Fabrication and Optimization of µECoG Electrodes and µfluidic Depth Electrode Delivery Devices

NSF Summer Undergraduate Fellowship in Sensor Technologies
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ABSTRACT

A third of all epilepsy patients who experience continuous seizures are treated with surgery to remove epileptogenic brain tissue. Currently, surgeons determine resectable tissue using electrocorticography (ECoG), a technique that detects action potentials or electrical differences within neurons in the brain. Current ECoG electrodes are not flexible enough to conform to the surface of the brain, while depth electrodes cause permanent damage to the brain. Additionally, the resolution of these sensors is not accurate enough to precisely locate the epileptogenic areas increasing the potential to leave behind epileptic tissue or to extend the resection to healthy tissue. In this project, we want to develop and optimize a microarray of sensors made of materials that are flexible enough, and sensitive enough, to increase temporal and spatial resolution. Additionally, a depth electrode delivery device will be developed to mitigate the damage done to neuronal tissue. The sensors are optimized to be used in rat models, which will determine the feasibility and safety of the device for clinical applications.

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1. INTRODUCTION

Epilepsy is a serious medical condition characterized by a sequence of uncontrollable seizures originating from abnormal neuronal activity. One-third of epilepsy patients not treatable with drugs may rely on surgical treatment as their only therapeutic option [1]. When a patient is being evaluated, physicians analyze their neuronal activity using a technique called ECoG. ECoG is an intracranial type of electroencephalography (EEG) that measures electrical impulses from neurons, and displays epileptic signal as high amplitude, high frequency oscillations [2]. ECoG tells neurosurgeons where to resect epileptic brain tissue. Due to lack of resolution, surgeons tend to be more conservative removing only the brain tissue that is necessary. As a result, there is a possibility that epilepsy tissue is not completely removed, which may cause the patient to have post-surgical seizures.

Current electrode sensor arrays are used to determine epilepsy regions of the brain, however there are two main limitations with these devices. The first arises from the material used in the array itself, where it is not flexible enough to completely conform to the irregular shape of the brain. Therefore, neurosurgeons are unable to access more than 60% of the cortical surface hidden in sulci and fissures, which can lead to missing recordings from crucial areas. Additionally, current arrays are not sensitive enough to accurately pinpoint the specific epileptogenic area. It is believed that sensor electrodes can be developed at a microscopic level to improve current technologies used to pinpoint the epilepsy regions in the brain [3].

2. MATERIALS AND METHODS:

2.1 µECoG Fabrication:

A µECoG device was made to be used in brain data recording of *in vivo* models. To develop such a device, one must stack several layers of materials (parylene, gold, photoresist, and aluminum) and then lift-off or etch specific areas to precision. Most of these processes take place in a clean room or instrument room, which implies that little-to-no particles are contaminating the samples and devices under fabrication. A step-by-step process as well as the materials necessary to complete fabrication are described in the following section.

2.1.1 Parylene Coating

Fabrication of the devices begins with the application of a 4-µm layer of parylene, a polymer that serves as an insulator preventing fluids from short-circuiting the closely-spaced gold channels, on a three inch wafer shown in Figure 2. The SCS PDS2010 Parylene

