FLEXIBLE ELECTRODE FOR ELECTROCOLONOGRAM MONITORING AND STIMULATION IN A MOUSE MODEL

An Undergraduate Research Scholars Thesis

by

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TABLE OF CONTENTS

Page

ABSTRACT

Flexible Electrode for Electrocolonogram Monitoring and Stimulation in a Mouse Model

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We have created a fabrication method for a flexible electrode for electrocolonogram monitoring and bowel stimulation on a mouse model. Specifically, the electrode can be used as a part of a system to monitor and/or stimulate the serosal surface of the colon in the caudal direction. This is done in order to monitor or induce the peristaltic movement that occurs down the bowel. The electrode has been designed to be flexible to handle the changing diameters of a mouse's bowel as well as fabricated to be thin and small enough to avoid any disruptions or problems to the mouse's health after implant. Physical validation of the electrode has proved that it is small and thin enough for mouse implant. Further validation of the electrode still needs to be done to ensure that the electrode can face the environmental conditions inside of a mouse model. The fabrication method utilized to create this electrode was demonstrated to be an effective method in creating thin and small Ecoflex electrodes for mouse implant. This electrode can be used in the future as a part of a closed loop bowel monitoring and stimulation system for a conscious mouse.

CHAPTER I

INTRODUCTION

The bowel plays an important role in nutrient absorption and fecal excrement. It consists of the large intestine, the small intestine, and the rectum. It is necessary for these organs to function correctly in order to get rid of solid wastes from the body. The bowel rids solid waste from the body by using mass peristalsis, which is a wave like contractual movement that pushes the waste down the bowel. Neurogenic bowel dysfunction, or NBD, is the term given for having nerve damage on the connections between the spinal cord and the bowel. NBD often occurs as a result of a spinal cord injury or multiple sclerosis. These injuries or diseases often cause nerve damage between the spinal cord and bowel, resulting in decreased bowel movement and bowel strength to push solid waste out of the body. This reduced intestinal movement can cause anything form fecal incontinence to constipation, severely reducing one's quality of life. Cures to NBD often involve taking laxatives or doing physical therapy to help the intestines to push out the waste, but these solutions do not bring back the natural strength of the bowel.

Recently, another cure has been researched involving electrical stimulation of the bowel to facilitate movement. There are several ways that this is being attempted. One common method is to stimulate the bowel along the intestine in a wave like manner in order to replicate the intestines' natural peristaltic movement. In this case, the electrode is aligned along the intestine such that stimulation can be done in the wave like manner. This has been shown to work in multiple occasions for different animals.

Another method of electrical stimulation to activate bowel movement solely relies on a unique condition of intestinal muscles. The intestine is made up of "adjacent rings of smooth muscles” that react to each other in a way such that if a nearby neighboring ring contracts, the following ring down the intestine will also contract. This occurrence causes the whole bowel to activate its wave like contraction with the stimulation of just the beginning end of the intestines, which is what the body naturally does in order to induce the peristaltic movement in the bowel. This occurs with just the nerve and muscle connections within the intestine itself, so NBD does not affect the intestines’ ability to do this. Using this unique ability of the bowel, this other method places an electrode at the upper section of the intestines, stimulating the intestine at one location and inducing further contractions down the intestine. The electrode in this case circles around the intestine instead of being placed along the colon in order to effectively stimulate a single location along the bowel. This method of electrical stimulation has only worked on an unconscious mouse model and needs further research for replication of natural bowel movements and validation on a conscious animal [1].

Electrical stimulation along the intestine in the first method mentioned requires enough power to stimulate the colon in multiple locations, while the second method of electrical stimulation only needs to stimulate at one location. This means that the second method has the potential of using less power over time than the first, ultimately making it more viable as a long term implant. In this paper, we develop a flexible electrode for monitoring and stimulating a conscious mouse’s bowel. This electrode can be used as a part of a closed loop system that can activate a mouse’s bowel in a natural rhythm, which can be seen in Figure 1 below.

