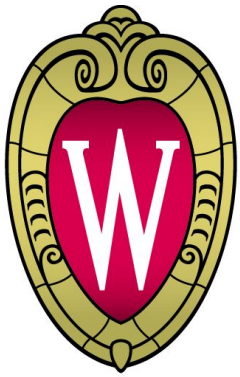

WISCONSIN

UNIVERSITY OF WISCONSIN–MADISON

Improving Diagnostic Technology of Acute Compartment Syndrome by Quantifying Intramuscular Glucose

Biomedical Engineering Design: 200/300

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Client: Christopher Doro, MD

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Abstract

Acute compartment syndrome (ACS) is a condition in which the pressure of the muscle compartment is increased significantly due to a traumatic event, most commonly a bone fracture. The consequences are a decrease in the pressure gradient of blood flow which essentially causes arterial blood to bypass the injured muscle, leading to cell anoxia, muscle ischemia and muscle death. Diagnosing ACS is problematic as current methods rely on the subjective assessments of clinical examinations and/or inaccurate intracompartmental pressure readings. These deliver unusually high false-positive diagnoses in trauma patients which often lead to unnecessary and invasive surgical treatment of a fasciotomy. Recently, however, it has been shown that alternative biochemical markers, such as glucose and pH, may lead to more a more accurate and concrete quantification of this trauma. While current technology exists to detect these markers in a canine model, these models are unfit for human patients, but there exists potential to translate these tools to a clinical setting. The aim of this paper is to explore possible routes - pH, glucose, and conductivity - to diagnose ACS in humans. Afterwards, the fabrication of the augmented glucose detection technology, experimental testing, and observed findings will be described. We aim to create a glucose probe that is biocompatible, accurate (detects 40 - 160 mg/dL glucose concentration range), invasive (2-8 centimeters into tissue), and able to continuously (1 sample/ 10 minutes) measure a biochemical marker associated with ACS. In creating a comprehensive diagnostic technology, we hope to eschew trauma patients from undergoing harmful and unnecessary surgery.

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Introduction

A. Motivation

Diagnosing acute compartment syndrome (ACS) traditionally utilizes subjective clinical examinations of trauma patients [1]. In recent years, an effort has been made to replace such qualitative measures with a method that's both accurate and reproducible. Intercompartmental pressure readings have shown some promise; however, quantifications may vary within the patient's compartments and the pressure threshold that diagnoses ACS is not well defined [2,3]. Most alarmingly, these readings may falsely diagnose patients 35% of the time, thus a number of patients will unnecessarily undergo a fasciotomy to treat their perceived ailment [4]. It is clear that a more accurate and definitive diagnosis for ACS must be explored to prevent unnecessary and intensely invasive surgeries.

A. Current Methods

Currently, clinical examinations in combination with a pressure reading taken from the compartment are the standard for diagnosing patient with compartment syndrome. This method is flawed, as it has an uninformative threshold, and is ultimately a subjective assessment performed by the medical professional. Experimental methods are also being tested in the areas of near infrared oximetry, pH, glucose, and partial oxygen pressure measurement, some with more success than others [5]. A promising alternative route for ACS diagnosis is the interstitial pH quantification. The Orian™ 8133BNWP ROSS™ Combination Spear Tip pH Electrode, a Fisher Scientific product, has been utilized to diagnose adult beagles with intentionally onset compartment syndrome. The probe's tip is 3 mm wide, 40 mm long, and detects pH 0 - 14 ∓ 0.01, and is therefore not directly applicable in the human medicine setting. Furthermore, Dr. Doro has shown that continuous glucose monitoring may unveil compartment syndrome in adult beagles [6]. A REAL-Time Guardian Continuous Glucose Monitoring System, Medtronic, (U.S. Patent No. 6,809,653) in conjunction with an Enlite™ Sensor, Medtronic, showed a significant difference in glucose levels between control and injured compartments. The sensor was 10 mm in length and must be calibrated for 90 minutes prior to use.

B. Problem Statement

Acute Compartment Syndrome (ACS) impacts many trauma patients and presents medical providers with perplexing dilemmas regarding the diagnosis and treatment of this condition. ACS diagnosis is most frequently based on clinical examinations and traditional measurements of intracompartmental (IC) pressure, but these techniques prove unreliable, and therefore, commonly lead to misdiagnosis and unnecessarily invasive procedures. While alternative biochemical markers, such as glucose and pH, have been linked to ACS in a canine model, the diagnostic technology has not been readily translated to the clinical setting. The goal of this project is to create a tool that accurately, continuously, and easily quantifies a chosen biochemical marker indicative of ACS in human subjects. An accurate detection of these markers may expedite ACS diagnosis and prevent patients from undergoing unnecessary treatment.

