April 2015

Continuous Glucose Monitoring using Titanium Dioxide Biosensors

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Continuous Glucose Monitoring using Titanium Dioxide Biosensors

A Major Qualifying Project:
Submitted to the faculty of
Worcester Polytechnic Institute
In partial fulfillment of the requirements for the
Bachelor of Science Degree

Submitted by:

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Date: April 2, 2015

Submitted to Project Advisor:

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Professor Hong Susan Zhou
Abstract

Glioblastoma Multiforme is a form of intracranial tumor that while only affecting 2-3 people per 100,000 per year, has an extremely high death rate with only 30% of patients surviving after two years. While early detection can extend this survival rate, GBM tumors mimic other complications and make it very difficult to detect via magnetic resonance imaging or computed topography scans. Therefore, an alternative detection method has been developed by taking advantage of the cancer cell’s altered metabolism. This mutated metabolism results in GBM patients having abnormally high lactate and low glucose levels.

One method of continuously monitoring the low glucose levels of patients is through a biosensor. This study looks to observe the effectiveness of a titanium dioxide electrode with copper nanoparticles deposited on the surface. Nine electrodes were synthesized from small pieces of titanium foil to be used for experimentation. Using cyclic voltammetry in various electrolyte solutions, the electrode’s effectiveness at detecting glucose was analyzed.

Acknowledgements

First and foremost, I would like to thank Professor Hong Susan Zhou for providing me with this research opportunity and for providing guidance throughout the project. I would also like to thank Zanzan Zhu for making this project possible by providing very valuable help throughout my work in the lab whenever I needed it and for guiding me through my experiments. Finally, I would like to thank Worcester Polytechnic Institute for the opportunity to participate in a research project of this caliber.
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1. Introduction

Glucose is one of the most essential carbohydrates in humans, being one of the main energy sources in the body and a precursor to countless other important substances, such as starch and cellulose. As essential as it is in providing life in humans, it can also be a key component in detecting or monitoring certain diseases, such as the widely spread diabetes. Glucose levels can also be used in monitoring tumor levels, especially in the very rare, but very deadly form of brain cancer known as Glioblastoma Multiforme, or GBM. GBM is a Grade IV astrocytoma that originates in the star-shaped cerebrum cells called astrocytes that affects 2-3 people per 100,000 per years with a 75% death rate within the first eighteen months (Stark, 2005). Treatment of GBM usually ranges from radiotherapy to total removal of the tumor followed by treatment with a chemotherapy drug.

It is essential that these tumors are identified as early as possible in order to increase survival rate. However, the most common forms of tumor recognition, magnetic resonance imaging and computed topography scans, may not always identify GBM due to how well the tumor mimics other complications; therefore, the patient may lose valuable time in detecting the tumor which may inevitably cost them their lives.

However, tumor cells have an altered metabolism that results in the body having abnormally high lactate and low glucose levels. This leads to an alternative way to detect GBM tumors—glucose monitoring. One form of continuous glucose monitoring is through the use of a biosensor. This MQP will study the effectiveness of using a titanium dioxide electrode with copper nanoparticles as a non-enzymatic biosensor for the detection of glucose. In order to test efficiency, cyclic voltammetric analyses will be performed under various conditions to determine how well the electrode can detect glucose levels.
2. Background Chapter

The purpose of this chapter is to further examine the role of glucose in the body and its role in diseases, especially the brain tumor, Glioblastoma Multiforme. Also discussed in this chapter are different methods of monitoring a person’s metabolite levels, both inside and outside of the body, including biosensors devices. Specifically, the focus of this work is on the use of titanium dioxide biosensors implanted directly into the body in order to monitor glucose levels in a patient.

2.1 Glucose

A carbohydrate, or a saccharide, is a biological compound that consists of carbon, hydrogen, and oxygen. Carbohydrates are categorized by their complexity (a simple one chain monosaccharide versus a large polysaccharide), reactivity (reducing or oxidizing), number of carbons, and their carbonyl grouping. The most common carbohydrate is glucose, a simple sugar with the chemical formula of C\textsubscript{6}H\textsubscript{12}O\textsubscript{6} found widely in both plants and animals. Fitting the previous categories, glucose is a simple monosaccharide, an aldohexose (or a six-carbon molecule containing an aldehyde group), and a reducing sugar (Reusch, 2013). Glucose exists in humans through three primary natural sources. The first is the consumption of plants, which use sunlight to convert water and carbon dioxide to glucose and oxygen through photosynthesis. The second is the breakdown of glycogen, a polysaccharide of glucose that is used as the main storage of glucose in the body. And the final method is through gluconeogenesis in the liver that creates glucose from non-carbohydrate sources when the supply of glycogen is consumed and glucose is not being provided through diet.
2.1.1 Role of Glucose in the Body

Glucose is the main energy source for the brain and nervous system, being the main substrate in cells’ energy production cycles, such as the tricarboxylic acid cycle (also known as the Krebs cycle or the citric acid cycle) and the mitochondrial electron transport chain. Through glycolysis, the body takes glucose and breaks it down to pyruvate and coenzymes used for energy (such adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH)). Pyruvate is further broken down in the Krebs cycle to release more energy coenzymes to be converted to ATP in the mitochondrial electron transfer chain.

Glucose also plays a role in synthesizing several other carbohydrates and important substances. In most animals, glucose is a pre-cursor to ascorbic acid, or vitamin C. Glucose can also bind to proteins or lipids in order to form glycoproteins or glycolipids. Adding glucose to these structures assist in folding to increase stability or can act as recognition sites (New World Encyclopedia, 2008). Also, the linkage glucose is essential in synthesizing other complex carbohydrates.