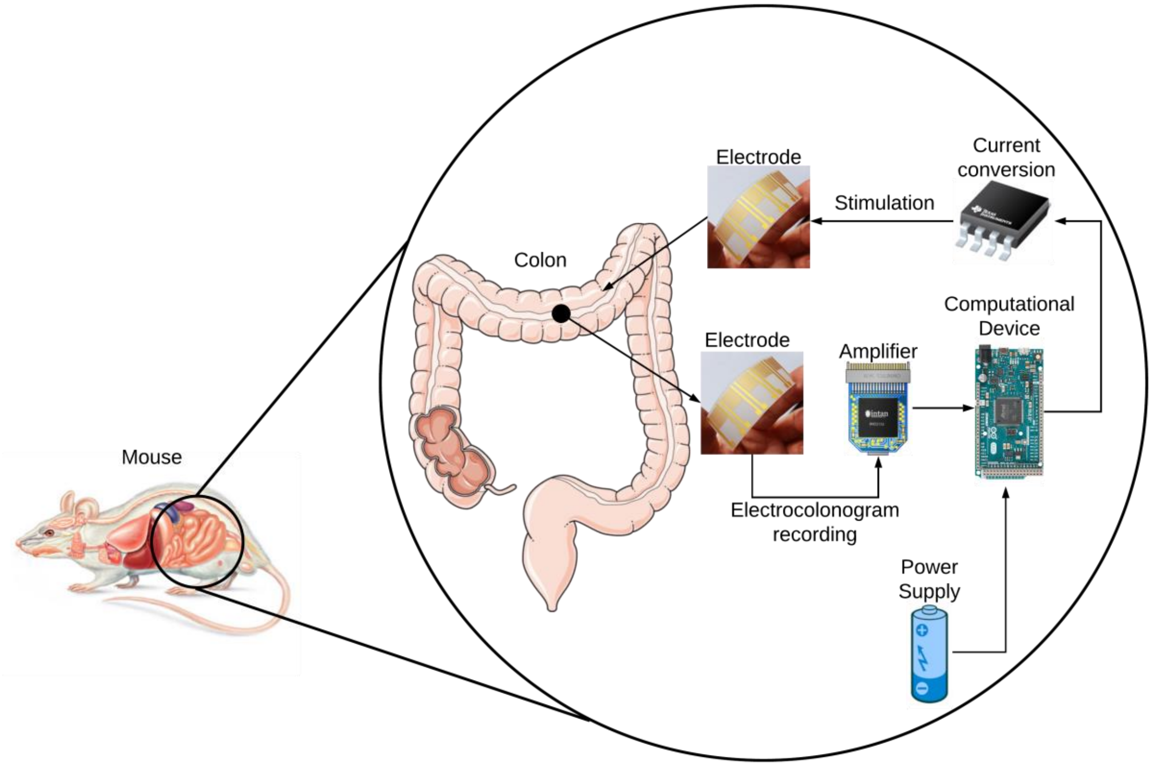


Figure 1. Diagram of the closed loop system. This paper focuses purely on the electrode part.

CHAPTER II

METHODS

Electrode Fabrication

Three attempts were done in to trying to fabricate these electrodes. In each of these attempts, the procedure was modified slightly, but the overall process was the same. The process can be seen in Figure 2 below.

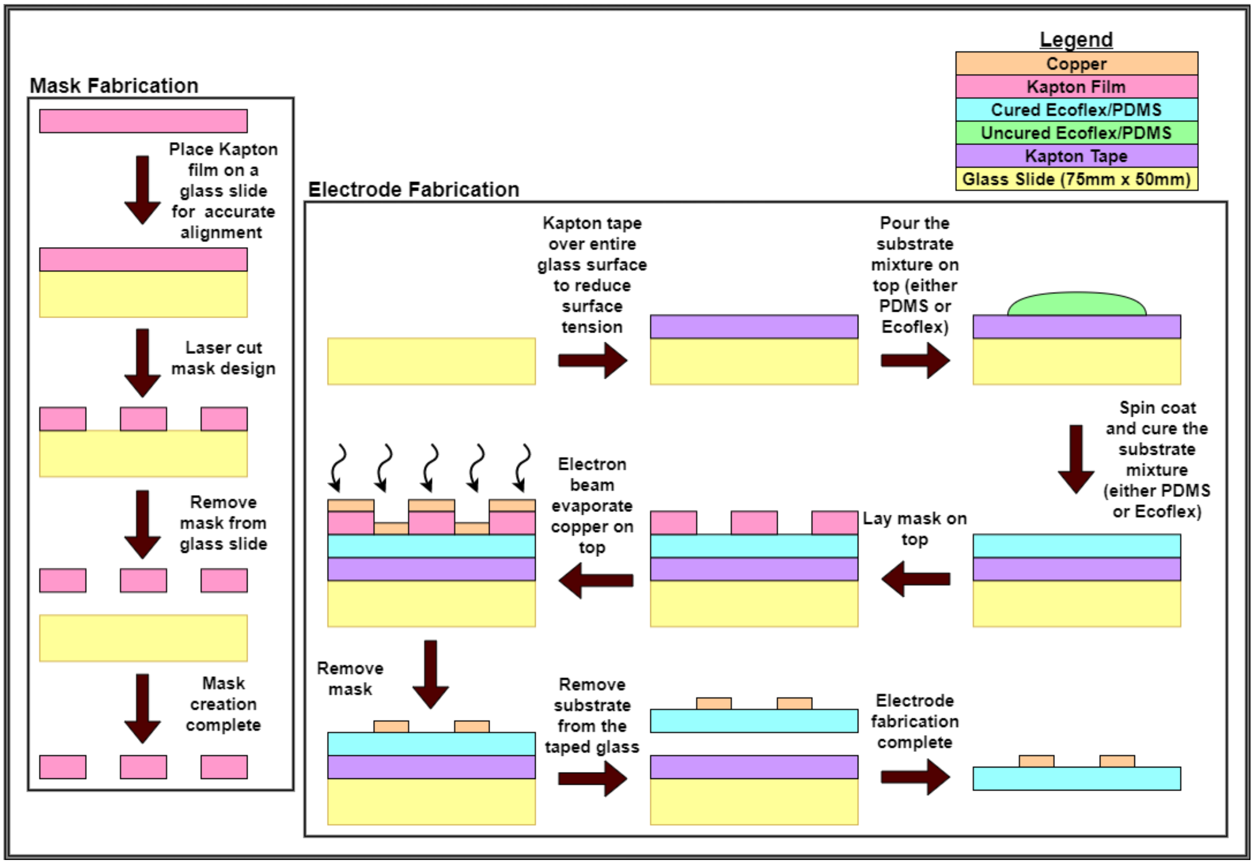


Figure 2. Process for making the mask and fabricating the electrode

First Attempt

First, two substrates were created. A silicone rubber called Ecoflex (Ecoflex 00-30, Smooth-On, USA) was used as the substrate material. The electrodes were created on a glass slide (75mm X 50mm). The following was not done in a clean room environment. Kapton tape was first laid across the entire glass slide surface of one side in order to reduce surface tension when the silicone rubber cures on its surface. Uncured Ecoflex mixture (1A:1B) was poured on top of the Kapton taped glass slide and spin coated for 30 seconds. One substrate was spin coated at 300rpm, and the other substrate was spin coated at 200rpm. The uncured substrate covered glass slide was then put aside in a petri dish to avoid any further contamination. To make the mask for the copper deposition, another glass slide was covered with Kapton tape. The wanted design was created in AutoCAD and printed on a laser cutter (PLS6.120D Laser Engraver, Universal Laser Systems Inc., USA), cutting the design into the tape. The plan was for the tape mask to be pulled off the glass slide and placed on top of the cured substrate, but due to the difficulty of pulling off the tape mask in one piece, this did not end up being the mask for the electrode. Once the substrate cured for at least 4 hours and the mask creation was attempted, the rest of the procedure was done in a cleanroom environment. The mask was replaced with Kapton tape being directly taped onto the substrate in the design wanted. This was done for the 300rpm spun substrate (the 200rpm spun substrate was saved for the next attempt). The masked substrate was then put in an electron beam evaporator (PVD 75, Kurt J. Lesker, USA) and copper was deposited at a rate of 1A/s for 250nm. After this, the mask was removed from the substrate, and the Ecoflex was carefully pulled off of the glass slide. The electrode fabrication is now complete and can be seen below in Figure 3.

