Development of Precise, Affordable Glucose Sensors

An Undergraduate Research Scholars Thesis

by

CHRISTOPHER EVAN WRIGHT

Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Jun Kameoka

May 2019

Major: Electrical Engineering

TABLE OF CONTENTS

ABSTRACT

Development of Precise, Affordable Glucose Sensors

Christopher Evan Wright
Department of Electrical and Computer Engineering
Texas A&M University

Research Advisor: Dr. Jun Kameoka
Department of Electrical and Computer Engineering
Texas A&M University

Diabetes mellitus is a chronic condition that affects millions of people around the world. This disease develops as a result of either disrupted insulin production (type 1) or altered insulin absorption (type 2), leading to increased blood glucose levels over long periods of time. High blood glucose levels lead to serious complications such as cardiovascular damage, nerve degeneration, and vision damage. As one of the most expensive diseases to treat, it is important to reduce the cost of detecting glucose levels as much as possible without sacrificing accuracy. It is common for patients with diabetes to utilize two classes of devices to monitor glucose levels: single use test strips and continuous glucose monitors.

To attempt to improve these devices, two devices were created which represent both classes of glucose sensors. The first was created using the method of molecular imprinting and is used as the single use test strips. Polyaniline (PANI) was used as the conducting polymer, soaked on a paper substrate. The second was fabricated using a similar technique also using PANI to create a flexible glucose sensing ink. Instead of using glucose as a template for MIP, GOx is immobilized in the PANI using its own oxidation reaction, allowing for the PANI monomers to form on any substrate that the ink is deposited on, rather than during the fabrication process.

ACKNOWLEDGEMENTS

I would like to thank Dr. Kameoka for his support and guidance throughout this research project and for being an amazing mentor in helping me meet my career goals. In addition, I would like to thank Onder Dincel, Zheyuan Chen, and Ting-Yen Chi for their advice during this project.

Furthermore, I would like to thank the Texas A&M faculty and staff for making my undergraduate experience truly excellent and for providing me with all the knowledge and tools that are necessary to advance my engineering career.

Finally, I would like to thank my family for all the amazing love and support they have given me throughout my undergraduate journey.

NOMENCLATURE

BG	Blood Glucose
CGM	Continuous Glucose Monitor
PANI	Polyaniline
GOx	Glucose Oxidase
APS	Ammonium Persulfate
HCl	Hydrochloric Acid
PE	Polyethylene
MIP	Molecular Imprinting
mg	Milligram
dL	Deciliter
L	Liter
Mol	Mole
DI	Distilled

CHAPTER I

INTRODUCTION

Millions of people worldwide are affected by diabetes mellitus. The most important metric of this disease is the patient's blood glucose (BG) levels, which can become elevated and cause severe complications if left uncontrolled. Therefore, each patient requires access to reliable sensors to collect data about what their BG level is at any given time. While commercial sensors are widely available, they are quite expensive for both insurance companies and for patients. The expensive nature is due to the high cost of fabrication, the materials used for the sensing enzyme, and the agents used to mask the sensor from the immune system. As more patients are diagnosed with conditions, such as type 1 diabetes, that require access to reliable glucose sensors, the need for less expensive sensors increases dramatically.

The goal of this project is to explore different ways of creating reliable sensors using highly repeatable and low-cost techniques. For many diabetes patients, there are two categories of sensors: single use test strips, and continuous glucose monitors (CGMs). Each type of sensor is explored and improved upon with affordability being the central goal of this project. Furthermore, a standard metric for identifying if the sensor is applicable for actual patient use was identified: "within 15% of reference value if ≥ 100 mg/dL or 15 mg/dL of reference value if ≤ 100 mg/dL." [1]

Glucose Enzyme Molecule

Nearly all glucose sensors on the market fall back on the same technology for sensing the BG levels in a sample. The sensors use an enzyme called glucose oxidase (GOx) to convert the concentration of glucose into an electrical signal. The GOx enzyme oxidizes the glucose

molecule into gluconic acid and hydrogen peroxide (H_2O_2) [2]. The in-situ reaction requires an input of an oxygen (O_2) molecule to donate electrons, but common sensors donate electrons from the negative electrode (anode). Equation 1 shows the oxidation reaction that is done by the GOx enzyme.

