoring of redox reactions using cyclic voltammetry [3-4]. As a result, carbon nanotube coatings have been found to improve electrode interface selectivity and sensitivity since they have superior charge transfer characteristics [5]. Traditionally, MWCNTs are electrochemically deposited by applying a current across the desired electrodes immerse in a solution of MWCNTs and gold sulfite, which aids with the uniform deposition of MWCNTs on the surface of the electrode. Additionally, recent study suggests the utilization of carbon nanotubes can improve the quality of neuronal cell adhesion and motility on electrodes [6].

Recently, we reported on the initial biocompatibility assay of our fabricated neural probe to confirm the lack of cytotoxicity [7]. The cell viability results suggest that the polyimide neural probe exhibits desirable characteristics for implant material coatings with a high viability (>80%) and low proliferation (< 40%) [cite]. Here we describe the characterization of cell adhesion and motility on the recording electrode sites of the fabricated flexible polyimide neural probe by employing cultured PC-12 cell line derived from a pheochromocytoma of the rat adrenal medulla. PC-12 cells were chosen because they have been shown to exhibit neuron-like properties in the presence of neural growth factor and make good analogs to brain tissue [8]. EIS was employed to characterize the intimate electrode-to-cell contact adhesion and motility.

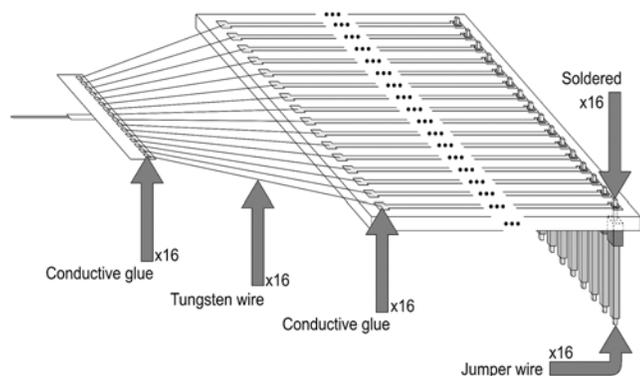
## 2 EXPERIMENTAL

### 2.1 Chemicals & Materials

Ethanol, Phosphate Buffered Saline (KCl 0.20 g/L, KH<sub>2</sub>PO<sub>4</sub> 0.20 g/L NaCl 8.00 g/L, Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O 2.16 g/L), acetic acid, Ham's FK12 Cell Culture Media, trypsin-EDTA, Falcon 4 chamber culture slides, Epo-Tek 301 epoxy, were purchased from Fisher Scientific. The hemocytometer was purchased from hausser Scientific Company. Adherent PC-12 cells were purchased from ATCC. MWCNTs were purchased from Cheap Tubes Inc. TSG-250 Gold Sulfite solution was purchased from Transene.

### 2.2 Device Packaging

The flexible polyimide neural probe is fabricated using standard CMOS techniques with gold electrodes and polyimide structural and passivation layers in order to improve performance in neuro-interfacing [9]. The optical image in Figure 1 displays a clear view of probe connected to the fabricated PCB. The shank of the probe is designed to have a length of 3 mm, thickness of 12  $\mu\text{m}$ , and width of 41  $\mu\text{m}$ . The shank is equipped with 16 recording electrode sites/channels.



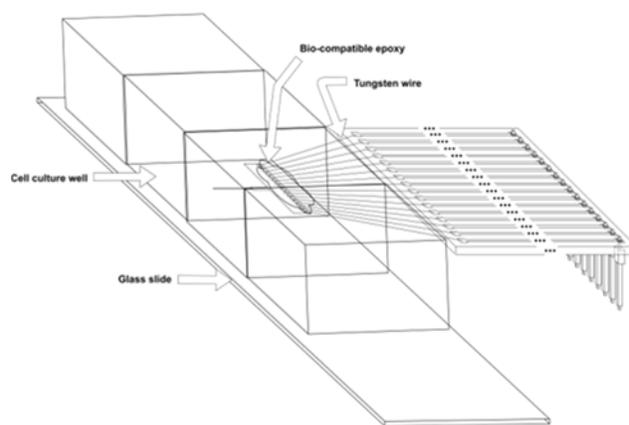
**Figure 1:** Flexible and optically transparent polyimide neural probe packaging.