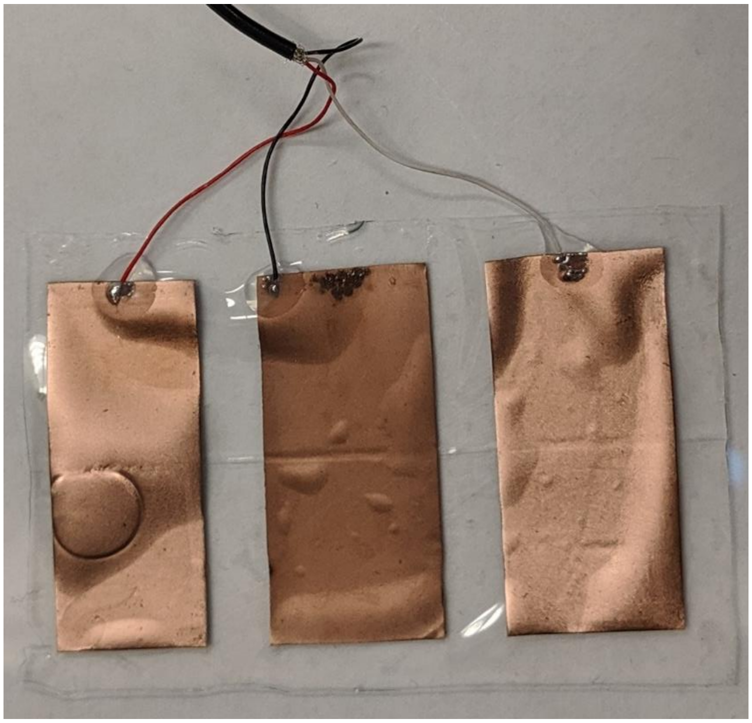


Figure 3. Completed Ecoflex electrode from the first attempt with wire connections

Second Attempt

Two substrates were first created, using a silicone rubber called PDMS. The following was done in a cleanroom environment. A 75mm X 50mm glass slide was cleaned with DI water and nitrogen gas and Kapton taped entirely on one side. The PDMS mixture was created using 10-parts base elastomer and 1-part curing agent. After mixing, the mixture was put in a desiccator for ~15 minutes to remove any air bubbles. The degassed mixture was then poured over the Kapton taped glass slide and spin coated for 30 seconds. One substrate was spin coated at around ~200rpm, and the other substrate was spin coated at around ~300rpm. The PDMS coated glass slides were then cured at ~100 degrees Celsius for at least 35 minutes. For mask fabrication, a Kapton film material, which does not have any adhesive, was used this time instead of Kapton tape. The film was cut to a size slightly larger than the dimensions of the glass slide and taped against the glass slide. The same AutoCAD design in attempt one was printed again on a laser cutter, cutting the mask design onto the film. The mask was then removed from its glass base and cleaned to remove any unwanted particles. After the PDMS substrates finished curing and the mask fabrication is completed, the mask was placed on top of the cured PDMS and taped down, being now ready for deposition. The 200rpm Ecoflex substrate created in attempt one was deposited on in attempt two. This electrode's mask was done out of Kapton tape, similar to attempt one, but the non-adhesive side was placed against the substrate such that removal of the mask from the substrate's surface is easier. The two PDMS electrodes and the one Ecoflex electrode was placed in the electron beam evaporator, and 420nm of copper were deposited at a rate of 0.8A/s. Once deposition was completed, the mask was taken off and the electrodes were taken off their glass slide. The second attempt at electrode fabrication is now complete and can be seen below in Figure 4.

