6-2022

Minimally Invasive 3D Printed Microneedles for Glucose Monitoring

Eduardo Quintero

Justin Wong

SANTA CLARA UNIVERSITY

Department of Bioengineering

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Eduardo Quintero and Justin Wong

ENTITLED

Minimally Invasive 3D Printed Microneedles for Glucose Monitoring

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE

IN

BIOENGINEERING

Unyoung Kim — **June 9, 2022**

Thesis Advisor — Date

06/14/2022

Department Chair — Date

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By

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SENIOR DESIGN PROJECT REPORT

Submitted to

the Department of Bioengineering of

SANTA CLARA UNIVERSITY

In Partial Fulfillment of the Requirements for the degree of

Bachelor of Science in Bioengineering

Santa Clara, California 2021-2022

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Eduardo Quintero and Justin Wong

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2022

Abstract

Continuous glucose monitoring (CGM) provides an instantaneous real-time display of glucose level, rate of change of glucose, alerts, and alarms for actual or impending hypo- and hyperglycemia. Continuous glucose monitors are able to stay implanted on the patient anywhere from 7-14 days, giving patients and doctors valuable information to help improve patient care for diabetics, and providing them with a way to continuously monitor their blood glucose level concentration throughout the day.

Conventional methods of glucose detection such as blood glucose meters require the patient to gather frequent blood samples to generate instantaneous results that are only accurate as of the time of day the reading was taken. These methods fail to take account of daily fluctuations in glucose levels that can arise from changes in diet and physical activity.

In an effort to optimize cost, reliability, and accessibility, this paper proposes a minimally invasive continuous glucose monitoring system that is able to detect glucose levels in interstitial fluid through the use of solid 3D printed microneedles. This CGM device would provide an easily affordable, accessible, and pain-free option for diabetic patients. Providing patients with a way to continuously monitor their glucose levels allows for more personal involvement with their health decisions and ultimately serves as a guide toward more effective diabetes management.

Keywords: Microneedles, CGM, Minimally-Invasive, 3D-Printed

Acknowledgements

We would like to thank Santa Clara University School of Engineering for the funding required to complete our research subject. In addition, we would like to thank our faculty advisor, Dr. Ashley Kim for guiding us throughout the whole process. We would also like to thank Dr. Maryam Mobed-Miremadi for letting us use her SLA 3D printer and materials to print different variations of our microneedle arrays. Lastly, thank you to Daryn Baker and Yohannes Kahsai for providing us with the necessary equipment and resources to conduct our research.

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Chapter 1: Introduction

1.1 Introduction

According to the Centers of Disease Control and Prevention (CDC), roughly 37.3 million people in the United States have diabetes (11.3% of the population); 28.7 million people, including 28.5 million adults are diagnosed with diabetes, and 8.5 million people are thought to be undiagnosed. Furthermore, in total, 96 million people aged 18 years or older have prediabetes (38% of the adult US population), and 26.4 million people aged 65 years or older (48.8%) have prediabetes [1]. Diabetes is also one of the leading causes of death in the United States, according to the American Diabetes Association, in 2019 diabetes was the seventh leading cause of death in the United States based on the 87,647 death certificates in which diabetes was listed as the underlying cause of death [2].

There are two types of diabetes, type 1 and type 2, that occur when the body is unable to store and utilize insulin effectively. This causes glucose to stay in the blood, making it hard for the body's organs to generate the energy needed to function normally. Limited energy sources can lead to heart disease, vision loss, extreme fatigue, neuropathy, and kidney disease [2].

Of all diagnosed diabetes cases, 5.8% were type 1 [3]. Although type 1 diabetes affects all age groups, the majority of individuals are diagnosed either at around the age of 4 to 5 years or in their early teens and early adulthood. Type 1 diabetes results from an autoimmune disease associated with the selective destruction of insulin-producing pancreatic β-cells that prevent your body from producing insulin [4]. The low insulin levels make it harder for your body to regulate blood glucose levels. Patients with type 1 diabetes must receive insulin from an outside source every day in order to regulate their blood glucose levels.

Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes [5]. Type 2 diabetes is caused by the inability of insulin-target tissues to respond properly to insulin. It can be caused over time due to unhealthy eating and exercise habits. The

main way to treat type 2 diabetes is through losing weight, eating healthier foods, and staying physically active [6].

For patients with type 1 and type 2 diabetes, it is important to regularly monitor their glucose levels. Understanding how one's blood glucose levels fluctuate throughout the day allows patients and doctors to better prepare treatments and lifestyle changes to manage diabetes.

1.2 Background

Continuous glucose monitoring (CGM) systems represent an important advance in diabetes technology that can facilitate optimal glucose control in type 1 and type 2 diabetes. Continuous glucose monitors allow for hundreds of readings per day that display real-time glucose levels, fluctuation rates, and directionality of glucose levels [7]. Using a small patch to obtain continuous glucose readings makes it easier, less painful, and more accessible for patients with diabetes. Users will no longer have to prick their fingers for blood samples that are only accurate as of the time they collected that sample. Continuous glucose monitors enable diabetic patients to gain a better understanding of how their glucose levels fluctuate throughout the day, and as a result, they gain a better understanding of how to change their lifestyle to better treat their form of diabetes.

Our goal is to build a minimally invasive CGM system through the use of microneedles, which are able to penetrate a few hundred micrometers into the skin. Using microneedles should reduce the amount of pain and swelling, minimize the risk of infection, and provide more accurate glucose readings compared to current CGM devices on the market. While current systems on the market penetrate the subcutaneous layer, our system will penetrate into the dermal layer of the skin as shown in figure 1-1, measuring glucose concentration in interstitial fluid.

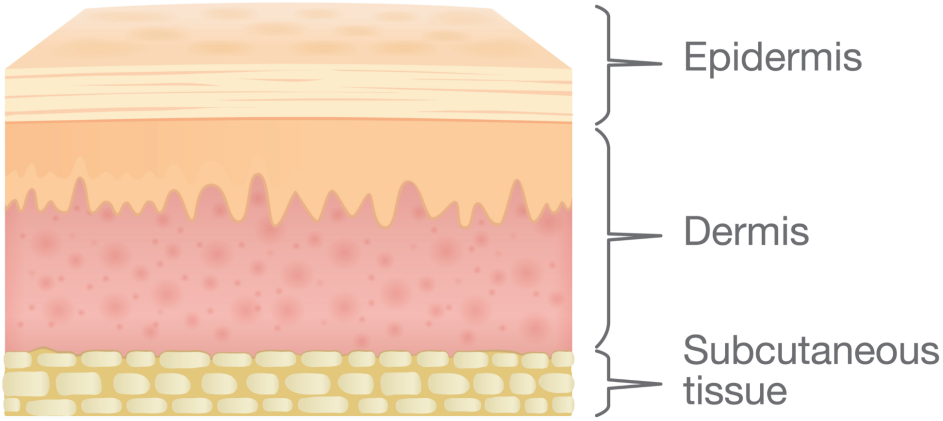

Figure 1-1: Layers of the Skin

1.3. Current CGM Devices on the Market

There are currently multiple continuous glucose monitors available on the market. Most CGM devices are electrochemical sensors that detect glucose concentration in blood samples. For these CGM devices, the sensor needle tip penetrates into the subcutaneous level of the skin, where it measures the glucose concentration in blood. Insertion of these glucose sensing needles and electrodes through the epidermis and dermis into the subcutaneous tissue damages cells, connective tissue, and extracellular matrix. These devices are often reported as invasive and associated with discomfort for the patients, which are potential limitations to continuous and consistent use of the device. They also have relatively high drop-out rates within their clinical studies [8].

