Integrating Iontronics with Cerebral Organoids

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Abstract

The goal of this project was to integrate a bioelectronic ion pump for use with cerebral organoids. Cerebral organoids are 3D models allowing researchers to study the development and function of various areas of the human brain. The ion pump allows researchers to deliver ions and charged molecules to specific areas of the organoid with a high level of control over the amount delivered as well as the ability to deliver the ion without its co-ion. The primary method of this project was to use an ion pump previously developed by Dr. Marco Rolandi’s group to deliver potassium ions to an organoid, causing an increase in electrical activity which was verified using calcium imaging. This integration was first attempted with an ion pump used for in vitro studies with cells. However, inconsistent fabrication of this device soon led me to use the smaller, simpler in vivo ion pump. Integration attempts did not yield successful calcium imaging results which indicates the need for further research to gain a deeper understanding of the organoid’s expected electrophysiological reaction and the device’s electrical behavior.
Table Of Contents

Abstract 1

Table Of Contents 1

Problem Statement 3

Background 5

Characterizing Organoid Activity 6
    Calcium Imaging 6
    Multi-electrode arrays (MEAs) 7
A Bioelectronic Device for Wound Regeneration 7
    Basic Principles of Electrochemical Bioelectronic Devices 8
        Figure 1: Device Schematic 9
        Figure 2: In Vitro and In Vivo Devices 10

Work Conducted 11

Integrating with In Vitro Device 11
    Objective: 11
        Figure 3: Leakage Assembly 12
        Figure 4: PDMS Inserts 13
    Materials and Methods:
        Figure 5: PDMS-Insert Molds 14
        Figure 6: In Vitro Clamp 15
    Testing: 16
In Vivo Device and Adapter Troubleshooting 19
    Experiments with Fixed Organoid 19
        Figure 9: Fixed Organoid 20
        Figure 10: In Vivo Device Current 21
    Failed Experiments with Live Organoid 21
        Figure 11: Failed Experiments 23
Device Characterization without Organoid (Mar. 3 2022) 23
    Figure 12: In Vivo Current at -0.8V 24
Problem Statement

I developed a device for the electrophoretic delivery of ions and other small charged molecules to cerebral organoids. I worked towards the delivery of potassium ions as a proof of concept. Whereas researchers currently hand pipette molecules of interest into cerebral organoids, electrophoretic ion pumps allow for computer controlled, highly specific delivery of molecules. In addition, the small size of the capillary outlet (~35um) allows for researchers to control what part of the organoid the ion pump delivers to. The effect of different ions and molecules on cerebral organoids is important for studying brain development in human embryos, neuron communication in human brains and neurodegenerative diseases.

Organoids are 3D in vitro tissue constructs, grown from stem cells and differentiated in vitro to model various organs. They can be used to model organ development, model diseases, test drugs and assist in the development of personalized and regenerative
medicine (Xu et al., 2018). Myproject used cerebral organoids which are grown from stem cells that are exposed to growth factors leading them to differentiate into neural cells. They are excellent in vitro models for the brain, allowing researchers to study specific areas of the brain and connections between regions of the brain in a noninvasive manner (Osaki & Ikeuchi, 2021).

Recently, research using organoids has benefitted from the use of various technologies to automate parts of the research process. Microfluidic devices such as autofeeders enable the development of better nourished organoids. Improvements in multi-electrode arrays (MEAs) allow for more detailed measurements of organoid activity. Developments in the field of iontronics could help further improve organoid technology.

Iontronics operate at the intersection of biology and electronics, creating wearable and implantable systems for monitoring and actuation. Iontronics use mobile ions as charge carriers instead of electrons (the charge carriers typical electronic devices use). Biological systems also use ions to communicate, helping iontronic devices more easily interface with biotic systems. Improvements in the materials used to create iontronic devices have also increased their biocompatibility. Ion pumps are a type of iontronic device that converts an electric signal to an ionic current using an ion selective membrane. This allows a specific ion to be delivered from a reservoir to a target. This ability of the ion pump to convert between electrons (the language of computers) to ions (the language of biology) makes the device a good candidate for enabling the computer controlled delivery of ions to organoids.
I started with an iontronic device being developed at UC Santa Cruz by Dr. Marco Rolandi’s and Dr. Mircea Teodorescu’s research groups to speed up wound regeneration. This device is optimized for integration with a mouse for *in vivo* work, so I will be responsible for adapting it for use with organoids. I planned for the organoids to grow in a 35mm petri dish and the ion pump to sit on top of a 3D printed adapter. Successful integration will be shown by the delivery of potassium ions. An influx of potassium ions sparks electrical activity in the cerebral organoid, so verification of the delivery of potassium ions is done via calcium imaging (a fluorescence-based method to verify the electrical activity of neural cells).

