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Monitoring the Electrochemical Activity of Biological Samples Using Scanning Electrochemical Microscopy

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Abstract

The goal of the project is to develop Scanning Electrochemical Microscopy (SECM) as a tool to study the biological effects of oxidative damage on rat pheochromocytoma cells (PC12 cells). SECM is a useful tool for the analysis of biological samples because the ultramicroelectrode (UME) tip can detect the presence of electrochemically active compounds such as neurotransmitters, particularly dopamine and norepinephrine, while simultaneously characterizing the topography of the cell. The topography of the cell was determined by maintaining a constant distance between the tip of the electrode and the surface of the cell. In the collector mode, the potential of the SECM microelectrode was set to detect dopamine release after stimulation with a 100mM potassium ion solution. A spike in current indicated the release of neurotransmitters from the cell surface. Successful stimulation was observed on differentiated PC12 cells, which were treated with nerve growth factor (NGF), and undifferentiated PC12 cells.

Impedance-Based Constant Distance SECM Imaging Gives a Topographical Image of a Surface

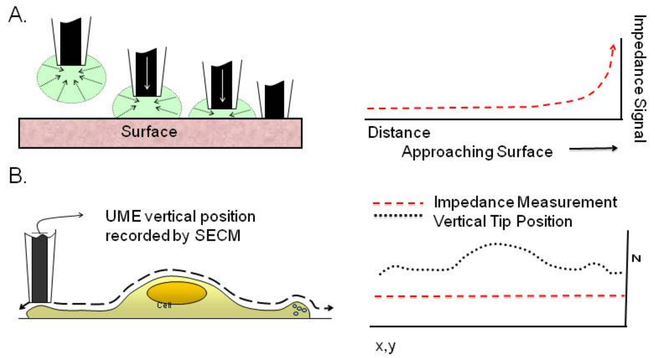


Figure 1. Schematic A shows that diffusion of ions to the electrode decreases as the electrode approaches a surface. Since the impedance signal is related to the concentration of ions in the solution, this signal changes as the electrode approaches a surface, giving a distance-dependent signal. Schematic B shows that this distance-dependent signal can be set and maintained by the SECM. As the electrode moves across a surface, the vertical position of the electrode is recorded by the SECM, which generates a topographical image of the surface.

Impedance-Based Topographical Imaging is Independent of the Applied Potential

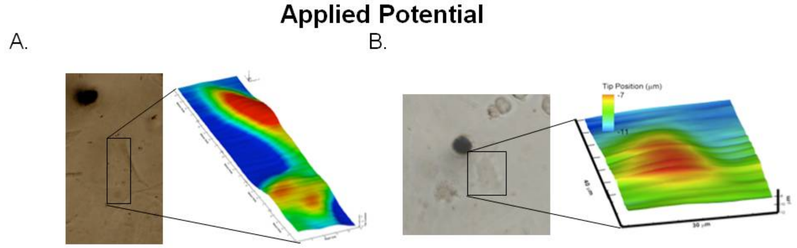


Figure 2. A 5µm carbon fiber disc electrode was used for the impedance-based constant distance imaging of PC12 cells. In Image A, the applied potential of the SECM was -1.2 V. At this potential, molecular oxygen is reduced to water. For Image B, the applied potential was +0.8 V. At this potential, dopamine is oxidized to dopamine orthoquinone.

