Supplemental Data

Neurite Outgrowth on Tunable Line Width Micro-Grids

The micro-grid pattern is formed by stamping poly-lysine onto a PDMS surface with one microline stamp, and then stamping in an orthogonal direction with a second stamp. All grids were created using the stamp with periodicty = 8.3 μ m. Micro-grids were created with line widths varying from 2.3 μ m to 5.7 μ m (Fig 1a). The width for a given grid is taken as the average width of the two orthogonal directions.



Figure 1. Poly-lysine Grids With Varying Line Width on PDMS (a) Fluorescent images of FITC-PLL on PDMS. For all patterns, $P = 8.3 \mu m$, while L is varied from 2.7 μ m to 5.7 μ m. For clarity, a horizontally printed line is outlined in the right image. Where the vertical lines intersect the horizontal lines, PLL is stamped twice, causing all intersections to appear brighter. Scale bars in (a) and (b): 25 µm. (b) Phase images of cortical neurons on PLL grid patterns after 48 hours. (c) Polar distribution of neurite outgrowth. Although growing neurites interact with the poly-lysine grid in all cases, on more narrow line widths the growth is straighter, and the outgrowth direction more rectilinear.

Individual widths are no more than 15% deviant from the reported average width for a given data point. Orthogonal alignment of the second stamp was achieved by hand, with an average deviation of 2.9°.

Directional neurite outgrowth was measured on the micro-grids. Outgrowth direction is defined as a straight line direction from soma to the tip of the longest neurite. As the width of the poly-lysine lines increased, outgrowth direction became more random (Fig 1b, c). On the narrowest lines, growth was directed in a rectilinear fashion, along either of the orthogonal directions. Where turning occurred, adjacent growth segments were entirely perpendicular.

Additional distinctive morphological characteristics also warrant mention. For example, the soma typically adopted one of two positions: Either centered on the gap in the grid, so as to have all outside edges touching a poly-lysine line (Fig 1b-center, Fig 8), or centered on a line, occupying the gap between two orthogonal lines (Fig 1b, left). These represented $54\pm4\%$ and $46\pm4\%$ of the total, respectively, on the thinnest grids (line width $\leq 3.5 \mu$ m), and $74\pm3\%$ and $26\pm3\%$, respectively, on the wider grids (line width $\geq 3.5 \mu$ m). Also, neurites on thick lines would commonly appear to be thicker, suggesting that the narrow lines may influence the size of neurite that would grow upon them.