Public Abstract
First Name:Won-Chul
Middle Name:
Last Name:Shin
Adviser's First Name:Kevin
Adviser's Last Name:Gillis
Co-Adviser's First Name:
Co-Adviser's Last Name:
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Neurons and neuroendocrine cells contain vesicles packed with hormones or neurotransmitters. Upon appropriate stimulation, a rise in intracellular Ca2+ concentration triggers the fusion of vesicles to the outer membrane of cells and release of vesicle contents into the extracellular space in a process called exocytosis. Understanding of the mechanisms and regulation of exocytosis has been an important topic of cell research for many years. In this thesis we developed three new nano- and micro- techniques to study exocytosis.

1. The extent that vesicles maintain a distinct identity and morphology after fusing with the plasma membrane is controversial. We used scanning ion conductance microscopy to image changes in the surface membrane of adrenal chromaffin cells after stimulation of exocytosis with a high K+ solution. Within several minutes after stimulation, punctate depressions were noted from 16% of the cells. The depressions were not randomly distributed, but appeared in clusters of two or more in small area and disappeared after several minutes. Increases in membrane surface area, consistent with the fusion and collapse of one or more vesicles into the surface membrane, were observed in 64% of the cells after high K+ stimulation. This study showed that scanning ion conductance microscopy can be used to follow the time course of surface membrane changes resulting from exocytosis and endocytosis.

2. It is important to be able to study neurons in culture in simple networks in order to address questions in neuroscience at the cellular and molecular levels. The simplest network is known as the autapse, where a neuron synapses onto itself. We used a microcontact printing method with PDMS stamps fabricated by “soft” lithography to create controlled microisland patterning of rat Hippocampal neurons to enable autapses to form. A collagen solution to promote neuron adhesion and growth was stamped on an agarose substrate that prevents adhesion of neurons. 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. Patch-clamp electrophysiological recordings demonstrated that the microstamp microisland culture neurons were viable by the traditional collagen spray microisland cultures. We conclude that the microcontact printing method is a simple and effective method for microisland culture of Hippocampal neurons.

3. Microfabricated devices were developed to electrochemically measure quantal catecholamine release from an array of individual cells as a step towards high throughput assays of exocytosis. Here we report patterning of cell-sized holes in ~ 15 m-thick films. These films are placed on transparent indium tin oxide (ITO) 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. Thus there is “self-alignment” of working electrodes and cell docking sites. Two approaches were used to fabricate insulating films containing holes. In one approach SU-8 photoresist was patterned using photolithography into 15 – 20m wide posts and then the elastic polymer poly(dimethylsiloxane) (PDMS) was molded over the posts using a compression method to create a thin PDMS film. A second approach is to directly pattern holes in a photoresist film spin coated onto the electrode array. We recorded amperometric spikes indicative of quantal exocytosis from bovine adrenal chromaffin cells following stimulation of cells with a depolarizing “high-K+” solution. We conclude that this approach represents a simple and effective way to target cells to electrodes.