Further research might include integrating the ion pump with an MEA to allow for fully autonomous, multiplexed actuation and monitoring of cerebral organoids. Additionally, the delivery of larger, charged molecules (such as neurotransmitter GABA) with the device can be studied, as well as the use of the device to simultaneously deliver different ions.

**Background**

I. **Organoids as a model of cerebral activity and differentiation**

Organoids are 3D *in vitro* (grown in a dish) models of organs grown from stem cells. As 3D cell cultures they provide more details than typical 2 dimensional cell cultures in a less invasive manner than *in vivo* (in life / in animal) studies. Organoids are grown from stem cells, which are undifferentiated cells that are exposed to differentiation factors causing them to develop into
more specialized cell types, similar to what happens as a human embryo develops. Among various other uses, organoids give us an excellent glimpse into the hard-to-study field of human development (Xu, 2018).

Cerebral organoids, in vitro models of the brain, can be excellent models of brain development. Using animal models or brain slices to study the human brain is difficult because they can be sensitive and not accurate reflections of the human brain. Meanwhile, actual studies in humans are limited by their invasiveness (Poli, 2019). To circumvent this, researchers have grown successful models of many parts of the brain, including the cortex. The human cerebral cortex is the site of much cognitive and social processing and studying its development is an important step to understanding the basis of many neurological disorders such as alzheimers and dementia (Rubenstein, 2011).

**Characterizing Organoid Activity**

Mature cerebral organoids and other neuronal cell cultures show spontaneous electrical activity (Osaki, 2021). This electrical activity enables researchers to use cerebral organoids as a model of the function of the human brain and determine how different environments, drugs and molecules affect its function. Thus, researchers must have a reliable way to measure electrical activity. Among current methods to do this, multi-electrode arrays (MEAs) and calcium imaging are two well-used and promising methods (Poli, 2019).

**Calcium Imaging**

When neurons and other excitable cells depolarize during signaling, voltage-gated calcium channels open, allowing the influx of calcium ions (Putney, 1999). Fluorescent dyes such as
Fluo-4-AM, can bind to these calcium ions and release a fluorescent signal. By using a fluorescent microscope, researchers can see this fluorescent signal oscillating (the amount of light increasing and decreasing), indicating that a specific neuron is firing. The fluorescent signals can be processed to discover temporal (time-related) patterns behind the electrophysiology of the neurons (Yin, 2020). For this project, we used calcium imaging to verify that the organoids we were testing were active as well as for initial experiments involving using the device to deliver potassium ions. Calcium imaging is great for real time, multi point analysis of single neurons of the organoid (Poli, 2019; Hamasaki, 2017).

Multi-electrode arrays (MEAs)

Multi-electrode arrays (MEA) are another method to allow researchers to study the electrical activity of neurons in a realtime, multipoint, noninvasive manner (Hamasaki, 2017). The MEA consists of a small computer chip with many small electrodes on it. The MEA used for our experiments has 26,400 electrodes in a 2x4mm rectangle (Maxwell Biosystems, 2020). Cells are grown (or organoids are transplanted) onto the MEA and the large number of electrodes detect a voltage potential as it propagates throughout a cell. This data is processed to show which neurons in an organoid are firing and when. The large number of electrodes allows for detailed measurements of electrically active cells (such as the neurons in organoids) over an extended period of time (Axion Biosystems, 2022). For this project, we hoped to use MEAs to take long term, real time measurements with a high level of spatial and temporal specificity with the potential to later enable automated computer (closed-loop) control of the ion delivery.

