

## Neural cell patterning on thin film boron doped diamond electrodes for neurotransmitter detection *in vitro*

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We present our progress in the development of a method to measure neurotransmitter release and electrical signals from neural cells in culture by patterning the growth of neurons directly onto an array of diamond electrodes. This provides a controlled experimental system to quantify electrochemical communication between neural cells. This *in vitro* model provides a more predictable system for the study of electrochemical communication between neurons than *in vivo* animal models or studies using brain slices. Using various microfabrication techniques it is possible to achieve small feature sizes enabling observations at the cellular level. Detection with an extracellular electrode provides an alternative, nondestructive method to patch clamping to detect electrical signals from cells. Patch clamping also does not allow for the simultaneous stimulation and recording of electrical signals in small groups of cells.

Neural cells have been previously patterned over electrode arrays in order to detect electrical signals produced by these cells. Neurons have been patterned over Au electrodes and commercially available microelectrodes arrays. [1,2] Difficulties include control of directionality of neuron communication, and articulation of neurons in culture which is very sensitive to culture and the geometry of the cell pattern. High signal to noise ratios are also problematic in the detection of action potentials from the small groups of cells or single cells.

Diamond has emerged as a newly studied electrode material for neurological measurements. Diamond electrodes have been shown to have a wide potential window and low baseline current in aqueous solutions and provide long term chemical stability. [5] Diamond electrodes potentially provide an ideal sensor material for *in vivo* detection of biomolecules. One of the most widely used electrodes in neurochemical sensing research is carbon fiber electrodes. Carbon fiber electrodes do not display the same long term stability as thin film diamond electrodes because the surface becomes passivated. Diamond thin films possess a simpler surface chemistry than carbon fiber electrodes making the surface easier to functionalize in order to achieve a homogeneous surface; the terminal functional groups of the diamond thin film can thus be more predictably modified to manipulate its electrochemical properties. The surface of thin film diamond electrodes may also discourage the adsorption of proteins, which is desired for continuous detection of biomolecules in aqueous solution. [6,7]

Many methods for patterning cells into particular geometries over a substrate have been investigated. Examples include patterns of Self-Assembled Monolayers (SAMs) of alkanethiolates on Au substrates and application of nonspecific adhesion proteins or proteins which specifically guide axonal and neural process growth applied in various geometries using elastomer stamps or microfluidic methods [3,4]. The terminal group of the diamond thin film determines hydrophobicity properties and possibly cell adhesion properties, this may provide a possible avenue for cell patterning. By creating patterns of different diamond surface chemistries, it may

be possible to guide the growth of cells on a diamond surface.

A thin layer of boron-doped diamond is grown on a conductive silicon substrate by chemical vapor deposition. An insulating material is applied in a particular geometry such that the exposed areas provide electrode surfaces. This will result in an array of electrodes, though, at this stage, the individual electrodes will not be electrically isolated.

Preliminary studies with a cancer cell line on a diamond thin film over a silicon substrate show that it is possible for eukaryotic cells to adhere to a diamond thin film and to undergo cell division.

The cancer cell line was patterned over the substrate to produce selective growth of the cells over the electrode surface. In general, a culture of cells may be induced to grow in specific geometries by differential application of adhesion proteins to a substrate. [8] We are currently applying a pattern of the cellular adhesion protein, fibronectin, to the substrate using microfluidic techniques. The pattern of protein will be visualized by fluorescently labeling the fibronectin through a biotinylation process and reaction with fluorescently conjugated streptavidin. Time-lapse microscopy will then be used to determine the differential time for adhesion of the cells to the protein pattern and the unpatterned substrate. Further blocking of attachment of cells to the unpatterned areas is possible by using a blocking agent for cell adhesion such as Bovine Serum Albumin (BSA).

A line of neuroretinal cells will be patterned over the diamond thin film electrode array in specific patterns such that release of neurotransmitters from specific groups of neural cells may be detected.

Our future goals include the integration of electronics into the electrode array in order to allow stimulation of small groups of neural cells (possibly single cells) as well as detection of electrical potentials from the cells. Future work also includes chemical modification of the electrode surface for detection of neurotransmitters as well as the fabrication of an array of singly addressable thin-film diamond electrodes.

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