$$Glucose + O_2 \rightarrow Glucono\ delta\ lactone + H_2O_2$$

Equation 1: GOx enzyme reaction

When these electrons are donated, they are used up in the oxidation reaction to form gluconic acid and hydrogen peroxide. Therefore, less electrons are in the conduction band of the material that the GOx is bound to, altering the electrical characteristics of the material. As less electrons exist in the material's conduction band, the resistance increases. Therefore, a simple resistivity measurement can determine the glucose concentration in the solution.

GOx is a fabulous tool for determining BG levels in patients. However, simply using the enzyme is not enough. The difficulty lies in binding the GOx to a conductive material from which electrons can be donated. This process of embedding GOx in a conductive material is called immobilization. Methods for GOx immobilization is a primary research question for developing glucose sensors that are lower cost, more accurate, or novel ideas altogether [3].

Molecular Imprinting Technology

Molecular imprinting (MIP) is a technique that can potentially mimic the glucose binding site in GOx. The theory is that while a polymer is forming from the building of monomer groups, another molecule known as the template molecule, is inserted to become embedded in the polymer solution. Afterwards, the molecules that were on the surface of the polymer can be removed, forming a site that is the exact shape of the template molecule used.

This technology and process can also be used for glucose sensing. Glucose can be used as the template molecule in a conductive polymer synthesis technique. As for the conductive polymer, polyaniline (PANI) is a cheap, effective material that is commonly used in industry. Therefore, using PANI as the conductive polymer and glucose as a template molecule, MIP can be used to create a glucose [4]. Figure 1 graphically shows the theory behind the MIP process.

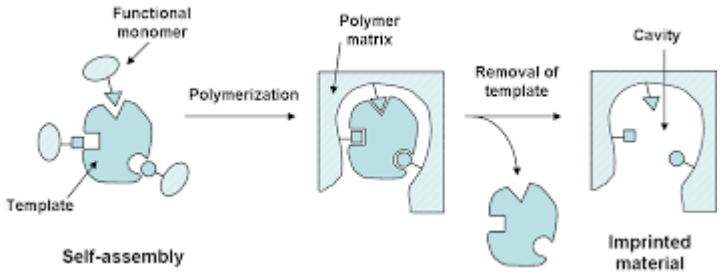

Figure 1: Molecular Imprinting Process [5]

Combining GOx and PANI

It is possible to combine the process of building the PANI molecule with a GOx sensor. During the polymerization process, GOx can be inserted in place of glucose as the template molecule. However, instead of removing the GOx after the PANI is formed, the GOx is immobilized in the PANI matrix.

Using this method, it is possible to use PANI as a conductive polymer for GOx immobilization. Furthermore, it is impossible to remove GOx from the matrix due to the size of the GOx molecule. Glucose can access and be oxidized by the enzyme, and the conductive PANI donates electrons to aid in this reaction.

Reusable MIP Test Strips

In this research project, MIP is used to create low-cost test strips for measuring the glucose concentration of a solution. The challenge of this research is creating a method which measures glucose precisely in a wide linear range. The range at which a patient with diabetes

mellitus might measure their BG is between 40-500 mg/dL or 2-30 mmol/L. A challenging aspect of this project is designing a sensor which linearly represents this range.

One problem with using PANI is that the material is not physically stable by itself if the dimensions are small. Therefore, the process is modified to include the addition of small paper strips to the solution. The PANI solution is soaked into the paper substrate, giving it increased physical stability while maintaining similar electrical characteristics. Then, electrodes are connected on either side of the paper that is soaked in PANI to measure the conductivity of the material, measuring the glucose concentration in the strips.

GOx Ink Continuous Glucose Monitor

The second device that this research has developed is a flexible ink that can be used to sense BG levels. This ink can be used in a variety of applications, such as smart tattoos embedded in a patient's skin.