A Bioelectronic Device for Wound Regeneration

This project involves the integration of an existing bioelectronic device with cerebral organoids. To understand the project, it’s important to have a basic understanding of the device’s original
application and function. The device is originally part of a Defense Agency (DARPA) funded project to help speed up wound healing. It is a type of electrochemical device, using basic electrical and chemical properties to monitor and actuate biological signals. By translating between ions (the language of biology) and electrons (the language of electronics), the device enables ions and other charged molecules to travel from a source solution to a target (Strakosas, 2019).

There were two similar device designs, one for \textit{in vivo} (in life) studies on mice and for \textit{in vitro} (in cell culture) experiments. Originally, this project intended to use the \textit{in vitro} device, however, I switched to using the \textit{in vivo} device design. Both device designs have similar operating principles and fabrication methods (H. Dechiraju, personal communication, January 21 2021).

\textbf{Basic Principles of Electrochemical Bioelectronic Devices}

\textit{Electrons} are negatively charged particles. As they travel through a conductive (metals) or semiconductive (for example silicon) material, they create a \textit{current}. The magnitude of the current depends on how easily they travel through the material (the \textit{resistance}) as well as the difference in charge between where they started and where they are going (the \textit{voltage}). The basic relationship between them is Voltage (V) = Current (I) \times Resistance (R) (Wong, 2009).

A simple analogy is that of a sled going down a hill. The height of the hill represents the voltage, the sled represents the electron, and how quickly the sled slides down the hill represents the current. Different surfaces on the hill represent resistance, for example a snowy hill will represent low resistance and a dirt hill will represent high resistance. The current (sled speed) will be largest with a low resistance (snowy hill) and high voltage (tall hill).
For the device to work, first a voltage is applied across a system with an electrode on each end in the source solution. Negatively charged electrons travel from the positively-biased electrode to the negatively-biased electrode. Because negative charges and positive charges attract each other, positively charged ions in the solution want to travel to the target to balance out the negative charge. A selective exchange membrane prevents ions from traveling back to the source solution once the voltage stops being applied (Williamson, 2015). This is shown in Figure 1, with a schematic of how the device for this project works. The figure shows electrons moving from a positively biased electrode to a negatively biased electrode, causing a positively charged ion to move from the source reservoir through a hydrogel-filled capillary (selective exchange membrane) to the target (an organoid in the center of the schematic).

![Device Schematic](image)

**Figure 1: Device Schematic**

The figure shows electrons moving from a positively biased electrode to a negatively biased electrode, causing a positively charged ion to move from the source reservoir through a hydrogel-filled capillary (selective exchange membrane) to the target (an organoid in the center of the schematic).
III.B. Fabrication Methods

*In Vitro Device Construction*

The *in vitro* device, shown in Figure 2, consists of a polydimethylsiloxane (PDMS) body with microfluidic channels leading to a PDMS cell culture insert where cells can be grown. The microfluidic channels are filled with hydrogels (acting as the selective exchange membrane) which only allow positively charged ions to pass through. The channels are bonded to a glass slide with nano-particle plated electrodes. The device can be connected to and controlled by a microcontroller which attaches to the device via gold electrodes (H. Dechiraju, personal communication, January 21 2021).

*In vivo Device Construction*

The *in vivo* device, shown in Figure 2, consists of a PDMS body with 4 reservoirs that can be filled with the source solution. Hydrogel-filled capillaries (acting as the selective exchange membranes) are inserted into the PDMS to allow a path from the source solution to the target. Electrodes are embedded in the PDMS, in contact with the source solution and attached to a PCB board. The PCB board can be attached via an adapter to a microcontroller, allowing for computerized control of the device (J. Selberg, personal communication, June 21 2021).
Figure 2: In Vitro and In Vivo Devices

The *in vivo* (left) and *in vitro* (right) devices are shown above. Both are constructed primarily from PDMS, use hydrogels as a selective exchange membrane, and have electrodes that allow the device to be attached to a microcontroller.

