

GUIDED CELL GROWTH AND TISSUE REGENERATION

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INTRODUCTION: Spinal cord injury is a devastating disorder of the central nervous system, which does not spontaneously regenerate but has the capacity to regenerate. In an attempt to promote regeneration after traumatic injury to the spinal cord, we have been investigating the stimuli critical to axonal guidance and testing these in biomimetic strategies of repair. Specifically, since axons are guided to their targets by a combination of attractive and repulsive, short-range and long-range cues, we have been investigating ways to incorporate these signaling molecules into tissue engineering constructs and then test their guidance potential in vivo.

To test the importance of the short-range, contact-mediated cues, we created a 3D hydrogel scaffold that had biochemical volumes of cell-adhesive ligands (i.e. RGD) separated by non-adhesive volumes of agarose, thereby mimicking the attractive and repulsive cues found in development.

To test the importance of long-range, diffusible cues, we designed immobilized concentration gradients of neurotrophins (i.e. NGF) and tested their regenerative capacity.

METHODS: Three-dimensional (3D) agarose gels were modified with RGD peptides by a combination of photochemistry and focused single photon laser as previously described.¹

Neurotrophin concentration gradients of nerve growth factor (NGF) were immobilized in poly(2-hydroxyethyl methacrylate) macroporous scaffolds using a gradient maker, as previously described.² Primary rat dorsal root ganglia neurons were used to test the guidance potential of both the RGD-agarose patterned gels and the NGF-concentration gradient gels.

RESULTS: Fluorescein-isothiocyanate (FITC)-labeled GRGDS was immobilized in agarose gels and visualized by confocal microscopy to have biochemical volume channels of approximately 170 μm in diameter. These channels had almost identical rheological properties to non-RGD modified agarose. Dorsal root ganglia neurons aggregated on the RGD-channels and extended cell bodies and neurites into the channels and not into the agarose that separated the RGD-channels. Scrambled RDG peptide channels, identically

synthesized, had no cells or neurites growing within, demonstrating the specificity of the interaction between neuronal cell integrin receptors and RGD.

A series of linear NGF-concentration gradients were immobilized in PHEMA scaffolds as determined by ELISA. Dorsal root ganglia neurites were guided by the NGF concentration gradient at 310 ng/ml/mm. When a second neurotrophin concentration gradient of neurotrophin-3 (NT-3, at 200 ng/ml/mm) was applied, neurite guidance was observed at a lower NGF gradient of 200 ng/ml/mm. Interestingly, both tyrosine kinase receptors, TrkA (for NGF) and TrkC (for NT-3) were shown to be co-localized on the dorsal root ganglia neurons studied, demonstrating a synergistic effect in terms of neurite guidance observed. The importance of the dual concentration gradient was demonstrated through a series of controls with constant concentrations of either NGF or NT-3 while the other was presented as a gradient.

DISCUSSION & CONCLUSIONS: Primary neurons were shown to be guided by both contact mediated, extracellular matrix analogs and long range, neurotrophin concentration gradients. These systems demonstrate the fundamentals that are important to guidance and are being tested in a nerve guidance channel for their potential applicability in an in vivo model of repair of the spinal cord.

REFERENCES:

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