The most commonly used CGM devices in the US are the Abbott Freestyle Libre, the Dexcom G6, and the Medtronic Guardian Connect. All three of these devices penetrate into the subcutaneous tissue where glucose concentration is measured in blood plasma. Medtronic's Guardian Connect is injected into the subcutaneous layer and is considerably painful for patients to use [8]. Compared to typical single-use glucose sensors, these devices offer the ability to provide continuous data for up to 2 weeks. This can be extremely beneficial for doctors to help create a better treatment plan for that particular patient. Although it is better to be able to continuously monitor glucose concentrations throughout an entire day with minimal handling,

the fact of the matter is these devices are still not widely used. This can be due to the high cost of these devices, especially if the patient does not have health insurance. The cost of the three most commonly used CGM devices currently on the market, along with their sensor wear time is shown below in table 1-1 [9].

	Sensor wear time	**Cost (pre-insurance)**	**Penetration layer**
Abbott Freestyle Libre 2	~14 days	$2,300/year	Subcutaneous
Dexcom G6	~10 days	~$3,800/year	Subcutaneous
Medtronic Guardian Connect	~7 days	~$4,760/year	Subcutaneous

Table 1-1: Current CGM Device Comparison

1.4. Current Issues

As briefly mentioned above, due to the fact that most CGM devices penetrate all the way into the subcutaneous layer of the skin, many of the users report pain, discomfort, and possible risk of infection as a result. The inflammatory processes that occur in the local tissue following implantation can be categorized into three phases: (1) acute inflammatory phase, which invokes migration of inflammatory cells and plasma proteins toward the site of insertion; (2) intermediate phases, which invoke phagocytes onto the surface of the implant in an attempt to destroy it; and (3) chronic inflammatory phase, resulting in the formation of a fibrotic capsule around the implant (i.e., scarring), lasting from several days to years [10].

As a result of biofouling, approaches to overcome the foreign body reaction have included the use of biocompatible materials to coat the sensor or reduce the amount of penetration into the skin.

Another major issue with the current CGM devices is their cost. Without health insurance, these devices can be outright too expensive for many families around the world. Paying thousands of dollars per year for a CGM device, on top of the prices of insulin, can be downright unattainable for many families.

As a result, in order to make CGM devices more prevalent in our society, they must first be more affordable and accessible to patients from every socioeconomic background. Furthermore, they must also reduce the pain and discomfort commonly associated with their usage.

1.5. Project Goal

The focus of this project is to create a minimally invasive continuous glucose monitor, which penetrates into the dermal layer of the skin for real-time reading of glucose levels. Our goal is to build a CGM system through the use of 3D printed microneedles, which are able to penetrate a few hundred micrometers into the skin. Penetrating only into the dermal layer of the skin using microneedles should reduce the amount of pain and swelling, minimize the risk of infection, and provide more accurate glucose readings compared to current CGM devices on the market. Unlike most glucose sensors that measure blood sugar levels, due to the fact that the microneedles will be designed to penetrate into the dermal layer, it will measure glucose concentration in interstitial fluid.

We hope that our minimally invasive CGM system will provide diabetic patients with a cheap and effective alternative for monitoring and managing their blood glucose levels. We believe that this device will allow diabetic patients to live their lives without pain or swelling and risk of infection. Also, patients will be able to closely monitor how their glucose levels fluctuate throughout the day and prepare better treatments to better manage symptoms.

1.6. Literature Review

Continuous glucose monitors are an up-and-coming technology that measures glucose levels in real-time. Current CGMs penetrate the skin by inserting a needle into the subcutaneous layer of

the skin and measuring the glucose concentration of the interstitial fluid. These systems utilize electrochemical reactions consisting of glucose oxidase, the gold standard enzyme for glucose measuring, and some type of redox mediator. [11]

The three most commonly used CGM devices in the US are Dexcom G6, Freestyle Libre system from Abbott, and Medtronic's Guardian Sensor 3 [12]. While these devices are effective in certain areas of glucose monitoring, they do have some flaws that need to be addressed. The Dexcom G6 has many advantages, but it can be very costly for users. The Dexcom G6 can cost upwards of $1,600 per year without health insurance, making it one of the more expensive options for continuous glucose measurements [13]. The Medtronic Guardian 3 has been shown to cause pain for patients because it penetrates the subcutaneous layer of the skin [14]. The freestyle Libre System is not a continuous monitoring system like the other systems listed above. Instead, the device relies on the user having to scan the sensor for a glucose level reading [13].

This is a continuation of a previous year's project. The article "Simple and Customizable method for fabrication of high-aspect-ratio microneedles using low-cost 3D printing", utilizes a print and fill method to fabricate microneedle arrays [15]. Through the use of a low-resolution 3D printer in Dr. Mobed-Miremadi's lab at Santa Clara University, a higher aspect ratio can be achieved. Needle arrays are fabricated with high resolution by printing with a higher needle height, shortening needle length by filling the base of the arrays with UV curable resin, and creating a silicone female mold from the resin-filled arrays.

While the previous group was able to successfully fabricate needle arrays, we believed that we could improve upon the resolution and needle strength. After performing a literature search, we found the article "Rapid 3D-print-and-shrink fabrication of biodegradable microneedles with complex geometries", which follows a print and shrink method. A 3D printed array is printed 60% bigger than the desired size. Then, an agarose female mold is made from the 3D printed array and put in an incubator to shrink. Once the shrinking process is done, a 1:10 PDMS mixture is poured into the shrunken agarose gel to create a new master key. This process is repeated two more times, however, the agarose gel is not shrunken. Also, PVP is used for the final needle product [16].

In their literature review, they came across a proof-of-concept minimally invasive CGM device by Ribet, et. al., 2018 [17]. This paper incorporated a three-electrode system circuit model to continuously monitor glucose concentration in the dermal layer of the skin. As a result, the previous group also incorporated a three-electrode system. As we performed the commercial glucose testing, we noticed that the glucose strip implemented a two-electrode system. As a result, we also implemented a two-electrode system for our microneedle arrays. This two-electrode system allows for a much more simple electrode manufacturing process while retaining the ability to detect glucose concentration in a sample.

One of the most important aspects of our CGM device is the fact that it is minimally invasive and penetrates into the dermal layer. Most current CGM devices penetrate into the subcutaneous tissue, causing users to feel discomfort, pain, and swelling [18]. This is a potential limitation to continuous use, as patients may not feel comfortable enough to integrate these devices into their daily lives. By penetrating only into the dermal layer of the skin, our CGM device will detect glucose concentration in interstitial fluid, not blood. Blood and interstitial fluid differ in a number of different aspects with respect to glucose. Interstitial fluid is the compartment in which substances such as glucose diffuse to the surrounding tissues/cells on a local level; whereas the bloodstream is the transport system of the body for transporting substances such as glucose over long distances [19]. Furthermore, interstitial fluid constitutes approximately 45% of the volume fraction of human skin, with blood vessels contributing 5% of the skin volume [20]. The time required for glucose to diffuse from the capillary to the tissue plays a vital role in the lag time between changes in blood plasma glucose levels and interstitial glucose levels.

Under stable conditions blood glucose results and interstitial fluid results show more or less identical glucose values with rates of change below 1 or 2 mg/dl per min, However, when more rapid changes in glucose levels are induced, either by exercise, diet, or other activities, glucose measurements results can differ considerably between blood glucose and interstitial fluid [19]. Due to these discrepancies, patients using CGM systems are required to perform blood glucose measurements to make their therapeutic decisions (insulin dose adjustments).

Generally, glucose measurements are based on interactions with hexokinase, GOx, or glucose-1-dehydrogenase [21]. The first generation of glucose biosensors was first proposed in 1962 by Clark and Lyons from the Children's Hospital of Cincinnati [22]. This glucose sensor utilized GOx immobilized in a polyacrylamide gel on an oxygen electrode [23]. Second-generation glucose biosensors replaced oxygen with non-physiological electron acceptors such as ferrocene, ferricyanide, methylene blue, and other electron mediators [24]. Third-generation glucose sensors are reagentless and based on direct contact between the enzyme and the electrode without any intermediate mediators. Therefore, third-generation glucose biosensors have led to the development of implantable, needle-based devices for continuous monitoring of glucose in vivo [25],[26]. Consequently, our CGM device is based on a third-generation glucose biosensor that uses different metal coatings which interact directly with enzymes to detect glucose concentration in interstitial fluid.

