Abstract— We aim to create a prototype olfactory sensor (“nose on a chip”) by interfacing olfactory sensory neurons (OSNs) with a microelectrode array (MEA) and observing extracellular electrical impulses in response to various odorants. Thus far, successful measurements have been recorded using bovine aortic smooth muscle cells (BAOSMCs) exhibiting spontaneous activity and in response to chemical stimuli. Specific challenges in using OSNs include prolonging cell life and improving the instrumentation used to measure action potentials. We have found that a membrane composed of either paraffin oil or polydimethylsiloxane (PDMS) successfully prevents dehydration while maintaining the viability of BAOSMCs for the duration of an experiment. A four-layer printed circuit board utilized a 52-channel microelectrode array with 52 instrumentation amplifiers and was integrated directly with common data acquisition software. Future developments include establishing methods to interpret and analyze the electrical responses in order to classify odor signatures.

Index Terms—action potentials, bovine aortic smooth muscle cells, olfactory sensory neurons, printed circuit board

I. INTRODUCTION

The use of biological cells for sensing is an expanding interest due to their high sensitivity, selectivity, and fast response times. We intend to apply biological sensing to the development of a prototype olfactory sensor. Olfactory sensory neurons (OSNs) are receptor cells which detect odors in the environment. Different types of OSNs respond differently to odorants. In theory, various types of OSNs can be used to identify specific odors. The electrical responses can be viewed as electrical signatures, each corresponding to each odorant. Studying the diverse electrical responses of OSNs also enhances the understanding of the biological process of odor detection [1]-[6].

The approach to cell-based odor detection discussed in this paper consists of interfacing cells with a microelectrode array (MEA) to measure their action potentials. Detecting action potentials of OSNs presents challenges due to the magnitude of their electrical responses and sensitivity to the ambient environment [7]. In the past Bovine Aortic Smooth Muscle Cells (BAOSMCs) – robust, electrically active cells – have been used to test the capability of the instrumentation to measure extracellular potentials [8].

We have utilized BAOSMCs in our studies in order to draw a direct comparison to our past experiments and quantify improvements in our design. The improvements in our hardware aimed to reduce the amount of noise in our signals and measure action potentials. In addition to testing instrumentation, we address ways to prolong cell life outside of a standard incubator by preventing evaporation using a membrane or supplying sterile water to the cell culture.

II. IMPROVING INSTRUMENTATION

To detect action potentials, a 52 channel, passive microelectrode array was employed. 52 channels were used in order to increase our chances of successfully measuring action potentials and to allow for an extensive temporal analysis of electrical responses. The MEA contains 53 electrodes; 52 electrodes are active channels, and one electrode serves as a ground electrode.

![Microelectrode Array](image)

Fig. 1. The redesigned populated printed circuit board used for experimentation.

A. Hardware

In order to measure extracellular potentials with micro-volt amplitudes, high impedance, low noise amplifiers were used. 52 instrumentation amplifiers with a gain of 100 V/V were implemented on a four-layer PCB to amplify detected signals. Each positive input to the amplifiers comes from each of the 52 active electrodes. The board possesses the ability to amplify the signals in either single-ended amplification or differential amplification. This versatility is achieved through switching the negative input to the amplifier between an adjacent electrode and a global electrode ground. The toggling is achieved through an array of dual in-line package (DIP) switches. An additional array of DIP switches gives us the
ability to connect several of the active electrodes to the ground electrode. This reinforces the electrode ground throughout the MEA. To further reinforce the electrode ground, an additional probe can be employed, which connects the electrode ground to the cells via an external wire placed into the cell solution. DIP switches are also utilized to allow us to connect or disconnect different grounds in our circuit (i.e. the electrode ground, power ground, and data acquisition grounds). The outputs of the amplifiers connect directly to two high-speed, 16 bit National Instruments Data Acquisition Systems (NI DAQs), which can easily be integrated with common software such as MATLAB and LabVIEW.

**Improvements**

Several of the previously mentioned components are updated from an older version of the board. The AD8222 amplifiers with inputs driven by BJTs were replaced with AD8224 (Analog Devices) amplifiers, in which JFETs drive the inputs. JFETs inherently have higher input impedances and less noise than BJTs. The input current drawn by the amplifiers creates a voltage drop between the amplifier input and the electrode it is attached to if the signal source has high impedance. In past experiments, this voltage adversely affected the cells on the MEA. This voltage drop was responsible for the intermittent functioning of the previous PCB. To eliminate this effect, the inputs are now capacitively coupled into the amplifier, and a return path to ground via a 1 MΩ resistor was added.

The previous PCB achieved the same versatility as the current board through arrays of external pins. Each pin acted as a small antenna collecting noise from the surrounding environment. To reduce the effect of ambient noise, these pins were replaced with DIP switches. DIP switches maintain the same functionality, but reduce the coupling and interference from external sources of noise. Additionally, power planes were added to the top and bottom layers of the PCB to provide a shielding effect for the internal layers of the board. Since the vast majority of signals run through the inner layers of the board, the power planes aim to significantly reduce noise.