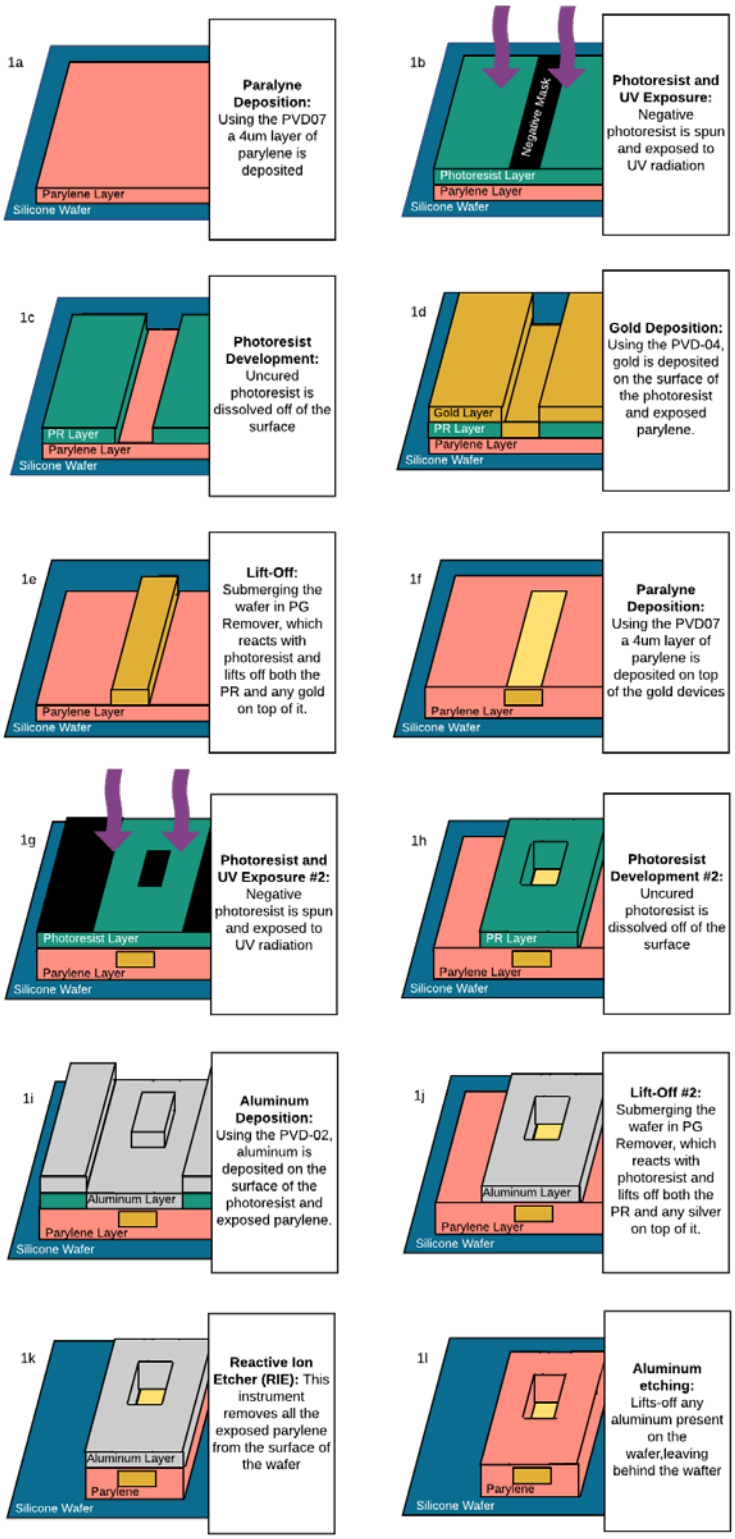

Figure 1*:a) Parylene Deposition, b) PR and UV Exposure, c) PR Development, d) Gold Deposition, e) Lift-Off, f) Parylene Deposition #2, g) PR and UV Exposure #2, h) PR Development #2, i) Aluminum Deposition, j) Lift-Off #2, k) RIE, l) Aluminum Etching*

Deposition (PVD-07) instrument is used to deposit an evenly distributed layer of parylene C onto the wafer surface. It does this by evaporating the parylene at high temperature and low pressure, and depositing it over the deposition chamber using cold temperatures. This step can be observed in Figure 1a.