1.7. Significance

The purpose of our project is to create a more affordable, accessible, and effective option for patients to better understand and manage their diabetes. This system would give patients the ability to access real-time blood glucose measurements and predict the direction their glucose levels are trending.

Chapter 2: Design Description

2.1. Solid Microneedles Design

Based on the previous group's data, we decided to move forward with the 4.5:1 aspect ratio in a 5x5 array [15]. Due to our print and shrink method, we decided to increase the size of our microneedle arrays by sixty percent compared to the previous group's models. The width, length, and needle heights came out to be 24mm, 24mm, and 4.5mm respectively. An image of the updated array on SolidWorks can be seen below in Figures 1-2. Our SolidWorks files were printed using an SLA 3D printer (Formlabs Form2 Rapid Prototyping Machine) and used for our two fabrication methods.

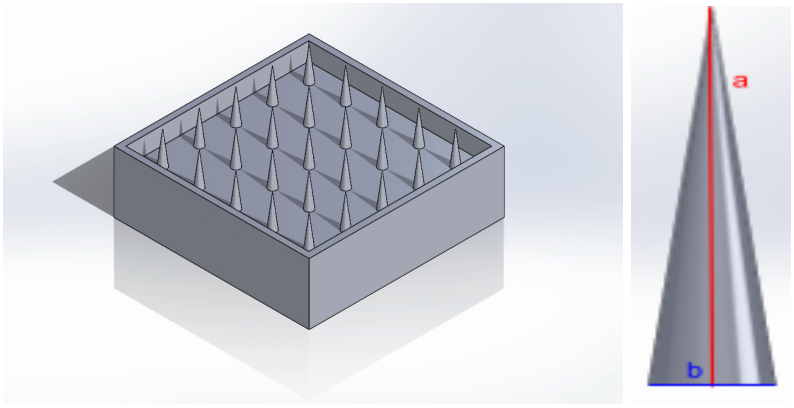

Figure 1-2: Solidworks Model of Microneedle Array

2.2. Glucose Oxidase Reaction

Glucose Concentration sensing on the solid microneedles will be conducted using a two-electrode system and via the glucose oxidase (GOx) reaction shown in equation 1

$$\text{Glucose} + O_2 + H_2O \xrightarrow{GO_x} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + \boxed{2e^-}$$

Equation 1: Glucose Oxidase Reaction

In this reaction, glucose is oxidized in the presence of oxygen, water, and GOx. This reaction forms gluconic acid and hydrogen peroxide. The hydrogen peroxide is then subsequently electrochemically oxidized at the working electrode, which produces a current proportional to the concentration of glucose in the sample, as seen by the 2 electrons produced in red.

This redox reaction will be utilized to compare the measurements from our microneedle array system using the electrochemical analyzer, compared to commercially available glucose meters in order to analyze the accuracy and precision of our system.

2.3. Two Electrode System

For our electrode system, we decided to implement a two-electrode system based on current commercial glucose sensors and for ease of fabrication. For the working electrode, we used carbon conductive paint. Since we decided to implement a two-electrode system, we combined the working and reference electrode and used a silver/silver chloride (60/40) paste. The purpose of each electrode is described in tables 2-1 down below.

Working	Reference	Counter
* Where the reaction of interest takes place * Magnitude of current reveals the concentration of analyte (Glucose) * Carbon Conductive paint	* Has a well-known electrical potential used to determine the potential of the working electrode * Reference electrode stays in chemical equilibrium * No current flows through * Coated with silver/silver chloride paste	* Source of electrons for the reaction occurring in working electrode * Current flows through the counter electrode to compensate for opposite current flowing through working electrode * Coated with silver conductive paint

Table 2-1: Electrode Description

Chapter 3: Methods, Results, and Materials

3.1.1 Print and Fill Fabrication of Microneedles

Using a gray UV curable resin, we filled up the microneedle array basin to the top of the basin wall so that only 1mm needles were exposed at the top of the resin. Next, we dip-coated the needles in Inhibit X in order to protect against cure inhibition and sprayed the exposed needles with Mann Ease Release 200.

We then prepared our Silicone mold casting that was used to create a female mold of our microneedle arrays. We mixed a set of platinum silicone medium cure rubber agents at a ratio of 1:1. Depending on how many molds you are making at once, we typically went with 7.5g of each silicone solution. We then set our microneedle array onto a small plate and carefully poured the silicone rubber mix so that it completely covered the microneedle array basin. After degassing our needles for at least 24 hours, we peeled off the female mold from our microneedles.

The resulting female mold has holes according to the 1mm exposed needle array. We then created a solution of 5%(w/v) sodium carboxymethyl cellulose to DI water, mixed the solution using a vortex mixer, and left it in a heated 60˚C water bath for an hour. After, we added bromophenol blue dye to the solution, mixed it again with a vortex mixer, and let it sit in a 60˚C water bath for another hour. Finally, we poured the solution onto the female molds, and after about 48 hours, the solution dried within the mold, and we obtained our final fabricated microneedles. An illustration of the print and fill method is shown below in figure 3-1 [15].

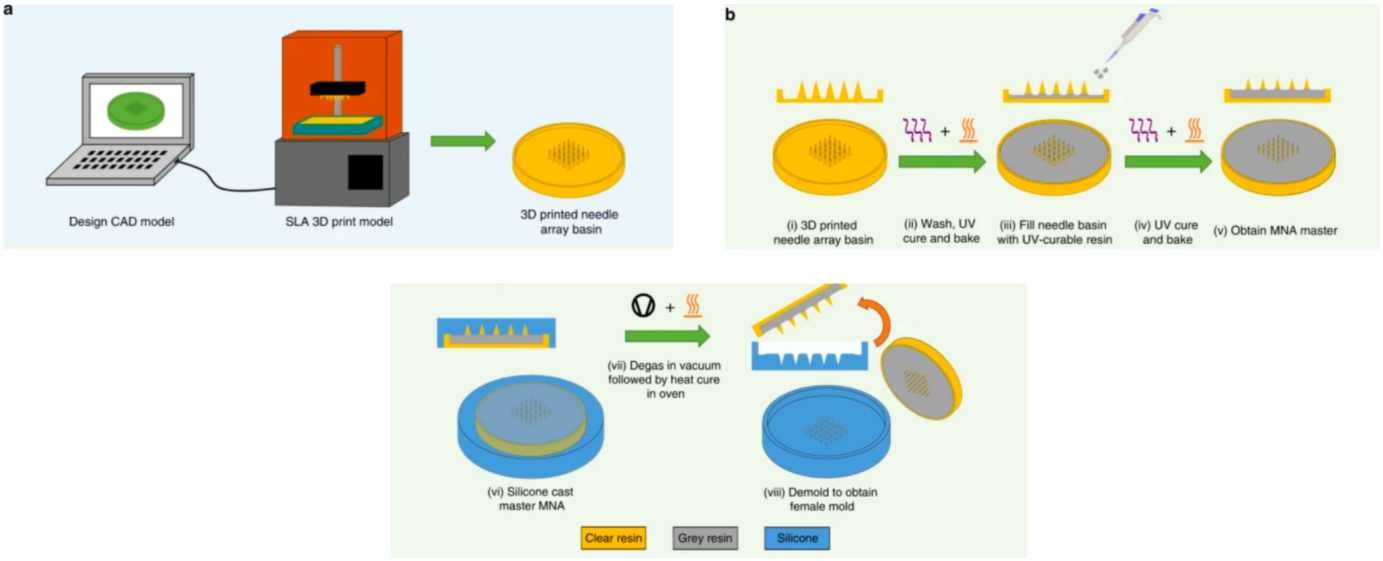

Figure 3-1: Print and Fill Method

3.1.2. Print and Fill Results

In our first attempts at fabricating the carboxymethyl cellulose solution, we noticed we weren't getting the gelatinous consistency we were expecting. Instead, we were left with a thin liquid solution that led to a very thin and brittle-like final product as seen in figures 3-2.