To create this ink, the fabrication process of the MIP PANI sensor is modified. Instead of adding glucose as a template molecule, GOx is added to immobilize in the PANI solution. Paper strips are not added as a substrate for the material since the PANI will be either embedded in the skin or deposited on a material. The ink could also be soaked into another substrate if desired.

The challenge with creating the ink is that the PANI should not fully form until it is ready to be deposited on another surface. So, acidic reagents which bind the aniline monomers together should be omitted from the solution. Instead, the hydrogen peroxide product from the GOx enzyme's reaction can be used to build the polymer allowing the ink monomers to be linked together using a simple glucose solution [6]. After the ink is saturated and the PANI immobilizes the GOx, the sensor is ready to react to glucose solutions.

CHAPTER II

METHODS

MIP Sensor Fabrication

There are three groups of steps that describe how to fabricate the MIP sensor. The first two steps are the preparation of the monomer solution and the oxidant solution. These two steps can be done in parallel before the third step, as shown in Figure 2 below. The total volumes of each of the solutions are kept equal to ensure a consistent amount of PANI is created. The third step is the combination of the two solutions, and the preparation of the strips.

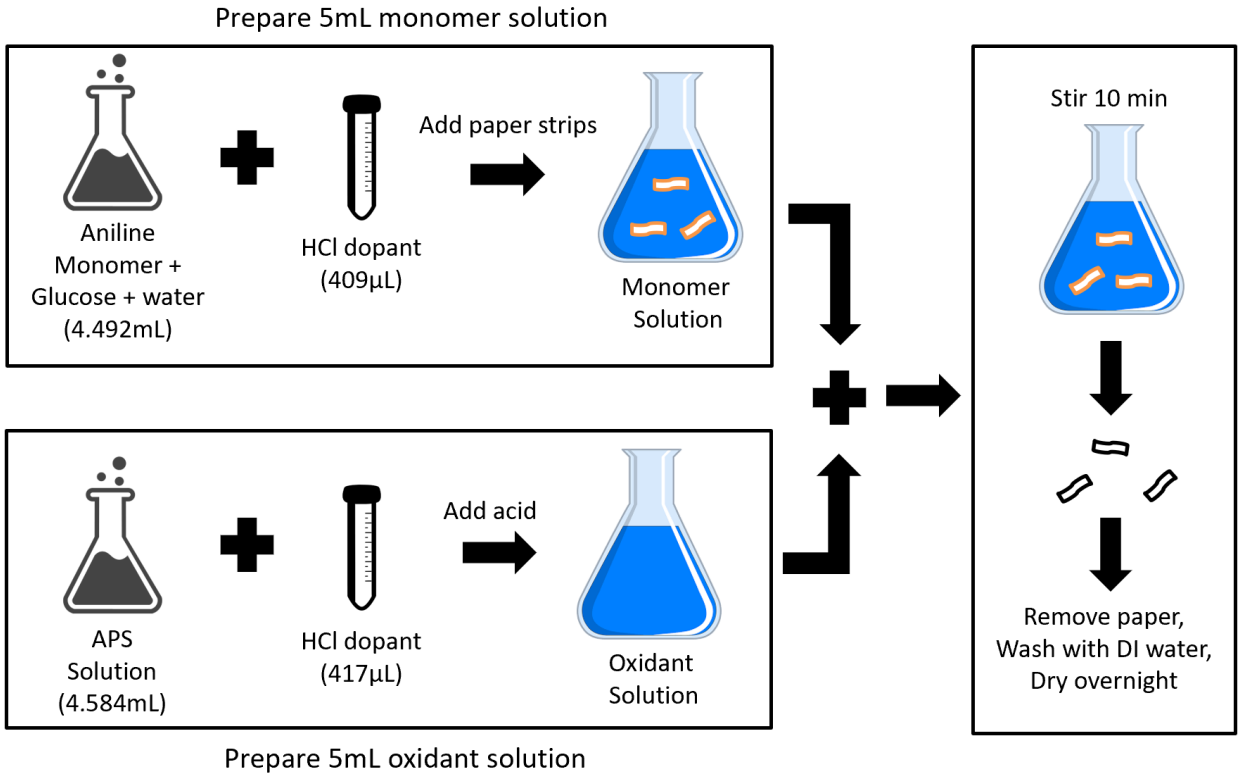

Figure 2: MIP Sensor Fabrication Process

Preparation of the Monomer Solution

The monomer solution was prepared using 0.1mL of 99.5% aniline solution, 0.409mL of 30M hydrochloric acid (HCl) solution, and 4.92mL of DI water to create a total of 5mL monomer solution.

Preparation of the Oxidant Solution

The oxidant solution was prepared using 0.4g ammonium persulfate (APS), 0.417mL of 30M HCl solution, and 4.584mL of DI water to create 5mL of oxidant solution.

