Collective Behavior of Neural Cells in Various Geometric Shapes Using Micropatterning Techniques
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Introduction: Micropatterning allows for the control of multicellular organization by confining cells into an area of desired geometry and area and culturing them in an in vitro setting. To date, many cell types have been tested using such techniques. Brock et. al 2003 seeded individual fibroblasts in various shapes and found that fibroblasts extended lamellipodia processes almost exclusively from corners, except for in the circle where growth was haphazard. This indicated that confining the cell into a corner regulated the growth of its processes. The processes of neurons, neurites, have similar cytoskeletal structure to the fibroblasts’ lamellipodia. According to an NPR report, roughly 50,000 Americans per year suffer a nerve transection injury, where the nerve is severed, with resultant loss of motor function or sensation. If, like lamellipodia, neurites have increased outgrowth velocity from the corners, one could manipulate growth in a way conducive to the reconnection of the severed nerve, irrespective of the pattern of transection. This study tests whether corners play some role in both the direction and speed, velocity, of the neurite outgrowth, employing a multicellular neural model. PC12 cells are often used as a neural model because, when treated with Nerve Growth Factor (NGF), they cease division and adopt a neural fate. Using this model, the effects of multicellular cell seeding as well as corners and angle sharpness on velocity will be explored, with the hypothesis that corners guide direction and more acute angles increase velocity.

Materials and Methods: Four shapes were tested for the studies: an equilateral triangle, an isosceles right triangle, and an isosceles 120° triangle, and a circle as a control. The major axis of all shapes was 500 μm. Using AutoCAD 2011 software, masks were created with 9 x 15 arrays of each shape, with each shape spaced 1 mm apart from adjacent shapes. Photolithography was done on polished silicon wafers of 3” diameter for a desired photoresist thickness of 250 μm, following the procedure provided by MicroChem. Exposure time varied between 85 and 90 seconds. After silanization, soft lithography was performed using Sylgard-184 polydimethylsiloxane (PDMS), following protocol consistent with Koschwanez et al. 2009. Cell seeding was performed using a protocol similar to Cho et. al 2008, with a density of 250,000 cells per stencil. The stencil was left for 2 days before removal, and PC12 media was supplemented with 50 ng/mL NGF and 1% Horse Serum/FBS to induce neuronal differentiation. Images were captured daily and neurite growth was measured using SigmaScanPro.

Figure 1. Micropatterned PC12 cells after 2 days of differentiation. Multicellular neurites emanated haphazardly from shapes (left four) and at roughly the same rate (right).

Results and Discussion:
Fabrication of the silicon wafers, PDMS stencils and subsequent cell patterning was successful with all shapes, with corner sharpness maintained. Cell seeding density was determined by culture of PC12 cells with 50 ng/mL NGF, at which time the density of 150,000 was shown to be optimal, though 250,000 showed no significant difference. After 2 days of differentiation, many shapes lost their form, potentially due to paracrine communication occurring between the seeded cells. As a result, many of the growths became haphazard as shown in Fig.1. There was no significant difference between the lengths in various shapes. Paracrine communication could regulate these neurite lengths in some way. The haphazard growth can be attributed to the presence of many neurons, whereas previous work used only one cell to have directed growth. In sum, the presence of multiple cells had more of an effect on neurite growth than did the topographic cues of corners and angle sharpness.

Conclusion: Multicellularity resulted in haphazard neurite growth with no significant difference in velocities, indicating this to be more important than presence of corners or angle sharpness. This can be confirmed in further studies with seeding of just one neuron per shape or with multiple fibroblasts per shape, as the lamellipodia and neurites are cytoskeletonally similar. Future studies may involve use of hydrophobic substrates to prevent excessive cell proliferation leading to loss of shape and more statistical analysis is needed to determine significance.