Work Conducted

Integrating with In Vitro Device

Objective:

The goal of this project is to use an ion pump to deliver ions to an organoid. There were two possible ion pumps to use for this project. The first was originally designed for *in vitro* applications, delivering molecules to cells. The second was originally designed for *in vivo* applications, delivering ions to wounds on a mouse. Originally, this project was going to be done with the *in vitro* device from the DARPA project, however, due to problems with the device this eventually transitioned to the *in vivo* device. My plan with the *in vitro* device was to modify the polydimethylsiloxane (PDMS) cell culture insert to make it compatible with organoids. PDMS is a transparent, flexible biocompatible silicone commonly used in bioelectronic devices.

In the *in vitro* device to test on cells, cells are grown on a PDMS insert. The original PDMS cell culture insert contained two 1.5mm biopsy-punched holes connected by a 1.5 mm microfluidic channel. By combining this insert with a parylene-coated, 3D printed leakage plug and well, cells were able to be grown directly on the PDMS cap. These three parts (PDMS insert, leakage plug and well) as well as the assembled assembly are shown in Figure 3.
Figure 3: Leakage Assembly
The 3 parts of a cell culture assembly. The cells grow on the PDMS insert (a), 3D printed well (b) and leakage plug (c) create a dish to grow the cells in (d). The PDMS insert can then be used in the in vitro device.

As organoids can typically grow to up to 3-4mm in diameter, I wanted a 4mm well for the organoid to sit in. There were two possible designs: having two 1.5mm holes with a 4mm hole in the center or just having the 4mm hole. The design with the 3 holes would allow for future integration with an autofeeder (a way to automate the feeding of cell cultures which typically has to be done 3-4 times per week). However, it was decided to stick with the single-well option for initial tests as it made sense to start with a simpler design. A photo comparing the 3 different styles of PDMS insert and a schematic showing how the PDMS insert integrates with the in vivo device is shown in Figure 4.
Figure 4: PDMS Inserts

The 3 types of PDMS inserts with their respective leakage plugs shown beneath (a-c). The two modified for organoid integration: the single 4mm hole adapter (a) and the 3 hole adapter (b). The original in vitro leakage plug (c) and a schematic (d) showing the expected integration with the in vitro device.

Materials and Methods:

The fabrication of the original cell culture assembly consisted of 3D printing a mold using Model V2 resin to be filled with PDMS, incubated, and then demolded. Model V2 resin is a type of material to make 3D prints out of, especially used for molds. The resin begins as a liquid, however exposure to a laser solidifies it, and creates a design layer by layer. An acrylic piece was used to create the microfluidic channel in the PDMS. Acrylic is a highly transparent, hard plastic. It is shaped from a 3D print file using a CNC (computer numerical control) machine which digs out a design from a stock piece of acrylic. The 3D printed mold is filled with PDMS while in contact with the acrylic piece and creates an indent for the microfluidic channels. The
PDMS is allowed to solidify before the finished PDMS product is removed. Two 1.5mm holes were biopsy-punched into the PDMS to allow for solution and media to flow through the setup. A biopsy punch can be thought of as a sterile hole punch with specified diameters. The leakage plug and well were both 3D printed using clear V4 resin and then coated with Parylene-C to ensure biocompatibility. Clear V4 resin is used in a 3D printer, similar to Model V2 resin. 3D prints with Clear V4 resin yield a relatively transparent product that can be made biocompatible by coating it with a polymer called Parylene-C.

Prototypes of this design were created by simply biopsy-punching an extra 5mm hole into existing cell-culture inserts. Later, I used Fusion 360 software (a software to create designs to 3D print) to create a negative mold of the desired PDMS cell culture insert. I redesigned the mold to minimize error created by using the biopsy punch. Because I was unable to access the CNC machine (the machine used to print acrylic designs, as well as other designs) to create the acrylic pieces, I 3D combined this component with the mold for my PDMS insert. The new molds are shown in Figure 5.

![Figure 5: PDMS-Insert Molds](image)

*The new PDMS-insert mold (a) uses one 3D printed piece whereas the old design combined a 3D printed component (b) with an acrylic component (c).*
When I went to test my assembly with the *in vitro* device, I realized that I would additionally have to redesign the clamp that held the *in vitro* device during experiments. The existing clamp had two 1mm holes to integrate with the holes and microfluidic channel in the original cell culture insert. Thus, to use the *in vitro* device with my new inserts with larger holes, I had to redesign a clamp. I 3D printed these new clamps with clear V4 resin. These new clamps are shown in Figure 6. The new design provided the same clamping effectiveness (verified by the *in vitro* device not moving around when the clamp was tightened).

