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UTILIZING CARBON NANOPIPETTES FOR INTRACELLULAR INJECTION AND ELECTROPHYSIOLOGY

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ABSTRACT

Since their discovery, researchers have been intrigued by the possibility of using carbon nanotubes and nanopipes for, among other uses, minimally-invasive intracellular delivery and sensing of cellular signals. However, the inability to efficiently interface these nanostructures with larger, maneuverable probes has hindered their use as cell probes. To overcome this issue, we have developed a means of integrating carbon nanopipes, with diameters ranging from 10's to 100's of nanometers, into the tips of pulled glass capillaries to form carbon nanopipettes (CNPs). CNPs are efficiently fabricated in large quantities and without the need for assembly. CNPs readily interface with standard cell physiology equipment such as micromanipulators, injection systems, and electrophysiology amplifiers. Here, we recount the application of CNPs as intracellular injectors and intracellular recording electrodes to demonstrate their capabilities as cell probes.

INTRODUCTION

Glass-based micropipettes are traditionally used for intracellular delivery or sensing cell signals. Although widely-used, they suffer from drawbacks such as fragility, a relatively large size, and inability to measure electrical signals concurrently with injection [1]. Glass micropipettes can also cause irreparable damage to the cell [2]. As an intriguing alternative, carbon nanotubes and nanopipes offer a minimally intrusive means for cell probing, injection, and sensing. Compared to glass micropipettes, these carbon nanostructures possess superior electrical, thermal, and mechanical properties. Additionally, carbon nanostructures have a high length-to-wide aspect ratio, making them minimally invasive to cell membranes and organelles during cell probing.

Several assembled nanotube-based cellular probes have been developed. Unfortunately, the probes are difficult to fabricate or require specialized equipment in order to use the probe. As an alternative to assembled nanoprobes, we integrated carbon nanopipes into the tips of glass micropipettes to form CNPs (Fig. 1). CNPs are efficiently produced in quantity and readily interface with standard cell physiology equipment. Since they are hollow and conductive, CNPs are capable of injecting fluids into cells and recording intracellular signals.

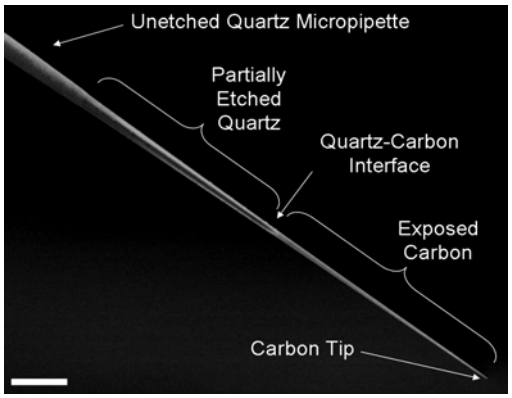

Figure 1: SEM micrograph of a CNP tip. Scale bar, 5 µm.

FABRICATION

CNPs are fabricated by depositing iron catalyst on the inner lumen of quartz capillaries and then pulling the capillaries into fine-tipped micropipettes [3]. A carbon film is then deposited only on the catalyzed sites inside the micropipette by chemical vapor deposition (CVD). The thickness and properties of the carbon film are controlled by the CVD process conditions [4]. The nanoscale carbon tip is then exposed by wet etching the quartz tip with buffered hydrofluoric acid.

The resulting probe consists of a conductive carbon film inside a glass micropipette which begins at the proximal end of the micropipette and terminates at the distal end as a carbon nanopipe tip. Although smaller dimensions are possible, the exposed carbon tips of CNPs produced for cell studies are typically between 50 and 250 nm in outer diameter and less than 1 µm in length. The carbon tips of CNPs can bend without breaking and can return to their original shape. Yet,

CNPs are rigid enough to penetrate into cells without damaging the membrane or hindering their growth [3].

