# Carbon Nanofiber Nanoelectrode Array for Closed-Loop Electrical Stimulation

**Thuy-Duong Barbara Nguyen-Vu, Hua Chen, Alan Cassell, Jessica Koehne, Hardeep Purewal, M. Meyyappan, Russell Andrews, and Jun Li**

Center for Nanotechnology, NASA Ames Research Center, Moffett Field, CA

Email: btnguyen@mail.arc.nasa.gov

## Abstract

***Carbon nanofibers (CNFs) can serve as an ideal material for the interface between solid-state electronics and biological systems. Such vertically aligned CNFs can be grown on underlying electronic circuits to be directly integrated in a multiplex microchip for neural electrophysiology. The chip contains multiple individually addressable nanoelectrode arrays that function either as electrical stimulation electrodes or electrochemical-sensing electrodes. The former is configured as a forest-like CNF array that exhibits extremely low impedance due to its three-dimensional structure, which can be further enhanced with a conformal polypyrrole coating. The latter is designed such that the CNFs are embedded in a dielectric material to form an inlaid nanodisk electrode array that demonstrates ultra-sensitive electroanalysis properties with low detection limits and the potential for extremely high temporal resolution. These properties are ideal for capturing neural signalling events facilitated through electrochemically active neurotransmitters. The feasibility of the CNF arrays as implantable electrodes has been investigated using in-vitro cell culture experiments.***

## 1. INTRODUCTION

The application of deep brain stimulation (DBS) has been proven to be an effective clinical treatment for a host of different neurological disorders despite the lack of a clear scientific understanding of its functional mechanism [1]. The process by which DBS is successful in alleviating the symptoms of disorders such as Parkinson's disease is complicated because it involves the most intricate of all biological systems, the brain. The brain is essentially the complex network of approximately a trillion nerve cells that are individually a composite of even smaller biomolecules operating on a nanoscale. It is of great potential to emulate the natural structure of the biological system and interface the neural network at that level. Thus, using nanotechnology can provide greater insight into the complexity of the brain and thereby enabling greater specificity and enhancement to the current deep brain stimulation technology.

To enable this interface, a novel method has been developed to fabricate vertically aligned carbon nanofibers (CNFs) into nanoelectrode arrays. CNFs can serve as an ideal material for the interface between solid-state electronics and biological systems based on their unique physical, chemical, and electronic properties [2]. Such vertically aligned CNFs can be precisely grown on underlying electronic circuits using techniques compatible with Si microfabrication. Consequently, the CNFs can be directly integrated into nanoelectrode arrays on a multiplex microchip for neural electrophysiology. The chip design composes of multiple types of arrays for both electrical stimulation and electrochemical monitoring of neurotransmitter, thus providing real-time feedback of neurological processes.

## 2. METHODS

### 2.1. CNF Array Fabrication

The carbon nanofibers are grown by catalytic plasma enhanced chemical vapor deposition (PECVD) on a silicon substrate, which is patterned with microcircuits to form the electrode arrays as shown in Fig 1. The as-grown CNFs are then encapsulated with $SiO_2$ by tetraethoxylorthosilicate (TEOS) CVD followed by chemical mechanical polishing (CMP) to expose the very tip of the CNF, which forms the nanoelectrode arrays for electrochemical sensing. The insulating material at some micropads can be completely etched away to reveal the forest-like structure of the as-grown CNFs for use as a stimulating

electrode. Depending on the function of each array, the micropads can be fabricated for the specific purpose of stimulation or sensing. The embedded CNF tips are electrochemically etched to activate the nanoelectrode for use as an electrochemical detector, whereas the exposed CNFs are coated with a conformal polypyrrole (PPy) layer by electrochemical deposition to improve the electrical performance for stimulation.

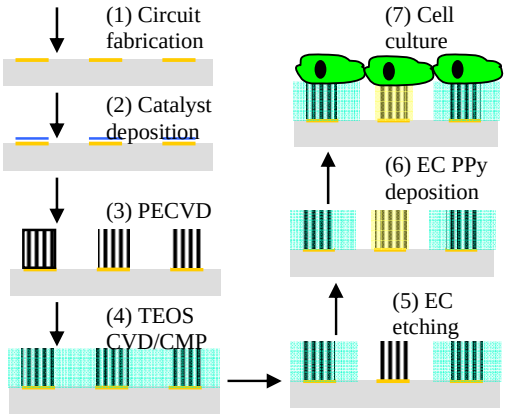


**Figure 1.** Schematic of the fabrication processes

### 2.2. PC12 Cell Culture

The biocompatibility studies were conducted using neuron-like PC12 cells derived from a transplantable rat pheochromocytoma. Cells were obtained from ATCC and incubated in DMEM growth medium with 10% heat-inactivated FBS, 5% heat inactivated horse serum, 2mM L-gluatmin, 100ug/ml of stretptomycin and 100U/ml of penicillin under 5% $CO_2$ and 95% $O_2$, The medium was changed every 2 to 3 days. Cells were cultured for 7 days on the UV irradiated nanoelectrode arrays to determine the biocompatibility of the as-grown CNFs vs. the polypyrrole coated CNFs substrate. The cells are assessed by fluorescence microscopy after being fixed and actin-labeled with fluorecein-phalloidin.