For ease of interfacing the flexible polyimide neural probe to the measurement equipment, a printed circuit board (PCB) consisting of interconnect lines is fabricated and connected to the probe contact pads via 200  $\mu\text{m}$  tungsten wires affixed with conductive silver wire glue to create a secure electrical connection. Finally, as demonstrated in Figure 1, a group of jumper wires were soldered to the other end of the PCB for further wire extension.

### 2.3 PC-12 Cell Culture

The completely assembled cell culture chamber integrating the flexible polyimide probe shank is shown in Figure 2. The flexible probe shank was sandwiched between

the borosilicate glass and the Falcon 4 chamber using biocompatible epoxy. Before re-mounting the chamber, the individual shank of the flexible polyimide probe was affixed to the borosilicate glass via a thin layer of the epoxy and then the contact pads and the tungsten wires were also covered with epoxy in order to avoid contact with the cell culture media.



**Figure 2:** Flexible polyimide probe-cell culture assembly using chambered glass slides.

### 2.4 Carbon Nanotube Deposition

The gold recording electrode surfaces were roughened with multi-walled carbon nanotube (MWCNT) in order to increase the electrode surface area for cell adhesion, in addition to lowering the overall electrode impedance. The deposited MWCNT provides a high catalytic surface area for electrochemical reactions. The MWCNTs were dispersed in gold sulfite electrolyte solution at a concentration of 1mg/mL. The prepared solution was sonicated for one hour to disaggregate and suspend the MWCNTs in solution. The probe shank was then submerged in the solution and each of the 16 electrodes were used as the cathode. A platinum wire electrode was used as the anode. These two electrodes were then connected to a function generator (Agilent 33250A) with a monophasic voltage pulse of 1.2 V, 10 Hz, at 50% duty cycle for 1-minute duration. After the deposition cycles, multiple layers of MWCNTs with gold nanoparticles were deposited on each of the gold recording electrode sites on the shank of the flexible polyimide neural probe.

### 2.5 Electrochemical Impedance Spectroscopy

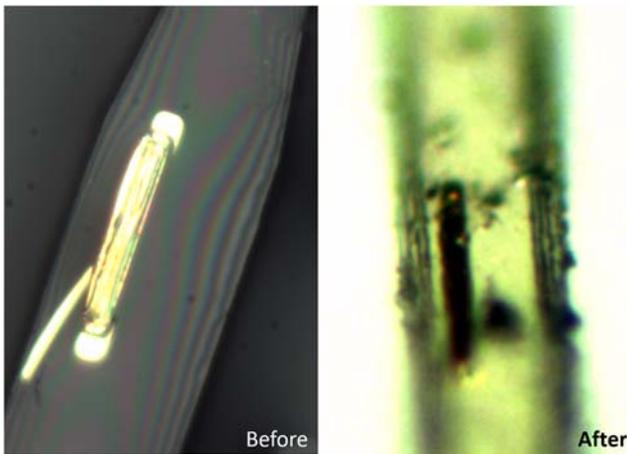
To characterize the pre and post-MWCNT electrode impedance of the neural probe, electrochemical impedance spectroscopy (EIS) measurement was acquired at room temperature using a potentiostat/ galvanostat (Autolab

PGSTAT204, Metrohm Autolab). The flexible neural probe shank consisting of the 16 recording electrodes was submerged in phosphate buffered saline (PBS, pH 7.4) solution. PBS was used to mimic the physiological environment. The experiments were performed in a three-electrode cell configuration with Ag/AgCl as the reference electrode, a platinum wire as the counter electrode, and each probe electrode (connected by each jumper wire) as the working electrode. The electrochemical impedance magnitude and phase measurements were taken using a 10 mV peak-to-peak waveform, at a frequency range between 0.1Hz to 10kHz. All measurements were made in duplicates.