**B. Software**

Signals detected and amplified in the PCB were integrated with MATLAB. A GUI was created to plot and store incoming signals. Additional features include stamping, searching for, and viewing data. When the user changes a parameter in the experiment, such as adding chemical stimuli, the time and description of the change is stamped. Rather than sifting through all of the data, Matlab searches for the relevant data (based on the stamps) and lists the data files, which can then be viewed by the user. The GUI also permits the user to filter the signals using a low or high pass FIR filter.

**C. Testing**

BAOSMCs were cultured for experimentation. The hardware was then tested by chemically stimulating BAOSMCs in order to measure and analyze their action potentials.

**Cell Culture**

Cryopreserved BAOSMCs (Cell Applications, Inc.) were thawed and cultured according to a protocol given by Cell Applications, Inc. A sub-culturing approach was adapted for subsequent cultures. Growth medium was removed from the culture flask and cells were rinsed with Hank’s Balanced Salt Solution (HBSS). Cells detached from culture flask upon a 2 to 3 minute treatment of trypsin, which was then neutralized with an equal amount of growth medium. The cells were then centrifuged for 5 minutes at 1500 RPM. The supernatant was removed and the cell pellet was re-suspended in 1 mL of growth medium. The cells were plated in a vial directly on the MEA. They were incubated at 37°C and 5.0% CO₂ and given 24 hours to adhere before the experiment was run.

**Experiment**

The PCB was powered by two 9.6 V batteries between VSS and VDD. The board was then attached to two NI DAQs and set up for single ended recording. Subsequently, the MEA was placed on the PCB, and baseline recordings were taken. The growth medium was then removed, and a small volume of 100 mM KCl was added simultaneously. Once a substantial number of samples were recorded, the KCl was removed and the cells were rinsed with HBSS. They were given fresh growth medium and incubated.
D. Results

**Before Chemical Stimuli**

![Before Chemical Stimuli Graph]

Fig. 3. Filtered extracellular electrical activity of the cells before they were stimulated with KCl. This serves as a baseline recording for comparison and verification that the PCB was capable of detecting action potentials.

**After Chemical Stimuli**

![After Chemical Stimuli Graph]

Fig. 4. Filtered extracellular electrical activity immediately after KCl stimulation. There is a noticeable difference in frequency and amplitudes of cell firing.

E. Analysis

BAOSMCs maintain a membrane potential of -60 mV to -70 mV by the selective opening and closing of K⁺ and Cl⁻ ion channels in their membranes [9]. The introduction of KCl to BAOSMCs should yield several expected results: the shape of action potentials should be more defined and the amplitude of the spikes should increase.

The expected changes previously mentioned are apparent in the juxtaposition of Fig. 3 and Fig. 4, which display low pass FIR filtered data. In the filtered data before chemical stimulation (Fig. 3) there appear to be two spikes with amplitudes of roughly 500 µV. The filtered data after chemical stimulation (Fig. 4) displays three well-defined spikes corresponding to action potentials with amplitudes of roughly 1000 µV. Since the expected changes were detected in our hardware, the improvements we implemented were successful, and we are detecting action potentials.

In comparison, Fig. 4 depicts a smaller signal to noise ratio than Fig. 5. Fig. 5 illustrates floating signals due to the lack of a return path to ground on the inputs to the amplifiers. Furthermore, the action potentials are unidirectional because the cells were in a hyperpolarized state because of the voltages induced on the electrodes. This was rectified by capacitively coupling the inputs to the amplifiers and adding a return path to ground.

Although our hardware is detecting spikes, it has proven to be difficult to implement spike detection algorithms. Potential challenges in detecting spikes via algorithms in the data include determining appropriate filtering techniques and thresholds [10], [11]. The locations of cells on the MEA pose problems for detection; they could be laying on top of one another or multiple cells may be located on the same electrode. This could cause some variance among the channels and inconsistencies in the data acquired.

III. Prolonging Cell Life

In the past, cells were not remaining active for experimentation due to evaporation of the cell media. A membrane approach was tested in order to prevent evaporation. The membrane needed to be hydrophobic, gas permeable, and biocompatible. Paraffin oil has been used in the past for in vivo olfactory neural experiments and was tested here as a membrane. Polydimethylsiloxane (PDMS) was also tested because it is known to be a highly gas permeable and biocompatible polymer [12]-[14]. Adding sterile water to the cell culture was a second approach attempted in order to keep cells viable for experiments in an ambient atmosphere [15].
A. Procedure

Membranes

The adapted cell culture protocol was used to plate BAOSMCs in petri dishes with additional growth medium, which was changed every 2 days. As a control, a thin layer of paraffin oil (Sigma-Aldrich) was placed on top of a small amount of growth medium in a petri dish without cells. Evaporation was measured by monitoring the mass of growth medium. The dish was weighed initially and placed in a mini-incubator for 24 hours before being weighed for a second time. The mini-incubator was built to maintain a temperature of 37°C.

In order to test the membranes, the cells were given 24 hours to adhere to a petri dish before paraffin oil was placed on the culture. Once the entire dish was covered the cells were kept in the mini-incubator and their weight was monitored every day. After obtaining each mass reading, the cells were observed under an inverted microscope to assess viability.