Combination of Solutions and Sensor Preparation

Polyethylene (PE) paper strips are added to the monomer solution to be soaked. The solution with PE strips were stirred using a vortex mixer for 5 minutes. Now, the aniline is ready to be synthesized. The solution begins as a clear liquid and will change colors to indicate the synthesis of PANI.

The oxidant solution is added drop by drop, with 10 seconds between drops. This is done while the monomer solution is being stirred with the vortex mixer. When the solution changes color to a dark blue, the addition of the oxidant is ceased, and the solution is stirred for 5 minutes.

Finally, the PE paper strips are removed from the solution, and are washed off with DI water. The strips are left to dry overnight and are ready for testing the next day.

Testing the MIP Strips

To test the strip's response to glucose, the strip was attached to copper tape using a silver nanoparticle paste deposited by brush. The copper tape allowed for measurement of the resistance across the strip without damaging the substrate. Glucose solutions of various concentrations were added, and the measured resistance was recorded.

GOx Ink Sensor Fabrication

Preparation of the PANI Solution

To prepare the glucose sensing ink, a simple solution of 0.25mL aniline solution and 20mg of GOx enzymes are added to create 5mL of total solution, using DI water as the solvent. Next, the solution is deposited on a porous surface to provide a solid substrate for the ink to be bound. To ensure total absorption of the ink, PE paper strips were soaked in the solution in a similar fashion to the MIP sensor.

Ink Saturation

Now, the aniline is synthesized on the paper substrate. Several drops of 20% saturated glucose solution were deposited on the strip to completely cover the exposed area. The paper strip can now be used as a glucose sensor, as shown in Figure 3.