### 3 RESULTS AND DISCUSSION

#### 3.1 MWCNT Deposition Results

To isolate the gold bond pads of the device from the liquid cell culture environment, the tungsten wires and the upper section of the probe were encapsulated with a biocompatible epoxy low water permeability. Six electrodes were characterized and MWCNT depositions on these six electrodes were subsequently utilized. The optical images of the flexible polyimide probe shank tip pre and post-MWCNT deposition are shown in Figure 3. The surface of the rectangular gold electrode appears smooth and reflective, whereas the MWCNTs coated electrode surface appeared rough and dark.

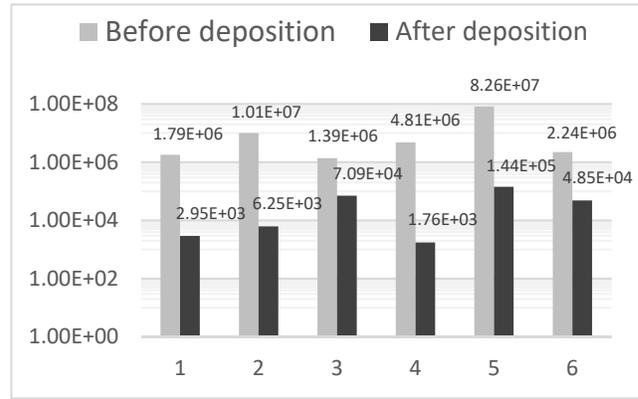


**Figure 3:** Recording electrode site before (left) and after (right) MWCNT deposition.

#### 3.2 Electrochemical Characterization

The impedance characterization of the electrode-electrolyte interface was assessed to determine the functionality of the electrodes via electrochemical impedance spectroscopy (EIS) [10]. The EIS measurements were performed on a total of six recording electrode sites. Figure 4 shows the

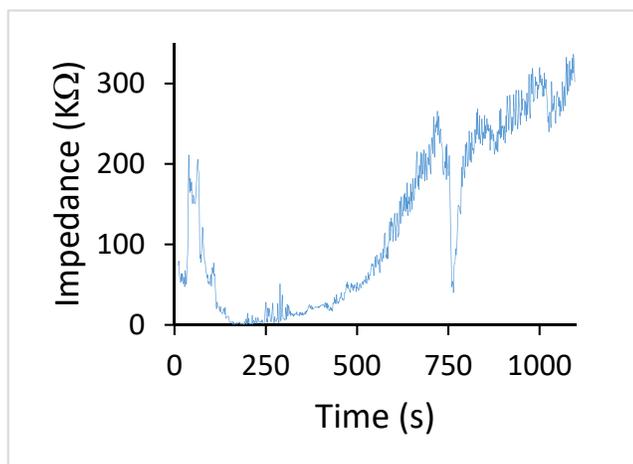
comparison of the gold and gold-MWCNT electrodes. The relatively large impedance value of 17.16 MΩ at 1 kHz on average is attributed to the electrode area being covered by a small amount of the biocompatible epoxy used to affix the flexible probe shank to the borosilicate glass during the construction of the cell culture chamber. The 60 seconds long deposition of MWCNTs resulted in a significant decrease in the overall impedance. The average impedance at 1 kHz after MWCNT deposition was 45.73 kΩ. Clearly MWCNTs contribute to the decrease of the electrodes chemical impedance. Thereby resulting in an enhancement of charge storage capability [11]. The observed average impedance after MWCNT deposition is an acceptable impedance value for electrochemical sensing of dopamine [4].



**Figure 4.** EIS measurement of six electrodes on a single probe shank before and after MWCNT deposition on a logarithmic scale.

#### 3.3 Cell Adhesion and Motility

EIS measurements were collected on one of the electrodes on the probe shank to study the electrical properties of cell-dependent adhesion in culture. Previous study of the electrical impedance characteristics of PC12 cell-dependent adhesion in cultured is represented by the work of Slaughter et al [12]. In the present approach, PC12 cells were cultured on the flexible polyimide shank consisting of 16 gold-MWCNT coated electrodes. The motility of the cells was electrically detected and monitored for a period of time as illustrated in Figure 5. It can be observed that the interaction and spreading of cells on the electrode surface results in a significant change in the measured impedance of the electrode after 20 h of initial cell seeding. The fluctuations observed in the impedance profile (approximately 18 minutes) are attributed to result of the attachment and motion of cells on the electrode surface. These changes continue as the cell layers become confluent.