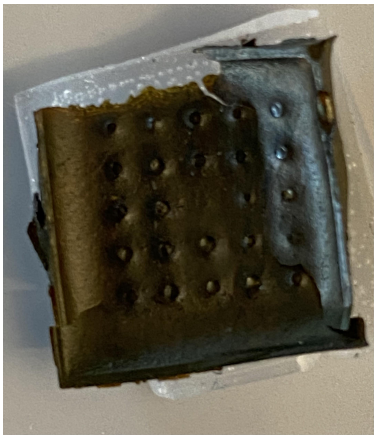

Figure 3-2: First Carboxymethyl Cellulose Solution

One possible reason, which was later improved upon, was the need to have a magnetic stirrer in the beaker when placed in the hot water bath. This allowed for better mixing of the solution and gave us the right consistency we were looking for.

After making some adjustments to our carboxymethyl cellulose fabrication process, we proceeded with making the final needle array products. The new carboxymethyl cellulose solution resulted in more defined needle arrays. However, upon careful removal from the silicone mold, we noticed that the structural integrity of the needles was compromised because the material was very fragile. Another issue with the print and fill method was the short shelf life as the needle arrays shrunk and shriveled up into an unusable product as seen in Figures 3-3.

Figure 3-3: Second Carboxymethyl Cellulose Attempt

One possible reason for the fragile final product was the cellulose solution was not completely mixed. When placing the cellulose in the water bath to mix, the temperature fluctuated between 45-60˚C. This could have affected the mixing between the water and carboxymethyl cellulose solution. While we did get the right consistency, we believe that leaving the solution in the water bath for a longer time could have led to a better-mixed solution. Also, using a more consistent hot plate could have improved our results.

3.2.1 Print and Shrink Fabrication of Microneedles

We used a stereolithography (SLA) 3D printer (Formlabs Form2 Rapid Prototyping Machine) using UV curable resin (White Resin V4, Formlabs Inc., Somerville, MA, USA) to fabricate our initial set of microneedles. When using an SLA 3D printer, there are inherent limitations with the resolution of the geometric structures which are 3D printed [27]. Due to these limitations of using a 3D printer for the generation of microneedles, there is a need to increase the resolution of the structures printed so that they are capable of puncturing into the skin.

The “Print and Shrink” method was developed by M. Ochoa, et al., 2015 as a way to develop a simple technique for fabricating microneedles of complex geometric shapes [16]. This method consists of transferring the pattern of 3D printed microneedles to a negative hydrogel mold, which is then subsequently shrunk by up to 40% within 12 hours. After the desired shrinkage/refinement is achieved, new needle patterns are then transferred to a biodegradable polymer, resulting in microneedles that are sufficiently sharp to penetrate porcine skin.

Figure 3-4 illustrates the “Print and Shrink” fabrication process for creating a set of microneedle arrays. First, the needle array is designed in SOLIDWORKS and fabricated by SLA 3D printing. This 3D printing process produces deposits on the object which are then subsequently dissolved by soaking the microneedle array in a 1 M NaOH solution for 24 hours under continuous stirring at 250 rpm. The microneedle array is then placed face up in a petri dish and the pattern is transferred to hydrogel by casting 1.5% w/v agarose onto the mold. After 30 minutes, the 3D printed microneedles are then removed from the hydrogel. The negative hydrogel mold is then sandwiched between two layers of agarose gel to form a protective layer to prevent aspect ratio distortions during the subsequent shrinking process. Next, shrink the hydrogel mold via evaporation in an oven set for 70˚C for 10 hours (Adjust shrinking time depending on the size of the hydrogel mold).

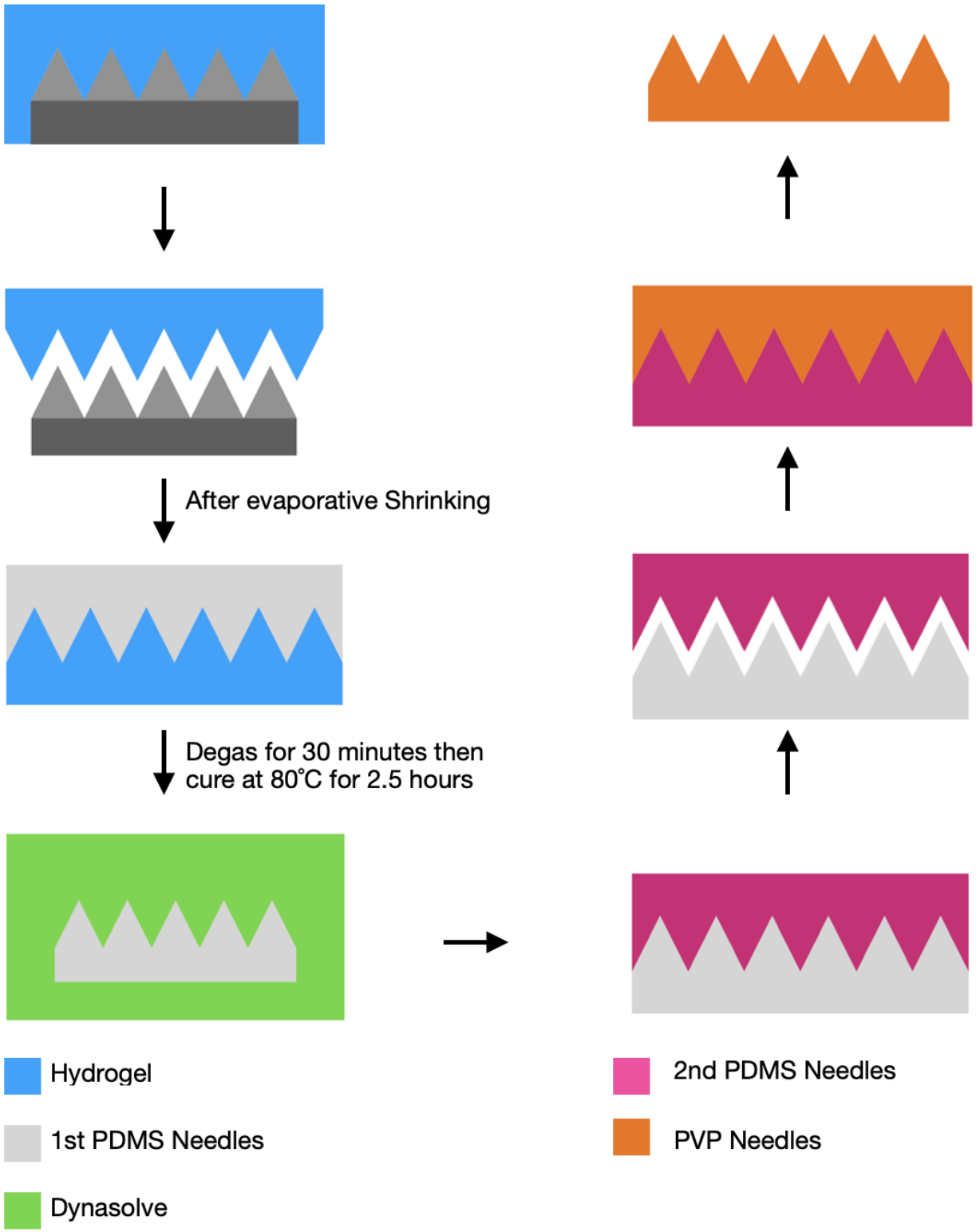

Figure 3-4: Print and Shrink Method

Remove the hydrogel mold from the oven and use it as a mold for transferring the microneedle array pattern to PDMS (polydimethylsiloxane, 10:1 Ratio, Sylgard 184, Dow Corning). Prepare the PDMS separately and degas for 15 minutes. Then, pour the 10:1 PDMS onto the negative hydrogel mold in a petri dish, degas for an additional 15 minutes, then incubate at 80˚C for 2.5 hours. The new PDMS needles are then sharpened by dipping them in a silicone-dissolving solvent (Dynasolve 218, Dynaloy LLC, Indianapolis, IN, USA) for 6 minutes. Further shrinkage can be achieved if the process is repeated. Figure 3-5.