The membrane approach was repeated using PDMS as an evaporation barrier. A 10:1 ratio of PDMS to curing agent was mixed and degassed in a desiccator until no air bubbles were visible. A small amount of the mixture was poured into the center of a petri dish until a thin layer covered the surface. The membrane was placed in an oven for 24 hours at approximately 50°C. After peeling the PDMS off the dish it was directly placed over the cell culture, which was then weighed and monitored.

Sterile Water

A cell culture plated in a petri dish that was divided in half was weighed and placed in the mini-incubator. A 5mL syringe filled with 4mL of sterile water (Sigma), intended for tissue culture, was held in place above one half of the cell dish using a syringe pump. Sterile water was continuously supplied to the culture at a rate of 3.0μL a minute overnight until the 4mL was consumed. The cells in the second half of the dish were left only with growth medium.

Membrane Permeability

A membrane of paraffin oil or PDMS was placed over dishes containing only growth medium. The pH of the media was measured before introducing additional substances. Droplets of Geraniol, eucalyptol, α pinene, and linalool (Sigma), which could eventually be used as odorants for OSNs, were placed on the membrane to observe permeability. If penetration was observed, pH measurements were taken. A 0.2 M solution of HCl was also tested because of its polarity and low pH value.

In order to test permeability to CO₂, growth medium dishes with membranes were placed in an incubator at 37°C and 5.0% CO₂. The color of the media was monitored over time for a color change from red to orange, which would indicate CO₂ contact with the growth medium due to pH sensitive dye in the media.

B. Results

![Graph showing the evaporation of cell culture versus time](image)

Table 1: Membrane Permeability

<table>
<thead>
<tr>
<th>Substance</th>
<th>Visible Penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraffin Oil</td>
<td>PDMS</td>
</tr>
<tr>
<td>0.2 M HCl</td>
<td>YES</td>
</tr>
<tr>
<td>Geraniol</td>
<td>YES</td>
</tr>
<tr>
<td>Eucalyptol</td>
<td>YES</td>
</tr>
<tr>
<td>Pinene</td>
<td>YES</td>
</tr>
<tr>
<td>Linalool</td>
<td>YES</td>
</tr>
</tbody>
</table>

This illustrates the permeability of liquid odorants through the membranes tested.

C. Analysis

Due to the hydrophobic nature of paraffin oil, minimal evaporation of the cell culture occurred. Cells remained viable for approximately 5 days before overgrowth and additional cell death was observed. Fig. 6 illustrates the extent to which cells remained healthy while minimal evaporation occurred. The permeability of paraffin oil permitted the diffusion of the selected odorants across the membrane and indicates a high probability of allowing other organic compounds to cross while preserving the growth medium. When placed in the incubator, CO₂ was able to penetrate, resulting in orange growth medium.

Although PDMS kept the cells alive less efficiently than paraffin oil, it is sufficient time for detecting signals. As Table 1 indicates, PDMS failed to be permeable to the liquid phases of the odorants tested, but did allow CO₂ to enter in the incubator. It was difficult to peel the polymer as a very thin membrane, therefore; the thickness of the membrane may have been the reason why the odorants were incapable of passing. Since PDMS is known to be highly gas permeable and experiments verified its permeability to CO₂ it would be beneficial to introduce the odorants to the membrane as gases instead of liquids.

In contrast to the membrane approach, continuously supplying sterile water to the cell culture failed to keep cells...
viable. In less than 24 hours, the cells were clumped together and inactive. Although the water was meant for tissue culture, it lacked ions and other nutrients.

IV. FUTURE WORK

Ultimately, we are working towards measuring the action potentials of OSNs. We plan on testing our improvements with neurons to further the development of an olfactory sensor. When working with OSNs it would be beneficial to introduce an artificial mucus membrane. It would resemble the environment of the nasal epithelium from which the OSNs are extracted and act as an odorant carrier.

In addition to being able to measure electrical signals from cells, it is important to be able to distinguish spikes from noise. Future developments include expanding spike detection techniques along with defining electrical signatures in response to different stimuli.

Imaging can be used to further verify that the signals observed are indeed action potentials. Calcium ion dyes would chemically indicate the influx of Ca$^{2+}$ into the cells during excitation. Voltage sensitive dyes would illustrate the change in membrane potential as the cells fired, but currently have longer loading times and higher phototoxicity than calcium ion dyes [16], [17].

V. CONCLUSION

The hardware improvements employed effectively identified extracellular electrical activity of BAOSMCs. The new GUIs allowed for a simpler way to view and maintain data obtained. Paraffin oil and PDMS successfully prevented evaporation of the cell culture and allowed for the cells to remain viable while being kept at 37°C outside of an incubator. Both materials are permeable to CO$_2$ and paraffin oil permitted liquid odorants to pass. Sterile water failed to maintain cell life by allowing the evaporation of nutrients.

ACKNOWLEDGMENT

We thank Dr. Elisabeth Smela, Dr. Jeff Burke, Marc Dandlin, and the Laboratory for Microtechnologies.

REFERENCES


