Platinum Electrochemical Detectors to Study Exocytosis and Stimulus-Secretion Coupling

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Abstract:

Neurons release neurotransmitters in a process called exocytosis, wherein packets of transmitter molecules are released from the cells upon stimulation. Adrenal chromaffin cells and mast cells, among others, also undergo exocytosis. Release of adrenaline from chromaffin cells or serotonin from mast cells can be measured using the electrochemical technique of amperometry, historically employing a carbon fiber electrode [1]. To gain information about the exocytotic mechanism, we have developed various planar amperometric electrode arrays on glass coverslips employing platinum as the electrode material. We have successfully measured electrochemical events using these electrodes, and have developed devices that include stimulatory molecules patterned onto the coverslips to study stimulus-secretion coupling in mast cells. Because the electrodes are fabricated on glass, the devices can also be combined with fluorescence imaging techniques, yielding more information about the exocytotic process.

Summary of Research:

Several important cell types, including neurons, chromaffin cells of the adrenal gland, and mast cells (responsible for such things as allergic immune response), release transmitter molecules via exocytosis—the release of membrane bound packets or “vesicles” of transmitter molecules. Exocytosis can be observed by amperometry, an electrochemical detection method. In amperometry, transmitter molecules released during an exocytotic event are oxidized by a nearby electrode, generating a measurable current signal. Historically, carbon fiber electrodes have been employed for this purpose [1]. We have fabricated a variety of planar electrodes and electrode arrays that take advantage of this electrochemical detection technique. Electrodes are fabricated of platinum (Pt) on glass coverslips, with fused silica (SiO₂) or photoresist insulation. The advantage of fabricating the electrodes on glass is the ability to see through the coverslips to the cells above, and allows additional information about cell function to be recorded via brightfield or fluorescence microscopy simultaneously with the electrochemical measurements.

Previously, we demonstrated that amperometric
signals from chromaffin cells can be measured using Pt electrodes patterned on a glass coverslip while simultaneously observing fluorescence from the cell between the electrodes. And, that the Pt electrodes behave similarly to carbon fibers [2].

Recently, we have made several advances in our electrode design and functionality. We have redesigned our original four-electrode arrays such that over three times as many experiments can be done with the same coverslip (Figures 1 and 2). The four-electrode arrays were created for simultaneous amperometric measurement from four electrodes placed about a cell, which allows us to determine the location of an exocytotic event on the surface of the cell [2]. The new four-electrode array design allows a much higher experimental throughput, with less manipulation of the coverslips.

Furthermore, we have developed electrode designs that incorporate stimulatory molecules onto the coverslip surface. We demonstrated direct measurements of single vesicle exocytosis of rat peritoneal mast (RPM) cells stimulated by Poly-D-Lysine (PDL). Planar platinum electrodes were insulated with fused silica and a circular region (~5 µm diameter) in front of the individual electrodes was selectively coated with PDL using dry lift-off of Parylene-C. RPM cells were placed on top of the electrode/PDL regions using a glass pipette and standard patch-clamp techniques (Figure 3). Exocytosis was monitored amperometrically as current spikes corresponding to oxidation of serotonin, which is secreted by RPM cells (Figure 4). This method provides direct measurement of single cell exocytosis stimulated by a localized stimulus patterned with micrometer precision.

Taken together, these devices make up a unique tool set to study the exocytotic mechanism on a cellular and sub-cellular level.

References: