A Three-Dimensional (3-D) Neural Co-culturing System for Drug Evaluation

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Statement of Purpose: Whole animal behavioral testing possesses complications in interpretation and single-cell screening of neurons makes robust pharmacological analysis difficult. To simplify experiments and reduce labor while increasing throughput, the pharmaceutical industry has employed multi electrode array (MEA) and 2-D dissociated culturing technology to enable analysis on intact networks of neurons (Whitson et al., 2006). However, 2-D dissociated cultures vary significantly from highly complex neural tissue, and may influence cellular and network level response to drugs. 3-D culture models consisting of multiple neural cell types may better approximate the cytoarchitecture, thus providing a more suitable alternative for *in vitro* drug testing (Williams and Herrup 1988). Therefore, the goal of this study was to develop a 3-D co-culture system, while demonstrating its application for drug testing using 3-D MEA technology.

Methods: Embryonic day 18 rat cortical neurons were mixed with post-natal day 1 derived astrocytes (between passages 4-10) in a ratio of 2:1 at a plating density of 2500 cells/mm^3 in Matrigel™ extracellular protein matrix (Catalog # 354263, BD Biosciences, San Jose, CA) approximately 500 μm thick and at a protein concentration of 7.5mg/mL. Co-cultures were plated on oxygen-plasma treated, and poly-D-lysine and laminin coated 3-D MEAs. Cultures were kept in a tissue culture incubator (37°C, 5% CO_2, 95% RH) and fed every other day with a defined co-culture medium (Neurobasal medium + 2% B-27 + 500 μM L-glutamine + 1% G-5; Invitrogen, Carlsbad, CA) pre-warmed to 37°C. Nylon mesh insert were used to keep the 3-D cultures attached to the 3-D MEA substrates, and teflon membrane-based cell culture caps were used to maintain the humidity of the cell culture chamber, preventing evaporative losses, changes in osmolarity, and infection. Electrophysiological activity of the cultures was measured by placing the 3-D culture containing MEAs in a Multi Channel Systems MEA60 preamplifier system (Multichannel Systems, Reulingten, Germany). Data were recorded with an amplifier gain of 1200, input voltage range of -4096 to 4095 mV, and a sampling frequency of 25,000 Hz. MC Rack (Multichannel Systems) and Neuroexplorer (Nex Technologies, MA) were used to characterize the electrophysiological activity of the cultures in terms of spike (action potentials) and burst (temporal clusters of action potentials) rates. Staining the cultures with viability and immunocytochemical markers at different time points up to 21 days *in vitro* distinguished live and dead cells, nuclei, neural cell types, and synapses. Two different drugs were tested at 21 DIV: bicuculline methiodide (BMI, 50 and 200 μM) – a seizure inducing drug, and gramicidine (0.01 μg/mL) – a cell-poring antibiotic - evaluating the 3-D neural co-culturing system for drug testing.

Results: Figure 1 shows spike rate (spikes/second) of two 3-D neural cultures in response to BMI and Gramicidin. Spike rate of the cultures predictably increased with the concentration of BMI, as BMI indirectly excites neural network activity by blocking the inhibitory synapses (Heuschkel et al., 2006). Likewise, gramicidin increased the electrophysiological activity (Doebler 2000). While MEA 1 and 2 respond in the same trend, the randomness of the networks formed in those cultures and the differing baselines partially explain the response variability. The drug induced responses may be influenced by the initial baseline activity and saturation limits that are different for different randomly formed neural networks. Furthermore, the rinses did not completely reverse the pharmacological effect, indicating at least two possibilities: these drugs have low dissociation constants, and/or given the three dimensionality of the cultures, the diffusion is mass transport limited.

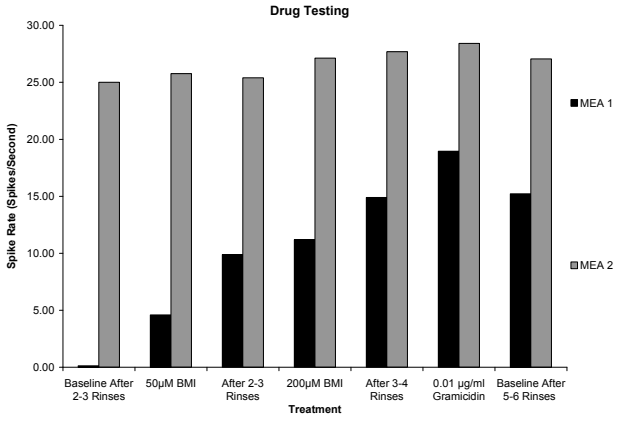

Figure 1. Pharmacological evaluation of the 3-D neural co-culture system

Conclusions: A 3-D co-culturing system was developed using biological materials such as neurons, astrocytes, Matrigel™ matrix, and synthetic materials such as nylon mesh, Teflon membrane and 3-D MEAs, for testing the pharmacological response of neural tissue. This system was evaluated by successfully testing the electrophysiological response to two different drugs at 21 days *in vitro*. The advantages of this system are that it allows for high through-put testing of drugs on neuronal networks in a 3-D micro-environment, with control over cell density, cell types, and media conditions. Future work would potentially include the application of this system as a test-bed for modeling disease states *in vitro* and testing various reparative drugs.

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