Quantitative Effects of NGF Encapsulating PLGA Microsphere on Neurite Outgrowth at Single Cell Level

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Statement of Purpose: In the U.S., 250,000 people currently suffer from some form of spinal cord injury. Ten thousand new spinal cord injuries occur each year **[1]**. One approach to treat nerve damage may be the implantation of biodegradable polymer "nerve conduits" seeded with new neurons inside and loaded with neurotrophic factors that promote and direct nerve growth while suppressing scar tissue. To achieve this goal, bioengineers will need to understand the factors involved in individual neuron growth in channels promoted by neurotrophic chemical gradients provided by time-release biodegradable compounds. The effect of nerve growth factor (NGF)-loaded Polylactic-Co-Glycolic Acid (PLGA) microspheres has been studied in the aggregate, however, little is known regarding the effects of a single degrading microsphere on a single neuron. A laser cell micropatterning technique allows the positioning of individual neurons and microspheres, enabling quantitative analysis (Fig. 1).

Methods: Embryonic day 7 chick forebrain neurons were cultured using a modified culture technique described by Heidemann and coworkers **[2]**. Cells were placed in suspension of culture media (L15+ CO_2 independent) and injected into the patterning chamber. The suspended neurons are patterned into microchannels coated with poly-L-lysine to aid in cell adhesion.

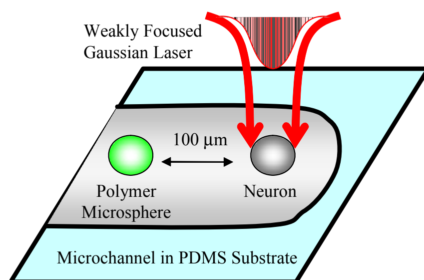

***Fig. 1:** The laser micropatterning technique developed by Bakken and coworkers [3]. provides the ability to position neurons and microspheres within the guidance channels with speed and ~1µm precision.*

To fabricate a spin-coated Poly Dimethyl Siloxane (PDMS) surface a mold was created by photolithography. Shallow channels (~30 μ m depth) were created to confine the neurite outgrowth regulated by the NGF diffusion in 1D as shown (Fig. 2).

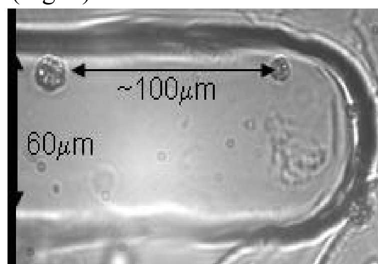

***Fig. 2:** Neuron (right) and microsphere (left) laser micro-patterned in a shallow micro-channel*

Results / Discussion: The quantitative results were shown in Fig. 3. Neurons have been shown to respond with preferential growth in the direction of neurotrophic concentration gradients released by NGF microspheres and neighboring neuron cells at rates 2 to 3 times, respectively, those of control groups. With NGF in solution and no gradient, neurite outgrowth is not statistically different from lack of NGF. The random nature of neuron growth necessitates a large number of data points to attain statistically significant generalized conclusions. The laser micropatterning technique provides the speed and precision necessary for quantitative experimental analysis. The NGF concentration gradient created by time-dependent NGF release of a degrading microsphere can be modeled theoretically with a system of differential equations. Parameters will be derived experimentally with the described technique, boundary conditions may be translated from microspheres in open space to more closely model intracellular spaces, and the mathematical model will be validated experimentally.

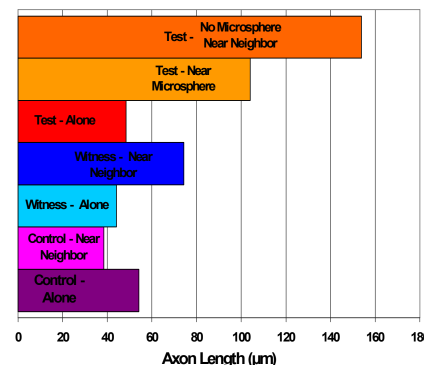

***Fig. 3:** Comparison of axon growth at day 8 for neurons in L15+ media with no NGF (Control), with NGF in media (Witness), and with NGF only in microspheres (Test) for neurons alone and influenced by nearby neighbor neurons.*

Conclusions: Laser patterned neuron- PLGA microsphere coculture system on a micro-fabricated, grooved surface can be used to systematically investigate the effect of gradually released NGF on the neurite outgrowth at the single cell level.

References:
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[2] Heidemann, S.R., et al., (2003) The culture of chick forebrain neurons, *Methods in Cell Biology* **71**:51-65
[3] 1. Bakken D., et al., (2005) Laser Micropatterning of Polylactide Microspheres into Neuronal-Glial Coculture for the Study of Axonal Regeneration, Macromolecular Symposia, **227**:335-344