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DIRECTING NEURITE GROWTH IN 3D COLLAGEN SCAFFOLDS WITH GRADIENTS OF MECHANICAL PROPERTIES

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ABSTRACT

Biomaterial scaffolds for nerve and spinal cord regeneration must not only promote neurite re-growth but also direct it. Several cell types, including neurons, respond to the mechanical properties of the substrate on which they are grown. We believe that gradients of mechanical properties can be used to direct neurons. To spatially control the mechanical properties, gradients of genipin – a naturally occurring, cell-tolerated crosslinking agent – are created in 3D through a compliant collagen gel using microfluidics. Gradients of mechanical properties are evaluated by measuring genipin-induced fluorescence, which we have previously correlated to mechanical properties. Growth of neurites was evaluated in gels of uniform stiffness and a gradient generated by incubation in 0 to 1 mM genipin for 12hrs to produce approximately an order of magnitude change in the shear modulus. Neurite growth was evaluated 5 days after gradient formation. Neurites demonstrated a directional bias against the gradient of stiffness. These results demonstrate that neurites can respond to subtle gradients of mechanical properties within a 3D scaffold and point to opportunities to manipulate properties for directed nerve and spinal cord regeneration.

METHODS

Microfluidic networks are generated in a poly(dimethyl siloxane) (PDMS) elastomer using standard soft lithography techniques. To generate gradients, a simple 'H' model is employed, where one channel will contain a 'source' solution and the other a 'sink' solution. Upon introduction of flow, a source-to-sink gradient is established in the cross channel. The 'H' network is bonded to a second network via conformal contact such that the cross-channel of the 'H' crosses over a small channel connected to a circular well (Figure 1).

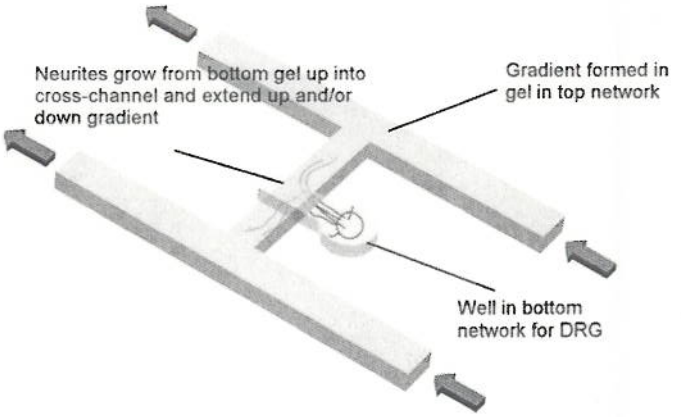

Figure 1: Schematic of the PDMS network. Cells are allowed to grow through the cell channel and into the cross channel.

Dorsal root ganglia (DRG) are dissected from E7 chick embryos. Both the cell channel and the 'H-model' network are placed on a glass slide and plasma treated to render the channels hydrophilic. A 2mg/ml type I collagen solution is prepared by neutralizing stock 3mg/ml solution with NaOH within a buffered solution. After pipetting 20μl of collagen into the bottom channel. the DRG is placed in the collagen in the circular well. The 'H-network' is then placed over the cell channel and collagen in flowed through both inlets with syringe pumps to fill the network. The network is then moved to the incubator to allow the collagen to self-assemble into a fibrillar gel. Following self-assembly, a gradient of crosslinks is established in the cross channel by introducing medium + genipin in one inlet and media alone in the

other inlet. Genipin-mediated crosslinking generates both changes in color and fluorescence [1], and the stiffness of the collagen is well-correlated to the intensity of fluorescence (Figure 2) [2].

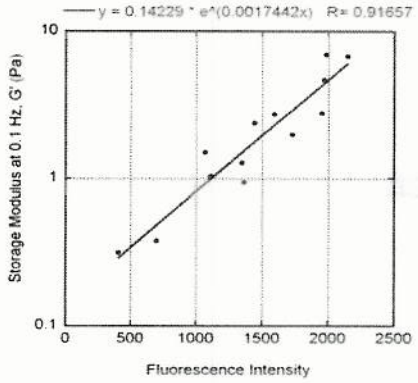

Figure 3: Correlation of genipin crosslinked storage modulus to fluorescence.