Undifferentiated PC12 Cells vs. Differentiated PC12 Cells

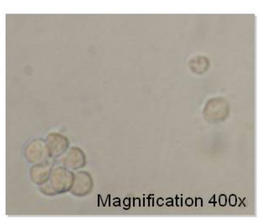
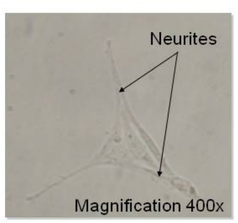
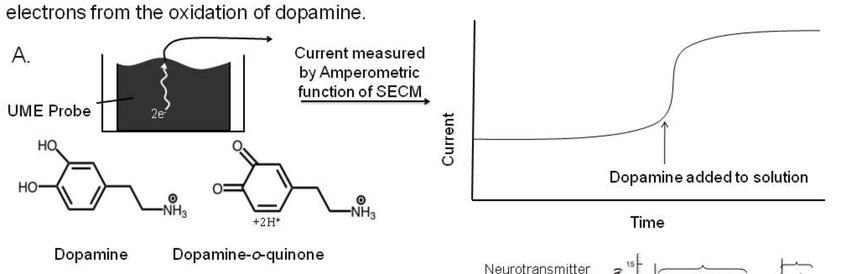


Figure 3. Undifferentiated PC12 cells (shown left) are round and spheroid. When treated with NGF, a peptide hormone, the cells become differentiated (shown right). Differentiation is characterized by the flattening of cells and the formation of neurites.



SECM Monitors Neurotransmitter Release using Amperometry in Collector Mode

In collector mode of operation, the SECM was specifically set to oxidize neurotransmitters at the tip of the UME. The UME was used to detect the release of individual vesicles that contain neurotransmitter. To optimize the chances of recording release, the UME was placed close to the cells' surface. Stimulants, such as K+, acetylcholine, and nicotine, were added close to the cells by means of a release pipette. These stimulants caused vesicles to fuse with the membrane and release dopamine. The change in current as detected by the UME was due to the flow of the electrons from the oxidation of dopamine.



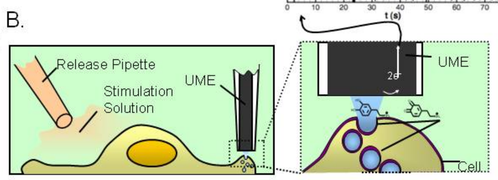


Figure 4. Schematic A shows the oxidation of dopamine at the UME tip at a potential of +0.8 V. The release of dopamine was detected by amperometry. Cartoon B shows the experimental setup and graph of neurotransmitter release.

Stimulated Release of Dopamine from Undifferentiated PC12 Cells

For each of the graphs below, cells were stimulated with a 100 mM K^+ solution. The red line shows the measured current and the black line shows the measured impedance. The spikes in current (red line) show the release of dopamine from the cells. The impedance signal (black line) changes when the cells are stimulated due to the different ionic strength of the solution containing the stimulant. Immediately following stimulation with K^+, the cells released dopamine. Cells were stimulated multiple times, but with each subsequent stimulation the observed amount of dopamine released decreased. Each of the upward arrows represents stimulation.

