Biology Inspired Design for Guided Axon Growth

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Axons are guided to their targets by a combination of both haptotactic and chemotactic cues that are either attractive or repulsive.[1] The haptotactic cues are the short-range cell adhesion molecules, often provided by the extracellular matrix, and include laminin protein and peptides which is predominant in the basal lamina of neurons. The chemotactic cues are the long-range diffusible molecules comprised of netrins, semaphorins, neurotrophins, among others. Our goal is to mimic these effects in the design of 3-dimensional polymeric matrices.

We have been specifically focused on understanding the role of concentration gradients of neurotrophins for cell guidance, first examining the role of soluble neurotrophins and more recently the role of immobilized neurotrophins. We are interested in whether guidance can be achieved with immobilized factors, as has been observed with soluble factors, and understanding the mechanism for this guidance behavior. To this end, we created well-defined concentration gradients of nerve growth factor (NGF) and neurotrophin-3 (NT-3) and examined guidance of neurites of both primary dorsal root ganglia (DRG) and pheochromocytoma cell lines (PC12). Guidance was observed in response to *soluble* gradients of both NGF alone (133ng/ml/mm)[2] and NGF/NT-3 (80 ng/ml/mm of each), where a synergistic effect was observed with NGF binding at tyrosine kinase A (trkA) and NT-3 at trkC.[3] We also created well-defined concentration gradients of *physically immobilized* NGF and NT-3 in poly(2-hydroxyethyl methacrylate) (PHEMA) matrices and asked the same guidance question. Here we observed axonal guidance of DRG neurons in response to immobilized NGF (300 ng/ml/mm)[4] and NGF/NT-3 (200 ng/ml/mm of each); synergism of NGF/NT-3 was confirmed by demonstrating binding of NGF and NT-3 to trkA and trkC, respectively.[5]

In order to better understand the mechanism of guidance while at the same time design a scaffold for implantation, we have been examining biodegradable chitosan matrices for the *chemical immobilization* of neurotrophin concentration gradients.[6] Since the superior cervical ganglia (SCG) neurons have only the trkA receptor, they are particularly well-suited to ask questions on cell guidance mechanisms as a function of NGF concentration gradients. To this end, we are currently immobilizing NGF concentration gradients on chitosan and examining guidance with SCG neurons.

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