2.1.2 Problems Caused by Glucose

While glucose is essential to human life, many complications can be attributed to glucose levels in the body. The most well-known disease caused by glucose levels is hyperglycemia, which is an excessive amount of glucose circulating in the blood stream. Hyperglycemia is most common in diabetes mellitus (DM), where the glucose level is high due to the lack of inulin being produced in the body (Type 1 DM) or the cells do not properly react to the insulin being produced (Type 2 DM). According to the American Diabetes Association, a person with a resting glucose concentration consistently in the range of 110-126 mg/dL (5.6-7 mmol/L), as opposed to
the healthy adult rest rate of 70-110 mg/dL (3.9-5.6 mmol/L), is considered hyperglycemic while a person over 126 mg/dL is considered diabetic (ADA, 2003).

On the opposite hand, hypoglycemia occurs when the body experiences a shortage in glucose and consistently has a resting concentration level below 70 mg/dL (Chase, “Low Blood Sugar”). Since the brain heavily relies on a steady income of glucose, a slight reduction in mental effectiveness can be observed as soon as a person reaches the hypoglycemic range. Prolonged hypoglycemia can result in neuroglycopenia, where the neurons in the brain become nonfunctional due to the lack of glucose.

Other diseases have been attributed to glucose that do not necessarily come from the levels of glucose. One example is von Gierke disease, or glycogen storage type I disease, where once glucose is converted to glycogen, it cannot be converted back for energy consumption, resulting in a buildup of glycogen in the liver and a shortage of glucose in the body (Haldeman-Englert, 2013). Most of the previous conditions mentioned, can be counteracted by monitoring the amount of glucose in a person’s diet. However, monitoring glucose levels can also be beneficial to observing phenomena that occurs in the body, such as a cell metabolizing more glucose than normal—one common trait of malignant cancer cells, specifically in Glioblastoma Multiforme.

2.2 Glioblastoma Multiforme

Glioblastoma Multiforme (GBM) is a form of intracranial tumor that is rare amongst people, but has rapid progression, a low survival rate, and mostly unknown etiology. GBM is a Grade IV astrocytoma that originates in the star-shaped cerebrum cells called astrocytes that affects 2-3 people per 100,000 per year with a 75% death rate within the first eighteen months (Stark, 2005). In a study in 2013, observing 1645 cases of GBM, 98.4% of the patients, mostly
males between the ages of 55-64, died. These cases included multiple different types of treatments from radiotherapy to gross total removal of the tumor (Dubrow, 2013). The standard treatment of GBM is initial radiotherapy, followed by gross total resection (often omitted in patients older than 70 years) followed by treatment with the alkylating oral chemotherapy drug temozolomide (Bleeker, 2012). Studies show that primary GBM tumors often recur within two centimeters of its original size after radiotherapy in 80-90% of patients. The introduction of temozolomide as a treatment in 2005 has increased overall survival from 12.1 to 14.6 months and increased the likelihood of two-year-survival from 10% to 26% (Bruce, 2014). As with most tumors, treatment is most effective when detected early. However, this is difficult due to the fact that GBM can only be identified by clinical neuroimaging and the tumor manifests with little evidence of precursors. A secondary form of the GBM tumor that arises from lower-grade gliomas, but these are less common, less aggressive, and appear mostly in younger patients.

2.2.1 Detecting Glioblastoma Multiforme

Typically, patients with GBM present symptoms in some or all of three categories: focal neurological deficit, increased cranial pressure, and seizures. Focal neurological deficits are problems with brain, spinal, or nerve function that affects a specific location on the body, as opposed to a general nonfocal problem (i.e. loss of consciousness) (Dugdale, “Focal neurological deficits”). Focal neurological deficits can be demonstrated in paralysis of limbs or facial movements, loss of muscle control, or changes in mood, speech, hearing, and vision. Once these symptoms occur, the GBM tumor can only be detected through neurological imaging.

Magnetic resonance imaging (MRI) and computed topography (CT) scanning are the most common ways to identify GBMs, but do not always work. As stated before, GBM tumors have little evident malignant precursors and can form in as little as three months. Because of this,
CT scans may miss small tumors if the scan is conducted in the early ages of GBM growth. MRI scans are more adept at identifying small tumors; however, GBM tumors can often mimic other complications, such as abscess, and can go undetected and prolong recognition (Bruce, 2014). These flaws in MRI and CT scanning can cause a patient to lose vital time in diagnosing their condition. Therefore, it is essential to either improve these methods or to introduce a new form of imaging.

2.3 Glioblastoma Multiforme Tumor Cells

In GBM tumor cells, several growth factor pathways are altered from normal brain cells. In 2010, GBM cancer cells were separated into four subtypes based on what gene was mutated: classical, mesenchymal, proneural, and neural. In classical GBM tumors, high levels of the surface protein EGFR (epidermal growth factor receptor) are found, which causes the cell to divide more rapidly. The tumor suppressor gene, NF1, is most commonly mutated in mesenchymal tumors and proneural tumors have mutated TP53 and IDH1 genes, which result in abnormal cell growth. The fourth subtype of GBM tumor, neural, had no one mutation that stood out more than the rest. These types also react differently to treatments, with classical having the highest survival rate after aggressive treatment, and proneural having roughly the same survival rate between aggressive and nonaggressive treatments (Evans, 2011). This information is vital to treating GBM, because it allows clinicians to know more about how to specifically treat this case and what to look for when identifying the tumor.

2.3.1 Growth Factor Levels in GBM Cells

In GBM cancer cells, high levels of growth factors are observed. For example, the increased production of EGFR in classical GBM tumors can be monitored by the level of the growth factor, EGF, which binds to EGFR in order to promote cell division. Other growth factors
such as PDGF (platelet derived growth factor), VEGF (vascular endothelial growth factor), and IGF (insulin-like growth factor) are also found to be abnormally high in malignant GBM cells (ABTA, 2014). This is important in initially detecting a GBM tumor in a patient. If a growth factor blood test shows high amounts of EGF or the other growth factors, then the patient can be recommended for neurological imaging in order to be certain of the tumor.