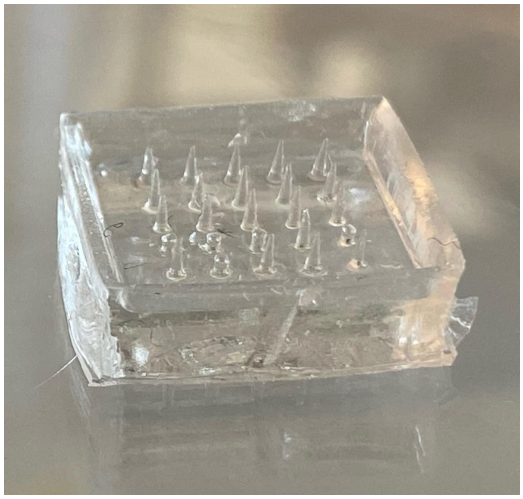

Figure 3-5: PDMS Needle Array

3.2.2 Print and Shrink Fabrication Results

While fabricating microneedles using the “Print and Shrink” fabrication method, we only made it to the first PDMS microneedles step. The PDMS step was largely inconsistent with the transfer of the microneedle array patterns. As shown in figure 3-6 and figure 3-7 below, not all microneedles were successfully transferred into a PDMS mold.

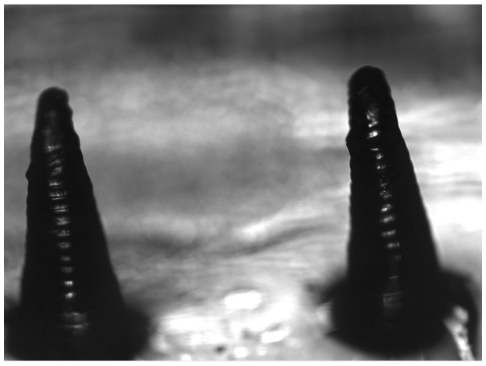

Figure 3-6: Successful microneedle pattern transfer to PDMS mold

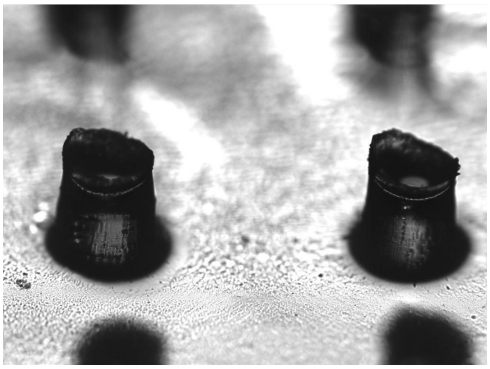

Figure 3-7: Unsuccessful microneedle pattern transfer to PDMS mold

One possible reason for this inconsistency can be due to the inconsistent shrinking rate experienced during the evaporative shrinking process. The paper by M. Ochoa, et al., 2015 was a conference paper, which means its method section was not as descriptive as an ordinary literature article. The fabrication method of the protective layer required for preventing aspect ratio distortion during the shrinking process was not very descriptive. As a result, it was difficult to completely replicate their results.

Another possible reason for the inconsistent microneedle array pattern transfer to a PDMS mold could be damaging the PDMS needles while the user is removing them from the negative hydrogel mold. When removing the PDMS needles from the negative hydrogel mold, if performed without caution, parts of the PDMS needles may break inside the negative hydrogel mold, leading to the results shown in Figure 3-7.

3.3. Electrode Fabrication

Our CGM system implements a 2 electrode system to perform glucose level monitoring in the dermal layer. The working electrode consists of a carbon conductive paint layer, while the reference/counter electrode consists of silver conductive paint.

Once your final needle product is obtained from either the “Print and Shrink” or “Print and Fill” method, pipette a small layer of carbon conductive paint onto two rows of the 5x5 microneedle array. Next, pipette a small amount of silver conductive paint onto the opposite 2 rows of the microneedle array, leaving a bare middle row with no metallic coating as shown in Figure 3-8.

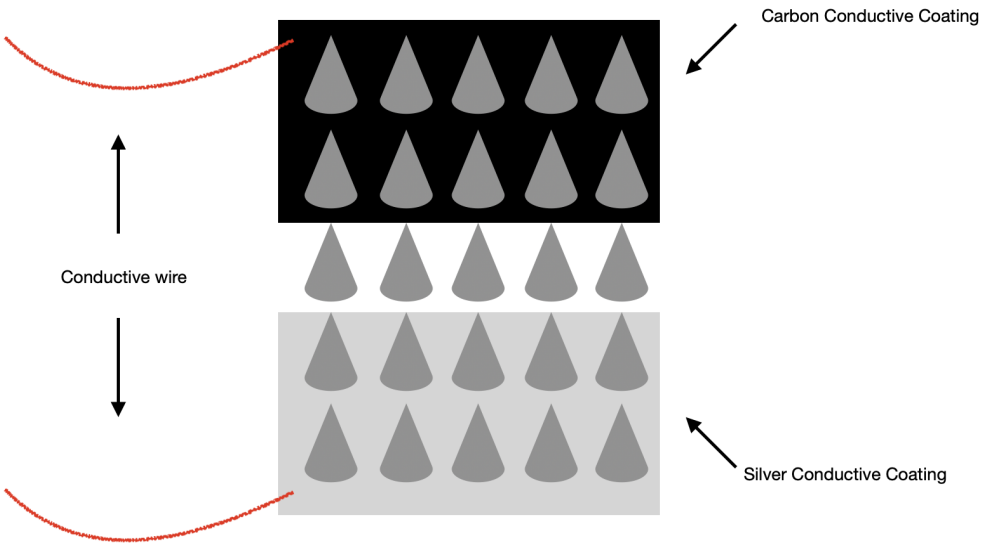

Figure 3-8: Electrode fabrication onto microneedle array

While the metal coatings are drying onto the microneedles, place electrically conductive wires onto the electrodes while making sure there is sufficient contact between the wire and metal coating to allow for optimal conductivity. Figure 3-9 shows the product after microneedle array fabrication after electrode fabrication