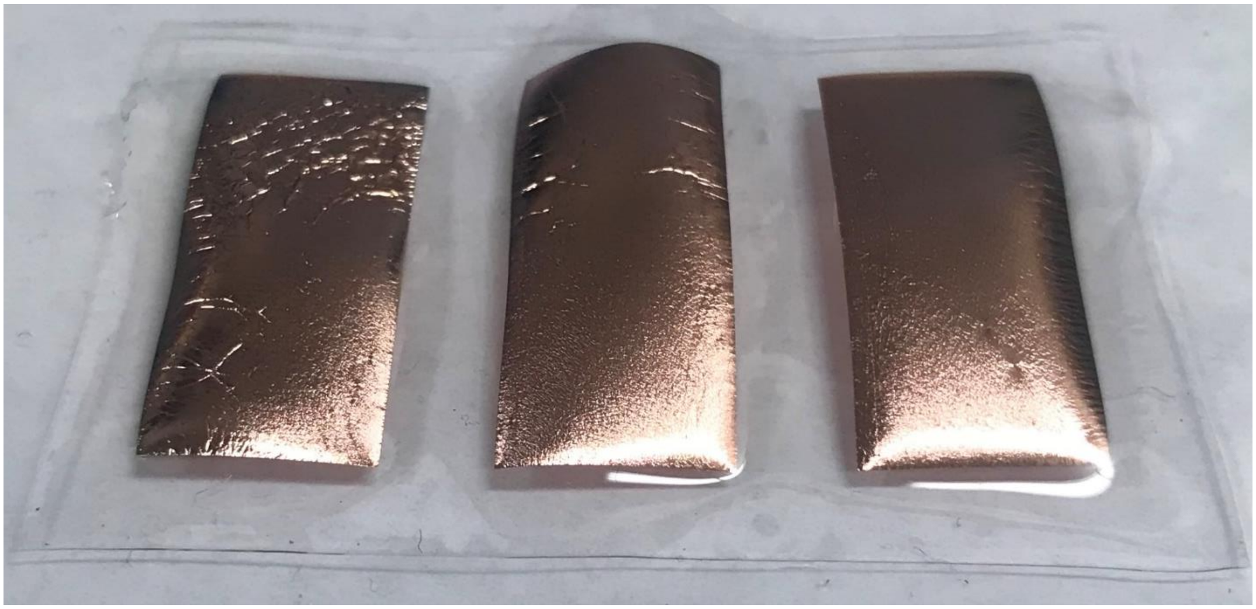


Figure 4. One of the completed PDMS electrode from the second attempt

Third Attempt

First, three Ecoflex substrates were created. The following procedure was done in a cleanroom environment. Similar to the second attempt, the glass slides were cleaned and Kapton taped. The uncured Ecoflex mixture (1A:1B) was poured onto the taped slides and spin coated for 30 seconds. Two different spin speeds were used for each of the substrates: 300rpm, 300rpm, and 725rpm. Mask fabrication was done the same way as in attempt two, using AutoCAD for making the design, laser cutter for cutting the design, and the Kapton film material. After the substrates had cured for at least 4 hours and the masks were fabricated, the masks were placed against the substrates and placed in the electron beam evaporator. 600nm of copper was deposited at a rate of 2A/s, with a 30 minute break in between after 300nm of deposition. Once deposition was complete, the samples were removed, the masks were taken off, and the electrodes were taken off the glass base. The result can be seen below in Figure 5.

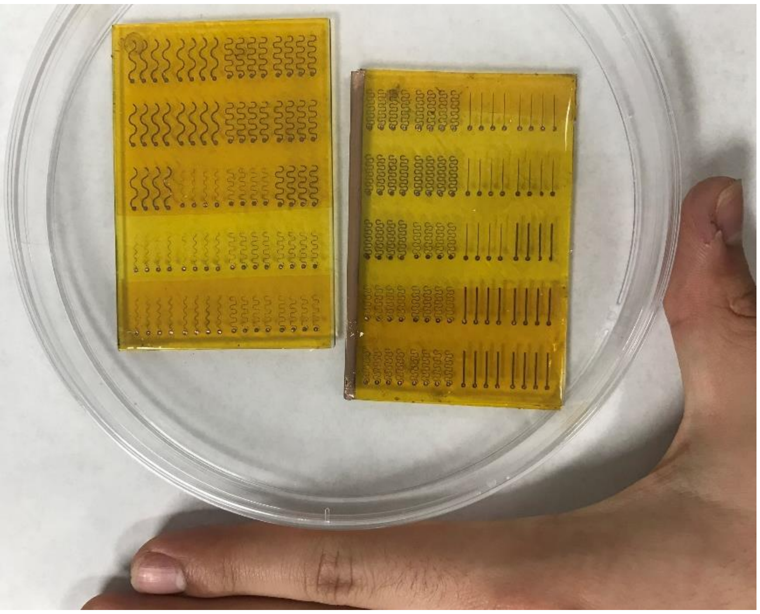


Figure 5. Two of the three sets of completed electrodes from the third attempt with size comparison to a human hand

Electrode Design

In the first and second attempt of electrode fabrication, the mask design was chosen for reading electromyography (EMG) signals, specifically from the bicep. This was done in order to verify if the method of fabrication used for the electrodes is valid for detecting biological signals. The AutoCAD design of this mask design is shown in Figure 6 below. To test the electrodes for EMG signal detection, the system in Figure 7 below was used.

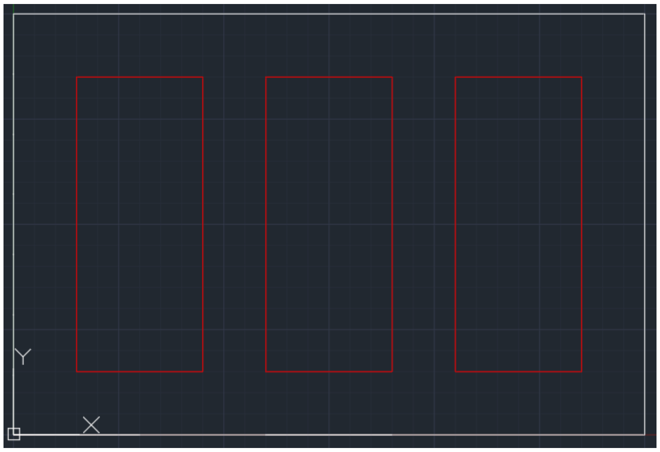


Figure 6. AutoCAD design for EMG electrode; dimensions of the rectangles are 15mm X 35mm.

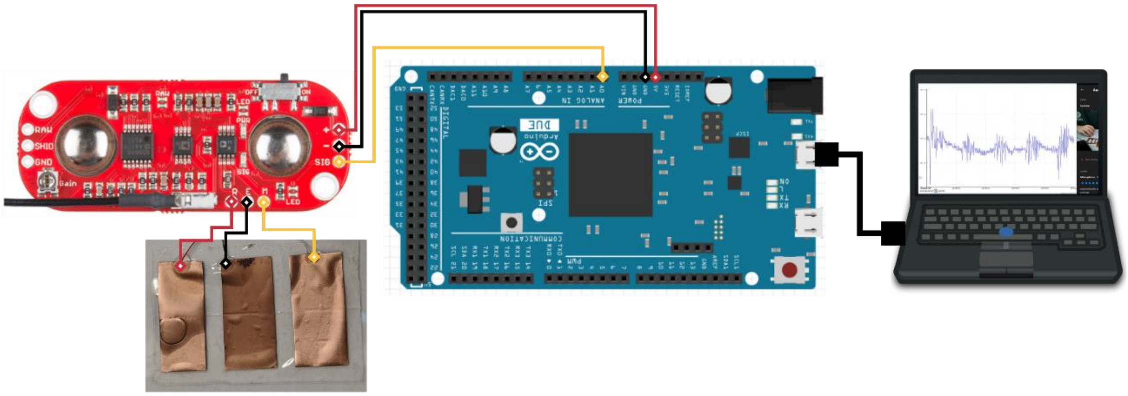


Figure 7. EMG signal electrode validation system; consists of the fabricated electrode (bottom left), MyoWare Muscle Sensor (top left), Arduino Due (center), and a computer (right)

The EMG signal electrode validation system was set to receive EMG data at the Arduino Due's default frequency of 960Hz and default resolution of 10 bits. The signal was set to display on Arduino's Serial Plotter in live time on the computer. The planned placement of the electrode for EMG signal detection was along the bicep of the right arm.