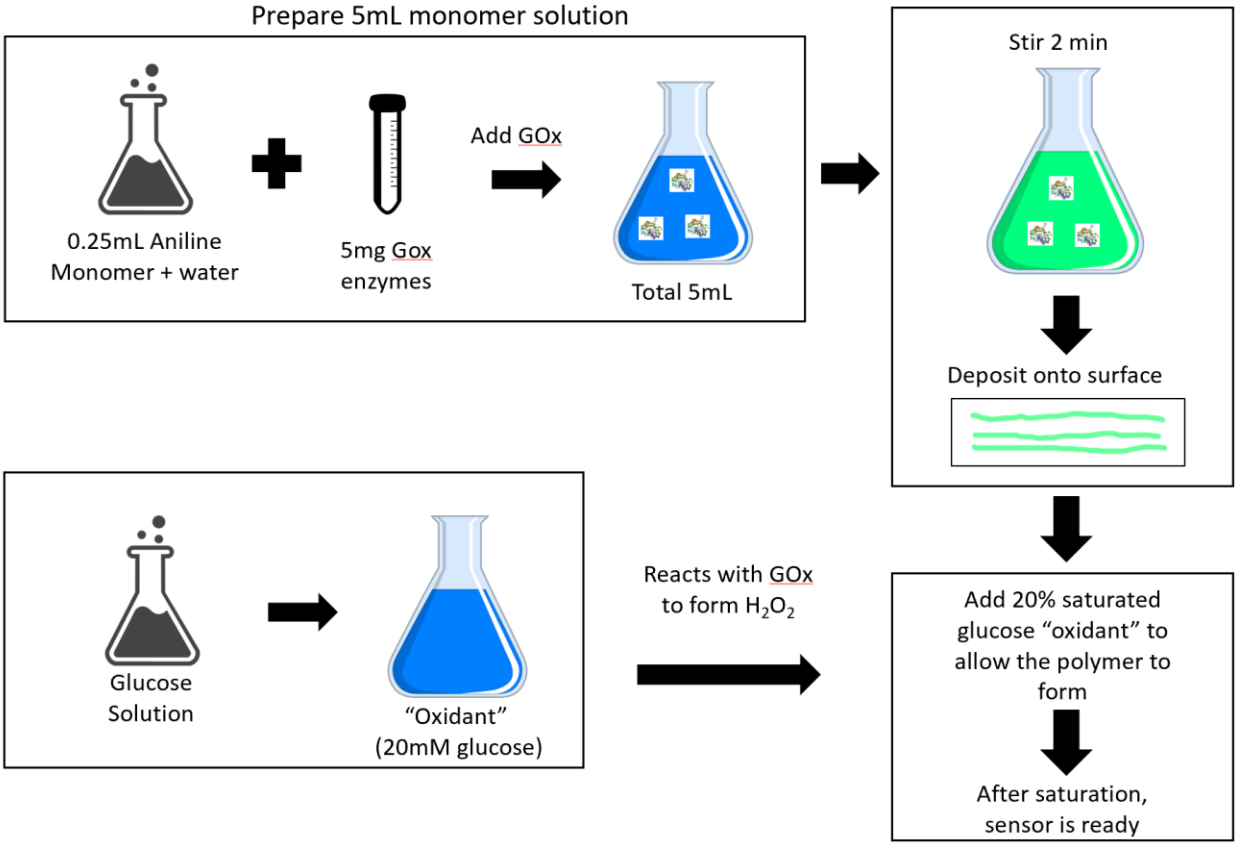

Figure 3: GOx Ink Sensor Fabrication Process

CHAPTER III

RESULTS

MIP Test Strips

Paper strips which accurately measure the glucose levels were successfully fabricated. These strips turned a green-black color, indicating that the PANI was synthesized on the surface of the strips.

Concentrations from 0-13 mmol/L were tested on the strips. Five total trials were taken with two different sets of fabricated sensors. Figure 4 below shows the results of the data.