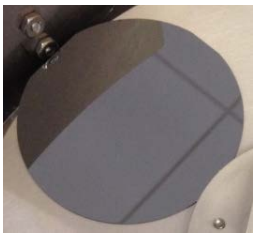

Figure 2: *Plain wafer to be deposited with parylene*

2.1.2 Photoresist and Mask Alignment

The wafer is then placed in a negative resist spinner and coated with a negative photoresist. A photoresist is a polymer that changes properties once it is cured by UV radiation exposure. A negative photoresist is one where areas exposed to UV rays will harden and cure, and anything that is not exposed will remain liquid and uncured, and thus can be washed off. Washing off the uncured areas makes it easier to develop microscopic patterns where the gold device will be formed. Once the wafer is coated with photoresist, the wafer is placed underneath a mask, and inside the MA-01 (SUSS MicroTec MA6/BA6 GEN-3 Mask Aligner). Using this instrument, one will be able to expose the material for a short amount of time to 80 mJ/cm^2 ultraviolet radiation to cure only specific portions of the photoresist. This process is shown in Figure 1b. The wafer is then removed from the MA-01, baked and developed (Figure 1c), before a metallization step. The result is shown in Figure 3.

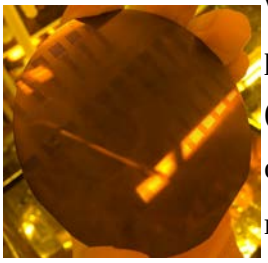

Figure 3: *Photoresist (PR) covered wafer with Mask Alignment pattern and PR development*

2.1.3 Gold Deposition and Following Steps

After photolithography, the wafer was placed in the chamber of the PVD-04 E-beam Gold deposition instrument, where 100 nm of gold is evaporated onto the surface of the wafer. Once the wafer has been covered in gold, it is placed in a bath of PG Remover, a stripper solvent, which uses specific chemicals to dissolve any photoresist, thus removing any gold that was adhered to the dissolved photoresist. The gold forming the μECoG device is preserved because it is adhered directly to the insoluble parylene that was exposed during the previous step of photoresist development. The PG Remover attacks the photoresist and lifts-off the gold above it. This leaves only the gold that is directly in contact to the parylene surface as shown in Figure 4. (Figures 1d & 1e)

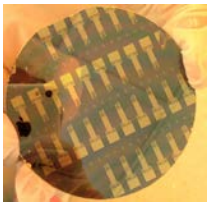

Figure 4: *Wafer after gold deposition and PR lift-off*

Once the gold has been deposited onto the surface of the wafer, parylene deposition (Figure 1f) and photoresist application are repeated. For the second mask alignment, a secondary mask is placed in the instrument and has to be aligned to the devices already on the wafer. The secondary mask has features that will allow exposure of the electrodes to the environment (Figure 5).

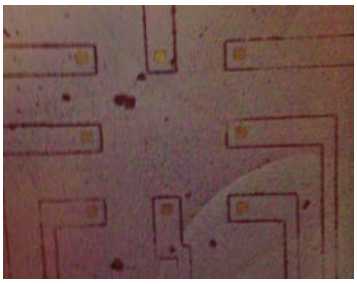

***Figure 5**: Microscopic design of the ECoG micro devices showing perfect alignment of the contact pads post second mask alignment*

Because both the devices and the new features on the mask are microscopic, it can be time-consuming to ensure that the old features are in the right location compared to the new features (Figures 1g & 1h).

2.1.4 Aluminum Deposition & Reactive Ion Etching (RIE)

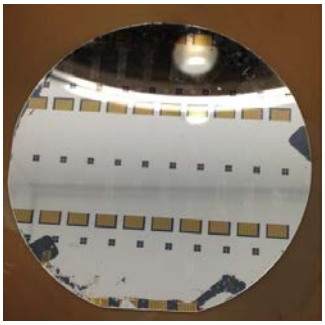

***Figure 7:** Wafer covered in aluminum, post lift-off, exposing contact pads for RIE*

Once the second photolithography step has been completed, aluminum (Al) is deposited (Figure 1i) and partially lifted-off (Figure 6). The purpose of the aluminum layer is to cover any area on the surface of the electrode that cannot be de-insulated. To explain more carefully, only specific portions of the μECoG electrodes should be exposed for proper function. In order to expose these portions, the parylene that was placed on top of the gold electrodes needs to be removed. This can be done with an instrument called a reactive ion etcher. This instrument creates a reactive plasma that etches any parylene that is exposed to the surface. However, any parylene that lies underneath the deposited aluminum metal remains on the surface (For raw image refer to Figure 6 and for step process refer to Figure 1k). Once any unwanted parylene has been removed, the wafer is taken to an acid bay to perform an acid bath. The acid bath etches off all aluminum still on the surface of the wafer. The result is a layer of gold sandwiched between two layers of parylene that function as insulators, with a small area of metal exposed to the

***Figure 8:** Wafer being exposed to plasma to perform parylene etching*

outside environment to be able to record data from the tissue and deliver it to a computer (Figure 1l) [4].

