# Precise control of neural network structural connectivity in neural cultures

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ACTIVITY-DEPENDENT stimulation, which has been utilized for both research and rehabilitation purposes, induces neural network reorganization through native mechanisms of neuroplasticity [1]. At the level of the single synapse, these changes are manifested by well-characterized local mechanisms such as spike-timing dependent plasticity (STDP); however, the neuronal sites of manipulation during external recording and stimulation are typically separated by multiple synapses and incorporate both feedforward and recurrent pathways, resulting in the distribution of plasticity across synapses. The relationship between neuronal circuit structural motifs and this polysynaptic plasticity has yet to be studied in detail. Due to the difficulty of exploring the influence of neural network architecture on neuronal interactions amid the complexity of *in vivo* environments, neuronal cultures can provide a reduced system to elucidate these specific relationships. To do so, experimenters must be able to reliably and precisely control the structure of the cultured networks.

Microcontact printing (μCP) has been used recently in conjunction with surface chemistry to pattern neural networks of arbitrary geometries *in vitro* [2]. More specifically, these techniques have been used to create low-density functional neuronal circuits on multi-electrode arrays (MEAs), but the directionality of the axons within the networks could not be controlled [3]. Single isolated neurons have been patterned with controlled directionality for axon differentiation by stamping self-assembling alkanethiols onto gold [4]. However, the small feature sizes necessary to develop high-fidelity control are difficult to reproduce with traditional microcontact printing techniques due to problems associated with high feature aspect ratios.

We have extended this latter work in combination with other μCP techniques such as submerged μCP and composite polydimethylsiloxane (PDMS)/h-PDMS stamps, which have both been shown to increase reproducibility of high aspect ratio features. This modification increases pattern feature fidelity at the single micron level and enables creation of neural networks of predetermined structural connectivity. Our current results indicate that the precise shapes of the individual neuronal patterns are critical for neuronal compliance to the desired configuration. The starburst shaped pattern previously used for axon differentiation encourages the correct placement of somas compared to a unipolar pattern, in which somas tend to adhere to protein regions intended for axons. The success of this project will enable the ability to culture neural circuits of predefined geometry and directionality on MEAs, providing precise control of the context of connectivity during the study of polysynaptic plasticity induced by external stimulation.

## REFERENCES

1. Jackson, A., J. Mavoori, and E.E. Fetz, *Long-term motor cortex plasticity induced by an electronic neural implant.* Nature, 2006. **444**(7115): p. 56-60.
2. Wheeler, B.C. and G.J. Brewer, *Designing Neural Networks in Culture: Experiments are described for controlled growth, of nerve cells taken from rats, in predesigned geometrical patterns on laboratory culture dishes.* Proc IEEE Inst Electr Electron Eng, 2010. **98**(3): p. 398-406.
3. Nam, Y., et al., *Gold-coated microelectrode array with thiol linked self-assembled monolayers for engineering neuronal cultures.* IEEE transactions on bio-medical engineering, 2004. **51**(1): p. 158-65.
4. Johnson, D.M., J.P. Abi-Mansour, and J.A. Maurer, *Spatial confinement instigates environmental determination of neuronal polarity.* Integr Biol (Camb), 2012. **4**(9): p. 1034-7.

Research supported by NIH Grant R01-DC009215.

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