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Vertically Aligned Carbon Nanofiber as Nano-Neuron Interface for Monitoring Neural Function

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Abstract

Neural chips, which are capable of simultaneous, multi-site neural recording and stimulation, have been used to detect and modulate neural activity for almost 30 years. As a neural interface, neural chips provide dynamic functional information for neural decoding and neural control. By improving sensitivity and spatial resolution, nano-scale electrodes may revolutionize neural detection and modulation at cellular and molecular levels as nano-neuron interfaces. We developed a carbon-nanofiber neural chip with lithographically defined arrays of vertically aligned carbon nanofiber electrodes and demonstrated its capability of both stimulating and monitoring electrophysiological signals from brain tissues *in vitro* and monitoring dynamic information of neuroplasticity. This novel nano-neuron interface can potentially serve as a precise, informative, biocompatible, and dual-mode neural interface for monitoring of both neuroelectrical and neurochemical activity at the single cell level and even inside the cell.

Keywords

carbon nanofiber; neural interface; neural chip; nano-neuron interface; electrophysiology

As neural interfaces, neural chips are used to decipher brain function and principles of neural information processing both *in vitro* and *in vivo*, enabling a direct communication

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between the brain and external devices for neuroprosthetics and neurorehabilitation.^{1,2} Neural chips have been developed, based on micro-/nano-fabrication technologies, as neural interfaces for long-term, simultaneous multisite detection and modulation of neural function.¹⁻⁴ High spatial and temporal resolution of information acquisition is important for neural decoding of neural circuits and understanding disease-related dysfunction of the brain. Micron-scale, high-density microelectrode arrays have been developed for detecting neural signals in the past two decades, enabling extracellular recording at a spatial resolution of tens of microns.¹ As the size of microelectrode is scaled down, double-layer impedance of the electrodes in physiological solutions rapidly increases; thereby detection of neural signals is not possible due to a concomitant increase of noise. Surface modification of nanostructures has been studied to reduce the impedance of microelectrodes for neural recording,³ and nanostructures were found to facilitate neural electrical activity by forming tight contacts between neurons and nanostructures.⁴

Over the years, a variety of materials and structures have been tested to improve spatial resolution of neural chips. Sub-micron-scale neural electrodes are fabricated by nanofibers,^{5,6} nanotubes,⁷ and nanowires,⁸ to overcome the intrinsic limitation of planar microelectrodes. The individual ultramicroelectrode electrodes are fabricated as 3D, pin-like structures allowing the electrodes to penetrate into the interior of the tissue to improve electrical coupling. The 3D shape provides a large active surface area for neural recording, although the spatial resolution is increased to sub-micron levels by scaling down individual electrode sites on the substrate. Moreover, 3D sub-micron-scale electrodes have been coupled with individual cells to measure the intracellular voltage,⁹ enabling high-spatial-resolution neural recording at the single cell level. In this work, we developed a 3D ultramicroelectrode array consisting of vertically aligned carbon nanofibers (VACNFs) that has the potential to perform dual-mode recordings of both neuroelectrical activity and neurotransmitter release. Individual VACNF electrodes were cone-like in shape allowing the electrodes to penetrate into tissues and to be pinned at individual cells. This novel device will make possible the dual mode nano-neuron interface of electrophysiological and neurochemical communication not only at the extracellular level with high spatial resolution, but also at the intracellular level by penetrating into single neurons.

Methods

Our VACNF arrays consist of 40 electrodes in a line with 15 μm spacing along a total length of 600 μm ; individual VACNF electrodes were cone-like in shape as shown in Figure 1. The fabrication process was briefly described in Supplementary Materials.¹⁰ All animal procedures were approved by the Columbia University IACUC. The culture technique and electrophysiology measurement procedure were described in Supplementary Materials. To evaluate biocompatibility, rat cortical neuronal cells were cultured directly on VACNF chips, and the cells appeared to be of good quality during the first week culture. After culture, cells were fixed and dehydrated for scanning electron microscopy (SEM) observation and immunostaining assays.