In the third attempt at electrode fabrication, the mask design was chosen in order to read electrocolonogram signals from a mouse model and/or stimulate the mouse's bowel. The general format used is shown in Figure 8 below. The circles on the right side of the design are there to help with soldering wire connections to the electrode. The dimensions of the entire electrode are 14mm X 10.6mm. The diameter of the circles are 1mm.

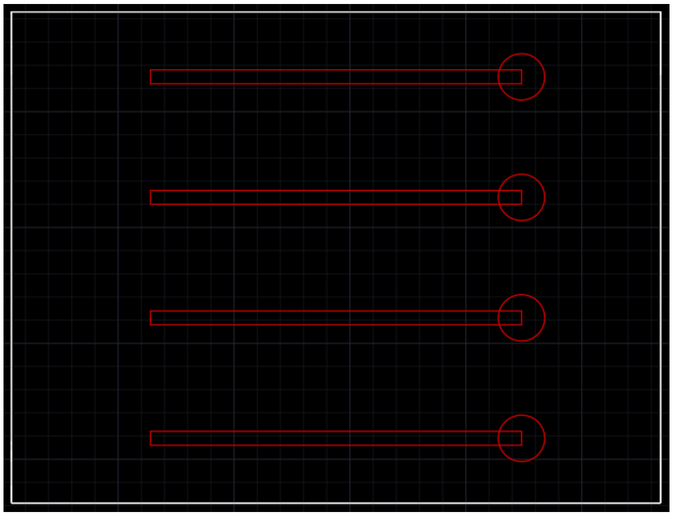


Figure 8. General format of the bowel electrode; dimensions of the trace are 8mm X 0.3mm.

In an attempt to possibly increase the flexibility of the electrode, the design in Figure 8 was modified with serpentine structures. These structures were designed according to the dimensions of a serpentine shown in Figure 9 below.

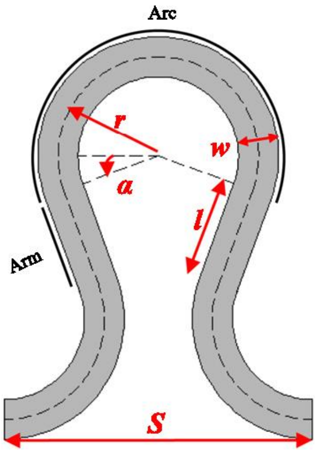


Figure 9. Parameters of serpentine structures. Values often used are w/r, l/r, and a [2]-[4].

Using Figure 9 as reference, three designs were created, each being the ideal serpentine for flexibility according to different papers. These designs are shown below in Figure 10, 11, and 12. Each electrode would have 4 of these structures, similar to the general design shown in Figure 8. Figure 10 has a length of 9.6318mm and width of 0.25mm, Figure 11 has a length of 8.8737mm and width of 0.125mm, and Figure 12 has a length of 8.7939mm and width of 0.1mm. Each of the designs also had a single lined version with the assumption that the width of the structure would be dependent on the highest resolution that the laser cutter can achieve, which is supposedly around 0.1mm. 8.4572mm, 8mm, and 7.9252mm were the corresponding lengths of the single lined designs for Figures 10, 11, and 12.

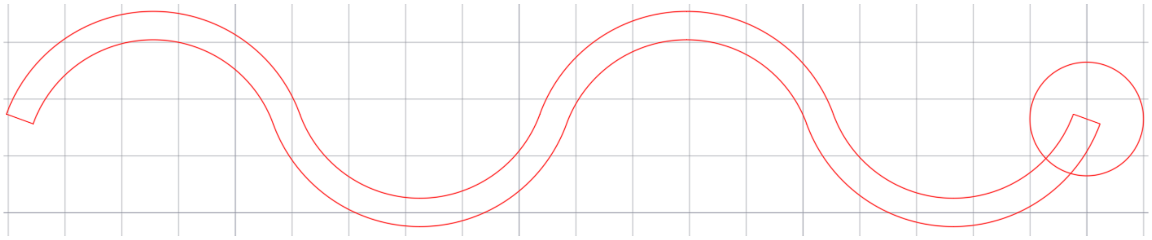


Figure 10. Best serpentine according to reference [2]: w/r = 0.2, l/r = 0, and a = -20 degrees.

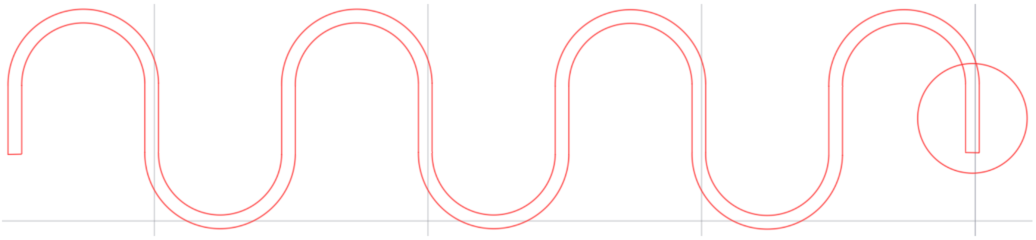


Figure 11. Best serpentine structure according to reference [4]: w/r = 5, l/r = 1, and a = 0 degrees.

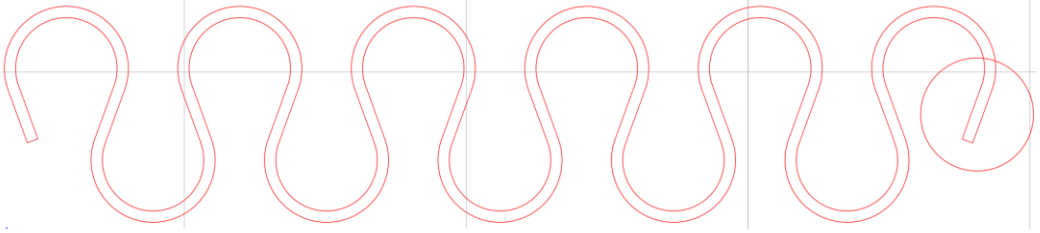


Figure 12. Best serpentine structure according to reference [3]: w/r = 0.2, l/r = 1, a = 20 degrees.

