Development of a Single Cell Neurotoxicity Assay

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Statement of Purpose: Our goal is to develop high-throughput neurotoxicity assays to investigate chemical effects on pioneer neuron axon pathfinding. Pioneering neuron axon pathfinding is a single-cell event and may be a rate-limiting event in tissue innervation, normal nerve development, and nerve regeneration. Existing neurite outgrowth assays cannot adequately investigate pioneer neurons response to neurotoxicants.

Cells communicate through paracrine and autocrine factors. Neurons appear to protect each other from apoptosis and toxins by providing survival factors known as trophic support. Therefore, to isolate single-cell responses requires isolating single cells from neighboring cells. High throughput assays require shrinking cell culture wells. As sample sizes get smaller and smaller, slight variations in cell number in each well creates significant measurement errors. We propose the solution is single-cell wells to assay cells individually.

Neurons do not grow well in low culture densities. Also, the effect of toxicity is amplified in small cell culture volumes. We propose a culture system for single cell assays that overcomes these challenges and supports neuronal culture viability and axon extension. We demonstrate the ability to interrogate individual neurons with chemicals released from single biodegradable microspheres.

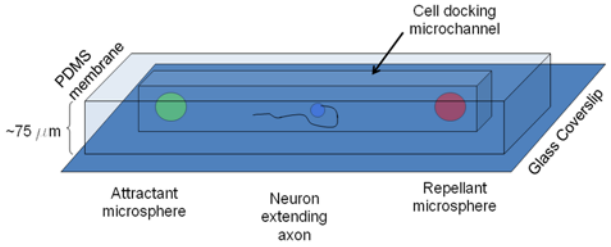

Figure 1. Schematic diagram of the goal of a single-cell docking microchannel neurotoxicity assay

Methods:
We microfabricated cell docking channels in poly(dimethyl) siloxane (PDMS) with soft lithography techniques. Neurons were laser patterned into the channels and cultured normally at low cell plating density conditions. Single neuron viability was observed in response to the chemicals released from the microspheres. Polymer microspheres encapsulating various chemicals thought to attract and repel growth cones were fabricated as models of neurotoxicants that could affect axon pathfinding. We used Semaphorin 3A (Sema 3A) as a model to mimic toxic chemicals that causes axons to "die back." Micropsheres incorporating Sema 3A were laser patterned adjacent to PC12 neuron model cells.

Results:

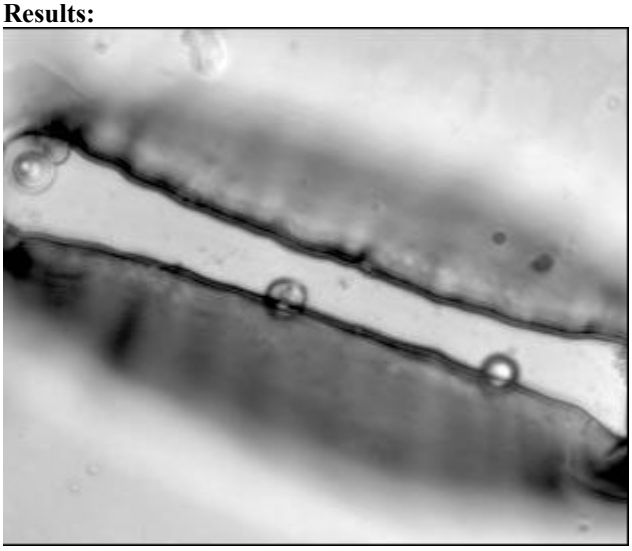

Figure 2. A single chick forebrain neuron one day after patterning after laser micropatterned between microspheres in a PDMS cell docking microchannel

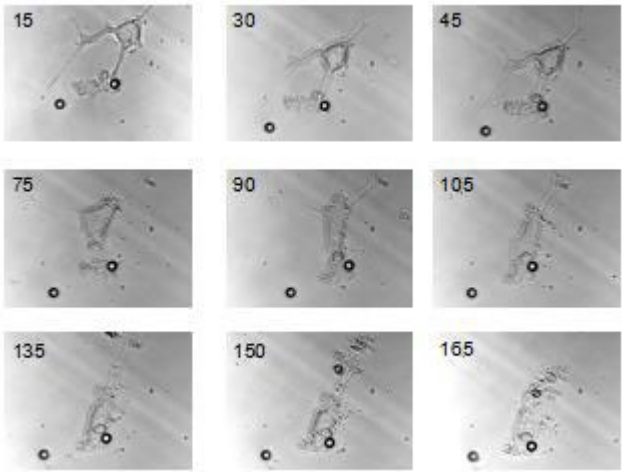

Figure 3. Low culture density differentiated PC12 neuron model cells showing "dying back" of neurites in response to microspheres releasing Semaphorin 3A. Numbers in the upper left corner of each picture represent minutes after patterning of microspheres.

Conclusions:
Primary chick forebrain neurons were shown to be viable after laser patterning into PDMS microchannels. We demonstrated that PC12 cells, a phenotypic neuron model, was sensitive to Sema 3A released from biodegradable microspheres. Future work will involve scaling this assay into a single-cell multi-well format.

References:
(Dickson, BJ Science 2002;298:1959-1964)
(Pirlo, RK. Biotech J. 2007;1:1007-1013)