Polypyrrole Micropatterns for Electrical and Topological Stimulation of Hippocampal Neurons

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Statement of Purpose:

Electroconductive materials have been studied for applications in the bioengineering field. Also, cells, including fibroblasts, PC12 cells, and osteoblasts, respond to electricity with enhanced growth and differentiation. At the same time, well-defined micron level structures have been shown not only to guide axonal direction but also to accelerate the rate of axon extension and polarization in embryonic hippocampal neurons. Thus, in these studies, we demonstrate a method to combine electrical and physical cues by fabricating electrically conducting polyppyrole (PPy) materials modified with microchannels.

Methods:

Pyrrole was polymerized electrochemically on 1 µm and 2 µm patterns written using electron beam lithography (EBL) on poly(methyl methacrylate; PMMA)-coated indium tin oxide (ITO) glass slides. Various pyrrole and polystyrene sulfate (PSS) concentrations, applied current and polymerization times were investigated in terms of microchannel thickness via SEM and surface roughness with AFM.

Embryonic hippocampal neurons were cultured on PPy micropatterned substrates and unmodified PPy controls with supplemented Neurobasal Medium. After 20 hour incubation, neurons were stained immunochemically with Tau-1 antibody and a secondary antibody. Fluorescence microscopy was used to analyze polarization, and the orientation of axons relative to the micropattern lines. A hippocampal cell was defined as polarized (i.e., expressing a defined axon) when one of its neurites, stained positively for Tau-1, at least twice as long as the other neuritis.

Results/Discussion:

The effects of PPy polymerization conditions were studied. As expected, higher pyrrole and PSS concentrations, higher applied current, and longer polymerization times resulted in increased microchannel thickness, and reduced gap width between channels (Figure 1). PPy micropatterns of 300 nm thickness were fabricated at the optimal condition for thin PMMA resists (10 nm thick) of 25 mM pyrrole and PSS, 144 µA for 30 seconds; on the other hand, the condition that 50 mM pyrrole and PSS, 144 µA for 30 seconds resulted in 800 nm thick and better-defined microchannel on thick PMMA resists (100 nm).

Embryonic hippocampal neurons polarized significantly more on 2 µm microchannels (40%) compared to 33% on 1 µm channels and 21% on unmodified PPy after 20 hour culture. Also, we observed improved axon guidance on thicker and more aligned micropatterns. Regarding the micropattern dimensions, the thicker and more defined microchannels are, the better the axon guidance and polarization (Figure 2).

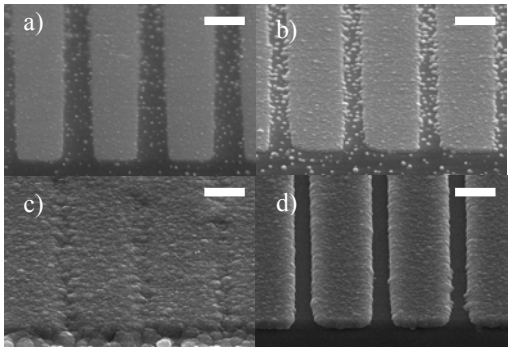

Figure 1. SEM Images of 2 µm PPy-PSS micropatterns fabricated on 10 nm PMMA-coated ITO slides; a) 12.5 mM pyrrole/PSS, 144 µA; b) 25 mM, pyrrole/PSS, 144 µA; c) 50 mM of pyrrole/PSS, 720 µA; d) polymerized on 100 nm PMMA coated ITO slide with 50 mM of pyrrole/PSS and 144 µA applied current. Scale bars=2 µm.

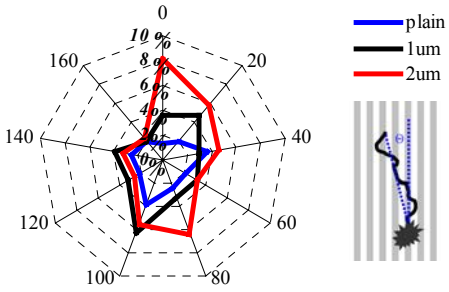

Figure 2. This plot represents the population of polarized hippocampal neurons along the specific axon angle (Θ) relative to the pattern lines after 20 hour culture. More polarization and better axonal orientation were observed on the 2 µm PPy microchannels.

Conclusions:

PPy microchannels were fabricated using EBL and electropolymerization. These materials have the potential to combine electrical cues and physical cues for nerve cells. Axons of hippocampal neurons were found to polarize faster on 2 µm channels compared to 1 µm channels and unmodified PPy surfaces and were guided along the 2 um channels.

References:

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