CHAPTER III

RESULTS

The substrates that were prepared in a non-cleanroom environment and used a Kapton tape mask contained deformities in the form of bubbling. This can be seen in Figure 13 below.

Figure 13. Indications of bubbling occurring in the electrodes prepared in attempt one.

In the left picture of Figure 13, the entire center electrode experienced bubbling, most likely being the cause of why that center electrode could not pass the multimeter continuity test when tested from opposite corners. Despite this, all the copper leads in the right picture passed the multimeter continuity test, even with their own excessive bubbling.

The PDMS electrodes fabricated in the second attempt inside a cleanroom environment contained a functional low resistance, but they did not have a strong bond between the copper and substrate, resulting in the copper flaking off of the PDMS substrate. This can be seen in Figure 14 below.

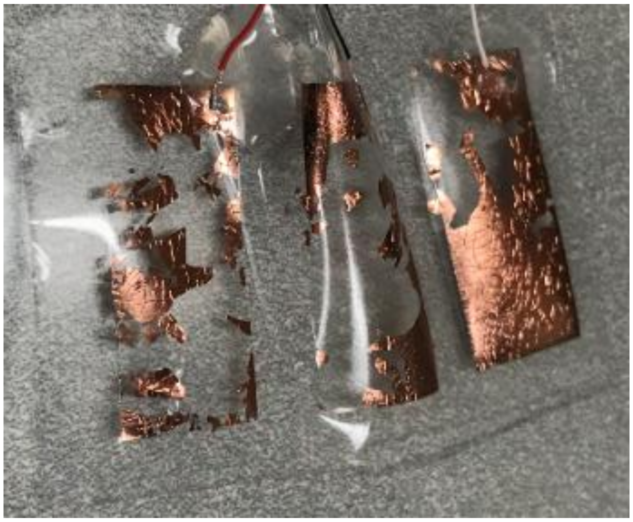


Figure 14. Copper flaking off of the PDMS substrate electrode.

The EMG signal testing system was connected with the electrodes from the second attempt, and a screen video recording was taken from the computer while a video recording of the EMG setup was running. The bicep was flexed at certain times throughout the recording. A screenshot of the results from the videos can be seen below in Figure 15.

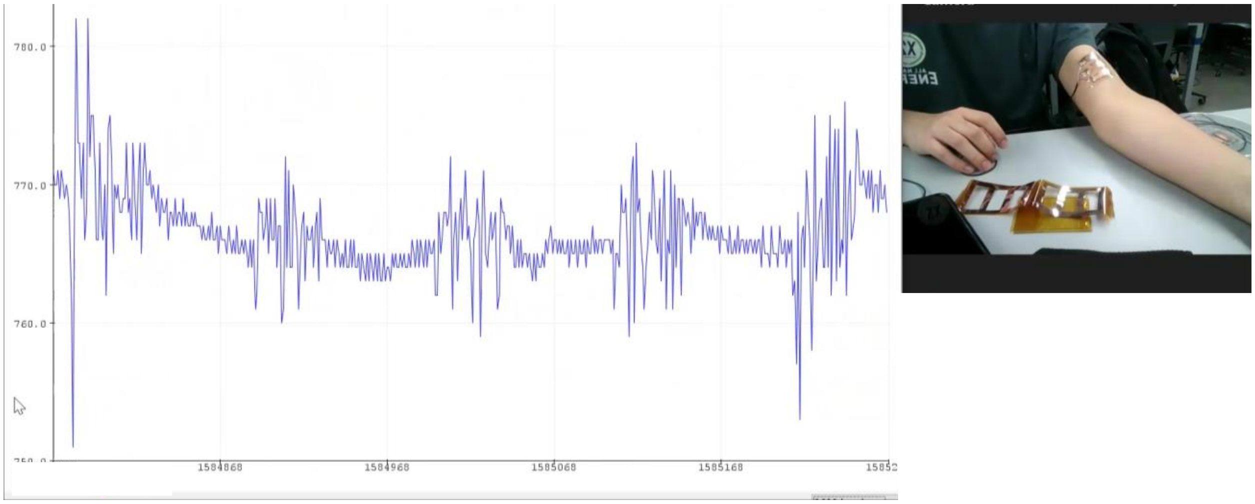


Figure 15. Raw EMG signals detected from the right arm’s bicep with the EMG signal testing system

The thicknesses of the substrate and copper deposition were measured using the electrodes fabricated in the third attempt. Specifically, the substrate thickness measured was for Ecoflex spin coated at 300rpm with 600nm of copper deposited. The electrode measured is shown below in Figure 16.

