Directing Neuronal Differentiation Via Neurochemical Release From Carbon Nanotube-doped Conducting Polymers

Cassandra Lynn Weaver[1,2], Xinyan Tracy Cui[1,2,3]

[1]Department of Bioengineering, [2]Center for the Neural Basis of Cognition, [3]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA

Statement of Purpose: Neural stem cells (NSCs) have been implicated in potential therapies for central nervous system injury and dysfunction; however, localization and functional integration of newborn cells into existing circuitry remains a significant hurdle that must be addressed before NSC therapies can become viable clinical options. Many soluble factors are involved in the complex process of NSC proliferation, migration and fate determination, and proliferating cells are sensitive to these factors in intricate spatial and temporal gradients.

We are investigating a controlled, local release system to deliver neurochemicals to *in vitro* NSC cultures in a temporally and spatially exact manner. In this work, conducting polymer films are synthesized and loaded with γ-aminobutyric acid (GABA), an inhibitory neurotransmitter involved in NSC migration and functional integration during development. Conducting polymers have the unique ability to undergo reversible re-dox reactions, and will incorporate or dispel charged particles when switching between states. We utilize this property to load and release GABA molecules from the film. As a method of increasing the stability and drug-loading capability of the films, GABA-loaded carbon nanotubes (CNTs) were incorporated in the polymer films. Film biocompatibility was assessed with neuronal and NSC culture, and released drug bioactivity was evaluated using cultured neuronal networks (CNNs) as sensors.

Methods: Conducting polymer films made of poly(pyrrole) (PPy) or poly(3,4-ethylenedioxythiophene) (PEDOT), with or without CNTs and/or GABA were grown potentiostatically on gold macroelectrodes. To synthesize the films, an oxidizing potential (1.2 V, 600 s) was applied to a solution of monomer with or without CNTs and/or drug. Control dopants were ions contained in phosphate buffered saline for PPy and poly(styrenesulfonate) (PSS) for PEDOT. To release the loaded drug, the films were stimulated using 30 cycles of cyclic voltammetry with voltage sweeps between -0.8 V and +1.4 V.

Mouse NSCs were cultured on the polymer surfaces in differentiation media for 7 d, fixed and stained for neuron marker β-tubulin III. The density of cell growth and percentage of neuronal differentiation was quantified. Rat cortical neurons were cultured on the polymer surfaces for 3 d, fixed and stained with β-tubulin III. Neuronal affinity for each surface was qualitatively assessed, and average neurite length was quantified.

Released drug bioactivity was assessed by applying solutions of released drug to a CNN grown on a multielectrode array (MEA). Neuronal firing was monitored with extracellular recordings from the 64 planar electrodes of the MEA. Solutions of known GABA concentrations were applied to the CNN as controls.

Results: Neurons were cultured on the polymer-coated macroelectrodes to evaluate the biocompatibility of the coatings. The CNT-doped films appear to encourage a higher density of neuronal attachment (Figure 1). GABA-loaded PEDOT surfaces (with or without CNTs) supported neurons with longer average neurite lengths than control surfaces. The enhanced neurite outgrowth may be due to the soluble GABA that leached out of the film, or immobilized GABA at the surface of the film. GABA has been shown to enhance neurite development in neuronal cultures (Barbin G. Neurosci Lett. 1993;152:150-4). Neuronal differentiation of NSCs cultured on the polymer surfaces was evaluated. A significant increase in cell density and percent of neurons was found on GABA-loaded PPy films.

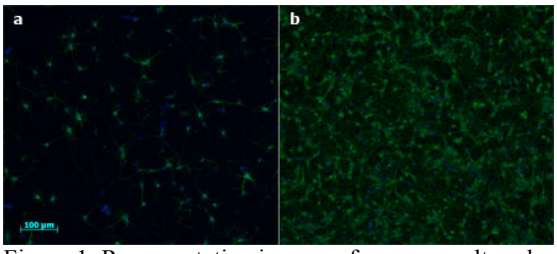

Figure 1. Representative images of neurons cultured on (a) PEDOT/PSS surfaces and (b) PEDOT/CNT surfaces.

To evaluate the effectiveness of electrically triggered drug release from the polymer films, a CNN cultured on an MEA was used as a sensor. Dose dependence of GABA on neural activity was characterized, and concentrations of GABA above 5 μM were found to abolish all spiking activity in the culture. Released drug solutions from the PPy/GABA film (250 μL) and PEDOT/GABA film (500 μL) were successfully sensed by the CNN (Figure 2).

Figure 2. Raster plots of CNN activity before, during, and after application of PPy-released GABA.

Conclusions: GABA-loaded conducting polymer films were successfully synthesized on macroelectrodes. Enhanced neurite growth and neuronal differentiation in neuronal and NSC cultures on the polymer surfaces were observed. Addition of CNTs did not contribute any toxic effects; on the contrary, the neuronal attachment and neurite extension were enhanced by the presence of CNTs. We showed that drug released from PPy and PEDOT retained its bioactivity, and PPy films release more drug than the PEDOT films per CV stimulus, as indicated by the ability of the CNN to sense smaller volumes of released drug solutions.