Abstract:
The separation between the axon tip and the cell body of neurons in vivo is not easily achieved by traditional culture techniques. Because of the importance of the axon in neuronal function, learning how the axon functions over long distances is crucial to our understanding of neurodegenerative diseases. We have developed a microfluidic chamber culture platform to separate the axon tip from the cell body in vitro. Using this platform, we have identified retrograde axonal transport of insulin-like growth factor 1 and functional interactions between immune effector cells and axons. With these data, we gain a better understanding of the pathology underlying neurodegenerative diseases affecting the motor neurons.

Summary of Research:
Neurons are the highly polarized cells of the nervous system required for execution cognition, movement, and sensation. A neuron has a cell body with short dendrites and a single long axon, which may be up to a meter in length. Cell bodies are housed in the gray matter of the brain and spinal cord, while axons of the white matter project to distal targets. This separation between the cell body and the axon tip is impossible to model using basic culture methods, in which neuron populations are cultured in a dish with no structured organization of axons or cell bodies (Figure 1). Though this type of “gang” neuron culture has provided researchers with data critical to our understanding of neurodegenerative diseases, gang cultures fail to adequately isolate the axon-specific contribution to disease.

We have produced a microfluidic neuron culture platform based on that by Taylor, et al. [1]. Two-layer molds were produced using SU-8 negative photoresist on a silicon wafer. Axon grooves produced by the first layer (3 µm) are 5 µm wide ridges that span the distance between the cell body and axon chambers, between 200 and 900 µm. The second layer (124 µm) produces the cell body and axon chambers, which are 7 mm long by 1 mm wide and are connected to 5 mm diameter round inlets. These dimensions allow cell bodies to enter the cell body chamber, but prevent them from crossing through the grooves to the axon chamber (Figure 1). The wafer mold is then flooded with polydimethylsiloxane (PDMS), degassed, and cured at 65°C for 4 hours. The PDMS is peeled from the wafer, trimmed, and then sealed to a glass coverslip. Neurons isolated from mouse embryos are plated in one chamber. Axons extend through the grooves and populate the axon chamber (Figure 2). This culture platform more accurately models neurons in vivo, where the axon and cell body are physically separated, and may be treated separately to better mimic the differences in neuronal microenvironments in vivo. Additionally, a pure axon population is isolated, and axon-specific events can be observed. As axonal pathology have been implicated in the
progression of most if not all neurodegenerative diseases, this system provides a powerful tool to identify roles for the axon in normal neuron function, survival and disease.

Our first focus is on the role of target-derived insulin-like growth factor 1 (IGF-1) on neuron survival and phenotypic maintenance. IGF-1 has been shown to support the neuronal survival in vitro and in vivo. We treated fluidically isolated axons with quantum dot-conjugated IGF-1, and identified fluorescence in the cell bodies of these cells, indicating that the IGF-1 is retrogradely transported in axons (Figure 3), while quantum dots alone are not. This suggests that transport is specific for IGF-1. We are currently working to identify signaling cascades activated by axonally-applied IGF-1, and how these signals maintain survival of the neuron and the structure of the axon.

Multiple sclerosis (MS) is an inflammatory demyelinating disease that affects motor pathways. Previous work in our lab has demonstrated a role for CD8+ T lymphocytes in the pathogenesis of a mouse model of MS. We used our microfluidic chambers to develop an in vitro model of the interaction between axons and T cells, taking advantage of the pure population of axons. Using this model, we find that T cells introduced to the axon chamber interact with axons in a wash out-resistant manner, suggesting that this interaction is real and specific (Figure 4). We are continuing with this line of inquiry to identify immune ligands on the axon, which may facilitate the injury of demyelinated axons in MS.

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