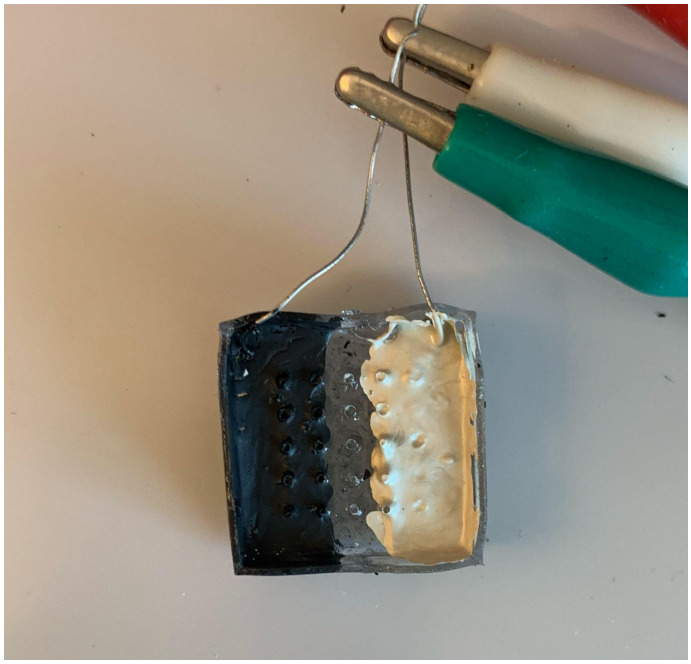

Figure 3-9: Final microneedle array after electrode fabrication

3.4 Materials

Commercial Glucose Meter (Keto Mojo Glucose Meter), Digital Microscope (Bysameyee USB Digital Microscope 40X to 1000X), Glucose (from Sigma Aldrich), PBS (from Alumni Science lab), Bromophenol Blue Dye (from Sigma Aldrich), DI water, Conductive Paste (Silver/Silver Chloride Paste from Sigma Aldrich), Mann Ease Release (Mann Release Technologies Ease Release 200 14 fl. oz.), Platinum Cure Silicone Rubber (Smooth-On Dragon Skin 10 Medium platinum cure silicone rubber), Inhibit X (from ReynoldsAM), UV-curable Resin (Crystal Clear Ultraviolet Curing Epoxy Resin for DIY Jewelry Making, Craft Decoration from Youngfocus), Rich-OPTO 3D Printer Resin UV-Curing 405nm Rapid High Precision Quick Curing Standard Glucose Test Strips (bkt Glucose Test Strips (x200)), Platinum Wire (PT005115 Platinum Wire from Goodfellow), Silver Spray Paint (MG Chemicals 842AR-140G Super Shield Silver Conductive Coating, 5 oz, Aerosol Can), Carbon Spray Paint (MG Chemicals - 838AR-340G 838AR Total Ground Carbon Conductive Paint, 12 oz Aerosol Spray Can), Silver Liquid Paint (MG Chemicals 842-20G Silver Print Conductive Liquid Paint, 20g Container), Transfer Pipettes (from Karter Scientific 206H3), Dynaloy Dynasolve 218 Cleaner Light Amber 1 Qt Bottle.

The costs and vendors for each item listed above can be found in figure 5-1in the Cost analysis section

Chapter 4: Glucose Testing

4.1. Commercial Glucose Testing

The accuracy of blood glucose meters is important for the calculation of insulin doses and the calibration of continuous glucose monitors. A study by Laya Ekhlaspour, et al., 2017, has shown that across 17 commercially available glucose meters from 9 different manufacturers, the accuracy across a wide range of glucose levels varies widely [28]. In order to test the accuracy of a commercial glucose sensor, we tested AUVON's blood sugar test kit. We tested values from 50-350 mg/dl in increments of 30 mg/dl. After conducting three separate readings for each concentration, we plotted the measured glucose concentration vs. expected glucose concentration. The resulting R-squared value represents the proportion of the variance for a dependent variable that's explained by an independent variable or variables [29]. As seen in figure 4-1, the R-squared value = .996, showing that the AUVON commercial glucose meter is accurate.

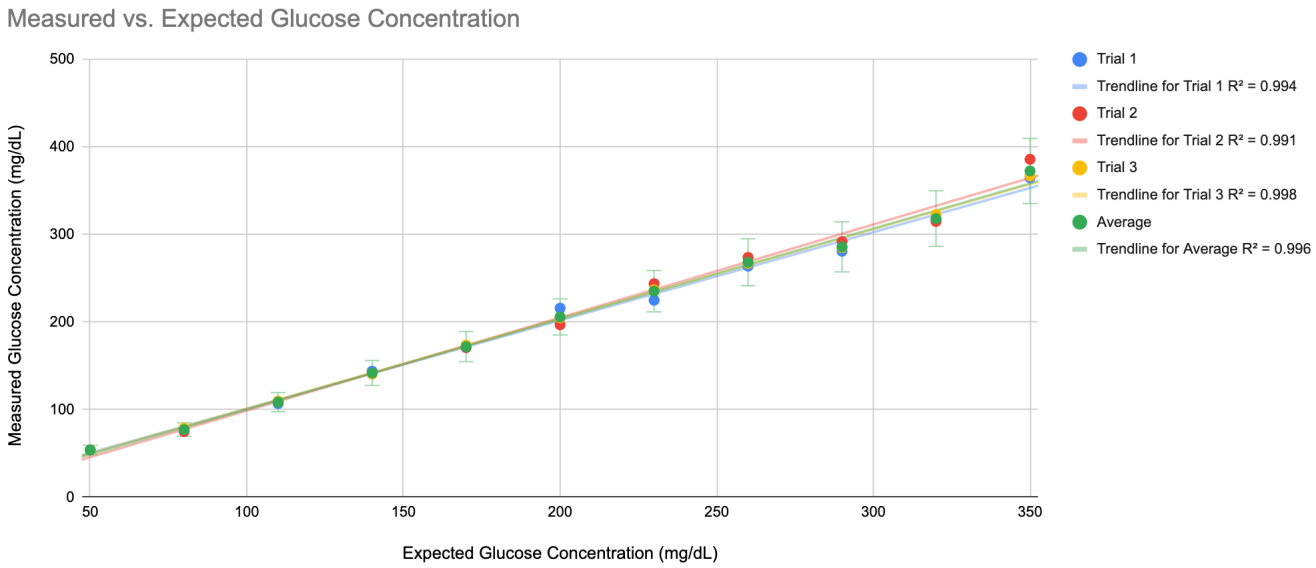

Figure 4-1: R^2 value of AUVON commercial glucose sensor

4.2. Auvon Commercial Glucose Testing with Electrochemical Analyzer

After the accuracy of the commercially available AUVON glucose meter was shown, a series of glucose testing was performed using a CHI 730D electrochemical analyzer (CHI Co., USA). These sensor strips implement a two-electrode system and thus needed an edge board connector in order to test using the CHI instrument, we used the CONN EDGE SGL FEMALE 10POS 0.156 connector from Digi-key (Digi-Key Co., USA) shown in figure 4-2.

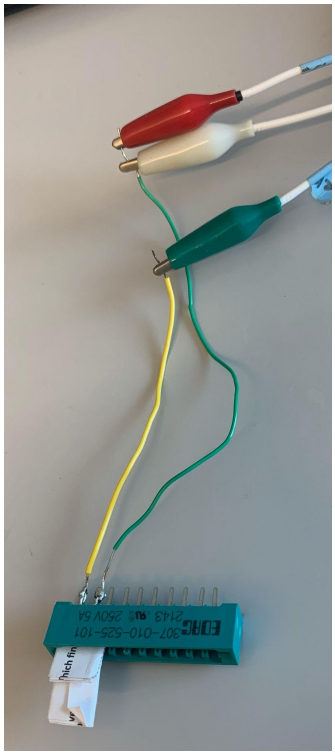

Figure 4-2: Female edge adaptor for two-electrode glucose sensor

Electrochemical analysis was performed from a range of different predetermined concentrations of glucose in 10X PBS solution. Amperometry was used to quantitatively determine current peak values and trends which correspond to different glucose concentrations through the usage of the glucose oxidase reaction.

Enzymatic amperometric glucose biosensors are the most common devices commercially available. Amperometric sensors monitor currents generated when electrons are exchanged either directly or indirectly between a biological system and an electrode [30]. In an amperometric glucose sensing technique, glucose values are estimated by measuring the electrical current generated by the reaction of glucose with an immobilized redox mediator, in this case, glucose oxidase [31]. There are generally three enzymes used for glucose measurements: hexokinase, GOx, or glucose-1-dehydrogenase (GDH) [32],[33]. We chose to use GOx because it has a relatively higher selectivity for glucose, is easy to obtain, relatively cheap, and can withstand greater extremes of pH, ionic strength, and temperature than many other enzymes. These conditions allow for less stringent conditions during the manufacturing process and relatively relaxed storage norms [34],[35]

Using the CHI instruments 730D we performed amperometric detection for glucose ranges from 50-140 mg/dl in increments of 30 mg/dl using the AUVON glucose testing strips. We used an initial voltage of 0.6V, a scan rate of 0.1 V/s, a sampling rate of 5 seconds, and a sensitivity of 1e-004. Figure 4-3 shows that as the glucose concentration increases, so does the maximum current output.