**Figure 6: In Vitro Clamp**

*The new PDMS insert design required a new clamp to maintain function of the device while simultaneously allowing access to the organoid. The old clamp is shown at the top, and the new clamp is shown at the bottom (a). The assembled stage, clamping the *in vivo* device to a piece of acrylic, ready for imaging (b).*
Testing:

To verify cell culture assemblies are working, the assembly must pass a leakage test. This test consists of filling an assembly with water, placing it in a 37°C incubator for 24 hours and then verifying that no water has leaked out. Four out of five of the cell culture assemblies I tested passed, which meant an organoid would be able to grow on the assembly and integrate with the \textit{in vivo} device.

No further tests were done. Although integrating the \textit{in vitro} device looked promising, the \textit{in vitro} device was frequently breaking and not yielding promising results in experiments being done by graduate students on the DARPA project. So, my adviser Dr. Marco Rolandi suggested I begin using the \textit{in vivo} device for my project, with the thought that the \textit{in vivo} device is significantly cheaper and less complicated to fabricate.

Integration with In Vivo Device

In Vivo Adapter:

Using the \textit{in vivo} device (originally designed to decrease wound healing time in animal models) to deliver ions to organoids posed a unique set of problems as the device was designed to lay flat against mouse wounds. As an organoid is more sensitive than the mouse wound and significantly smaller, integrating the device directly would not work.

\textit{Adapter 1}:

The first method I tried was to create an adapter to sit on top of a 35mm petri dish. The adapter consisted of a 3mm piece of acrylic with a 1mm ridge to sit on top of the petri dish, a hole to
allow capillaries from the device to reach through to the petri dish and several smaller holes for air and media exchange. PhD candidate Kate Voituk helped me create the acrylic piece from my Fusion 360 file. I then extended the length of the \textit{in vivo} capillaries from 1mm to 10mm to reach the organoid and the media. I characterized the capillaries by testing the current response of the capillary with a 0.8V voltage for 5 minutes. The current response (approximately 45nA) was similar to the current characterization experiments done on DARPA capillaries, indicating the capillary should perform well. However, I still ran into issues with this path because the longer capillaries made the device difficult to assemble and use. In addition, the longer capillaries changed the electrical characteristics of the device. To solve this, I 3D printed a lower adapter. A figure of the first adapter set up and an \textit{in vivo} device with longer capillaries is shown in Figure 7.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{in_vivo_adapter.png}
\caption{First \textit{in vivo} adapter}
\end{figure}

\textit{The first in vivo adapter involved an acrylic petri dish topper (a) and extending the length of the capillary tubes (b) (the selective exchange membranes of the ion pump) to 10mm in length to reach the organoid.}
Adapters 2, 3 and 4:
I decided to lower the adapter, at first creating a bulky 3D printed adapter (adapter 2) and then creating an inverted tophat shaped adapter (adapter 3). The new adapter worked well as it allowed me to shorten the length of the capillaries back to the original 1mm, allowing me to leave the actual in vivo device unaltered. When testing the setup with a fixed (dead) organoid, I found that the organoid moved around a lot, preventing me from positioning it directly under the adapter. I further modified the adapter (adapter 4) to create walls reaching the bottom of the petri dish, creating a well for the organoid inside of the petri dish.

Adapter 5:
I found that adapter 3 worked well, however, the shape of the adapter prevented the in vivo device from sitting flat as the cable connecting to the PCB board was unable to lie flat. I modified this by adding a rectangular cutout section to enable the device and the cable to lie flat. In this modification, I also decreased the diameter of the organoid well to enable it to sit closer to the capillary outlets of the in vivo device.

Figure 8 shows adapters 1-5.

Figure 8: Chronology of the in vivo adapters
Adapter 1 (a) is made of acrylic while adapters 2-5 (b-e) are printed with clear V4 resin. Adapter 1 sits at the top, adapter 2 (b) reaches down 5mm, adapter 3 (c) sits 1mm from the bottom and
adapters 4 and 5 (d and e) touch the bottom. Adapter 5 (e) contains space for the device wiring while the other adapters do not.