2.1.5 Next Steps

Once the devices have been fabricated, they can be floated out of water by simply submerging the wafer underwater. The final microelectrodes are shown in Figure 8. The device will be connected to an extension ACF tape and the gold contacts will then be used to record data from a rat model and deliver information to a computer for further analysis.

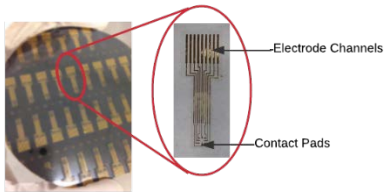

Figure 6: *Wafer with final ECoG microelectrodes*

3. EXPERIMENTAL RESULTS

3.1 Impedance Testing:

The electrodes were first tested *in vitro*. An electrode with an impedance of the range of kΩ is considered to be working properly. A range of AC voltages were applied up to 10mV RMS, and the current was measured to calculate the impedance. From the manufactured device, it was found that only two electrodes worked properly. The impedance values are shown in Figure 9 graphed to the range of frequencies. The phase of the respective channels is also shown in Figure 10. Channel 4 appeared to behave closer to what was expected although both channels had relatively good data.

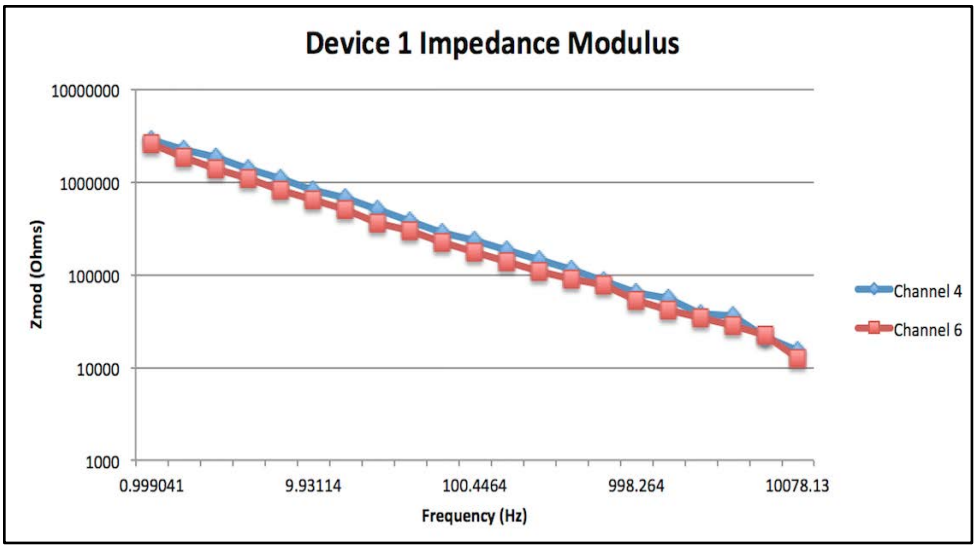

Figure 7: *Impedance Modulus of Channel 4 and 6*

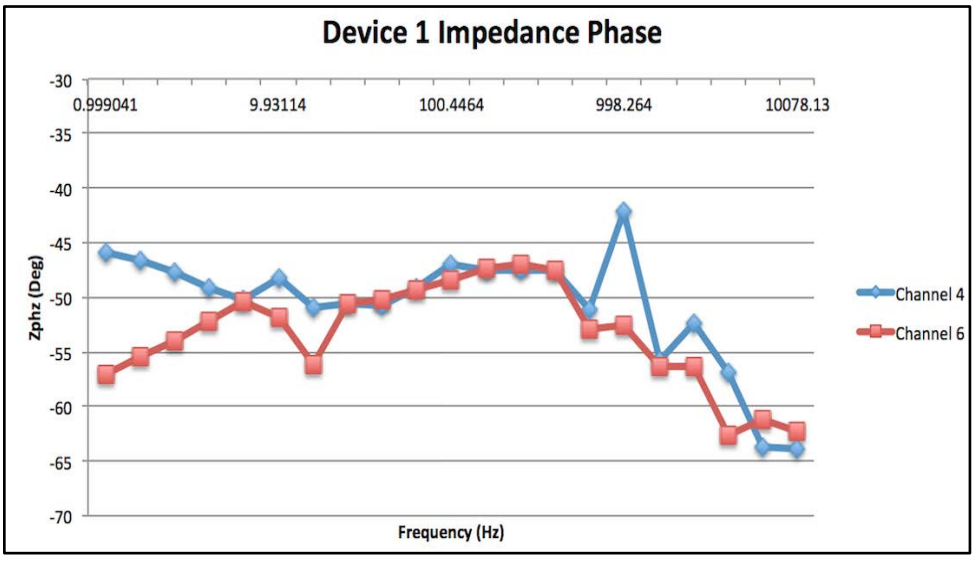

Figure 8: *Impedance Phase of Channels 4 and 6*

Another important test performed is stability testing. The environment of the brain is surrounded by blood, cerebrospinal fluid, interstitial fluid, etc. This means that if fluid leaks between the parylene layers, it could short-circuit the