INTRACELLULAR INJECTION

Using standard microinjection equipment, CNPs can inject fluids, such as calcium-mobilizing second messengers, into breast cancer cells (SKBR3) via pressure injection (Fig. 2) [5]. Cells are targeted (Fig. 2a), then fluorescently monitored before (Fig. 2b) and after (Fig. 2c) injection to measure second messenger-mediated intracellular calcium release as a function of time (Fig. 2d). Upon injection of intracellular solution (Fig. 2d, arrow), we observed a negligible change in calcium levels (Fig. 2d, black). However, upon injecting the second messengers, inositol trisphosphate (IP_3) (Fig. 2d, cyan), nicotinic acid adenine dinucleotide phosphate (NAADP) (Fig. 2d, blue), and cyclic adenosine diphosphate ribose (cADPr) (Fig. 2d, magenta), we observed increases in fluorescent intensity 6 times that of basal levels. Since intracellular calcium is a sensitive indicator of cell toxicity, the return to basal calcium levels indicates that CNPs do not cause intracellular calcium release and that cells remain healthy after probing and injection.

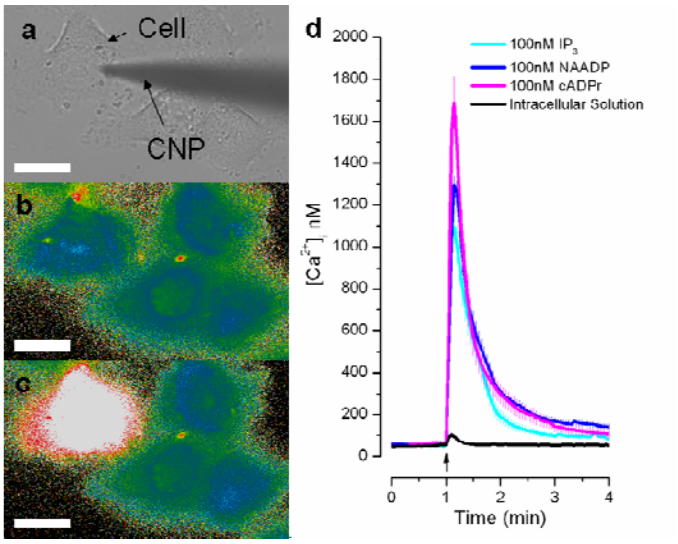

Figure 2: A messenger-loaded CNP targets a cell (a). Basal ratiometric fluorescent calcium levels are monitored before injection (b, blue and green in all cells). Messenger is injected and causes calcium to be released inside the targeted cell (c, white, left cell only) while non-targeted cells remain at basal levels (c, blue and green, right cells). The fluorescence intensity before, during, and after injection is monitored over time (d). Scale bars, 15 µm. Traces are mean +/- s.e.m of 6 cells.

CELL ELECTROPHYSIOLOGY

Using standard electrophysiology amplifiers, CNPs can probe intracellular domains of mouse hippocampal neurons (HT22) and record cell signals across their membranes (Fig. 3) [6]. After the CNP penetrates the cell, a seal is formed between the cell membrane and the glass near the tip so that the cells resting membrane potential can be measured (~-54 mV). Upon extracellular administration of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, the CNP records a hyperpolarization in the cell membrane potential (Fig. 3, black triangles) due to the influx of Cl^- ions into the cell. Hyperpolarization can be completely blocked (Fig. 3, blue squares) by first incubating the cells in 20 µM bicuculline (BIC), a competitive antagonist of the $GABA_A$ receptor.

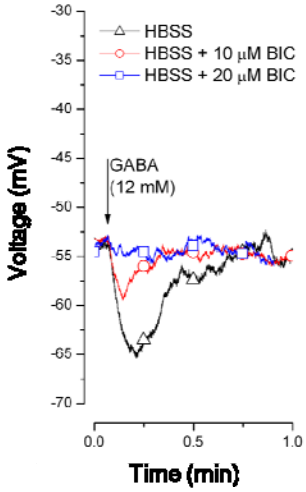

Figure 3: Cell electrophysiology with CNPs.

With their proven injection and electrophysiology abilities, it may be possible to utilize CNPs for electrophysiology measurements during cell injection – something not possible with their glass counterparts – which would be a valuable tool for biological and pharmacological studies. Although our experiments thus far have focused on individual cells, CNP-technology can be adapted to produce arrayed nanoprobes that would allow simultaneous probing of multiple cells.

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