A Chemical Sensor Using Neurons and a 3-D Micro-fluidic Chip

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ABSTRACT

In recent years, there has been a merger of microelectronics and biological sciences to develop integrated nano and micro-scale biosensors or biochips. The implementation of portable, rapid and economic methods for detecting different biological species on a chip will benefit from the development of electronic means for the analysis of cells. Neurons are very attractive as chemical sensors due to their sensitivity to specific toxins and their unique electrical properties. The use of closed well micro-fluidic devices for the growth of neurons has not been explored extensively. In this work, we will describe surface preparation techniques to enhance the neuronal cell viability and growth on microfabricated surfaces. We have fabricated micro-fluidic bio-chips for the trapping of neurons and to examine their growth. Neural cells are maintained in a chamber on the chip with fresh nutrient media continuously flowing through the chamber. The temporal viability of the neural cells within the chip will be reported. The long-term goals of the project include electrically measuring the viability of the cells inside the micro-fluidic chambers.

INTRODUCTION

The possibilities of interfacing neurons with micro-fabricated structures bring about very exciting opportunities to develop biosystems with a wide variety of applications, ranging from sensing to living neural networks. Neuroscientists have long been studying the electrical impulses of neurons as bundles, however micro-fabrication techniques have allowed for the stimulation and recording of individual neurons [1-4]. Recently researchers have begun exploiting the sensitive electrochemical transduction capabilities of neurons for use as biological and chemical sensors [5,6]. A fundamental issue in the development of these systems remains outstanding, i.e. the ability to reliably pattern neurons in specific locations such that they remain viable and make connections with neighboring neurons. We envision a micro-fabricated system where neurons are placed and confined in closed 3-D channels and wells, recording and stimulation devices are fabricated and integrated within the channels, and nutrients are supplied to the cells to keep them alive. The electrical activity of these cells can be examined. Both cellular metabolic activity and action potential impulses may be observed in such a device. Changes in the electrical activity would be studied under exposure to toxic chemicals and bio-warfare agents.

EXPERIMENTS AND RESULTS

Cell Culture

Experiments described in this paper were performed using Dorsal Root Ganglia (DRG) and Sympathetic (Symp) Neural Cells extracted from 7-8 day old chick embryos. Cell concentrations were optimized to provide sufficient cell coverage on-chip, without interfering
with cell growth and analysis techniques. A final quantity of 6x10^4 cells/culture dish (35mm) was used allowing for approximately one cell/100µ^2 on the chip surfaces.

Cell Viability Studies

We began with basic experiments to determine the optimal surfaces for cell viability, attachment and growth. Patterned substrates of platinum on silicon oxide were prepared. The surfaces were cleaned with acetone, rinsed with DI and dried with a flow on N₂. Cells were plated in neural media on multiple chips in a series of 35mm culture dishes at a concentration of 6x10^4 cells/dish and placed in an incubator at 37°C and 5% CO₂. Some dishes were maintained with the initial neural media. The neural media in other dishes were refreshed every other day by replacing half the media with new media. During each observation a single chip was removed and exposed to a solution of trypan blue in neural media for 15 minutes and then rinsed with neural media. Trypan blue is known to infiltrate dead neurons while the live neurons are impervious to the solution. Percentages of dead and live cells were determined under a Nikon Olympus BX51 light microscope.

Two types of experiment were performed to establish cell growth and viability. Figure 1 shows a plot of the data obtained, with and without nutrient replenishment. This data show that the cells can be maintained on-chip for an extended period of time. In fact, the limiting factor that ended these experiments was growth of contaminants in the petri dishes in which the substrates were maintained. The 15-20% cell death can be attributed to dissection and handling of the neurons. However in this experiment, the neural cells did not attach to the surfaces and neural processes did not develop.

Cell Attachment and Growth

This experiment was performed to determine the optimal surface for cell attachment and process growth. Three substrate surfaces were examined, i.e. silicon oxide, gold, and platinum, the last two being patterns of these metals on oxidized substrates. Two types of surface cleaning were used: 1) acetone/DI followed by exposure to ultraviolet light (UV) and 2) O₂ plasma cleaning. In some cases, an organic coating of polyornithine and laminin (PORN/L) was applied as this is expected to increase cell attachment and process growth [7]. Polyornithine is known to alter the surface charge, providing a more suitable substrate for the negatively charged neurons. Laminin is a major component of extracellular matrix, thus also increasing the adhesivity of the surface for cell attachment and process growth. The neurons were plated and observed as described above. Cell attachment and process growth were analyzed using a high power microscope to quantify the # of cells/area, cell coverage/area, number of processes/cell and length of processes.
These factors were examined and quantified after 12, 24, 48, 72, and 96 hours (same chip and same location), although only a 96 hour observation is provided in Figure 2. Typically, the neural cells preferred metallic surfaces, in particular the platinum. Also from these figures, it is apparent that cell attachment and growth is better for DRGs as compared to the sympathetic cells. The PORN/L coated samples improved the cell attachment and process development dramatically over the uncoated samples. In figure 3, the DRGs are seen to have faster process development over the sympathetic cells. Table 1 summarizes the results obtained on the growth of cells and the number of processes for DRG neurons. Figure 4 provides a photo of (a) a DRG neuron and (b) a sympathetic neuron attached and growing on a SiO$_2$ surface that was coated with PORN/L.