device, preventing it from working properly. For this reason, the team does a stability test where the sensors are left in saline for a prolonged period of time and impedance is measured after certain time points. As seen in Figure 11 the impedance of the device was 350kΩ after one hour, it decreased to around 320kΩ after three hours, however, it had increased to 400kΩ after an overnight soak. This test should indicate that over time the functionality of the electrodes decrease, although at an unknown rate, but the impedance appears

that the impedance increased after an overnight soak when it should have done the opposite. The source of this error is attributed to evaporation of the water leaving behind ionic components and thus increasing the impedance of the electrode.

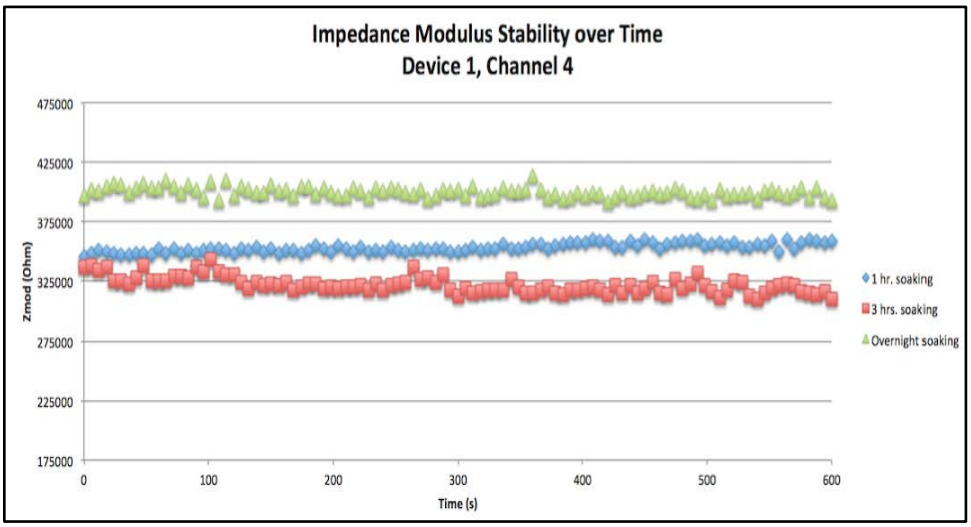

Figure 9: *Impedance Modulus Stability over Time at Different Timepoints*

3.2 *In vivo* Testing

Out of the first batch of electrodes, only one electrode could successfully be tested in an animal model. This electrode had an impedance of 370 kΩ. A craniotomy on a rat was performed, and a data recording procedure followed. An image of the surgical animal is shown in Figure 12. Successful data was collected in Figure 13, indicating that while the rat was asleep, delta waves could be recorded. The main purpose of the project was to be able to show a proof of concept in the fabrication and usage of μECoG sensor electrodes, which was proven with the collection of neuronal brain signals.

