Inducing directional axon growth with laser micropatterned neuroattractant-repellant eluting PLGA microspheres in PDMS microchannels

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Statement of Purpose: Cell polarity and motility mechanisms are important issues in areas such as nerve regeneration. In vitro investigation techniques provide insight into in vivo behavior. It remains a challenge to control the direction of axon guidance in vitro. Suzuki and coworkers [1, 2] describe stepwise laser thermal etching of agarose passages to allow axon growth. However, thermal etching may modify the defined surface, affecting the advancing growth cone. Alternately, a mechanism to use microcontact printing of adhesive surface molecules to guide axon growth has been proposed by Stenger and coworkers [6] and then modified by Vogt and coworkers [7]. They reported that under their conditions, to this point, surface adhesivity alone was not sufficient to maintain polarity. To address these issues, we propose a novel approach which preserves the spatial resolution, micro-scale surface features, and adhesive/matrix molecules. Neurons respond to basal concentration levels of soluble factors for survival as well as the concentration gradient for growth cone guidance [3]. Growth cones also respond to soluble and surface-bound neurorepellants [4]. Therefore, our proposed method utilizes biodegradable polymer microspheres releasing attractant or repellant factors to guide axon growth along a single direction as shown schematically in Fig. 1.

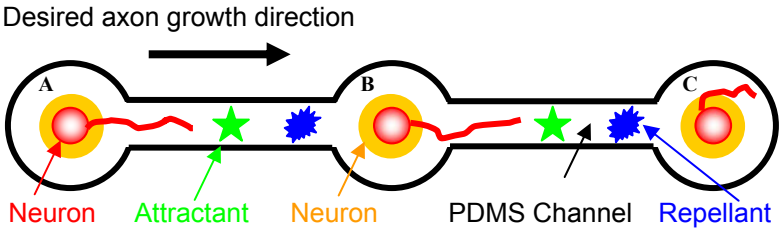

Fig. 1. Schematic of the attractant-repellant mechanisms for directional axon-growth guidance within PDMS microchannels

Methods: Neurons (Fig. 1) were laser micropatterned into poly(dimethylsiloxane) (PDMS) microchannels. Neuroattractant-releasing microspheres (Attractants) were deposited in the desired forward growth direction. Neurorepellant-releasing microspheres (Repellants) deposited adjacent to neurons repel initial axon growth from the undesired growth direction.

The cell culture surface was cleaned and coated homogeneously with poly-L-lysine and laminin for neuron adhesion and axon outgrowth. Microchannels were created by spin-coating PDMS on a silicon wafer mold with photoresist (SU-8) features.

Polymer microspheres were formed using a modified double emulsion water/oil/water (W/O/W) method. 400mg of polylactide-co-glycolide 85/15 (PLGA; Absorbable Polymers) were dissolved in 2mL of dichloromethane. To prepare Nerve Growth Factor (NGF) encapsulated microspheres, 80 mg of bovine serum albumin (BSA; Sigma) and 100 µg of NGF (Harlan Bioproducts) were mixed in 200 µL of PBS. Other growth factor combinations were prepared similarly (e.g. Brain derived neurotrophic factor (BDNF) and the neurorepellant Ephrin). The protein mixture was then added to the polymer solution and homogenized, creating the first emulsion. 5% Polyvinyl alcohol (PVA; Sigma) was added and homogenized, forming the second emulsion. The second emulsion was added to 0.1% PVA solution and stirred to evaporate the organic solvent. The microspheres were centrifuged, rinsed, freeze dried, and stored under vacuum.

Results/Discussion:

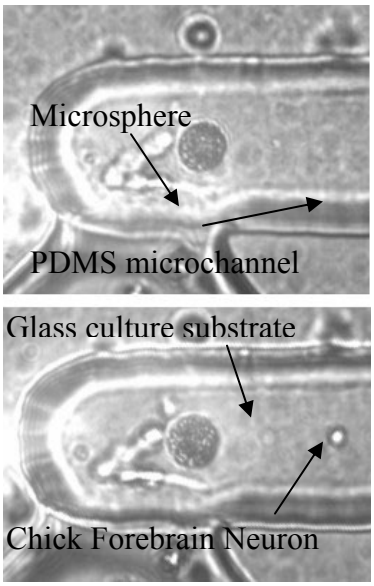

Fig. 2. PDMS channel with PLGA Microsphere before and after micropatterning a chick forebrain neuron

Conclusions: Neurons and microspheres releasing neuroattractants and repellants were laser micropatterned on defined substrates achieving soluble factor diffusion. Future work involves using the system to manipulate axon polarity for the precise evaluation of the mechanism of growth cone guidance response to chemical gradients. The optimum microsphere deposition time point, position, encapsulation concentration, and degradation release rates will be determined by experimentation.

References

1. Suzuki, Y. Lab on a Chip, 2005. 5(3): p. 241-247.
2. Suzuki, Y. J Nanobiotechnology, 2004. 2(1): p. 7.
3. B.I. Rosner, Ann. of Biomed. Eng., 2003. 31(11): p. 1383-1401.
4. A.C. von Philipsborn Develop., 2006. 133(13): p. 2487-2495.
5. C. Deister, J. Neur. Eng., 2006. 3: p. 172–179
6. Stenger et. al. J. of Neurosci. Meth., 1998
7. Vogt et. al. J. of Neurosci. Meth., 2004