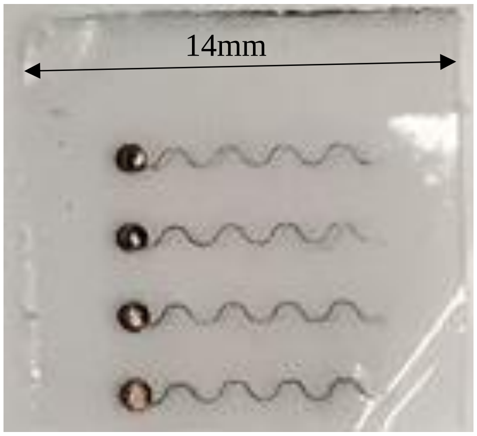
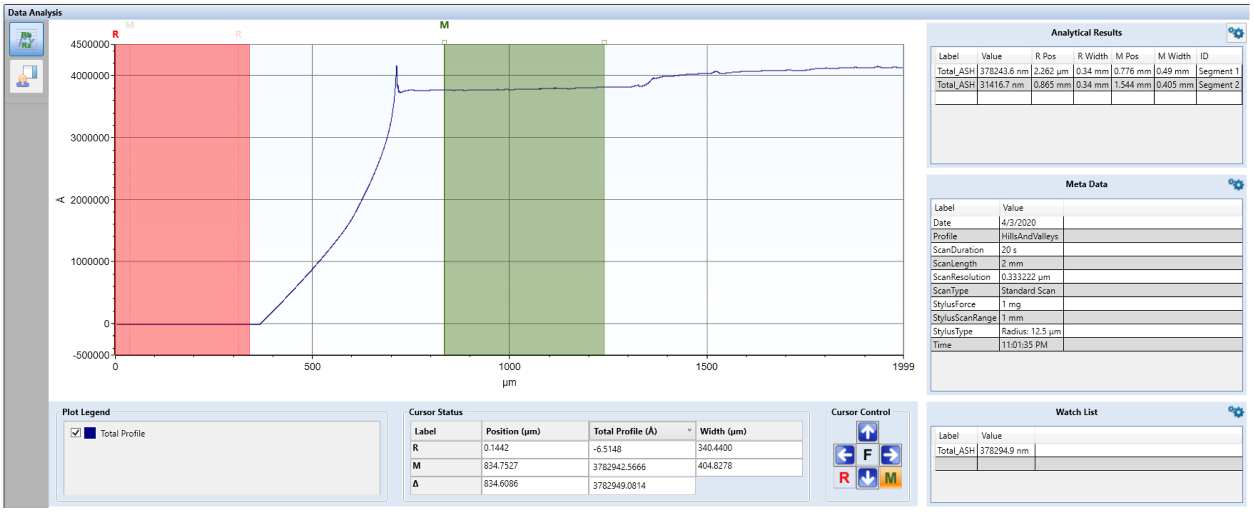


Figure 16. Electrode fabricated in the third attempt that was used for thickness measurements.

For the thickness measurements, a profilometer (Bruker DektakXT Surface Profilometer) was used. The data gathered for the substrate thickness can be seen below in Figure 17, and the data gathered for the copper deposition thickness can be seen below in Figure 18. The Y axis represents the height measured by the profilometer as it goes a certain distance across the electrode, which is shown by the X axis.



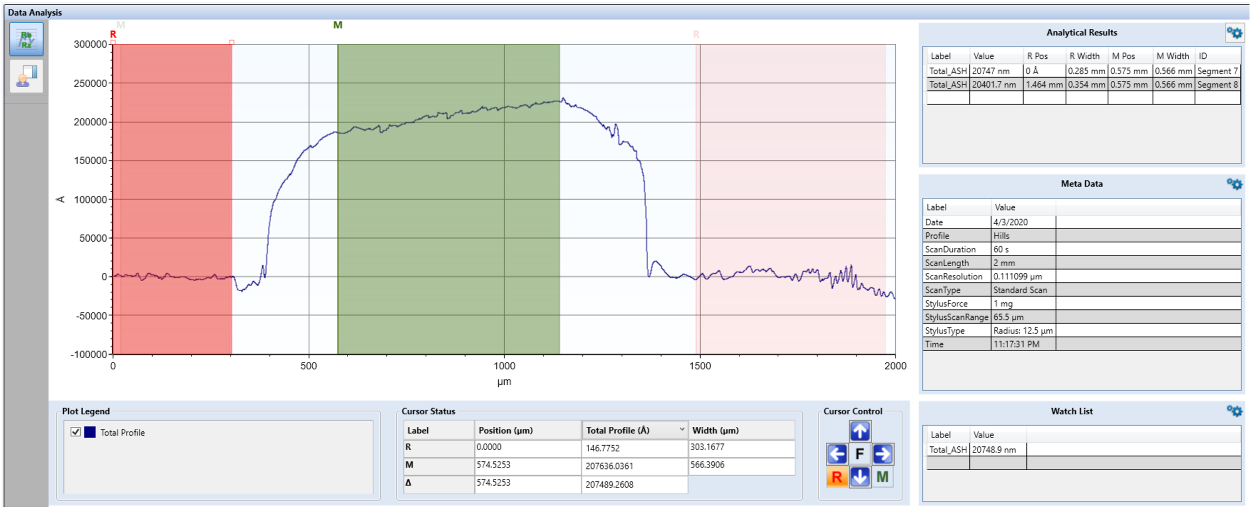
Label	Value	R Pos	R Width	M Pos	M Width	ID
Total_ASH	378243.6 nm	2.262 µm	0.34 mm	0.776 mm	0.49 mm	Segment 1
Total_ASH	31416.7 nm	0.865 mm	0.34 mm	1.544 mm	0.405 mm	Segment 2

Label	Value
Date	4/3/2020
Profile	HillsAndValleys
ScanDuration	20 s
ScanLength	2 mm
ScanResolution	0.333222 µm
ScanType	Standard Scan
StylusForce	1 mg
StylusScanRange	1 mm
StylusType	Radius: 12.5 µm
Time	11:01:35 PM

Label	Position (µm)	Total Profile (Å)	Width (µm)
R	0.1442	-6.5148	340.4400
M	834.7527	3782942.5666	404.8278
Δ	834.6086	3782949.0814	

Label	Value
Total_ASH	378294.9 nm

Figure 17. Graph visual of the measurement of the Ecoflex substrate thickness.



Label	Value	R Pos	R Width	M Pos	M Width	ID
Total_ASH	20747 nm	0 Å	0.285 mm	0.575 mm	0.566 mm	Segment 7
Total_ASH	20401.7 nm	1.464 mm	0.354 mm	0.575 mm	0.566 mm	Segment 8

Label	Value
Date	4/3/2020
Profile	Hills
ScanDuration	60 s
ScanLength	2 mm
ScanResolution	0.111099 µm
ScanType	Standard Scan
StylusForce	1 mg
StylusScanRange	65.5 µm
StylusType	Radius: 12.5 µm
Time	11:17:31 PM

Label	Position (µm)	Total Profile (Å)	Width (µm)
R	0.0000	146.7752	303.1677
M	574.5253	207636.0361	566.3906
Δ	574.5253	207489.2608	

Label	Value
Total_ASH	20748.9 nm

Figure 18. Graph visual of the measurement of the copper deposition thickness.