Background

A. Preliminary Research

A compartment is a section within the body that includes a group of muscles and their nerves surrounded by a layer of inelastic fascia. These compartments are found all over the body, and there are multiple in each extremity. Capillary beds across the compartment create a perfusion gradient, allowing blood to flow from high pressure to low pressure. This blood flow provides the muscles in the compartment with the nutrients they need to remain functioning. ACS is created when a serious injury, commonly a bone fracture or deep bruising, causes the inside of the nearby muscle compartment to swell. Since the fascia is unable to stretch, the pressure inside of the compartment rises, which eliminates the perfusion gradient. Blood is no longer able to flow into the muscle because the pressure inside the compartment is no longer lower than the arterial pressure. This syndrome leads to cell anoxia, muscle ischemia, and eventually muscle death if gone untreated. A fasciotomy is performed to decompress the compartment, and if this is not performed in time, the patient will have permanent damage from Volkmann's Muscle Ischemia, which is death of the muscles within the compartment [12].

B. Design Research

The client, Dr. Doro, has done previous studies showing pH and glucose are effective biochemical markers that will accurately diagnose ACS. In Dr. Doro's studies, a crude meat and cheese probe measured the intercompartmental pH in anesthetized beagles with compartment syndrome. This method measured the change of pH in a dying compartment. In his research, he found the pH in an injured muscle can drop well below 7.1, a pH that would be fatal to a human [5]. In a separate experiment, he used a Medtronic Enlite™ Sensor to measure the glucose of the canines. He concluded that the glucose level in the compartment essentially goes to zero [6]. Therefore, he highly encouraged we try to build the probe measuring one of these biochemical markers. However, beyond Dr. Doro's initial research, he later informed us potassium ions are a strong indicator of an increase in conductivity in the compartment. This is because in all dying muscle cells, potassium ions are released from the cells in mass quantities, which causes a major increase in conductivity. Therefore, potassium ions are also a strong biomarker to diagnose compartment syndrome.

The bulk of the background research that was used to formulate the preliminary designs came from studying current devices that measure pH and glucose. There are no devices designed for the purpose of this project, so they all would require some degree of modification. As stated earlier, the Orian™ 8133BNWP ROSS™ Combination Spear Tip pH Electrode is a Fisher Scientific product commonly used to measure the pH in meat and cheese. In order for this technology to fit the given requirements, a prototype would have to be created that was thinner and deeper. The continuous glucose sensor that was researched is one made by Medtronic used to monitor the blood glucose levels of diabetics. This sensor, the Medtronic Enlite™ Sensor, works by creating a redox reaction in the surrounding fluid of the wire that is inserted into the body. This redox reaction allows for an electrical signal to be transmitted to the sensor outside of the body, giving a glucose reading [8]. This device, however, is not long enough to satisfy the given requirements, and it requires calibration. From this research, it was concluded that in order

for this technology to be compatible with the problem statement and design specifications, a probe would have to be created that uses this technology but is longer, more rigid, and does not require calibration.

C. Client Information

The client, Dr. Christopher Doro, is an Orthopedic Surgeon at the UW Hospital. He has conducted his own research on compartment syndrome, mainly including the experimentation on dogs to test the effectiveness of biochemical markers, pH and glucose, as standards to diagnose compartment syndrome. He has concluded that these biochemical markers work well and has asked us to create a probe that effectively measures them in humans.

D. Design Specifications

The most important requirement for the design is the probe is able to improve the current false positive rate of 35% for compartment syndrome by monitoring a chosen biomarker continuously and directly with readings being taken at least once every 10 minutes. Additionally, the probe must be long and rigid enough to reach the muscle tissue (~1-5 cm) within a compartment. The probe should also not create more discomfort for the patient nor a greater risk of infection, therefore the probe should be no larger than a 16 gauge needle. Medical staff must be able to use this device quickly, easily, and safely while in the chaotic environment of an emergency room. Finally, the device should ideally consist of two different parts; a disposable part that is cheap and readily available, and a reusable analyzation component that is reasonably affordable. The total budget for this project is $10,000. Additional and more specific design requirements can be found under the Product Design Specifications in Appendix A.