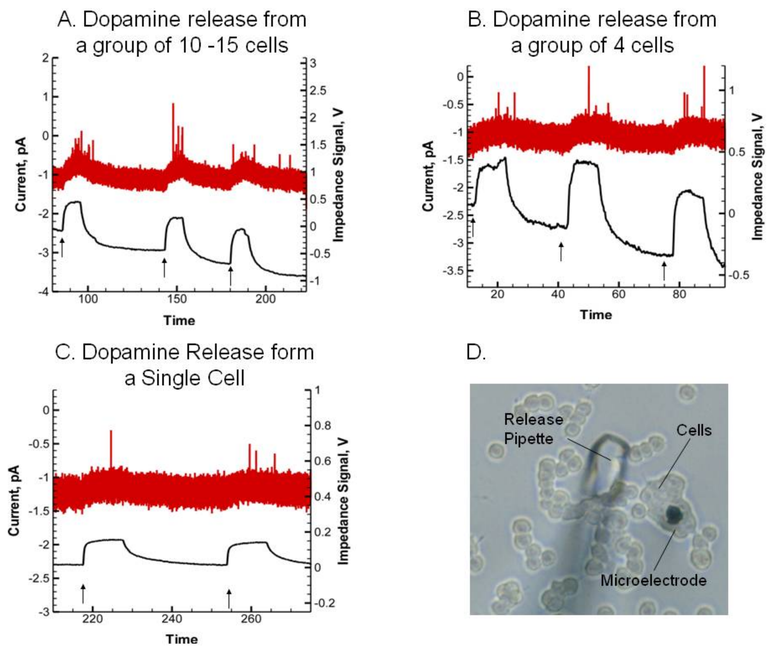


Figure 5. Each of the graphs was obtained from an experiment using a 5µm carbon fiber disc electrode to detect the release of dopamine from undifferentiated PC12 cells. The potential of the SECM was set to +0.8 V. Graph A and B show the dopamine release from two separate groups of undifferentiated cells, while Graph C shows dopamine release from a single undifferentiated cell. Image D shows PC12 cells with the release pipette and microelectrode.

Stimulated Release of Dopamine from Differentiated PC12 Cells

These three graphs show the current relating to dopamine release from differentiated PC12 cells. Greater dopamine release was observed from a group of cells than from a single cell. The amount of dopamine the cell(s) release(s) decreased with each consecutive stimulation, since the cells had depleted their original supply of dopamine. The same group of cells was stimulated 15 minutes later and dopamine release was detected.

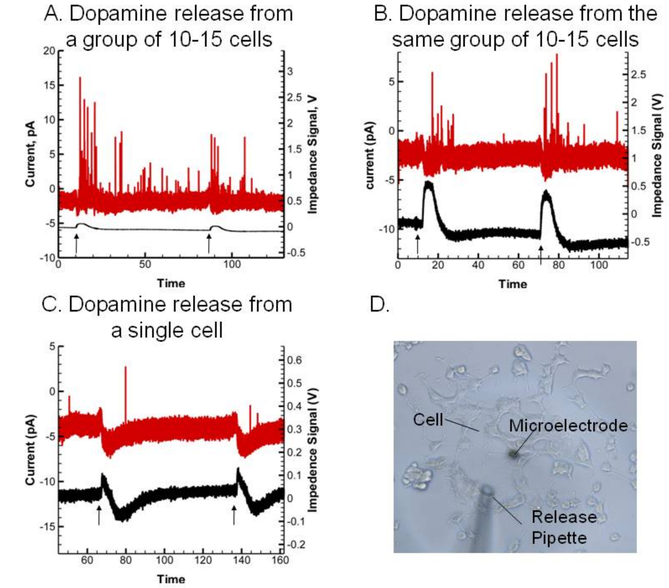
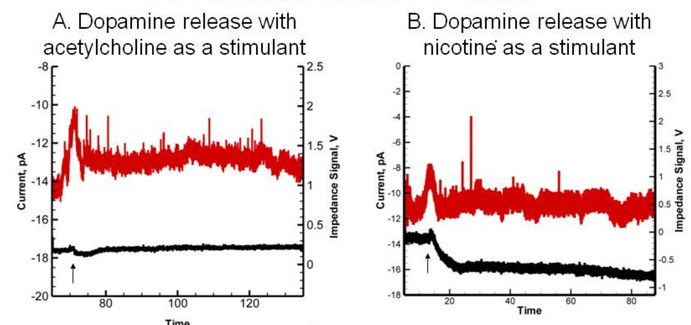


Figure 6. Each of these graphs was obtained from an experiment using a 5µm carbon fiber disc electrode to detect the release of dopamine from differentiated PC12 cells. The potential of the SECM was set to +0.8 V. Graph A shows the current relating to dopamine release from a group of differentiated cells. Graph B shows the current relating to dopamine release from the same group of cells 15 minutes later. Graph C shows dopamine release from a single differentiated cell with the UME positioned over the cell body. Image D shows PC12 cells with the release pipette and UME.

Acetylcholine and Nicotine Stimulated Release of Dopamine from Undifferentiated PC12 Cells



Future

In the future, we wish to continue to detect the release of dopamine upon stimulation with acetylcholine and nicotine while the impedance remains constant and simultaneously image PC12 cells and monitor neurotransmitter release from healthy cells.

Acknowledgments

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