Thus, the gradient of crosslinks – and, hence, stiffness - can be visualized as a gradient of fluorescence (Figure 3).

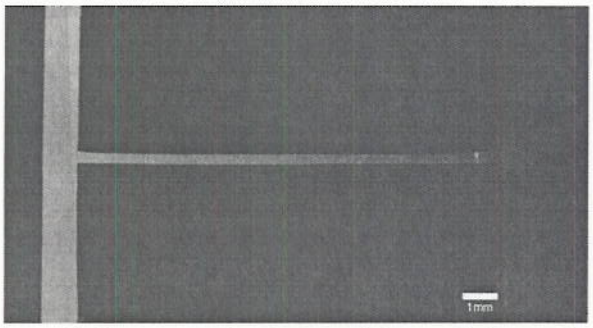

Figure 2: Representative image of the genipin gradient visualized though genipin fluorescence.

RESULTS AND DISCUSSION

DRG's are introduced into our microfluidic system. Gradients are made with 1 mM genipin flowing through one inlet and media without genipin flowing through the other inlet. Controls are performed with no genipin as well as uniform crosslinking at 1 mM genipin. Neurites grow through the cell channel and into the cross channel (Figure 4).

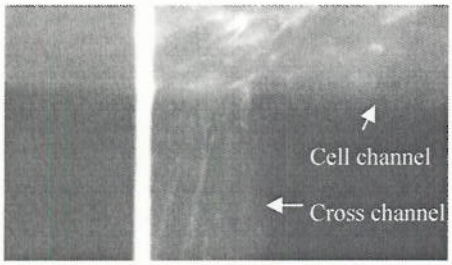

Figure 4: Representative image of neurite growing through the cell channel into the cross channel. Cells are stained with phalloidin for visualization.

Significant numbers of neurites were observed to traverse up and into the cross channel of the 'H' model. In control cases (Figure 5a) neurite extension was equal in either direction of the cross channel. However, when a gradient of stiffness was introduced, neurites extended non-uniformly into the cross channel (Figure 5b), preferring to grow in the direction of lower compliance.

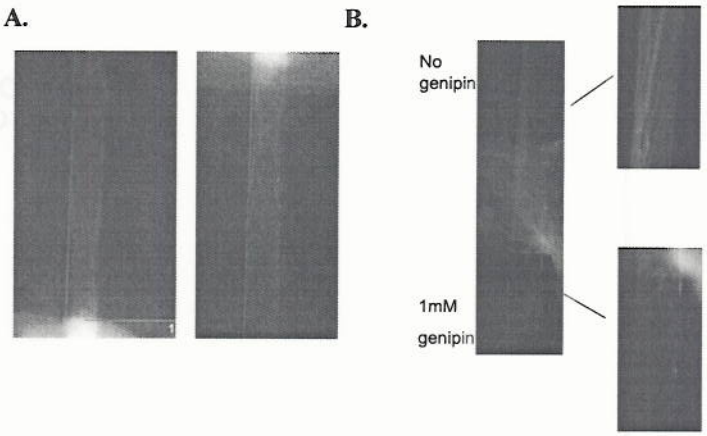

Figure 5: Images showing neurite growth in networks. Panel A shows neurite growth in control conditions on both sides of the crosschannel. Panel B show neurite growth toward the more compliant region, which has not been crosslinked by genipin.

CONCLUSIONS

The environment following spinal cord injury is not conducive to nerve regeneration. One approach is to implant a biomaterial scaffold to "bridge" the gap between severed neurons. To provide an optimal regenerative environment, the scaffold should not only support axon growth but also direct it. Anisotropy has been suggested as a means of directing this growth [3]. We have demonstrated that smooth, continuous gradients of mechanical properties can be introduced into collagen gels with microfluidics, and that these gradients direct neuron growth. We are combining the mechanical anisotropy with haptotactic anisotropy to further customize these collagen scaffolds.

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