Figure 17 shows that the Ecoflex substrate thickness was measured to be 378.2949 micrometers, which is within the expected slightly less than 380 micrometers range. Figure 18 shows the copper deposition height across of one of the circular ends of the copper leads of the electrode. The thickness of the copper came out to be 20.7489 micrometers, which was not the expected amount of copper deposited. The electron beam evaporator's quartz crystal monitor stated that

600nm of copper was supposed to be deposited on the electrodes. Despite this disagreement, the thickness of 20.7489 micrometers is within the desired range of less than 30 micrometers.

In addition to the measurements on the electrodes from the third fabrication attempt, the surface was observed with a standard microscope. An image of the surface can be seen below in Figure 19.

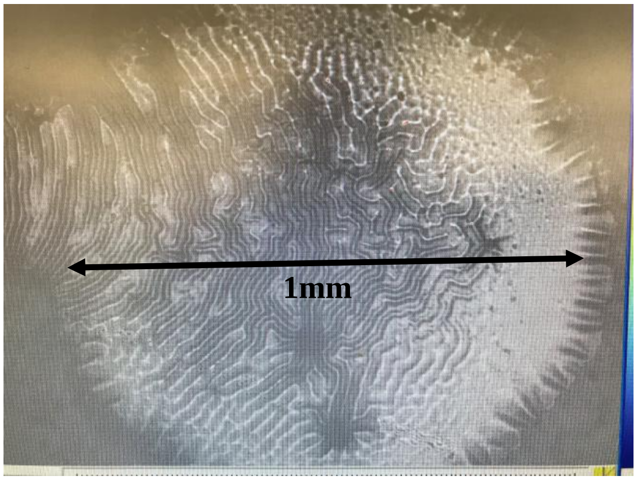


Figure 19. An image of the surface of the electrode at an area of copper deposition.

As can be observed in Figure 19 above, the surface appears to be rough and non-uniform, which was not expected. These kind of features were seen across the entire electrode in the areas of copper deposition.

A summation of the conditions, parameters, and results of each fabricated electrode set can be seen in Table 1 below.

Table 1. Summation of the results for each of the electrode fabrication attempts

	Attempt 1	Attempt 2			Attempt 3
Substrate preparation environment	non-cleanroom	non-cleanroom	cleanroom	cleanroom	cleanroom
Substrate material	Ecoflex	Ecoflex	PDMS	PDMS	PDMS
Spin speed	300rpm	200rpm	300rpm	300rpm	300rpm, 300rpm, 725rpm
Mask material	Kapton tape	Kapton tape	Kapton film	Kapton film	Kapton film
Electrode design	EMG	EMG			Bowel electrode
Deposition thickness	250nm	420nm			600nm
Deposition rate	1 angstrom/sec	0.8 angstrom/sec			2 angstrom/sec
Bubbling	Yes	Yes	No	No	No
Flaking	No	No	Yes	Yes	No
Multimeter continuity test	2 out of 3 copper leads passed	Passed			NA
EMG signal test	failed	failed	passed	passed	NA
Thickness measurements	NA	NA			Substrate: 378.2949 micrometers Copper: 20.7489 micrometers
Notes	Thickness measurements in Attempt 3 were only done with one of the 300rpm spun electrodes.				

CHAPTER IV

CONCLUSION

From the results of the three attempts, five aspects were observed: deformities, flaking, EMG signals, surface features, and thicknesses. Deformities such as bubbling appear to be caused by electrode fabrication in a non-cleanroom environment or use of Kapton tape for the mask. Although, with enough copper deposition, bubbling may not be much of a concern, with one of the electrodes still functional despite bubbling. The main reason to avoid bubbling would be for consistency, which will be needed in the future.

Using the fabrication method stated in the beginning in Figure 2, PDMS is not usable as an electrode substrate. PDMS does not bond to the copper deposition well, causing copper flaking with the method pursued. In order to use PDMS as a substrate, a different method must be utilized. Ecoflex is still viable using this fabrication method, but further validation is necessary before testing in a mouse model.

The resulting raw EMG signals produced from the right arm's bicep by the EMG signal testing system corresponded with standard surface EMG signals [6]. Each bicep contraction observed in the video responded with an EMG signal accordingly. These results prove that the fabrication method for these electrodes creates functional EMG electrodes, which is a step closer to reading intestinal signals. Only the PDMS electrodes were able to detect EMG signals, so further testing with Ecoflex electrodes will be necessary without the interference of bubbling. Further validation may also be needed to determine if the electrodes are capable of detecting electrocolonogram signals specifically.

The rough surface features observed in the final batch of electrodes was not an expected result. The reasoning for this can be many things, but it is most likely caused by the elasticity of the substrate and the heat caused by the deposition from the electron beam evaporator. It could also be due to the higher deposition rate and copper thickness done on the last set of electrodes. These rough features may help support elasticity of the copper leads, since hill and valley features have been show to help provide additional elasticity in flexible electrodes [5].

Thickness measurements of the electrode have proved that the fabrication method can create small and thin enough electrodes for mouse implant. A reason for the disagreement in measurement values for the copper thickness may be due to the roughness of the surface causing a greater thickness in profilometer measurement than the quartz crystal monitor measurement taken during copper deposition.

We have developed a fabrication method for flexible bowel electrodes for a mouse model. The electrodes were determined to be thin and small enough for safe mouse implant and functional for EMG signal detection. Due to the unforeseen events surrounding the COVID-19 virus in spring 2020, complete data was unavailable at the time of publication for this URS thesis. Further validation of this method of fabrication will need to be done to determine if it produces valid electrodes for implant in a mouse for bowel monitoring and stimulating. In the future, we plan to further examine and validate the electrical and mechanical properties of the electrode as well as its lifespan and strength such that this method of electrode fabrication can be determined as valid for the eventual project of creating a closed loop bowel stimulation system on a mouse model.

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