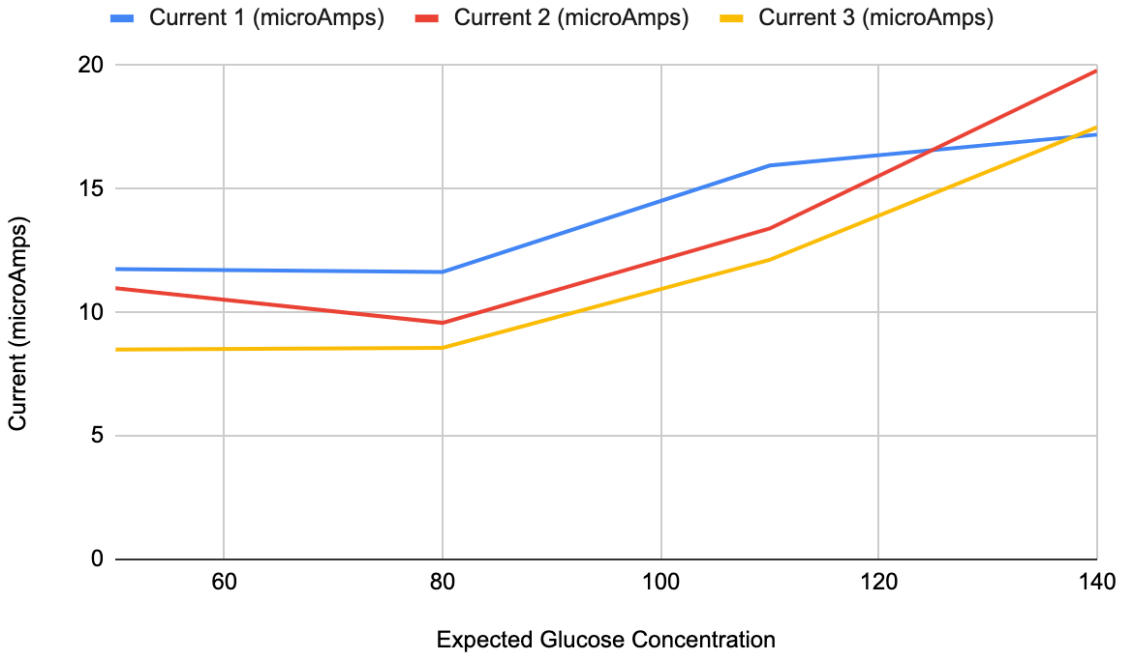

Figure 4-3: Results of AUVON commercial glucose sensor with electrochemical analyzer

4.3. Dropsens Glucose Testing

Before testing our microneedles, we wanted to mimic the conditions on our microneedles onto an electrode strip. For this testing, we used Metrohm Dropsens 110 testing strips which implement a three-electrode system. The working electrode consists of carbon, the counter electrode also consists of carbon, and the reference electrode consists of silver. Unlike other glucose sensors, Metrohm's Dropsens sensor does not have GOx predeposited, as a result, GOx needs to be deposited onto the working, counter, and reference electrode.

We wanted a 50u/uL solution of GOx, so we measured out .0063 grams for every 200uL of 10x PBS solution. A stock solution of 10% glutaraldehyde was diluted to the desired 2% via a 5x dilution. 40ul of the 10% was mixed with 160ul of DI water to create the 2% solution. The diluted Glutaraldehyde solution (2%) was mixed with GOx (Toyobo Ltd., Toyobo) solution (50 U/ul) at a volume ratio of 1:2 respectively. Then 20 ul of the Glutaraldehyde + GOx mixture was deposited on the working electrode. 20ul of this GOx + glutaraldehyde solution was then pipetted onto the working electrode of the Metrohm Dropsens sensor.

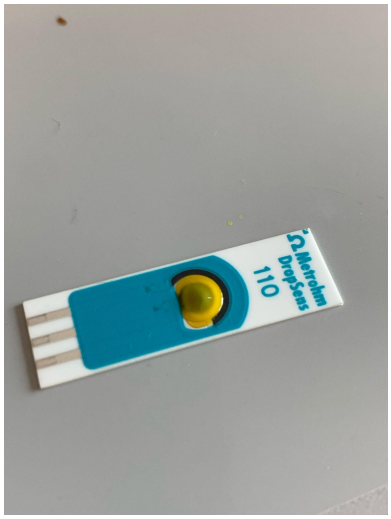

Figure 4-4: GOx deposited onto electrodes of Metrohm Dropsens 110

Next, 30ul of the predetermined glucose solutions (40-160 mg/dl; increments of 40 mg/dl) were pipetted onto the GOx + glutaraldehyde mixture and gently pipette mixed on the working solution as shown in figure 4-4.

After running the CHI 730 instrument for the specified glucose concentrations shown in table 4-5, it is apparent that as the glucose concentration increases, so does the maximum current output shown in figure 4-6.

Glucose Concentration (mg/dL)	Current (nA)
40	3.364
80	3.508
120	4.352
160	4.907

Table 4-5: Glucose concentrations tested and the maximum current output for each respective concentration

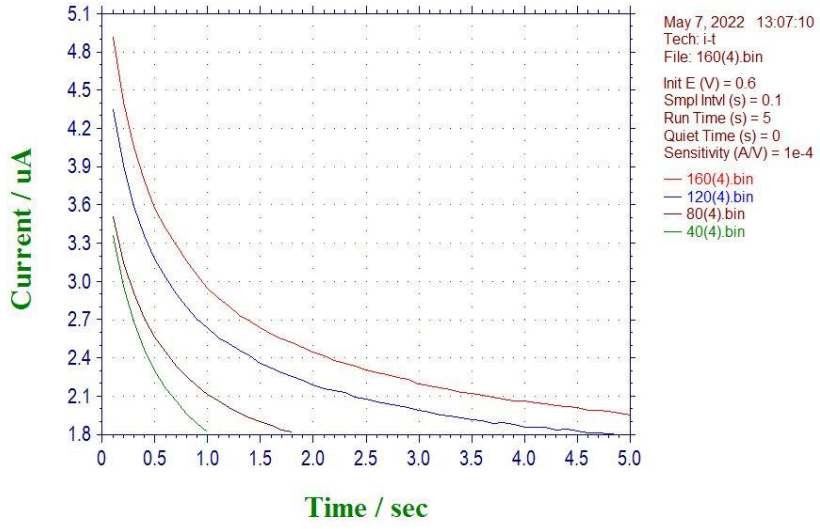

Figure 4-6: Graph overlay of electrochemical analyzer results using Dropsens 110

4.4.1 Microneedle Array Glucose Testing

After painting our final needle arrays, we were able to proceed with glucose testing using a similar procedure as the DropSens method above. We wanted a 50u/uL solution of GOx, so we measured out .0063 grams for every 200uL of 10x PBS solution. A stock solution of 10% glutaraldehyde was diluted to the desired 2% via a 5x dilution. 40ul of the 10% was mixed with 160ul of DI water to create the 2% solution. The diluted Glutaraldehyde solution (2%) was mixed with GOx (Toyobo Ltd., Toyobo) solution (50 U/ul) at a volume ratio of 1:2 respectively. Then 20 ul of the Glutaraldehyde + GOx mixture was deposited on the working and reference/counter electrodes [36]. 20ul of this GOx + glutaraldehyde solution was then pipetted onto the working and counter/reference electrodes.