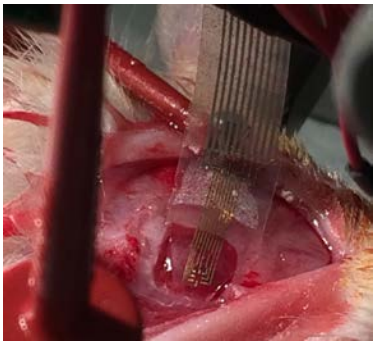

***Figure 10**: μECoG device on the surface of an animal model for in vivo testing*

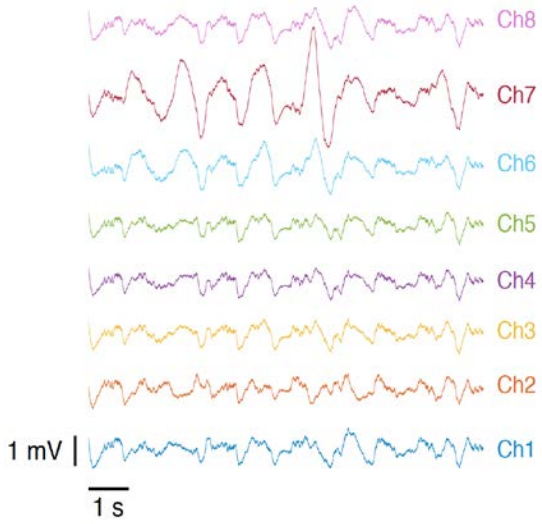

***Figure 11:** Collected Data from Animal Model showing Feasibility of the μECoG Device*

4. DISCUSSION:

Current ECoG devices, while functional, lack appropriate resolution and biocompatibility. Surgeons may not have enough resolution information of the brain activity to dissect all of the necessary tissue to cure some patients from epilepsy. Also, poor biocompatibility leads to the possibility of inflammation. The purpose of this project was to develop a set of electrodes that could record data from a much smaller area to increase resolution and to improve biocompatibility and flexibility. After performing the *in vivo* testing it was found that the electrodes are fully functional and can record data from an animal model. Biocompatibility and

flexibility of the device was ensured due to the use of parylene. In general, the fabrication of μECoG is relatively complicated and has not been optimized to its full potential, however over time, the fabrication process should be optimized.

MICROFLUIDICS

5. INTRODUCTION

Epileptic tissue may not only be located on the surface of the brain, but can also be found deep into the grey matter of the brain. Current medical procedures include the insertion of depth electrodes into the brain to record data from several locations in the thickness of the brain. This allows neurologists to know how deep the epileptic tissue is located in case extraction of the tissue is an option. Additionally, depth electrodes can be used to record data for extended periods of time post-surgically. However, the material currently used to fabricate depth electrodes causes harm and leads to scarring. Scarring of the brain prevents neural connections from working properly and reduces the signal recording by the electrodes over time.

The lab will attempt to develop a new set of depth electrodes to reduce damage to the brain, thus reducing scarring. Instead of using metals, the team considered using softer materials as their detection media. Softer materials are relatively flexible, which allows for a smoother entrance into the tissue without causing significant damage. However, because it is quite flexible and the wires are small in diameter, they are prone to bend upon contact with tissue.

A device delivery mechanism would then have to be developed to insert the wire into the brain. Using Navier's Stokes theorem, it was determined that the fluidity and viscosity of a fluid can be used to generate enough force to embed the wire into the tissue without. The approach involves the development of Polydimethylsiloxane (PDMS) microfluidic devices containing strategically placed channels. The idea is to create flow and control channels by bonding two layers of PDMS together. A control layer would cause a block of fluid in the flow layer, thus increasing the pressure of the fluid within the flow channel. Once the air is released the control channel valve is opened and the fluid rushes along with the wire into the tissue. Additionally, if desired air can be reintroduced to close the valve and pinch the wire, thus controlling the depth electrode itself.