Preliminary Designs

The following designs will be used to puncture the muscle compartment. These designs will be applicable to any given compartment. Depending on the compartment in question, the probe, electrode, or wire will penetrate directly into the muscle compartment where the black lines (*Fig. 1)* are drawn.

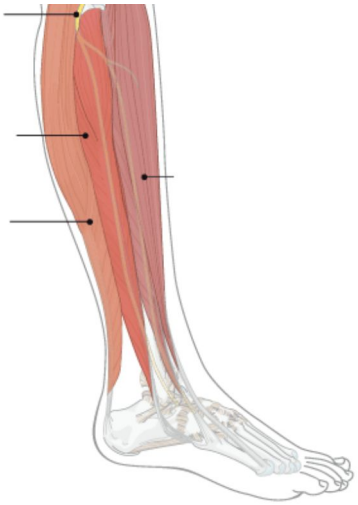

Figure 1: Surgical reference of where the prototypes will be inserted into the patient. [7]

A. pH Probe:

The pH probe design was based on the client's previous research with measuring pH in the muscle compartments of beagles. Since even a slight drop in pH is associated with a dying compartment, pH can be used to effectively diagnose ACS in a trauma patient. The rudimentary probe used in the client's research was extremely large, 3 times the width of the ideal needle referenced in the design specifications. A pH probe works by securing two silver alloy electrodes into the glass bulb (tip) of the probe, one to directly measure the hydrogen ion concentration of the compartment and the other for reference of a hydrogen ion concentration equal to a neutral pH of 7. The hydrogen ions in compartment will perfuse through the glass bulb due to a concentration gradient across the working electrode, thus generating current between the working and reference electrode. A processor takes into account the generated current and may relate this to pH after calibration.

For the design, since there are two electrodes in the glass bulb, the probe will remain at an unchangeable 3mm. The probe length will be extended to 8 cm to reach into any compartment in the patient. The probe will rely on a stylet entry system for insertion into the body. This stylet will be non conductive and rigid to ensure there is no contamination before entering the compartment and help with the insertion of the probe. A rigid needle and a surrounding case, comprising the stylet system, will puncture the body into the muscle compartment. The inner needle will be removed, making a gap in the hollow case. The probe will be inserted into the imbedded portion. By using this technique (*Fig. 2*), although the probe is still an unpleasant 3mm wide, the fragile probe will be at a lower risk of breaking apart.

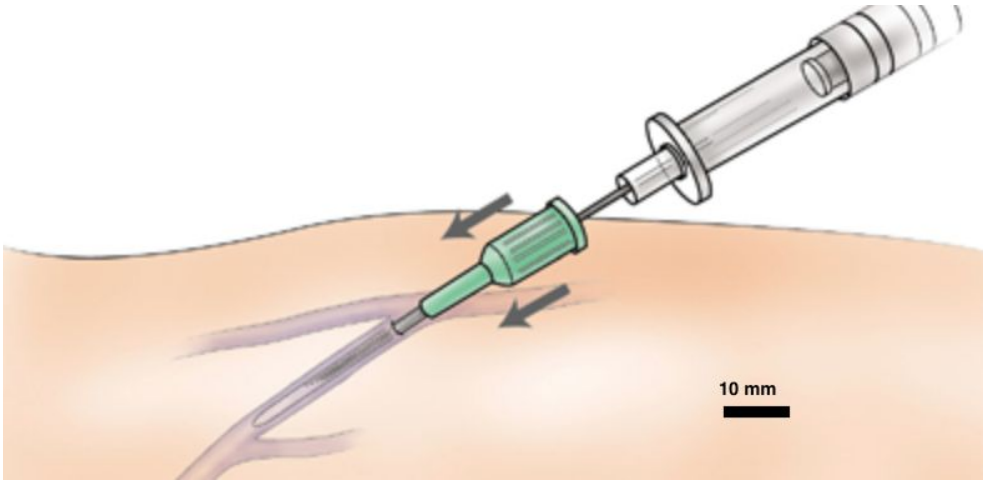

Figure 2: This figure represents a stylet entry system that would be utilized with the pH probe. The green portion is the case, and the device above would be the rigid needle that would be removed to make room for the probe. Instead of being inserted into a vein, as pictured, the stylet would puncture the muscle compartment. [10]

Muscle can survive at a pH significantly lower than the systemic pH level, and an injured muscle drops to a pH around 6.27 [9]. A pH reading significantly below this would be indicative of compartment syndrome.