**Figure 5.** EIS time-dependent profile of the impedance magnitude of one MWCNT coated gold electrode of a flexible polyimide probe shank cultured with PC-12 cells.

## 4 CONCLUSION

We successfully demonstrated the reduction of the gold recording electrodes' impedance via the deposition of MWCNTs. The observed impedance values at physiologically relevant frequency 1 kHz were on average 45.73 kΩ, which is suitable for electrochemical sensing. Moreover, PC-12 cells were successfully cultured on the MWCNT coated recording electrodes and the results indicate that the PC12 cells that become attached to the MWCNT continue to exercise their motility forces that eventually lead to strong cell attachment to the gold recording sites, thereby resulting in an intimate cell-to-electrode contact and further support better electrical impedance performance of the PC-12 cells.

## REFERENCES

- [1] Lisdat, F., & Schäfer, D. (2008). The use of electrochemical impedance spectroscopy for biosensing. *Analytical & Bioanalytical Chemistry*, 391(5), 1555-1567.
- [2] Franks, Impedance characterization and modeling of electrodes for biomedical applications. (2005). *IEEE Transactions on Biomedical Engineering, Biomedical Engineering*, IEEE Transactions on, IEEE Trans. Biomed. Eng, (7), 1295.
- [3] Xiang Z, Yen S, Lee C, et al. Ultra-thin flexible polyimide neural probe embedded in a dissolvable maltose-coated microneedle. *Journal Of Micromechanics And Microengineering*, 24(6), 065015, doi:10.1088/0960-1317/24/6/065015
- [4] Huffman, M. L., & Venton, B. J. (2009). Carbon-fiber microelectrodes for in vivo applications. *The Analyst*, 134(1), 18-24. doi:10.1039/b807563h

- [5] Keefer, E. W., Botterman, B. R., Romero, M. I., Rossi, A. F., & Gross, G. W. (2008). Carbon nanotube coating improves neuronal recordings. *Nature Nanotechnology*, 3(7), 434-439.
- [6] Burbliès, N., Schulze, J., Schwarz, H., Kranz, K., Motz, D., Vogt, C., & ... Behrens, P. (2016). Coatings of Different Carbon Nanotubes on Platinum Electrodes for Neuronal Devices: Preparation, Cytocompatibility and Interaction with Spiral Ganglion Cells. *Plos ONE*, 11(7), 1-22.
- [7] Tyson, J., Tran, M., & Slaughter, G. (2017). Biocompatibility of a Novel Quad-Shank Neural Probe. *Solid State Electronics*.
- [8] Das, K. P., Freudenrich, T. M., & Mundy, W. R. (2004). Assessment of PC12 cell differentiation and neurite growth: a comparison of morphological and neurochemical measures. *Neurotoxicology And Teratology*, 26397-406.
- [9] Slaughter, G., Robinson, M., Tyson, J., & Zhang, J. (2017). Process development to overcome the challenge of a difficult to integrate neuroelectronic device. in *Optical Microlithography XXX*, edited by Andreas Erdmann, Jongwook Kye, Proceedings of SPIE Vol. 10147 (SPIE, Bellingham, WA, 2017) 101470W.
- [10] Williams, J. C., Hippensteel, J. A., Dilgen, J., Shain, W., & Kipke, D. R. (2007). Complex impedance spectroscopy for monitoring tissue responses to inserted neural implants. *Journal of Neural Engineering*, 4(4), 410-423. doi:10.1088/1741-2560/4/4/007
- [11] Rahman, A. R., Register, J., Vuppala, G., & Bhansali, S. (2008). Cell culture monitoring by impedance mapping using a multielectrode scanning impedance spectroscopy system (CellMap). *Physiological Measurement*, 29(6). doi:10.1088/0967-3334/29/6/s20
- [12] Slaughter, G., Bieberich, E., Wnek, G., Wynne, K., & Guiseppi-Elie, A. (2004). Improving neuron-to-electrode surface attachment via alkanethiol self-assembly: an alternating current impedance study" *Langmuir*. 20 (17), 7189-7200.