2.3.2 Metabolism in Healthy Cells

One of the most crucial characteristics of all cancer cells, GBM tumors included, is that they do not follow the same cellular metabolism as normal cells. As stated briefly in Chapter 2.1.1, in healthy cells, glucose is converted to pyruvate through glycolysis, releasing two ATP and two NADH molecules. From here, the path of pyruvate is dependent on the amount of oxygen available. If oxygen is present, the cell undergoes respiration where pyruvate is converted to acetyl-CoA and acetyl-CoA enters the Krebs cycle which produces more energy cofactors (ATP, NADH, and reduced flavin adenine dinucleotide, FADH$_2$) that enter the mitochondria for oxidative phosphorylation, ultimately producing an additional 34 ATP molecules. Since oxygen is vital in oxidative phosphorylation, this process cannot occur in anaerobic conditions. Healthy cells will take the pyruvate formed from glycolysis and convert it to lactate (or alcohol in plants and yeast) through fermentation. Fermentation produces a molecule of oxidized NAD which is recycled into the glycolysis stream, but no additional ATP (Gregory, “Cellular Respiration”). These metabolic pathways are shown in Figure 1.
Overall, in healthy cells, both pathways begin with glycolysis and 36 molecules of ATP are produced in the presence of oxygen and 2 molecules are produced in the absence of oxygen.

2.3.3 Metabolism in Cancerous Cells

Cancerous cells do not follow the same pathways as normal, healthy cells. Whether the tumor cell is in oxygen-rich or oxygen-deficient environments, it only follows fermentation pathway, as shown in Figure 2.
This removal of the normal aerobic pathway is the primary cause of cancer. Lactic acid fermentation is much less energetically efficient than aerobic respiration. Instead of the 36 ATP normally produced in oxygen-rich environments, only 2 ATP will be produced. However, lactic acid fermentation occurs about 100 times faster than oxidative phosphorylation and, consequentially, results in an abundancy of glycolysis byproducts. These byproducts can then be used in other biosynthesis pathways responsible for producing the building blocks necessary for assembling new cells, resulting in the rapid reproduction of cancerous cells (Wickramasekera, “Cancer Cell Metabolism”). As stated by in a lecture by Dr. Otto Warburg, “cancer…has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar…” (Warburg, 1956). This rapid rate of glycolysis and absence of oxidative phosphorylation is known as the Warburg effect.
Since lactic fermentation occurs much more rapidly than oxidative phosphorylation, glucose is consumed and lactate is produced much quicker in cancerous cells than in healthy cells. This difference becomes greater depending on the malignancy of the tumor, as shown in Figure 3.

![Figure 3: Glucose consumption in tumors of varying degrees of malignancy (Carter, “Microbial Metabolism”).](image_url)

Since Glioblastoma Multiforme is a grade IV tumor, it is considered extremely malignant. Therefore, GBM cells would be able to be detected in patients who exhibit the symptoms mentioned in Chapter 2.2.1, the growth factor levels mentioned in Chapter 2.3.1, and most importantly, the low glucose and high lactate levels discussed in this chapter.
2.3.4 Glucose and Lactate Levels in GBM Cells

The large difference in glucose and lactate levels between healthy and cancerous cells can be vital in identifying and treating patients with GBM. As stated in Chapter 2.1.2, the normal resting level of glucose is in the range of 3.9-5.6 mmol/L, while the normal resting level of lactate is in the range of 0.5-2.2 mmol/L (Dugdale, “Lactic acid test”). Patients with GBM tumors can have lactate levels as high as 40 mmol/L (Hirschhaeuser, 2011) and glucose levels between 1 and 2 mmol/L (Jiao, 2014). Since glucose is the prerequisite in lactic acid fermentation, it is especially vital in monitoring GBM tumors. Glucose can be controlled through diet and the effect of low-glucose diets has become a key focal point of GBM treatment (Jelluma, 2006; Champ, 2014). In the Jelluma et al. study, it was concluded that while glucose withdrawal usually results in lowered ATP production in most astrocytes, it actually increased ATP production in GBM patients. Overall, monitoring glucose levels can be very effective at monitoring the progression of the tumor.

2.4 Monitoring Glucose Levels in the Body

Due to the fact that high glucose levels accelerate the progression of glioblastoma growth, it is imperative that patients monitor their glucose levels. Since diabetes is such a widespread condition that also requires constant glucose monitoring, there are already many ways to go about this task. One method involves pricking one’s fingertip with a sharp needle and placing a drop of blood on a test strip. The test strip is then placed into a meter which gives results quickly. Newer meters allow the patient to use other test sites, such as one’s thigh, forearm, or upper arm. However, these areas are not as accurate as the fingertip, which shows changes more quickly—especially when levels are fluctuating such as during exercise or after eating. The newest method removes the need to continuously perform self-testing. Continuous glucose
monitoring (CGM) provides a nearly constant glucose level reading by monitoring the glucose concentration in the interstitial fluid between cells and sending the results to a handheld receiver. While CGM devices still need to be calibrated occasional through finger-sticking, it greatly reduces the hassle of monitoring glucose levels by letting the person just periodically glance at a device rather than perform a test (DHC, “How and When…”).

2.4.1 Problems in CGM

A CGM device consists of three parts: the monitor, the transmitter, and the sensor. The monitor displays the readout, the sensor is inserted into the tissue beneath the skin, and the transmitter relays the information from the sensor to the readout. One problem with CGM devices is the tradeoff between high sensitivity and high specificity. Highly sensitive devices sound an alarm every time the glucose level is outside of the normal range. This is effective at ensuring the safety of the patient, but can often have false alarms. Highly specific devices have fewer false alarms, but can overlook some important occurrences (Yee, 2010). Therefore, manufacturers need to choose between tradeoffs when designing the device.