6. MATERIALS AND METHODS

Fabricating a PDMS-PDMS layer device is relatively easy. One must first make a mold for each one of the layers. This was done using a 3” wafer and spinning SU08 photoresist. Similar to the process performed in μECoG mask alignment, the device-molds were made by photolithography onto the wafer surface, which were used to make channels on PDMS layers.

Manufacturing of a batch of microelectrodes begins with the mixture of PDMS and curing agent. One mixed two cups of 15g of PDMS and 1.5g of curing agent in a cup to create a 10:1 ratio and stirred to ensure even distribution of curing agent. The cups must go into a desiccator to remove all of the bubbles that originated in the mixing process. Once the bubbles were eliminated, one cup was poured onto an aluminum boat with a wafer. The other PDMS mixture was spun on a silicon wafer at 1000 RPM, 100 RPM/s for 60 seconds. Both layers were placed on a hot plate for a designated time. Temperatures and time were studied to determine the best parameters that would enhance the bonding between the two layers. Once both layers were partially cured, each flow channel is cut and carefully placed on top of its corresponding control channel. The new device is now baked overnight at 80 ℃ for full curing and bonding. Holes can then be made onto the PDMS devices to open the inputs and outputs of the channels. Lastly, the devices are glued to a glass slide using a oxygen plasma, which completes the control layer and allows easier handling for testing. In Figure 15, one may see different PDMS microfluidic devices.

Figure 125: *Example macro/microscopic images of PDMS devices*

The devices are then tested to ensure that there is correct flow of the fluid through the channels. The next set of tests involves using the device to attempt to deliver the wire into the rat’s brain. At this moment in time, the biggest priority of the testing involves ensuring that the wire is successfully embedded into the brain.

7. EXPERIMENTAL RESULTS:

Three experiments were performed to test what are the optimal conditions for PDMS - PDMS layer bonding. The first experiment involved the use of oxygen plasma bonding to create strong silicon bonds between the two PDMS layers since it works with PDMS and glass bonding. The plasma bonding did not work as expected, and thus one innovated another possible approach to optimize bonding between the layers by exploring the curing level of the layers. The less cured the PDMS, the better a bond it will create, but the harder it is to handle. The second experiment focused on changing the partial curing temperatures and times in which partial curing to find the transition point of the flow layer and best parameters. A transition point is considered to be the point where the PDMS is as gooey as possible, but is still manageable. Finally, the third experiment involved testing different times for the control layer's partial curing, to find the transition point.

The nature of the project is to be able to bond two layers of PDMS to have two levels of channels interacting with one another, while being separated by a thin layer of PDMS. As seen before, plasma bonding works to bond PDMS to glass as the oxygen causes silicon to expose hydroxyl groups and become available for bonding. Therefore, the theory suggested that since PDMS has silicon, it should be able to bond to itself using this technique. It appears that regardless of the parameters, plasma bonding did to bond the two layers of PDMS.

The second set of experiments focused on understanding the transition period of the flow layer and determining if the bonding of the two layers will change depending on the curing parameters selected. The samples were prepared and partially cured at the temperatures and times indicated by the respective columns in Figure 15. The predicted result/description is indicated by "Description" column. The first result is indicated in the "Did it cure?" column. A flow layer that is not partially cured cannot be handled for bonding to the control layer, thus those parameters are not feasible for the PDMS devices. Only five set of parameters shown in Figure 16 exhibited sufficient curing to be handled. However, after attaching the flow layer devices to the control layer, there was no satisfactory bonding. We made a map of the flow layer parameters which partially cured properly, which ones did not and the expected result of untested parameters. This gives us a rough estimate of the transition values for the flow layer of PDMS.

