157e Patterned Substrate-Mediated Gene Delivery Using Microfluidics

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The combination of gene delivery and tissue engineering has potential to promote the regeneration of tissue lost due to injury or disease. Delivery of genes encoding for tissue inductive factors can be employed to promote desired cellular responses, such as migration and proliferation, which lead to tissue formation. The ability to spatially regulate transgene expression could be used to pattern cellular responses and create tissues with complex architectures. We propose that substrate-mediated gene delivery can be employed to spatially regulate transgene expression on a surface. Substrate-mediated delivery involves immobilization of DNA complexes to a cell adhesive surface for subsequent uptake of cells cultured on the surface. We hypothesize that non-specific interactions between DNA complexes and a tissue culture surface could retain immobilized complexes in the pattern, and transfect cells only in that region. To regulate complex deposition, poly(dimethylsiloxane) (PDMS) molds were fabricated by soft lithography. A silicon master with lines ranging from 100-1000 μm wide, 5-10 mm long, and 250 μm high was created and PDMS was cured on the silicon master. The cured PDMS was oxygen plasma treated and used as a microfluidic device to deposit complexes into pre-defined regions of a polystyrene surface. The patterning of complexes was confirmed with rhodamine-labeled plasmid complexed with Lipofectamine 2000. HEK293T cells cultured on the substrate were transfected within the pattern. The activity of the complexes was correlated to deposition time, complex volume, and DNA concentration in the complex. Transfection efficiencies inside a 1 mm wide pattern were comparable to standard substrate-mediated transfection results for HEK293Ts (~70% transfected cells) with minimal cytotoxicity. A co-culture system with transfected HEK293T cells and primary dorsal root ganglia (DRG) neurons can be utilized to investigate neurite outgrowth in response to patterned gene expression in vitro. This ability to pattern cellular responses within a developing tissue may be a powerful tool to organize tissue formation for numerous applications.