Next, 30ul of the predetermined glucose solutions (40-160 mg/dl; increments of 40 mg/dl) were pipetted onto the GOx + glutaraldehyde mixture and gently pipette mixed on the microneedle array.

4.4.2 Microneedle Array Glucose Testing Results

After running tests for each concentration level (40-160 mg/dL), we received the results as seen in figure 4-7 down below. We noticed that the maximum current, which is used to quantify the amount of glucose concentration in each solution, did not follow the trendline that we expected. As our concentration increases, we would expect to see the maximum current increase as well. However, this was not the case for our experiments as the maximum current values fluctuated throughout our experiment.

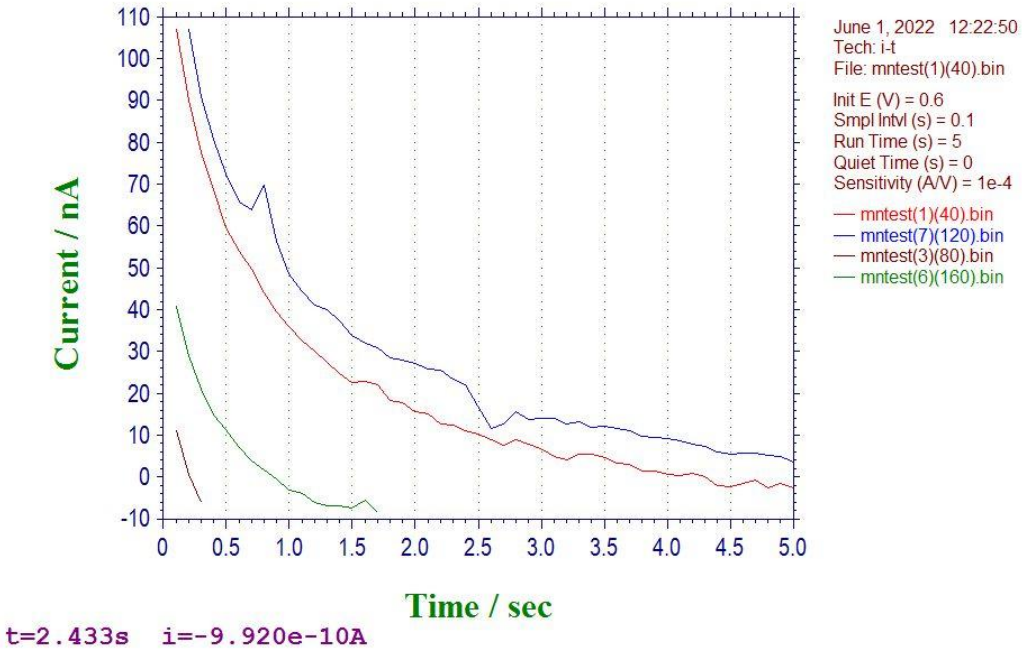

Figure 4-7: Electrochemical analyzer results from microneedle array glucose testing

Chapter 5: Cost Analysis

Figure 5-1: Component list and order cost

Component	Total Number of Units	Vendor	SKU number	Order Cost*
Commercial Glucose Meter AUVON DS-W Blood Sugar Kit	1	Amazon	DS-W5050011	$22.93
Digital Microscope Bysameyee USB Digital	1	Amazon	B07SR7YPV5	$24.02

Microscope 40X to 1000X				
Conductive Paste Silver/Silver Chloride Paste	1	Sigma-Aldrich	901773-50G	$283.66
Mann Ease Release Mann Release Technologies Ease Release 200 14 fl. oz	1	Amazon	B002YEBO1O	$22.37
Inhibit X	1	Reynolds Advanced Materials	Inhibit X	$72.15
Silver Liquid Paint MG Chemicals 842-20G Silver Print Conductive Liquid Paint, 20g Container	1	Amazon	842-20G	$53.00
Silver Spray	1	Electronic Inventory	842AR-140G	$145.34

Paint MG Chemicals 842AR-140G Super Shield Silver Conductive Coating, 5 oz, Aerosol Can		Online		
polydimethylsiloxane, 10:1 ratio, Sylgard® 184, Dow	1	Amazon	184	$165.00
PVP10-100G	100g	Sigma-Aldrich	PVP10-100G	$49.00
Dynasolve 218	1 Quart	Ellsworth	DYNASOLVE 218	$243
Glucose Oxidase from Aspergillus niger,Type X-S, lyophilized	60kU	Sigma-Aldrich	G7141-50KU	$160.30

powder				
Edgeboard Connector	5	Digikey	151-1341-ND	$23.90

Chapter 6: Professional Issues and Constraints

6.1. Ethical

As this medical device continues to develop and potentially be implemented for commercial use, there are a few ethical considerations that we must account for. One of the more important considerations is the accessibility and affordability of the device. Current CGMs can be very costly and hard to access for families in a lower financial bracket, making it important to create a more affordable device that is easily accessible to all diabetic patients.

6.2. Health and Safety

One of the issues with current CGM devices is that they need to penetrate the subcutaneous layer of the skin, causing increased pain and swelling upon penetration. Our sensing device hopes to penetrate into the dermal layer, one level higher than the subcutaneous, in order to make life easier for users. Another factor to take into account is that the materials used to fabricate the device must be biocompatible since the device will be inside the skin for up to 14 days at a time. Research will need to be done to ensure that the device is not toxic and cause any further damage or irritation to the skin upon penetration. The needle array must also be sterilized before use to minimize the risk of infections. It is also important to make sure the users are not exposed to any dangerous current levels in case the device malfunctions.

6.3. Environmental Impact

The environmental impact of this device can be applied to fabrication and disposal methods. The use of 3D-printed needle arrays eliminates the waste of materials compared to other techniques that require polishing or edging. The material used for the printing of our needle arrays, Polylactic Acid (PLA), is derived from renewable resources and is biodegradable within 6-9 months.

For the print and fill method, silicone was used to create a negative mold of the 3D printed arrays. While silicone is not biodegradable, it is incredibly durable and can be used multiple times. For the final product, carboxymethyl cellulose was used. This material is a biodegradable polysaccharide that does not cause any harm to the environment.

For the print and shrink method, we use an agarose gel to create a negative mold of the 3D printed microneedle array. PDMS was used for the final product

6.4. Science, Technology, and Society

This device will give diabetic patients a more affordable, easy-to-use alternative for continuous glucose monitoring. The potential of this device can give patients, physicians, and researchers a better understanding of how diabetes affects each individual by providing more data for analysis. This should allow for physicians to prepare better treatments for their patients and minimize the amount of doctor appointments for the patient. Having the ability to continuously monitor glucose levels can give patients a better understanding of how their daily routines, exercise, and glucose levels are affected throughout the day. Furthermore, this device can improve healthcare systems in underdeveloped countries with little to no resources.

Chapter 7: Summary and Conclusions

7.1. Conclusions

In conclusion, our project focused on fabricating a glucose monitor that could continuously monitor one's glucose levels, which in turn can lead to better lives for diabetic patients. Allowing patients and physicians to track glucose levels throughout the day can lead to better treatments and lifestyle changes. We were able to fabricate a solid microneedle array using two different methods and implement a two-electrode system to measure various glucose levels. The fabrication of the needles has the potential to make continuous glucose monitoring more accessible, easy to use, and less invasive for diabetic patients.

7.2. Future Works

Although we were able to produce successful microneedle arrays, our procedure was inconsistent. Some possible causes for this were the 3D printed needle array dimensions and the agarose “sandwich” used during the shrinking process. We believe that by increasing the dimensions of the 3D printed arrays, the PDMS needles will be more defined and consistent.

Another area to work on is the fabrication of the electrode system. The carbon paint was very thin which made it difficult to apply and keep a conductive wire on the array. Researching a different carbon conductive paint would be the next step to optimizing the fabrication of the electrode system.

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