Enhancement of Nonviral Direct Conversion to Neurons Using Substrate Topography

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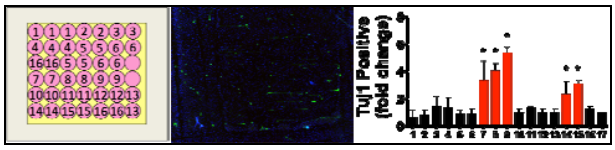
Statement of Purpose: Patients suffering from neurodegenerative diseases including Parkinson's and Alzheimer's may see temporary improvements with pharmacologic intervention or deep brain stimulation, but the therapeutic benefits inevitably decline with the progressive loss of neuronal function. Cell replacement therapy has been proposed as a long-term solution, but identifying an appropriate cell source has proved challenging. Direct conversion is the process whereby differentiated cells are reprogrammed into another lineage without passing through an intermediate proliferative stem cell-like stage[1]. The nonviral direct conversion of fibroblasts into functional induced neuronal cells has endorsed the prospect of autologous cell therapy, but low efficiency and poor understanding of the process precludes translation[2]. The physical microenvironment of cells is known to influence aspects of both nonviral transfection and neuronal differentiation[3,4], so we explored the effects of micro- and nanotopographical features on the transfection, reprogramming, and neuronal subtype specificity associated with nonviral direct neuronal conversion. **Our aim was to enhance nonviral neuronal conversion efficiency through modulation of cellular physical microenvironments using substrate topography, in order to generate a more robust source of functional neurons for cell replacement therapies.**

Methods: We used a multi-architecture chip (MARC) array to incorporate, on a single chip, 17 distinct topographies including both isotropic and anisotropic features, in nano- to micrometer dimensions, with various aspect ratios and hierarchical structures. The patterns were initially generated via nano-imprinting lithography, then assembled onto a single mold, and finally replicated in PDMS by soft lithography for experimental use. The MARC platform enabled a high-throughput systematic investigation of patterned nano- and microscale gratings, pillars, pits, and lenses iterated combinatorially through feature size and spacing, under otherwise identical experimental conditions. We delivered plasmids encoding neuronal transcription factors (*Brn2, Ascl1,Myt1l*) to primary mouse fibroblasts seeded on the chip with a bioreducible linear poly(amido amine) gene carrier. The low toxicity and high transfection efficiency of this polymer allowed repeated dosing to sustain high transgene expression levels. Serial 0.5 μg cm^{-2} doses of reprogramming factors delivered at 48-hour intervals produced $Tuj1^+$ (neuron-specific class III β-tubulin) cells, a subset of which expressed MAP2 (microtubule-associated protein 2), tau, and synaptophysin. A synapsin-red fluorescent protein (RFP) reporter helped to identify mature, electrophysiologically active cells, with > 90% patch-clamped RFP^+ cells firing single, multiple, or spontaneous action potentials. Conversion efficiency was calculated via image cytometry of immunostained $Tuj1^+$ cells normalized to the number of cells seeded.

Results: Quantification helped identify three anisotropic hierarchical patterns and two isotropic micropatterns that significantly increased the neuronal conversion efficiency. Each positive hit yielded at least a twofold improvement ($p<0.05$, t-test), with the best hierarchical pattern giving a fivefold increase in $Tuj1^+$ cells. Interestingly, the component features that comprise the positive hierarchical patterns did not yield the same improvements when isolated, suggesting a synergistic dependency between certain micro- and nanoscale features. A subset of patterns also promoted an increase in nonviral transfection, as quantified with a GFP reporter construct. However, deconvolution of changes in transgene expression and conversion efficiencies suggests that improved transfection alone is insufficient to explain the enhanced conversion on the patterns identified as positive.



MARC chip containing replicates of 17 distinct patterns, including micro- and nanoscale gratings (shown), pillars, pits, and lenses. Five (shown) improve conversion.

Conclusions: Using arrayed micro- and nanopatterns on the MARC platform, we systematically screened and identified specific topographical features that increase the efficiency of nonviral direct conversion to neurons. Higher neuron density increases survival, speeds maturation, and promotes synchronous function; the addition of topographical features may increase both the actual density of neurons through improved transfection and enhanced conversion, as well as the effective density through increased neurite contact along intersecting features. Ultimately, a convergent optimization of environmental cues, such as substrate stiffness, biochemical inputs, and topography, will identify the ideal conditions to maximize the generation of mature, functional neurons that may be suitable for clinical applications. Cell therapy is the next frontier of regenerative medicine, and these results demonstrate the potential of the physical microenvironment to enhance the efficiency of nonviral direct conversion, a potential source of safe and functional autologous neurons.

References:

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