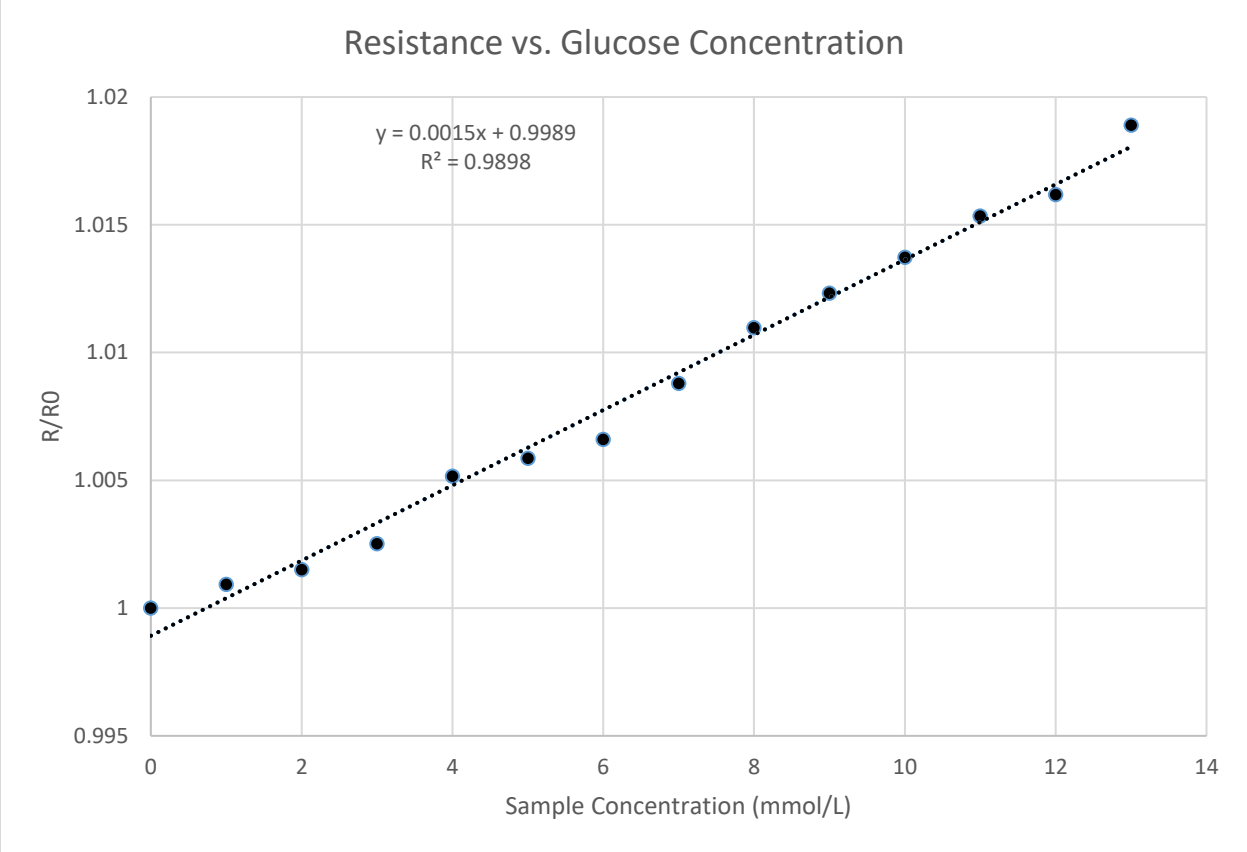

Figure 4: Graphical results of testing the MIP test strip sensors

The graph was obtained by first acquiring the percentage change of the resistance after the sample was added to the strip. Next, this percentage was normalized to the change when a water sample was added.

Future improvements could include comparing how much the PANI is doped versus the quality of the results. Comparing how much glucose is added to the strips versus the linear range would also help determine how much template glucose should be used. In future work, averaging a very large sample set of sensors would help better characterize the calibration curve.

GOx Ink Sensor

Results for this method could not be obtained, despite several changes to the experiment. The ink took several days to synthesize, which is undesirable for either patient deposition or paper fabrication. Also, the sensors that were successfully fabricated on paper strips were very unstable, so reliable measurements could not be obtained due to high capacitance of the sensor.

One possible downfall of my method was the pH level of the ambient solution. This can affect the amount of the reaction that is catalyzed, resulting in a very slow rate of production of the oxidant for PANI synthesis. This low rate can lead to failure of PANI synthesis.

To correct the pH level and facilitate a better environment for hydrogen peroxide production by GOx, less acidic dopants can be used, or different solvents could be explored. The bottom line for this method to work is that PANI must be synthesized. So, the optimal conditions for this should be met that produce hydrogen peroxide at an acceptable rate. Moreover, the enzyme is very sensitive to temperature, which can affect the hydrogen peroxide production rate as well. Future work in creating the GOx ink sensor should focus on creating the environment for the enzyme to produce the optimal amount of oxidant at an acceptable rate. Possible resources for this could be companies that currently fabricate the sensors that are used on the market.

CHAPTER IV

CONCLUSION

The average cost to produce a commercial BG test strip is 16 cents per strip. Patients with type 1 diabetes mellitus use an average of 8 test strips per day, amounting to $1.28 per day, or $467.20 per year, per patient [7]. Table 1 below shows the breakdown of the cost to use this process. Using this table, the total cost of producing one MIP test strip is 5.9 cents per strip. Using these test strips instead of the common commercial options costs a mere $172.28 per year. That amounts to a savings of $294.92 per year. Considering that 1.25 million people are affected by this disease in the United States, the potential savings to the healthcare system is upwards of $368 million [8].