2.4.2 Biosensors

The sensor in CGM devices is an example of a glucose biosensor. A biosensor is a compact analytical device made up of a transducer and a biological element that interacts with the desired analyte and relays the response via an electrical signal. The biological element can be an enzyme, a nucleotide, a protein, etc. and the transducer works in a physiochemical way to convert, or transduce, the signal resulting from the analyte interaction to a signal that can be quantified (Robertson, 2014). This concept is illustrated in Figure 4.
The biological element of the biosensor recognizes and interacts with the desired analyte. The transducer then takes the signal received from the interaction (heat change, electron movement, etc.) and converts it to a measurable form.

### 2.4.3 Glucose Biosensors

Leland C. Clark is credited as the “father of biosensors” by publishing a paper on his oxygen electrode in 1956. The Clark electrode, as it was named, measured the activity of oxygen on a catalytic platinum surface. Clark wanted to find a way to make his electrode “more intelligent” by expanding its use to measuring analytes inside the body (Setford, 2005). In 1962, Clark added an inner oxygen semipermeable membrane, a thin layer or glucose oxidase (GOx), and an outer dialysis membrane. Using this “enzyme electrode,” as it was named, Clark found that the decrease in oxygen measured was proportional to the glucose concentration (Yoo, 2010).

The basic concept of glucose biosensors is that the GOx on the enzyme (along with a redox cofactor acting as the initial electron acceptor) catalyzes the oxidation of glucose by the following reactions:

\[
\text{Glucose} + \text{GOx(FAD)} \rightarrow \text{Gluconolactone} + \text{GOx(FADH}_2) \tag{1}
\]
\[ \text{GOx(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOx(FAD)} + \text{H}_2\text{O}_2 \] (2)

The hydrogen peroxide then reacts with the platinum catalyst anode on the electrode, which releases two electrons. The sensor then uses the total electron flow to determine the glucose concentration. Succeeding glucose biosensors that followed this principle faced two main problems—restricted oxygen solubility in biological fluids and other species, such as ascorbic acid or uric acid, interfering with the electrons. In order to counteract the problems, “second-generation” glucose biosensors changed to replace oxygen with redox mediators, such as ferrocenes, quinines, or thionines. These mediators formed reduced mediators other than hydrogen peroxide. Overall, the same general principle applied to both generations, but second generation sensors removed the problems associated with oxygen and hydrogen peroxide. Today’s biosensors are referred to as “third-generation” sensors and are attempting to become reagentless (to avoid high toxicity mediators) and directly transfer electrons from the enzyme to the electrode (Yoo, 2010). Due to the reduced toxicity, some of these biosensors can be implantable, such as the CGM devices previously mentioned.

2.5 Implanted Glucose Biosensors

It has been generally described how biosensors interact with analytes in order to provide the necessary data; however, the materials used in the biosensors are vital to the success of the analyte detection—especially for biosensors implanted directly into the body. Since the discovery of biosensors, there has been little success in long-term implantable sensors. Overall, the main complications in long term use are: the body’s response to foreign bodies, the degradative effect of the proteases in the extracellular matrix on the biosensors, enzyme denaturation, changes in the membrane’s properties (such as density) due to time and temperature changes, and material failures (Kotanen, 2012).
2.5.1 Immune Response to Implanted Biosensors

Implantation of these devices cause the body’s immune system to react through inflammation, blood clotting, and tissue contracting around the sensor. The body also responds by sending macrophages, platelets, and other defense mechanisms to the infection site that encapsulates and degrades the enzyme—this is known as biofouling. Biofouling serves as the main cause of the biosensor’s loss in sensitivity. Biofouling can cause the biosensor to see a 50-80% decrease in sensitivity. The biofouling process is illustrated in Figure 5.

![Figure 5: Biofouling progress on the biosensor due to the body's immune system (Yun, 2009).](image)

The immune system’s first response is to send proteins, phospholipids, peptides, etc. to surround the biosensor. These proteins then interact with the cell, which produces tissue such as hemoglobin, albumin, and immunoglobulin to encapsulate the biosensor and prepare for attack of the phagocytic cells to degrade the biosensor (Yun, 2009).

2.5.2 Problems with Enzymatic Biosensors

In enzyme-based biosensors, enzyme degradation poses as a huge threat to the success of the biosensor. Long term stability is one of the biggest problems with enzymatic glucose sensors, which, as stated before, typically use the enzyme glucose oxidase. While glucose oxidase is
relatively stable compared to most enzymes, it still faces the same problems—constant exposure to thermal and chemical deformation, changes in temperature, pH, and toxic chemicals, and changes in humidity levels. It has been observed that glucose oxidase loses its catalytic activity outside of the pH range of 2-8 or temperatures above 40°C (Park, 2006). While the normal pH level and temperature of the human body is 7.5 and 37°C, it can sometimes spike resulting in an environment that will denature glucose oxidase, ruining the biosensor’s efficiency.

Another problem with enzymatic biosensors is the difficulty in production. In order to attempt to preserve the enzyme’s catalytic ability for as long as possible, multiple enzyme layers must be placed on the electrode in a carefully optimized method called enzyme immobilization (Park, 2006). The quality of the sensors depends on the immobilization of the enzyme and therefore it is a difficult procedure to ensure the reproducibility of each biosensor. This is a great challenge, especially in mass manufacturing of the device.

A final problem with enzymatic biosensors is the presence of oxygen. Oxygen competes as an electron-mediating site and can affect the activity of the sensors (Park, 2006). Some enzymatic devices have attempted to solve this problem by having the electron transfer occur directly on the electrode surface (third generation biosensors).