PDMS Bonding Tests				
Description	**Partial Cure Temp (℃.)**	**Partial Cure Time (min)**	**Did it Cure?**	**How many devices, post-bake, bonded successfully?**
Control	80	30	Partially	2/4
80 Max	80	40	Yes	0/5
80 Min	80	20	Yes	1/5
Expected Fail	50	10	No	N/A
50 Min	50	20	No	N/A
Paper Test (50)	50	30	No	N/A
50 Max	50	40	No	N/A
Temp 1	40	30	No	N/A
Temp 2	60	30	No	N/A
Temp 3	70	30	Yes	0/5
Temp 4	90	30	Yes	0/6

Table 15: *PDMS Flow Transition Tests*

Partial Cure Temperature (℃.)						
90						Pass: Partially Cured, Data available
80						Extrapolation to Pass
80						Might Pass, Uncertain
70						
60						Failed: Uncured, Data available
50						Extrapolation to Fail
40						Might Fail, Uncertain
	10	20	30	40		
	Partial Cure Time (mins)					

Table 16: *PDMS Flow Transition Extrapolation and Assumptions*

The third experiment, which has not been completed, attempted to look for the transition time where the control layer will go from uncured to cured. The first attempt to this experiment tested different times at 80 ℃. However, it appears that all the tested times shown in Figure 1 had a cured control layer, thus one was not able to find the transition time at 80 ℃. The experiment

was then extended to test the control layer every 30 seconds by doing a wafer touch test. In this test one was looking to find the time where the PDMS was too uncured, just right and too cured. The results are shown in Figure 18 where the ideal time is between 5 and 7 minutes.

PDMS Bonding Tests: Control Layer at 80 ℃			
Description	**Partial Cure Time (min)**	**Did it Cure?**	**How many devices, post-bake bonded successfully?**
Control	30	Yes	0/3
	27	Yes	2/3
	20	Yes	0/3
	15	Yes	0/3
Expected Fail	10	Yes	0/3

Table 17: *PDMS Control Transition Tests*

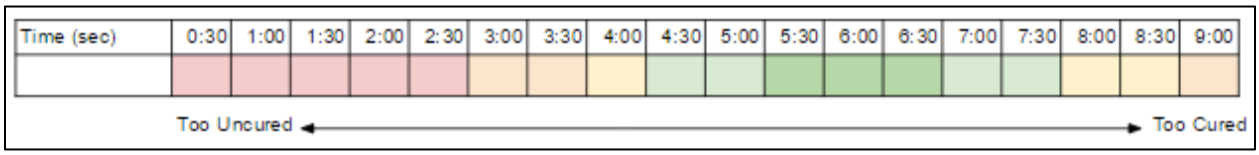

Table 18: *PDMS Control Layer Curing over Time Transition Test*

After finding the transition time of the control layer, the bonding of the two layers was again tested. A set of six experiments were performed using two optimal flow layer time and temperatures along with six set of parameters (temp: 70 ℃ or 80 ℃ time 5, 6, 7 minutes) for the control layer. While almost all of the experiments appeared to work, the best results were attributed with to the two experiments whose control layers baked for 6 minutes. These experiments were repeated with actual devices and the recommendation was selected to be control layer: 80 ℃ for 6 minutes and flow layer: 80 ℃ for 25 minutes for optimal bonding of the devices.

Sample	Control Temp (°C)	Control Time (min)	Flow Temp (°C)	Flow Time (min)
A	80	5	80	25
B	80	6	80	25
C	80	7	80	25
D	70	5	70	30
E	70	6	70	30
F	70	7	70	30

Table 19: *Final Tests and the selection of the two best results (B & E) for further experimentation*

8. DISCUSSION

Current depth electrodes, while functional, cause serious scarring in the brain due to their stiffness. For this reason, there is significant effort to device a softer depth electrode, which would require a delivery device. In this set of experiments, one attempted to develop a PDMS delivery device that can be controlled by a valve technology. The biggest challenge was the bonding of the two PDMS layers in order to ensure full functionality of the device. It was determined that the best set of parameters for the layers is 80 ℃ at 6 minutes for the control layer and 25 minutes for the flow layer. Testing of the PDMS devices is still needed to ensure that they can deliver the flexible wire into an animal model.

9. ACKNOWLEDGEMENTS

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