

NANO- AND MICRO- SCALE STUDIES OF EXOCYTOSIS

Won-Chul Shin

Dr. Kevin Gillis, Dissertation Supervisor

ABSTRACT

Neurons and neuroendocrine cells contain vesicles packed with hormones or neurotransmitters. Upon appropriate stimulation, a rise in intracellular Ca^{2+} concentration triggers the fusion of vesicles with the outer membrane of cells and release of vesicle contents into the extracellular space in a process called exocytosis. In this thesis, we developed three new nano- and micro- techniques to study exocytosis.

1. We used scanning ion conductance microscopy (SICM) to image changes in the surface membrane of adrenal chromaffin cells after stimulation of exocytosis. Punctate depressions were noted in clusters of two or more. Increases in membrane surface area, consistent with the fusion and collapse of one or more vesicles into the surface membrane, were observed 64% of the cells.

2. We used a microcontact printing method with PDMS stamps by “soft” lithography to pattern microislands of rat hippocampal neurons to form autapses. Neurons on microstamped microislands survived and grew neurites for more than 21 days and resembled microisland cultures formed by the traditional method of spraying collagen on agarose coated substrates.

3. Microfabricated devices were developed to electrochemically measure quantal catecholamine release from an array of individual cells. Here we report patterning of cell-sized holes in ~ 15 nm-thick films. These films are placed on transparent indium tin oxide electrodes to insulate the unused part of the electrode whereas the holes in the film both determine the location of the working electrode and serve as pockets for cell trapping. We found that this approach represents a simple and effective way to target cells to electrodes to record amperometric spikes.