In Vivo Device and Adapter Troubleshooting

Experiments with Fixed Organoid

Senior Researcher Mohammed Mostajo-Radjii prepared a 4 week old human cortical organoid. I placed this in a 35mm petri dish with phosphate-buffered saline (PBS) solution. I used this organoid as a trial run to test my adapter setup while imaging with the in vivo microscope. I found it difficult to use adapters 3 and 4 for images because the slightest movements caused the organoid to shift under the PCB board, out of view of the microscope. Adapter 5 worked better because the rectangular cutout limited the amount of movement caused by the wires from the microcontroller. Figure 9 shows the fixed organoid lined up with the in vivo device.
Figure 9: Fixed Organoid

The fixed (dead) organoid lined up with the in vivo device. The organoid is circled in red and the capillary outlets (where the ions come from) are circled in blue.

I further tested the setup by delivering K+ ions with the device by doing a test run using 100mM KCl as the target solution. To run this test, I used the software setup for the DARPA project and applied 2V for 2 minutes, followed by 0V for 1 minute (a typical test for the in vivo device). After the test run, I repeated this for the organoid. The results (shown in Figure 10) indicated that while the in vivo adapter was working well, the different salt concentrations in different cell media, buffers and other target solutions might influence the effectiveness of the device.
Figure 10: In Vivo Device Current

Results from a typical in vivo test with 100mM KCl target solution compared to fixed organoid in PBS buffer as a target. A voltage of 2V was applied for 2 minutes, followed by 0V for 1 minute (a typical test for the in vivo device). The results indicated that while the in vivo adapter was working well (the two tests show similar current responses), the different salt concentrations in different cell media, buffers and other target solutions might influence the effectiveness of the device (the slight differences in current could be exacerbated in other tests).

Failed Experiments with Live Organoid

Senior researcher Mohammed Mostajo-Radjii and I first attempted experiments with a live organoid on January 18, 2022. Calcium imaging was performed on 2 controls (one organoid without any ions delivered, one organoid with 2.5uL of 10mM KCl hand pipetted into the media) and one experimental organoid (one round of 2V applied voltage for 2 minutes). Unfortunately, none of these organoids showed any electrical activity. There are four reasons described below why this experiment could have failed.
First, the organoids were still quite young (5 weeks), so the neurons would not necessarily be expected to show spontaneous electrical activity yet as neurons in organoids typically become mature enough to do this at around 10 weeks old (M. Radjii, personal communication, January 18, 2022).

Second, the effect of potassium on a cell can quickly cause cell death. This means we may have been too slow to start the imaging when we hand pipetted in the potassium. Through practice this timing can be improved, and recording can begin before the device begins delivery or before K+ is pipetted in.

Third, the device adapter may have been contaminated from previous experiments. Extra potassium on the base of the device and device adapter could have killed the organoid before imaging began. This is remedied in future experiments by rinsing the adapter with DI water and UV treating it before experiments. The *in vivo* device will also be washed although it cannot be UV treated as UV rays interfere with the effectiveness of the selective exchange membrane.

Fourth, the device itself was possibly broken. Although the previous device had been used in fixed organoid experiments, its current response began showing signs that it was short circuiting (high current responses with a low voltage and not returning to 0 current with 0 voltage). In addition, the embedded wires began showing signs of rust. These defects (while not common) have happened in *in vivo* devices in prior DARPA experiments. The defective device and short circuit current are shown in Figure 11.
Despite successful tests using the same device 2 days prior, experiments on Jan. 18 failed. This was in part due to a failed in vivo device (a) that caused a short circuit current (b). The current shown is higher than expected for 10mV (an extremely low voltage) indicating that it was not delivering ions. In addition, the current did not drop to 0 when the voltage was turned off (at 120 seconds) indicating that ions continued to flow despite the device being off.

