Length-Scale Mediated Differential Adhesion of Neurites and Astrocytes

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Purpose: Despite the fact that most eukaryotic cells have dimensions of 10 μm or more, neuronal processes (axons, dendrites) have micron or submicron dimensions. Directing the growth of neuronal processes as part of, for example, a scaffold-based mechanism to mediate spinal cord injury will require technologies to create structures patterned at both micron and submicron length scales. A challenge is to create surfaces that preferentially control neurite adhesion/growth while reducing or preventing the simultaneous adhesion of inhibitory cells associated with glial scarring. We have used e-beam methods to create surface-patterned PEG-based hydrogels. They resist non-specific protein adsorption and can control cell adhesion. Here, we explore the differential response of neurons and astrocytes to cell-adhesive surfaces patterned with sub-micron cell-repulsive hydrogels spaced at micro length scales. Importantly, we show that there is a window of length scales over which these structures can control neurite growth while preventing astrocyte adhesion and astrocytic process development [1].

Methods: Si substrates were patterned with discrete hydrogels using established procedures [2-4]. Spincoated thin films of PEG [6800 Da] were irradiated in a LEO 982 FEG SEM (1 kV) with controlled e-beam position and dwell time. Samples were then washed in water to remove unirradiated PEG leaving Si except where gels had been patterned. Prior to culture, the samples were exposed to a conditioning solution of laminin in Hank's buffered salt solution (10 g/ml). Patterned surfaces were then exposed either to primary mouse dorsal root ganglia (DRG) or to Neu7 astrocytes for 24 hr. The samples were fixed, dried, and examined by SEM.

Results: Our important independent variable - the gel spacing - was varied from pseudo-continuous (overlapping gels) to 10 μm. Fig. 1 shows that such gels swell by a factor of about 5 and resist laminin adsorption. Fig. 2 shows an SEM image of DRG neurites growing on silicon where portions of two different gel arrays are visible. DRG neurites can grow from the unpatterned laminin-coated Si (center) into the square array of PEG gels spaced 2 μm from each other (left). They are unable to grow into an identical array with gels spaced by only 1 μm (right). Neu7 astrocytes were unable to adhere/spread significantly to either array (not shown). In fact, astrocyte adhesion and spreading is insignificant until the inter-gel spacing is increased to 3 μm or more. Fig. 3 illustrates the length-scale dependence of neurite growth and astrocyte adhesion as a function of inter-gel spacing. There is an important window where neurite adhesion is permitted and neurite growth can be controlled while astrocyte adhesion is prevented.

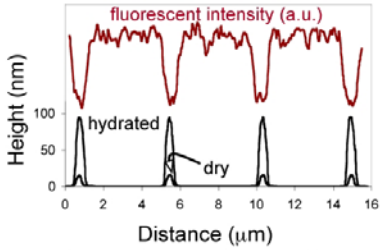

Fig. 1 - Laminin adsorbs onto the silanized Si but is repelled by the PEG gels.

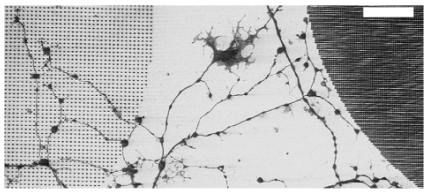

Fig. 2-DRG neurites grow from unpatterned Si into arrayed PEG gels spaced by 2 μm (left) but not into gels spaced by 1 μm (right)(bar=20 μm).

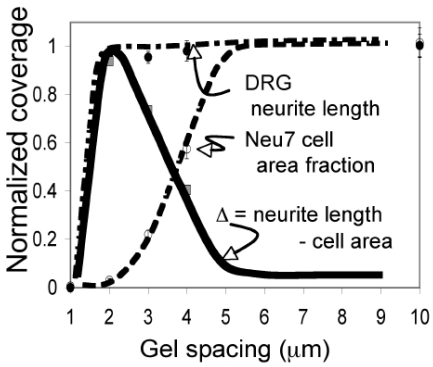

Fig. 3 - PEG gels arrayed at microscale spacings differentially enable neurite growth while inhibiting astrocyte adhesion.

Conclusion: Modulating the length scale of cell adhesive/repulsive features can differentially control the development of subcellular processes. In the case of neuronal processes, this method may prove valuable in a materials-based therapy for spinal cord injury by hindering glial scarring while promoting neurite growth.

References:

1. P. Krsko et al., Biomaterials, 2008. in press.
2. Y. Hong, P. Krsko, et al. Langmuir, 2004. 20: p. 11123.
3. P. Krsko, et al., in *Nanofabrication: Technologies, Devices, and Applications II*, W.Y. Lai, L.E. Ocola, and S. Pau, Editors. 2005, SPIE: Bellingham. p. 600201.
4. P. Krsko, et al., Langmuir, 2003. 19(14): p. 5618.

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