

ELECTROCHEMICAL IMAGING OF QUANTAL EXOCYTOSIS IN SINGLE CELLS

Syedmehdi Orouji

Dr. Kevin Gillis, Thesis Supervisor

ABSTRACT

Microelectrodes are widely used in detection of exocytosis events. In order to detect both the time and release location of quantal exocytosis from a single cell, four square microelectrodes located in a 20 μm square microwell were fabricated through photolithographic techniques. A 30 nm thin gold films were used as the material for the microelectrodes and the microwell was fabricated using SU8 thick photoresist. In order to test the quality of microelectrodes, we used cyclic voltammetry technique and the test analyte ferricyanide prior to the amperometry recording. A high density of chromaffin cells were placed in the solution reservoir on top of the electrode arrays, and individual cells were targeted in to the microwells automatically. Poly (L-lysine) was coated on the microelectrode to promote the cells adhesion. Following of that, exocytosis events were triggered by introducing a high potassium concentration to the bath solution. The data obtained from cell recordings were compared with the simulation data obtained from FEM modeling and the locations of release sites were identified. It was observed only sites of quantal releases with relatively high amount of charge (3.59 ± 0.58 pC) can be identified. In order to expand the area on the cell in which the electrochemical imaging is attainable, a simulation-guided electrode re-design was tested using FEM simulations. The results from simulations showed that the improved design, with curved-like electrodes, is predicted to increase the area of detection by approximately 45% compared to the design used in cell tests.