2.5.3 Non-enzymatic Biosensors

In order to combat the problems associated with enzymes, non-enzymatic catalysts have been the focus of recent studies. Examples of non-enzymatic catalysts used in studies are metals such gold, platinum, and copper or polymer films such as polypyrrole (Cui, 2007; Ozcan, 2008). Each of these materials shows electrocatalytic activity towards the oxidation of glucose without the use of an enzyme.
However, while these approaches solve the problems faced by enzymatic devices, they are not completely problem-free on their own. One of the biggest issues with using metals, as opposed to enzymes, as an electrode is the occurrence of surface poisoning. While platinum was found to have one of the highest catalytic activities towards glucose oxidation, it is very susceptible to self-poisoning due to the absorption of carbon monoxide, chlorides, or other intermediates on the surface of the catalyst (Jin, 2007). In order to improve self-poisoning resistance, platinum was modified with heavy metals such as lead, bismuth, or tungsten trioxide (Kokkinidis, 1984; Wittstock, 1998; Zhang, 1997). However, this now introduces the problem of dissolution and toxins associated with these heavy metals which makes it impractical for \textit{in vivo} usage. Heavy metal toxicity is a serious problem where the heavy metals interact with oxygen in the body and can affect normal enzymatic activity, which can affect nearly every organ system (Adal, 2014). Therefore, tradeoffs between catalytic activity and the ability to be used in the body must be considered.

\textbf{2.5.3 Titanium as a Glucose Biosensor}

Titanium has been used in surgeries since the 1950s and is currently one of the most widely used metals in surgical implants. This is due to its high strength, low elastic modulus (very similar to that of actual bone) and, most importantly to implant use, high corrosion resistance and tissue compatibility (Wang, 1996). Also, since titanium is non-ferromagnetic, patients with titanium implants can still undergo neuroimaging such as MRI (NASS, “Magnetic Resonance Imaging”). This is imperative for patients with GMB who need to frequently monitor tumor growth. Due to all of these reasons, titanium is an ideal candidate for use as an implantable biosensor.
Many studies have already been conducted using titanium-based biosensors for glucose detection, both non-enzymatically (Cosnier, 1997; Ikeda, 1993; Khan, 1995) and for enzymatic stabilization (Rahman, 2010). Overall, titanium biosensors have been found to avoid the surface poisoning that most biosensor electrodes faced when using other metals. This results in the titanium biosensors lasting longer in the body without losing its catalytic activity. Titanium dioxide nanotubes were found to function as low as 5°C and as high as 50°C (Yin, 2010). This covers a much greater range than enzymatic biosensors and easily functions within all biologically-possible temperatures within the human body.

The main focus of this study will be on the use of copper-deposited titanium oxide as an electrode in glucose detection. Cyclic voltammetry will be used in order to measure the catalytic activity between the electrode and the glucose solutions prepared.

2.6 Conclusion

Glucose, being responsible for a vast majority of the energy production in cells, is one of the most important carbohydrates in the human body. While it is responsible for keeping the body alive, cancerous cells such as glioblastoma multiforme, can take advantage of the cell’s metabolism to result in a disease with a very low survival rate. It is essential to monitor GBM tumors through neuroimaging, such as MRIs and CT scans, and continuous glucose-level monitoring, which can be achieved by biosensors. While most biosensors use enzymes to detect the oxidation of glucose, difficulties in stability and other problems has resulted in a push for non-enzymatic biosensors that use modified metal or polymer electrodes. Titanium is an ideal candidate due to its excellent biocompatibility and will be used as the focus of this study.
3. Methodology

The purpose of this chapter is to describe the methods used during this experimentation. This includes the synthesis of the titanium dioxide electrodes, the deposition of copper nanoparticles on the surface of the electrode, and descriptions of the procedures used to test the electrodes’ effectiveness at detecting glucose.

3.1 Synthesis of Titanium Dioxide Electrodes

The first step in creating the titanium dioxide electrodes was to oxidize titanium foil into titanium dioxide. Small pieces of titanium foil (roughly one inch by one inch) were obtained from the laboratory as the starting material. To ensure that the surfaces of each foil piece was ideal, a thorough polishing process was followed. First, the titanium foil was polished by hand using polishing paper with varying grit sizes. Each side of the foil was polished for 30 minutes using 220 grit polishing paper, followed by 400 and 800 grit paper. This extensive polishing procedure ensured that the foil was as smooth as could be obtained by hand polishing and that there was no foreign material on the foil.

Following the hand polishing, the titanium foil was also chemically polished with acid. 5mL of hydrofluoric acid was added to 15mL of nitric acid in a small beaker to form the strong acid solution to be used for polishing. This nitric-hydrofluoric acid solution is very common in industrial descaling of stainless steel and titanium. The titanium was submerged in the acid solution for 10 seconds until the solution turned a shade of yellow/orange and released a vapor of the same color. The titanium was then immediately submerged in deionized water, completing the polishing process and ensuring the highest current potential.

Following polishing, the titanium was anodized to synthesize the titanium oxide electrodes. Anodizing thickens the natural oxide layer on the surface of the metal, which would
form the titanium oxide electrode. The foil was anodized through potentiostatic anodization in an electrolytic solution. Some electrolytic solutions that can be used to anodize titanium are a dimethyl sulfoxide-hydrofluoric solution, a formamide-water/ammonium fluoride solution, or an ethylene glycol-ammonium fluoride solution (Shankar 2007). Shankar et al.’s work reported that using the ethylene glycol-ammonium fluoride solution resulted in the best nanotube array configuration; therefore, that was the best solution to use to anodize the titanium. The titanium anode was suspended in the electrolytic solution along with a small piece of platinum mesh to act as the cathode. The system was then attached to a power supply at 20 volts for one hour, continually being stirred by a magnetic stirrer set at a rotation speed of 190. After the anodization was complete, both the titanium dioxide electrode and the platinum mesh were washed with deionized water. Finally, the titanium was placed in a furnace set at 350°F for 1.5 hours to complete the synthesis of the titanium dioxide electrode.