Device Characterization without Organoid (Mar. 3 2022)

The in vivo device is characterized and tested at multiple points in the fabrication process. First, the hydrogel-filled capillaries (the foundation of the ion pump) are tested by applying a voltage of -.8V for 5 minutes. Typically, the capillaries show a current of around 50nA. Further characterization and testing is done after the capillaries are inserted into the PDMS body, the reservoirs are filled and the PCB board is soldered onto the device. Here, ideally the device will deliver one potassium ion for every electron passed through the voltage, however in reality the device efficiency is usually much lower, around 30% (Strakosas, 2021).
Upon testing the device I intended to use for experiments, I found that the device was short circuiting due to too much target solution getting in between the PCB board and the device. This can be solved by wiping the device dry with a kimwipe. In addition, to prevent the hydrogels from drying out, it is necessary to keep them soaking in solution when not in use. However, I found that with too much solution, the device will rust, increasing its propensity to short circuit.

Measurements showing current v. time are shown in Figure 12. I applied -.8V for 2 minutes and 0V for one minute, expecting that the 100mM KCl would have the largest current, followed by BrainPhys (the media the organoids grow in) with the short circuit test showing 0 current (I tested by taking the ends of the capillaries out of the solution, creating what would have ideally been an open circuit).

Figure 12: In Vivo Current at -0.8V
This figure shows multiple ion recitification curves obtained by applying a voltage of -.8V for 120 seconds. The results were unexpected, as they showed that the measurements obtained with
an open circuit (in red, labeled short circuit) were similar to those obtained with a closed circuit, indicating that the device had short circuited.

To prevent the device from short circuiting in future attempts, I devised a new adapter. I cut out a box and a circle from 2 sheets of red medical grade silicone. Stacking these and pressing them into a petri dish provided a watertight well to put a small amount of solution to soak the capillaries without short circuiting the device. This adaptation is shown in Figure 13.

![Figure 13: Silicone Well](image)

By pressing 2 sheets of medical grade silicone onto a petri dish, a small watertight well (a) can be created. Because only the capillary outlets will touch the liquid (b), the device will ideally not be exposed to liquid, causing it to rust and short circuit.

Organoid Characterization

Experiment with Neurons

Because of the failures involved using the organoids with the device, we decided to verify that K+ had the desired effect on neurons and that the fluorescence microscope was capable of detecting the change in fluorescence. For this project, I chose to begin by delivering K+ because
of its use in literature for inducing epileptiform activity in brain slices (Ructecki, 1985; Williamson, 2015). The epileptiform activity should look like an increase in spontaneous electrical activity. Because we were unsure whether the cerebral organoids were active yet, we verified the expected behavior of neurons exposed to potassium by pipetting in 2.5uL of 1mM KCl solution and calcium imaging the reaction of chicken neuroblastomas. In one well, we saw one neuron fire which indicated that we will likely need to wait for more mature neurons to grow in the organoid in order to see similar results with K+. More research is also needed to discover what concentrations of K+ will excite the neurons without killing them. The cell firing is shown by the fading green light in the calcium imaging in Figure 14.

**Figure 14: Chicken Neuroblastoma**

*Reaction of chicken neuroblastoma to 2.4uL 1mM KCl. As the neuroblastoma circled repolarizes after firing, calcium leaves the cell, causing the fluorescence to fade over time.*

Organoid Experiments (Mar. 7 and 8 2022)
When the organoids were 10 weeks old, immunostaining by Research Assistant Frederika Sullivan of organoids from previous experiments (shown in Figure 15), as well as the movement of the organoids from Sasai-3 media to BrainPhys media, led me to believe that the organoids would be mature enough to get promising results from calcium imaging.

**Figure 15: Immunostained Organoids**

*This figure shows 3 cerebral organoids stained to identify the presence of neurons and neuronal progenitors. MAP2 (in green) binds with neurons, and its presence in each organoid indicates that the organoids have mature neurons and should show spontaneous electrical activity. This image, the immunostaining and slicing was done by Research Assistant Frederika Sullivan.*

To test whether the organoids were mature enough to show spontaneous electrical activity, as well as to identify a K+ molarity change with which we would expect to see a difference in electrical behavior, I ran calcium imaging on four organoids, adding varying amounts and
concentrations of KCl. Research Assistant Frederika Sullivan prepared organoids for calcium imaging by adding 3uL of Flo-8 calcium chelator to a 6 well plate containing the organoids chosen for the experiment. The Flo-8 enters the neuronal cell body and binds to calcium, releasing a fluorescent signal when calcium enters the cell during neuronal polarization. Following a 30 minute incubation period, Frederika placed the organoids and 300uL of their media into the well created by the red sheets of silicone (as seen in Figure 16). Each organoid was then recorded for 2-3 minutes to check for spontaneous electrical activity. Following that, either 20uL of 1mM KCl or 1uL of 20mM KCl was pipetted into the well and it’s effect recorded via calcium imaging.

