Microfabricated Electrode Arrays to Study Exocytosis

CNF Project # 848-00
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Abstract:

Neurons release neurotransmitters in a process called exocytosis, wherein membrane bound packets of transmitter molecules are released from the cells upon stimulation. The mechanics of this process are poorly understood, but are known to involve three proteins collectively called the SNARE complex. Chromaffin cells of the adrenal gland share this release mechanism, and thus constitute a model system for neuronal release. Quantal release adrenaline from chromaffin cells can be measured using the electrochemical technique of amperometry, historically employing a carbon fiber electrode. To gain information about the exocytotic mechanism, and the release and diffusion kinetics of catecholamine during exocytosis, we have developed a method of electrochemical imaging employing microfabricated devices based on amperometry. We have successfully measured electrochemical events using Platinum, Gold, and transparent Indium-Tin-Oxide (ITO) electrodes. By comparing the experimental electrochemical signals with signals generated by Monte Carlo simulations, we can gain information about the release and diffusion kinetics of catecholamine during exocytosis. These devices have been combined with fluorescence imaging, yielding more information about the exocytotic process, and fast scan cyclic voltammetry (FSCV) which permits identification of some neurotransmitters. We are also working towards manipulating cells with the electrode arrays using Dielectrophoresis (DEP).

Summary:

Several important cell types, including neurons and chromaffin cells of the adrenal gland, release transmitter molecules via exocytosis—the release of membrane bound packets or “vesicles” of transmitter molecules. In amperometry, exocytosis is observed by electrochemical detection, usually employing a carbon fiber electrode [1]. Transmitter molecules released during an exocytotic event are oxidized by a nearby electrode, and the oxidation current is recorded.

We have fabricated electrochemical detector (ECD) arrays of four Pt, Au, or ITO electrodes on microscope coverglass to perform simultaneous amperometry by four electrodes at four different positions. The resulting current traces reveal the time course, quantal size, and position of vesicle fusion on the cell membrane. The average quantal size in individual cells on Pt electrodes was 0.5 pC to 3.5 pC, as expected for chromaffin vesicles [2]. The charge measured by the individual electrodes depends on the release position on the cell surface, allowing the release location to be pinpointed.

Each ECD is fabricated using 405 nm photolithography and lift off (Pt, Au) or etching (ITO) techniques. The ECD circuit consists of four wires, insulated by glass or photoresist, where the tips of the wires are exposed at the detector site, located at the corners of an ~ 12 x 12 µm square. The ECD is also coupled to an inverted fluorescence microscope, allowing us to observe the locations of release events both electrically and via fluorescence imaging. The positions of the release events determined from the ECD were in good agreement with the fluorescence locations [2].

Recently, we successfully performed amperometry using ITO ECDs. This gives us the ability to see what is directly above an electrode, via fluorescence if desired, and may be useful in studying transmitter release in neurons. The Au ECDs have also been successfully used for FSCV, where rapid oxidation and reduction of transmitter molecules results in a redox “fingerprint” that can be used to identify certain types of transmitter molecules [1].

A major drawback of the ECDs has been the need to place cells atop the ECDs “by hand” using a micropipette. This is tedious and time consuming. We are testing methods to position cells using Dielectrophoresis (DEP). DEP is the process by which a non-uniform electric field induces a dipole in a particle (a cell in our case), causing it to move.

References:


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- Study of neurotransmitter release mechanism using microfabricated electrode arrays (ECDs) and fluorescence.
- Possible to localize events using electrochemical information.
- Transparent electrodes allow observation of fluorescence through electrodes while recording electrochemical information.
- Fast cyclic voltammetry for identification of transmitter molecules.
- Dielectrophoretic (DEP) force to move and trap cells.

Figure 1, top left: 3- and 4-electrode Platinum ECDs.
Figure 2, bottom left: Transparent electrodes (white outline) with photoresist insulation (black outline).
Figure 3, above: A 9.6 µm polystyrene bead trapped between ECD electrodes using DEP.