3.2 Deposition of Copper Nanoparticles

It has been observed that depositing nanoparticles onto biosensors can improve its function. A study from 2009 looked at how nanoparticles effect can affect electrochemical biosensors (as well as optical and magnetic biosensors) and found that nanoparticles, such gold or platinum, can increase signal amplification, minimize noise interference, or increase sensitivity (Sanvicens 2009). Therefore, copper nanoparticles were deposited to the titanium dioxide electrode in order to increase the effectiveness of the biosensor. The nanoparticles were deposited through amperometric methods at two different conditions. A 15mL solution of 0.05M CuSO₄/0.5M H₂SO₄ was prepared. Oxygen was removed from it by slowly bubbling nitrogen gas through the solution for 20 minutes. The titanium electrode was suspended with a platinum counter electrode and a reference electrode in the CuSO₄/H₂SO₄ solution and was attached to a
An amperometric method was carried out through an Autolab computer program at two different operating conditions to see which method was optimal. The first set of electrodes was run at -0.4V for 220 seconds (Method 1) while the second set of electrodes was ran at -0.2V for 100 seconds (Method 2). Once the amperometry was complete, each copper-deposited electrode was rinsed with deionized water and dried with nitrogen gas.

3.3 Cyclic Voltammetry Tests

To test the electrodes, cyclic voltammetry was used using same three-electrode configuration mentioned in the previous paragraph: the titanium dioxide foil as the reference electrode, platinum as the counter electrode, and the reference electrode provided with the galvanostat. Cyclic voltammetry measures the working electrode’s current while increasing the current to a set point and then decreasing the current back to the starting point. Different scan rates, potential ranges, and number of cycles can be set for each experiment. Since cyclic voltammetry was the primary source of measuring the electrode’s efficiency, the analysis was performed at every step of the process.

Before the copper nanoparticles were deposited on the titanium dioxide electrode, a cyclic voltammetric analysis was performed on the pure electrode. A 15mL 0.01M phosphate buffered saline (PBS) solution was prepared and bubbled with nitrogen gas for 20 minutes prior to use as the electrolyte in this analysis. The three electrodes were suspended in the PBS buffer and cyclic voltammetry was performed at a potential range of -1.4 to 0.3V at a scan rate of 0.05 V/s for 5 cycles. The same experiment was performed for the copper-deposited electrodes. In addition to testing the copper-deposited electrodes in the PBS buffer, the experiment was repeated using 0.1M NaOH that was bubbled with nitrogen as the electrolyte. These experiments were used to determine which copper-deposited titanium dioxide electrodes were the most
suitable to be used for glucose detection. Those electrodes were then repeated at scan rates of 0.01, 0.03, 0.05, 0.07, and 0.09 while holding the potential range constant in order to ensure that each electrode followed the expectation that the magnitude of the current peaks should increase with increasing scan rates. This expectation is shown by the Randles-Sevcik equation, 

\[ i_p = 268,600 n^{3/2} A^{1/2} D^{1/2} C^{1/2} v^{-1/2} \]  

(where \( i_p \) and \( v \) represent the current peak and the scan rate; other variables represent the number of electrons transferred (n), the electrode area (A), the diffusion coefficient (D), and the concentration (C)).

### 3.4 Testing Glucose Detection

The next step after the ideal electrodes were determined was to experiment on the electrodes’ ability to detect glucose began. This analysis was also performed through cyclic voltammetry using the same three-electrode system as mentioned before; however, instead of a pure electrolyte solution of PBS or NaOH, a glucose-NaOH solution was used. A 1mM glucose-0.1M NaOH solution was prepared for the first set of experimentation. The cyclic voltammetric analysis was then carried out at potential ranges of -0.8 to 0.3V and -0.6 to 0.4V. Each trial was conducted three times in order to ensure reproducibility. A large peak in the cyclic voltammetric graph would represent the electrode detecting the oxidation of glucose.

A more diluted glucose-NaOH solution was prepared for the next round of experimentation, this time using 0.5mM glucose. The same procedure was repeated, running the cyclic voltammetry three times at potential ranges of -0.8 to 0.3V and -0.6 to 0.4V. By decreasing the concentration of glucose in the glucose-NaOH solution, the magnitude of the current peaks would be expected to decrease since less glucose is there to oxidize and be detected by the electrode. This expectation also corresponds with the Randles-Sevcik equation.
The final experiment performed aimed to directly see the effect increasing the glucose concentration had on the magnitude of the current peak. Three NaOH solutions were prepared and bubbled with nitrogen—one containing no glucose, one with 1mM glucose, and one with 5mM glucose. The cyclic voltammetric analysis was performed in succession using each of the three solutions (rinsing with deionized water between each trial) at a potential range of -0.2 to 0.8V. This final experiment would show the correlation between the magnitude of the current peak and the concentration on a single plot and, ultimately, the electrodes effectiveness at detecting glucose.
4. Results and Discussion

This objective of this study was to develop a copper-deposited titanium dioxide electrode that could be used as a biosensor for patients in need of continuously monitoring their glucose levels. In order to analyze the effectiveness of the electrode, multiple cyclic voltammetry and amperometry experiments were conducted on the electrodes in different electrolyte solutions. This chapter will report the results from the aforementioned experiments.

4.1 Synthesis of Titanium Dioxide Electrode

The first step in developing a method for monitoring of glucose level inside of a person was the creation of the titanium oxide electrode. Following the synthesis of titanium dioxide nanostructures on titanium foil through anodic oxidation, nine pieces of the titanium foil electrode were synthesized. To ensure that each electrode was developed as expected, electrochemical analysis was performed on each foil piece. They cyclic voltammetric scans for each of the nine titanium dioxide pieces can be seen below in Figure 6.

![Cyclic voltammetric scan of each of the nine synthesized titanium electrodes in 0.01M PBS buffer.](image)

Figure 6: Cyclic voltammetric scan of each of the nine synthesized titanium electrodes in 0.01M PBS buffer.
The main objective in analyzing each of the nine pieces was to ensure that none of the pieces differed drastically from the rest of the electrodes. As it can be seen in the figure, each of the nine scans adapted the same general shape, although the magnitude of the peak currents differed slightly. Since each scan resulted in the same shape, it can be inferred that the anodization process was generally successful, with a few exceptions.