B. Conductivity Electrode:

The second design is utilizing the conductivity in the compartment based on the increase of potassium ions. Since it is known there is a positive correlation between conductivity and potassium levels, this strategy can be used to diagnose ACS. Currently, conductivity probes calculate how well ions conduct electricity in a compartment based on their concentration.

The electrode consists of an anode (negative end) and a cathode (positive end) that interact together to measure the rate of flow of ions between them. The particular electrode the design is based off of [11], consists of two electrodes 5mm apart and 1 mm below the housing unit of the electrode housing unit. The electron housing unit is 155mm long and has a diameter of 12mm. The housing unit is then connected to the analyzer via cable. It can measure conductivity from 10 microsiemens/cm to 1 siemon (concentration of ions per cm space). For the modified design, the anode and cathode will be separated so the diameter of insertion is smaller. They will be inserted into the body using two 18 gauge (1.02 mm), pull away needles, so when the electrodes are inserted into the body, the needles can be removed from the body by pulling the tabs and having the needles rip down the side via a perforated line. The needles will be connected exactly 2.96mm apart, so the 5 mm electrode distance is maintained in the compartment, and and 9.5 mm from the tip of the electrode to ensure the connection point is above the skin. The electrodes will be left in the compartment for continuous monitoring and connected to an analyzer outside of the body.

C. Continuous Glucose Monitor

The continuous glucose monitoring design is based off of the technology currently in use for diabetic patients. This monitor is made up of three components: the wireless monitor, the transmitter and the sensor. A perforated 18 gauge needle (1.02mm in diameter) will house the thin flexible wire during insertion to the muscle compartment, similar to the conductivity electrode design. Once at the target location inside the muscle compartment, the tabs at the top of the needle will tear apart and be removed from the body, leaving only the coated transmitting wire in the body to maximize patient comfort. Two needles and two wires are required per patient. One needle will be positioned in the injured compartment, and another in the healthy compartment on the opposing limb.

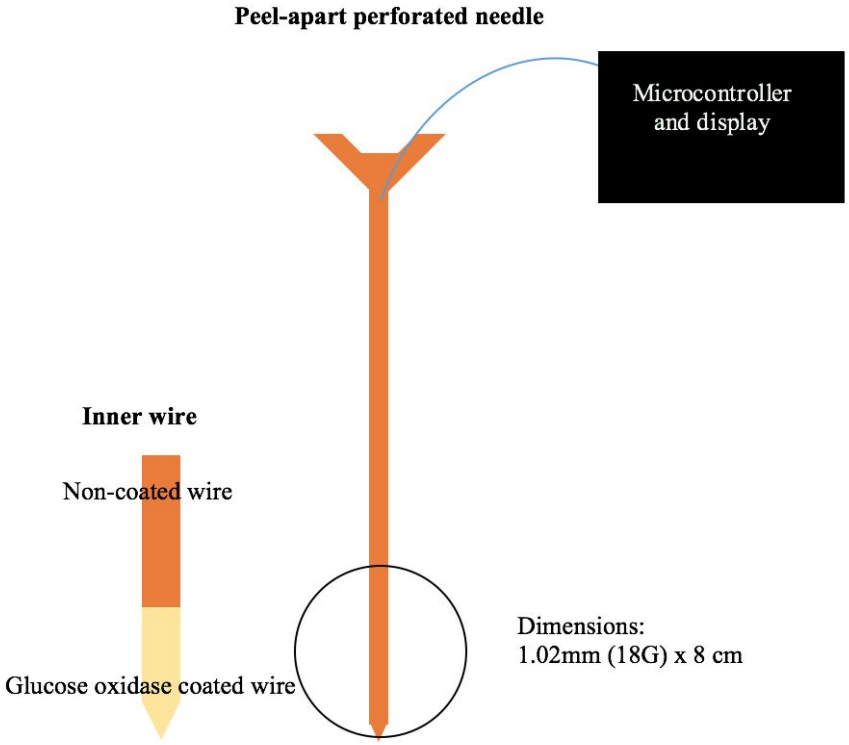

Figure 3: Schematic of components of continuous glucose monitor design. A working prototype requires two needles/wires.