Table 1: Breakdown of the costs to fabricate each strip.

Material	**Amount**	**Cost**	**Amount Used***	**Cost Per Strip**
Aniline	500mL	$90.90	0.0625mL	$0.01136
APS	500g	$149.00	0.10225g	$0.00762
HCl	500mL	$50.50	0.2065mL	$0.02086
Distilled Water	4L	$39.40	2.5mL	$0.01539
Glucose	100g	$30.50	0.0125g	$0.00381

Note: *Amount used is per set of sensors, which is 4 in this case.

Adding all values in the rightmost column, the total cost of to fabricate each strip is $0.059. These results show that using MIP to fabricate a low-cost glucose test strip is possible, and the widespread adoption of this method can result in massive savings. Using the method described in this document also costs much more than using bulk materials. Potentially, the cost

per strip could be as low as one cent per strip, compounding the savings that could be observed. The data shows that there is no loss in precision when using these sensors, so there is a high potential for commercial use.

The glucose sensing ink has great potential for use, but there are many details to be worked on. Aniline is a cytotoxic and harmful material to humans if it enters the body. Therefore, methods to safely deposit the ink on the skin without entering the bloodstream are necessary. The difficulty in this is that the ink should have access to the blood to sense glucose levels, but the ink can cause damage to blood cells. However, the ink could still be used to fabricate test strips or be used in continuous glucose monitors if the ink does not penetrate the skin.

Advances in glucose sensing technology can make diabetes mellitus a much more manageable disease. With access to much cheaper sensors, more patients can afford to take measurements more often. Overall, this can lead to much higher quality of life and allow the money to be spent on research for a cure.

REFERENCES

1 . Klonoff, David C., MD, FACP, FRCP, and Bbb. "BLOOD GLUCOSE MONITORING SYSTEM SURVEILLANCE PROGRAM." Diabetes Technology Society. June 23, 2017. Accessed March 04, 2019. https://www.diabetestechnology.org/surveillance.shtml.

2. "Glucose Oxidase." Glucose Oxidase - an Overview | ScienceDirect Topics. Accessed April 01, 2019. https://www.sciencedirect.com/topics/neuroscience/glucose-oxidase.

3. House, Jody L., Ellen M. Anderson, and W. Kenneth Ward. "Immobilization Techniques to Avoid Enzyme Loss from Oxidase-based Biosensors: A One-year Study." Journal of Diabetes Science and Technology. January 2007. Accessed April 01, 2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2769612/.

4. Farid, Mohammad Masoudi, Leila Goudini, Farideh Piri, Abbasali Zamani, and Fariba Saadati. "Molecular Imprinting Method for Fabricating Novel Glucose Sensor: Polyvinyl Acetate Electrode Reinforced by MnO2/CuO Loaded on Graphene Oxide Nanoparticles." Food Chemistry. July 29, 2015. Accessed April 01, 2019. https://www.sciencedirect.com/science/article/pii/S0308814615011620.

5. Satanaka, *Molecular Imprinting*. 2006. 1,159 x 449 pixels. https://commons.wikimedia.org/wiki/File:Molecular_imprinting.png.

6. Sun, Zaicheng, Yanhou Geng, Ji Li, Xiabin Jing, and Fosong Wang. "Chemical Polymerization of Aniline with Hydrogen Peroxide as Oxidant." Synthetic Metals 84, no. 1-3 (1997): 99-100. doi:10.1016/s0379-6779(96)03855-6.

7. Spero, David, BSN, RN. "Why Do Test Strips Cost So Much?" Diabetes Self-Management. September 18, 2013. Accessed March 04, 2019. https://www.diabetesselfmanagement.com/blog/why-do-test-strips-cost-so-much/.

8. "Type 1 Diabetes." American Diabetes Association. Accessed April 01, 2019. http://www.diabetes.org/diabetes-basics/type-1/.