The main discrepancy amongst the scans is that four of the electrodes (the purple, lime green, and innermost red and blue curves) have cathodic current peaks at about 0.2V less than the other five scans. This inconsistency can be explained due to a change in the counter electrode during the voltammetric analysis. During experimentation, the original platinum mesh (that was used in the four scans mentioned above) broke and became unusable. This mesh was replaced with a platinum coil that was used as the counter electrode for the remaining five experiments. Although the electrode changed, the scans were still similar enough to each other to believe that all nine pieces were suitable to be used in further experimentation.

### 4.2 Deposition of Copper Nanoparticles

Once all nine titanium electrodes were determined to be appropriate for additional testing, copper nanoparticles were deposited onto the nanostructures through amperometry. The copper nanoparticles were deposited onto three of the electrodes (chosen at random) for each of the two methods described in the Methodology chapter. The electrodes will be referred to as Electrodes 1-3 and Electrodes 4-6, for Methods 1 and 2, respectively. Following the deposition of the nanoparticles, another cyclic voltammetric analysis was conducted in 0.1M NaOH solution. The scans for each of the two methods can be seen in Figures 7 and 8 on the following page.
Figure 7: Cyclic voltammetric scan for copper-deposited titanium electrodes using Method 1 of deposition.

Figure 8: Cyclic voltammetric scan for copper-deposited titanium electrodes using Method 2 of deposition.
Once again, both Figures 7 and 8 illustrate that the copper-deposited titanium electrodes maintained similarly shaped curves throughout the electrochemical analyses while the magnitude of the peak differs. Again, this validates the assumption that the copper nanoparticles were successfully deposited onto each of the six titanium pieces. However, since the cyclic voltammetric analyses were conducted at differing potential ranges (-0.2V to 0.8V for Method 1 and -0.5V to 0.3V for Method 2), the two methods cannot be directly compared to each other. However, Figures 7 and 8 seem to suggest that the two methods would result in two uniquely shaped voltammetric curves, allowing for further analysis of which method results in the more superior glucose detection.

4.3 Determination of Most Efficient Electrodes

Based on the experiments in pure NaOH and pure PBS, the nine electrodes were narrowed down to two candidates to be used for the remainder of the experiments. These electrodes (Electrodes 1 and 4) were based on which had the most prominent cathodic peaks in the prior cyclic voltammetric scans (Figures 7 and 8). Once the two ideal titanium electrodes were selected, a second cyclic voltammetric analysis was conducted. The goal of this analysis was to observe how the voltammetric scan of the electrodes reacted to varying the potential scan rate. In theory, the magnitude of both the anodic and cathodic current peaks should increase proportionally to the square root of scan rate, as shown by the Randles-Sevcik equation, \( i_p = 268,600 * n^{3/2} * A * D^{1/2} * C^{1/2} * v^{1/2} \). Both Electrode 1 and Electrode 4 underwent the voltammetric analysis in 0.1M NaOH at a potential range of -0.8V to 0.3V while setting the scan rate to 0.01, 0.03, 0.05, 0.07, and 0.09V/s. The results can be seen in Figures 9 and 10.
Figure 9: Cyclic voltammetric scan of Electrode 1 at varying potential scan rates.

Figure 10: Cyclic voltammetric scan of Electrode 4 at varying potential scan rates.
As expected based on the Randles-Sevcik equation, the magnitude of the peaks increased as the potential scan rate increased. This demonstrated that the electrochemical properties of Electrode 1 and Electrode 4 were behaving as would be predicted by the theory behind the electrochemistry.

4.3.1 Electrode Stability Issues

Once the ideal two titanium electrodes were selected, no further experimentation was conducted for approximately twelve days. Following that short break, the same experimentation depicted in Figures 9 and 10 were repeated in order to compare results. These results can be seen in Figures 11 and 12.

![Cyclic voltammetric scan of Electrode 1 at varying potential scan rates after inactivity.](image)

Figure 11: Cyclic voltammetric scan of Electrode 1 at varying potential scan rates after inactivity.
The biggest observation amongst these Figures is the difference in voltammetric scans for Electrode 1, shown in Figures 9 and 11. After the inactivity, it appeared that Electrode 1 completely lost its cathodic current peaks and almost entirely lost its anodic current peaks as well. Because of this, it became apparent that Electrode 1 could no longer be used for experimentation with glucose. There were two most likely reasons that this drastic change occurred. The first reason may be that the copper nanoparticles oxidized over time due to improper storage of the electrode or just overall poor deposition from Method 2. The second reason could be that the titanium dioxide nanostructures changed their structure over time which would be the fault of an imperfection during the synthesizing of the titanium electrode before the copper deposition. When looking at the voltammetric scan of Electrode 4, it can be seen that the change was much less drastic. While there was a slight change in the magnitude of the anodic and cathodic peaks, the shape generally remained the same. This ultimately allowed Electrode
4 to be the only electrode synthesized that could be used in the further experimentation of glucose detection.

4.4 Glucose Detection

With an ideal electrode determined, the voltammetric analyses were no longer conducted in pure 0.1M NaOH or pure 0.01M PBS, but with glucose-NaOH solutions. The first electrolyte solution used was 1mM glucose in 0.1M NaOH. The cyclic voltammetric scan of Electrode 4 was run in this solution at two different potential scan rates, -0.8V to 0.3V and -0.6V to 0.4V, three times in order to ensure stability and reproducibility. The results are shown in Figures 13 and 14.