**Figure 16: Organoid in Well**

An organoid inside a small well containing 300uL of it’s BrainPhys media. This experiment was used to characterize the spontaneous electrical activity of the organoid as well as gain insight into the change in molarity necessary to increase activity of the organoid.

The resulting videos were studied for changing fluorescence, which was seen in 3 of the 4 spontaneous recordings, 3 of the 4 20uL of 1mM KCl additions and 0 of the 3 1uL of 20mM additions. This led me to conclude that the organoids were mature and the increase of K+ by 66 uM in the surrounding media would increase the electrical activity of the cerebral organoid. The lack of results from 1uL 20mM additions was confusing, emphasizing a need for further characterization experiments.
Conclusion and Future Work

This project began with the idea of using an electrochemical ion pump to deliver ions and charged molecules (including neurotransmitters) to cerebral organoids. I was responsible for adapting the ion pump, developed in Dr. Marco Rolandi’s lab at UCSC, for use with human cortical organoids (grown from stem cells and differentiated into 3D models of the human brain) by Dr. Mohammed Mostajo-Radjii’s group at UCSC. Dr Rolandi’s ion pump device can deliver a specific ion (for example K+ or GABA) from a source electrolyte to a target electrolyte by applying a voltage.

Dr. Marco Rolandi’s lab uses two devices: an in vitro device for use with cells and an in vivo device for use with mice. I first tried to modify the in vitro device by altering the insert of the device that cells normally grow on to be compatible with 3D organoid models. This also involved modifying a clamp to enable the organoid to sit flush against the device. While these alterations were successful, the in vitro devices began falling apart and delaminating during both my experiments and experiments run by other researchers in the Rolandi Group. This led to me transitioning to using the in vivo device.

Integrating with the in vivo device involved creating an adapter to allow the device to sit on top of a petri dish where the organoid was being grown. Several rigid in vivo adapters were developed and although these adapters successfully allowed the device to make contact with an organoid in media, ion delivery was not able to be verified. Future projects may lean towards using the soft adapters used during organoid characterization to minimize rust and short circuiting (two issues ran into while testing the rigid setups).
I laid the groundwork for characterizing the impact of K+ on a cerebral organoid by using a pipette to add KCl to the media of the organoid during calcium imaging. A resulting increase in electrophysiological activity was achieved, however further characterization tests are necessary to verify that this increase was the result of the increased K+ present.

Progress was also made on characterizing the amount of K+ delivered by the device on a given run, however the *in vivo* ion pump devices are finicky and further devices must be fabricated and tested to verify these results.

With the aforementioned further characterization, seamless integration of the *in vivo* ion pump device with a cerebral organoid is possible. Following this, future avenues of exploration include integration with a multielectrode array (MEA). This would allow for the possibility of longterm studies, as the electrophysiology of the organoid could be measured from inside an incubator.

Further research would also be useful in expanding the variety of molecules the device can deliver to include a wider range of neurotransmitters that would specifically be of interest in cerebral organoids modeling the human cortex. Using the ion pump to deliver these molecules to a cerebral organoid would give real-time results, giving insight into what is happening in the human brain.

In addition, the *in vivo* device has the potential to deliver 3 different ions or molecules independent of each other. Once again, using the ion pump with an electrophysiological characterization technique such as calcium imaging or MEAs could help model the more complex nature inside the brain where multiple ions and molecules are present at once.
Finally, research into creating a computer program to run closed-loop control of an ion-pump organoid, MEA integration could allow for longterm, handsfree research projects studying the interactions between cerebral organoids and various ions and neurotransmitters.

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