The conclusions of this experiment were the surface cleaning and preparations for optimal cell attachment and growth, which were determined to be acetone clean with a DI wash followed by UV exposure and coverage by the PORN/L organic layers. These procedures were to be used for preparation of the micro-fluidic device surface in the future. Although the DRG cells provided increased process growth, it was decided to use symp cells for the micro-fluidic device as the symp cells can be obtained with fewer associated glia cells. The micro-fluidic device has a volume on order of nL and would quickly become clogged by multiplying glia cells.

Table 1: Summary of results from the DRG cell growth experiments.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>12 hrs</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>72 hrs</th>
<th>96 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of processes per area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>-</td>
<td>0.50</td>
<td>-</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>Au</td>
<td>-</td>
<td>1.25</td>
<td>1.50</td>
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<td>1.00</td>
</tr>
<tr>
<td>Pt</td>
<td>-</td>
<td>1.50</td>
<td>0.75</td>
<td>0.50</td>
<td>-</td>
</tr>
<tr>
<td>Length per process [µm]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SiO$_2$</td>
<td>-</td>
<td>20.50</td>
<td>-</td>
<td>13.00</td>
<td>-</td>
</tr>
<tr>
<td>Au</td>
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<td>42.50</td>
<td>45.00</td>
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<tr>
<td>Pt</td>
<td>-</td>
<td>11.50</td>
<td>23.30</td>
<td>31.00</td>
<td>-</td>
</tr>
</tbody>
</table>
Micro-fluidic Device

The first revision of a micro-fluidic device [8] was used to incorporate neuron cells within the device and determine their viability in-chamber. Figure 5 shows the microfluidic chip used which has specialized chambers for cell trapping using dielectrophoresis and electrodes for cell metabolism measurements. The surfaces of the device are SiO2 with Pt electrodes. Dielectrophoresis (DEP) was used to trap cells from within the flow in the chamber. The effects of DEP force [9,10] is understood by knowing the size of the particle, the complex permitivities of the particle and the surrounding media, and the magnitude and frequency of the AC electric field applied. If the DEP force is positive the particle will move toward the maximum electric field gradient. Alternatively, if the DEP force is negative the particle will move toward the minimum electric field gradient. If the electric field and flow velocity are controlled sufficiently, the cells will continue to move into the DEP chamber and become trapped by the DEP force at the edges of the electrodes. During the experiment, cells were introduced to the chamber at a concentration of $10^6$ cells/ml in 2% neural media. A sinusoidal signal with amplitude of 10V at 1MHz was used to trap the cells at the edges of interdigitated electrodes. Figure 6(a) shows an image of neural cells, which have been stained with 3,3′ dihexyloxacarbocyanine iodide, flowing through the DEP chamber. A long exposure was used to observe continuous flow lines.
DEP entrapment of the cells in the chambers was accomplished and Figure 7 attempts to demonstrate this using images extracted from a video at different time points. Once the cells were trapped by DEP the flow was reduced to 2psi (approx. 3µl/hr) to replenish cellular nutrients. Initial investigations show the cells remain alive in-chamber for up to 15 hours, Figure 6(b).

Figure 6: (a) Labeled cells flowing through the DEP chamber over an interdigitated electrode array. (b) close-up of a live cell inside the chip.
CONCLUSIONS
In this paper, we have reported initial studies on the growth and attachment of neuron cells on micro-fabricated surfaces. Cell viability on open chip has been determined to be extremely high. Surface attachment and process development has been investigated resulting in an optimal surface structure and preparation technique. The final micro-fluidic device is a SiO₂ substrate with Pt electrodes covered with the PORN/L layers. Symp cells are used as they provide sufficient cell attachment and process development on these surfaces without a high content of associate glia cells. Initial microfluidic experiments have been performed showing the flow of neural cells into the devices, dielectrophoretic trapping of the cells in the device, and optical viability assessment. Future work includes demonstration of the cell growth inside the chips and investigations of the cell properties using electrical means.

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REFERENCES