Results

One of the potential advantages of VACNFs as neural interface electrodes is that their covalent carbon bonding structure can provide inherent biocompatibility and stability in physiological solutions. As shown in Figures 2A & B, neuronal cells appeared healthy, forming intimate contact with carbon nanofiber electrodes. This indicates that carbon nanofibers can potentially serve as a permissive substrate for interfacing with neurological systems. In addition, as shown in Figures 2C & D, dissociated cultures of neurons and astrocytes displayed a healthy morphology on the VACNF chip.

The 3D VACNF electrode has a nano-scale tip, which can pin individual cells. Figures 3A & B show a neuron sitting directly over a carbon nanofiber electrode. The nano-scale electrode may be engulfed by cell membrane and finally penetrate into the cell through endocytosis. This could allow the VACNF electrodes to detect intracellular signals or to deliver molecules into cells. Moreover, when penetrating into hippocampal tissue (Figure 3C) (Supplementary Materials), the 3D VACNF electrode was able to detect individual action potentials from single neurons (Figure 3D), which were eliminated by 1 μ M tetrodotoxin, demonstrating their biological origins (data not shown).

As a neural interface for monitoring neural activity, the VACNF electrode array was able to monitor the dynamic behavior of neuronal network activity. The phenomena of paired-pulse facilitation and depression (PPF/D), a well-studied example of short-term neuroplasticity, are believed to be responsible for cognitive abilities involving temporal processes. PPF/D ratios were generated by delivering two successive stimuli of the same intensity but with different inter-stimulus intervals. Evoked responses were recorded simultaneously from all electrodes (Figure 4C). The ratio of the amplitude of the field potentials evoked by the two successive stimuli indicate paired-pulse facilitation when larger than unity or paired-pulse depression when less than unity. Facilitation occurred when the inter-stimulus interval was increased over 300ms probably due to the residual calcium in the nerve terminals after the first stimulus.

Discussion

We report here the development of vertically aligned carbon nanofiber electrodes as a nano-neuron interface. Compared with planar microelectrode arrays, sub-micron VACNF electrodes can be fabricated in a high density due to the small electrode size and their 3D structure. This higher spatial resolution and density of VACNF arrays may help to acquire high quality neural information allowing a high-level of neural control with minimal training requirements for neuroprosthetics and neurorehabilitation. Moreover, the nano-scale tip of the VACNF can pin individual cells and may even penetrate into single cells. In this study, cells randomly sat on the electrode, and the electrode tip could then be engulfed by the cell membrane during endocytosis. To increase the yield of this nano-neuron junction, additional techniques could be implemented, such as cell micro-manipulation and 3D probe design of the electrode arrays. Beyond measuring potentials inside a cell, the VACNF electrode as a nano-neuron interface could carry ligands for proteins, enabling dual mode recording of both electrophysiological and chemical signals inside cells. This nano-neuron interface has great

potential as a precise, informative, and biocompatible neural interface, and may aid in understanding how neural circuits process information at the single cell and even intracellular levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

SEM images of (A) a VACNF array and (B) a single VACNF electrode from the same array.

Figure 2.

(A) An SEM image of cortical neuronal cells cultured on a VACNF neural chip (5 days *in vitro*, DIV); (B) an SEM image of a single VACNF electrode entwined by a neurite; (C) and (D) fluorescent images of mixed cortical cultures grown on a VACNF chip and stained for GFAP (red, for astrocytes), type-III β tubulin (green, for neurons) and DAPI (blue, for nuclei).

Figure 3.

(A) and (B) An SEM image of a neuron sitting on a single VACNF electrode; (C) light micrograph of a hippocampal slice (12 DIV) on a VACNF array chip; (D) action potentials recorded from one electrode.

Figure 4.

(A) Light micrograph of a hippocampal slice (22 DIV) on a VACNF array; (B) a schematic of the hippocampal anatomy depicting the recording locations; (C) evoked field potentials recorded from all 40 VACNF electrodes in response to two successive stimuli of 100 μA with 40 ms inter-stimulus-interval through electrodes E27 and E29 within the mossy fiber region; (D) paired-pulse ratios from electrode E03 located in the CA1 region as a function of inter-stimulus intervals.