![Figure 13: Repeated Cyclic Voltammetric scan of Electrode 4 in 1mM Glucose-0.1M NaOH solution at -0.8V to 0.3V.](image-url)
By running each scan three times and seeing the same scan each time, it was demonstrated that the electrode maintained its stability throughout the experiment. Figure 8 shows a noticeably greater magnitude of cathodic and anodic current peaks, reaching approximately $0.250 \times 10^{-2}$ A and $-0.700 \times 10^{-2}$ A, respectively. These large peaks occur due to the reduction of glucose (or oxidation in the case of the anodic peak). These figures illustrate the fact that Electrode 4 succeeds at detecting the oxidation and reduction of glucose when it is introduced into the solution, which is imperative when attempting to monitor glucose levels in patients.

### 4.4.1 Complications in the Glucose Detection

While the electrode did detect the glucose in the 1mM glucose-0.1M NaOH solution, some problems arose in the succeeding experiments involving the varying of the glucose concentration. The 1mM glucose solution was further diluted to 0.5mM glucose with 0.1M NaOH and cyclic voltammetry was repeated at the two potential ranges, again repeated three times each for reproducibility. The resulting scans can be seen in Figures 15 and 16.
Figure 15: Repeated Cyclic Voltammetric scan of Electrode 4 in 0.5mM Glucose-0.1M NaOH solution at -0.8V to 0.2V.

If the electrode detected the glucose as expected, then increasing the concentration of glucose should also increase the magnitude of the current peak. This is proportional correlation is shown yet again by the Randles-Sevcik equation. However, as it can be seen in the two figures, this does not occur. By decreasing the concentration of glucose to 0.5mM, most of the peaks either
stay the same or actually increase in magnitude, as opposed to the expected result of approximately halving their peaks.

One possible explanation to the phenomenon may be due to inaccurate dilution of the glucose-NaOH solution. In order to examine the possibility of this fault, new solutions were made from 10mM glucose and 0.1M NaOH. Once the new glucose solutions were made (this time 1mM and 5mM glucose), another cyclic voltammetric analysis was conducted in order to observe the behavior of increasing glucose concentration. These glucose concentrations were chosen to observe the effect of a five-fold increase in concentration as opposed to the two-fold increase in concentration used before. The analysis was run at a new potential scan rate in order to account for any abnormalities that may have occurred as a result of the low potentials. The result is seen in Figure 17.

Figure 17: Cyclic voltammetric scan of Electrode 4 in varying glucose concentrations.
Once again, the expected trend is that increasing the glucose concentration would increase the current peak. However, as it can be seen, the cathodic peak stays roughly the same while the anodic peak decreases when the glucose concentration is increased. While this nullifies the claim that improperly made solutions caused the unpredicted trend, it brings forth the idea that the titanium electrode itself may have a defect that caused this occurrence.
5. Conclusions and Recommendations

The results in the previous section demonstrates that although the electrode did not react fully as expected in the detection of increasing glucose levels, many of the preliminary results were successful. Each of the 9 titanium foil pieces underwent the anodization and copper deposition processes and resulted in the expected formation of the titanium electrodes. The cyclic voltammetric experiments determined that Electrodes 1 and 4 were the most effective at developing strong cathodic and anodic current peaks. Once glucose was introduced into the electrolyte, the electrode also proved to be effective as detecting glucose, as could be seen by that change in peak between the pure NaOH electrolyte experiments and the glucose-NaOH electrolyte experiments.

However, while the electrode did succeed in detecting glucose, it did not follow the trends that the Randles-Sevcik equation predicted. Instead of the current peak increasing with the increasing concentration of glucose, the electrode acted inversely. Whether this was due to poorly formed electrodes or improperly made electrolyte solutions was not determined. Another issue that arose was the instability of the electrodes. Electrode 1 changed so drastically after inactivity that it could not be used for glucose detection; even the final electrode, Electrode 4, altered over the inactivity, although not nearly to the same extent as Electrode 1.

In continuing this research, there are three vital recommendations to follow in order ensure that electrodes work as expected. The first is to confirm that every electrolyte solution is made properly. Each of the solution made for this research were made by weighing out dry glucose and mixing it into aqueous NaOH or PBS solution to result in the desired concentration. However, human error may have been the cause for the results coming out not quite as expected.
The second recommendation is to perform each experiment over a shorter period of time. Due to time constraints, the entirety of the experiment was spread out over the course of more than three months, including a twelve day period of total inactivity. This time span may have caused the electrodes to lose their effectiveness over time. As an alternative to this recommendation, experimentation to increase the stability of these electrodes can also prove to be vital in developing effective electrodes.

The final recommendation is to perform a greater number of glucose detection tests on the electrodes. The electrodes in this study were only tested in a few electrolyte solutions of differing glucose concentrations. By testing a wider range of glucose concentrations (as well as having more electrodes to test), it can be determined whether the trend found in the results of this study was a chance phenomenon or if every electrode would follow this same trend. This would result in an electrode that would be more effective at detecting low levels of glucose.

There are alternative ways that continuous glucose monitoring can be studied. As mentioned before, different combinations materials and nanoparticles can be used as biosensor electrodes to affect the efficiency of detecting glucose. If titanium desired to be the main metal of interest, then different nanoparticles, such as gold or silver, can be deposited on the surface. Alternatively, different metals, such as platinum, can be studied to see how well it can be used to detect glucose.

Overall, reducing human error, reducing inactivity, and increasing the number of experiments would all be very beneficial in determining whether or not copper-deposited titanium dioxide electrodes can efficiently be used as biosensors to aid in continuous glucose monitoring. However, regardless of the selectivity of the electrode towards glucose, many more tests must be done before it can be successfully used as an implantable biosensor. It is not
enough for the biosensor to detect glucose, but it needs to be able to remain stable in the conditions that are present in the human body to test if the conditions will affect the efficiency of the biosensor. Also, it needs to be ensured the body’s immune system will not attack and break down the biosensor too quickly. These in vivo requirements of the biosensor will also need to be tested once the selectivity towards glucose is at a desired level.
References