## 3. RESULTS AND DISCUSSION

In previous reports, we have demonstrated the fabrication of the freestanding vertically aligned carbon nanofibers on solid substrate [3-5]. PECVD growth conditions can be controlled to vary the length and diameter of the CNFs. The CNFs can also be directly grown on a patterned microelectronic device, which enables the technique to be well integrated with silicon technology. Fig. 2 shows the SEM images of the CNFs grown on a 200x200 $um^2$ microcontact pad patterned with UV lithography.

**Figure 2.** SEM images of CNF bundles grown as the array-in-array format on a micro-contact pad. Scale bars are 50 and 2 μm, respectively.

The forest-like structure of the as-grown CNFs has demonstrated attractive electrical properties. Due to the large surface area of the three-dimensional array structure, the electrode exhibits a high specific capacitance of 0.4mF/$cm^2$ and very low impedance. This structure is further enhanced with the electrochemical deposition of the polypyrrole (PPy), an electronic conducting polymer. The capacitance has been found to increase by 100 times to 40mF/$cm^2$ with a PPy film of only 24nm. Correspondingly, the impedance decreased and is negligible when compared to the Ohmic resistance of the solution and the impedance of the Pt counter electrode. In addition, PPy coating is conformal to the individual CNF and increases its mechanical strength. The PPy coated CNF retains its vertical alignment after submersion in solution and is strong enough to withstand cell culture. The SEM image in Fig 3 shows the PPy coated CNFs penetrating the cellular membrane. Both features result in an extremely efficient electrode that can be utilized for more specific and effective stimulation of the neural cells.

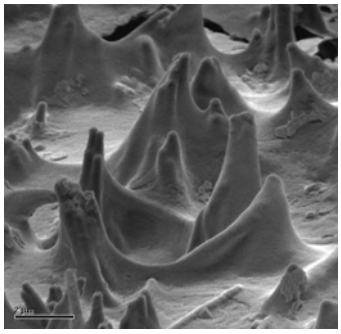

**Figure 3.** SEM image of PPy coated CNF penetrating the surface of the cell. The scale bar is 2um.

The embedded CNFs that form the nanodisk electrode array have been shown to be an extremely sensitive electrochemical detector. Such an array has demonstrated a detection limit in the nanomolar region with dynamic range of 8 orders of magnitude [6]. The nanoscale feature of the CNF exhibits ideal nanoelectrode behavior, which makes it perfectly suited for an ultra-sensitive detector of

low concentrations electro-active molecules such as catecholamine neurotransmitters. The nanodisk array is able to harness the unique signal at the individual CNF while enhancing the amplitude through the summation of the signals corresponding to number of CNFs exposed in the array. We demonstrated that this electrochemical method can be used to measure dopamine at the 60nM level. Furthermore, the physical dimension of the CNFs also makes them attractive for high temporal resolution detection, which is necessary to capture the transient signals of neurotransmitter release.

Lastly, we report that the PC12 cells can be cultured on the CNF arrays. The cells form a distinct monolayer on the array surfaces that are coated with a thin layer of collagen, as shown in Fig 4. The collagen helps to facilitate cell adhesion to the nanoelectrode surface. These results are encouraging pertaining to the feasibility of the CNF arrays as a biocompatible substrate for implantable electrodes.

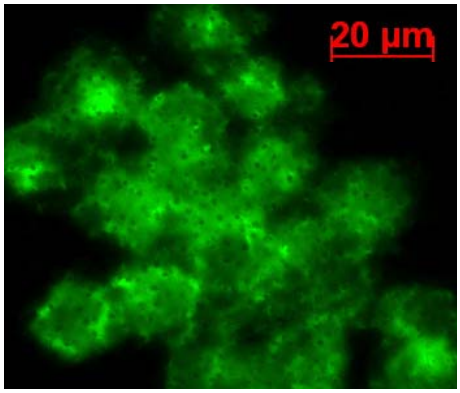


**Figure 4.** A monolayer of PC12 neural cells formed on the surface of CNF array coated with a thin layer of collagen. The scale bar is 20 µm.

## 4. CONCLUSION

The canbon nanofiber with its unique physical and electrical characteristics provide a wonderful opportunity to investigate at an intimate level the complexity of the brain. The ability to use CNFs in a congruent manner to microelectronics and directly interface with the biological systems on a nanoscale provides enormous potential for elucidating the intricacy of the neural network. Additionally, the capacity of the CNFs allows for versatility of the function, such that the same CNFs can be utilized in a stimulating electrode array or in an electrochemically sensing electrode array on the same integrated platform. A closed-loop microchip with real-time feedback of neurological processes upon electrical stimulation can be used to improve implantable devices currently employed for deep brain stimulation treatment of neurological disorders.

### References

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