

Patterning Neurons with Microcontact Printing on Silicon Oxide Substrates

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Abstract:

Signal transduction and processing between groups of neurons is yet to be fully understood. Guiding cell growth to create defined *in vitro* neural networks with specific synaptic connectivities will allow for the analysis of signal transduction and processing. Therefore, the present study aimed to use microcontact printing to pattern neurons *in vitro*. Microgradient protein patterns on silicon oxide substrates were created using laminin and poly-L-lysine. Primary rat embryonic cortical neurons were subsequently seeded onto these patterns. Neuron morphology and growth were analyzed using fluorescence and scanning electron microscopy. Although several scanning electron microscopy (SEM) pictures appeared quite promising, much remains to be investigated before a comprehensive understanding of the behavior of neural networks can be gained.

Introduction:

The process by which neural connections form is of particular interest. It is not entirely known how and why connections between specific neurons occur. Therefore, it is necessary to study neural networks on a small scale. Neural guidance through protein patterning techniques, such as microcontact printing, can be used to create neural networks to analyze network formation, individual neuron growth, and signal transduction and processing [1].

Microcontact printing is a process that was established for the purpose of creating micro-sized patterns on substrates. It is becoming more and more widely used because it is a simple, customizable process. A hydrophobic polymer, such as polyolefin plastomer (POP), is used as the “stamp” and a hydrophilic protein, like poly-L-lysine (PLL), acts as the “ink.” The stamp contains a micropattern that has been created by hot embossing. By first soaking the “stamp” in “ink” and then pressing it onto the substrate, the protein pattern is transferred. The choice of protein should be cell specific; for example, laminin is used to promote neural cell adhesion and guidance. Ideally, cells will only attach to regions where protein is present.

Experimental Procedure:

Microcontact printing with POP stamps, which were produced through a hot embossing process from a Teflon® master, was performed on silicon oxide substrates. Silicon oxide was used in order to enhance contrast for imaging. A protein solution of fluorescein isothiocyanate labeled poly-L-lysine (FITC-PLL, 10 µg/mL) and laminin (5 µg/mL)

diluted in Hank’s balanced salt solution (HBSS) was mixed on ice. The POP stamp was immersed in this solution for 20 minutes, dried with nitrogen gas, and then pressed onto a silicon oxide substrate that had been pre-hydrophilized by flame. After two minutes, the stamp was removed, thereby transferring the protein pattern to the substrate. Patterns were inspected with a fluorescence microscope to ensure good, usable quality. Primary rat embryonic cortical neurons were seeded at 16,000 cells/cm² onto these patterned substrates and grown for three days. At three days, cells were fixed with 5% glutaraldehyde in 20 mM HEPES and dehydrated in increasing concentrations of ethanol for SEM observation.

Results and Conclusions:

Microcontact printing of microgradient patterns onto silicon oxide was successfully achieved, as shown in Figure 1. Ideally, a neuron’s soma should attach to the circular node, which has a 10 µm diameter, and its neurites should extend only along the gradient pattern and not in any other direction.

However, it was difficult to induce this type of neuronal growth under the given conditions. Many of the SEM images that were taken reveal numerous neurons growing in many directions and not on the pattern. Fortunately, a few images, such as those shown in Figures 2 and 3, adequately displayed neurons growing along the pattern.

The SEM image in Figure 2 is not ideal because neurite branching occurs in several directions and both soma are not

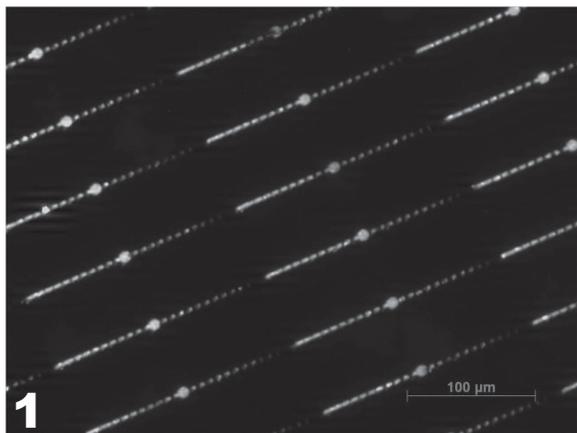


Figure 1: Fluorescence image of a microgradient pattern produced by microcontact printing of FITC-PLL (10 μg/mL) and laminin (5 μg/mL) on silicon oxide (20X objective).

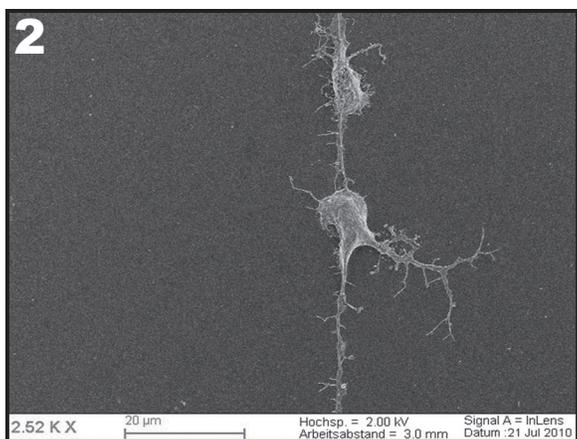


Figure 2: SEM image of two neurons growing on microgradient patterns.

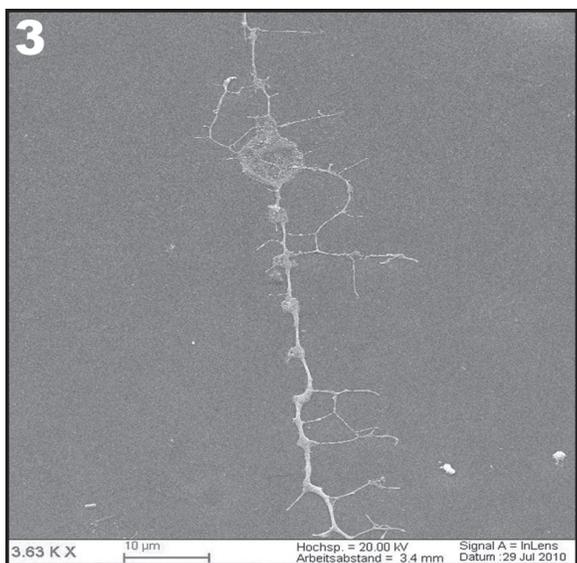


Figure 3: SEM image of a neuron growing in alignment with the microgradient pattern.

localized on the nodes. Figure 3 shows excellent neurite attachment and growth along the pattern.

It will be necessary to improve the stamping process to obtain better neuron alignment with the microgradient patterns. Several factors could have contributed to the fact that the majority of the neurons did not grow in alignment with the pattern. For example, smudging the stamp during the microcontact printing process may have caused the cell adhesive proteins to spread across the surface, which would promote neuron growth in every place where laminin or poly-L-lysine was present.

Future Work:

Enhancing the microcontact printing process will result in better SEM images that show alignment of the neurons to the pattern and more confined neural growth. These images can then be used to more comprehensively analyze how neurons grow and how networks develop in response to a gradient protein pattern. Future work, which has been preliminary investigated, includes using cell-repulsive materials, such as agarose, to pattern neurons to create neural networks. The early stages of this project were carried out in conjunction with the present study, but a lot remains to be explored. This idea could lead to exciting applications in the field of bioelectrical and neuroengineering.

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References:

- [1] Offenhäusser, A et al. "Microcontact printing of proteins for neuronal cell guidance." *Soft Matter*, 3, 1-9 (2007).