The platinum-iridium (Pt/Ir) alloy wire will be coated with glucose oxidase in the innermost 2 centimeters of the wire. This will ensure the following reaction will take place only in the muscle compartment. Glucose oxidase spurs a reaction with the surrounding tissue and fluid, converting glucose into hydroxide, which will react with the wire and generate an electrical signal to transmit to the sensor above the skin [8]. The readings from both compartments will be read and the difference between the healthy and unhealthy glucose levels will indicate compartment syndrome. Since a muscle undergoing muscle ischemia will not be receiving nutrients from the blood, glucose levels essentially drop to zero in a limb with compartment syndrome. It is expected that the two electrode system will negate a need for a threshold value

that could lead to problems with false positives and misdiagnosis. Also, since glucose is so variable depending on diabetes status of the patient, diet, time since meals, etc., the reference value will eliminate patient variability.

Preliminary Design Evaluation

A. Design Matrix Criteria

Seven categories were created to rank the potential compartment syndrome detector designs. Each category was given a certain weight to signify its importance in determining the final preliminary design. These categories were then inputted into a design matrix (*Table 1*) and evaluated to determine the best design.

Accuracy and Precision: (25/100)

This ranking carries the most weight considering the current limitations of compartment syndrome diagnosis include generating false-positives via oxygen pressure measurements. The main concern is with whether or not the technology will work. The new device must replace the current standard of diagnosis by quantifying a novel biochemical marker. Because various biomarkers may correlate differently with acute compartment syndrome, it's crucial that a chosen biomarker is consistent across patient populations, easy to detect, and is homogenous within a compartment. The pH and potassium conductivity probes scored highest in this category. Both pH and potassium levels are very consistent throughout the population making systemic, reference values easily identifiable. For instance, pH ranges from 7.35 to 7.45 [9] and extracellular [K^+] ranges from 3.5 to 5 mM [11]. During muscle ischemia pH drops down as low as 6.29 [9] while [K^+] rises up to 16 mM [11]. These large changes in biomarker levels combined with low margins of error on their respective probes give both these probes the maximum score in this category.

Ease of Analysis: (20/100)

Refers to how easily this device is able to read and report data as well as ease of calibration and continuous use. The nurse or doctor should ideally be able to continuously monitor the chosen biomarker level without performing additional, time-consuming steps. The glucose probe received the highest score in this category because there are pre-existing glucose monitors capable of providing continuous, digital read-outs of glucose levels within the body. They are also already capable of tracking glucose over time, and displaying the results both graphically and numerically. There are also many options to calibrate a glucose probe such as using a finger-prick test or having it factory calibrated.

Safety: (20/100)

Refers to the current standard of care and how comfortable the patient will be during the use of the probe, as well as the safety of the nurse. In current method for determining if a patient has compartment syndrome the nurse uses an eighteen gauge needle. It is required the new probe is up to or exceeding the current standards. The new probe should also be comfortable for the patient to have inserted into their compartment. Lastly, the nurse needs to be able to safely insert the probe into the patient without causing harm to their own body. The glucose probe received the highest score because of the ability to use a small needle, however there were concerns with using two needles and possible entanglement. The conductivity technology was given a slightly score because it also requires two entry points like the glucose probe, but both entry points would need to be significantly larger than glucose (18 gauge needle vs 22 gauge needle). The pH probe was immediately counted out at this stage in the matrix. Since safety is a category with high importance, the 16G max requirement was a strict guideline that could not be overlooked. The 3mm probe would have been too large to insert into the patient and would lead to an unnecessary amount of pain and increased risk of infection which would compromise the safety of the patient.

Ergonomics: (15/100)

Refers to the ease and efficiency of use of the device. The doctor should be able to make this reading quickly and insert the needle with one hand so the patient can be secured with the other. The device should be small and be able to be operated by one trained professional in the ER. The glucose probe and the conductivity electrodes received the same score, they both require two needle insertions but they are relatively small needles and the operator should already have experience.

Ease of Fabrication: (10/100)

Refers to how easily our design team will be able to fabricate the sensor. The sensor will likely be composed of a probe that will be inserted into the patient and an analyzer that will read the measurements from the probe. Our team must be able to either buy or fabricate these two components using our current skill set. We will have access to the student shop. The potassium conductivity sensor received the highest score because all materials necessary for the sensor would be able to be purchased, making fabrication as simple as connecting the parts together. The glucose probe would require the electrode portion to be manufactured, since there are no currently existing electrodes long enough.

Reusability: (5/100)

Refers to the device having a main analyzation system that is able to be used repeatedly while the sterile sensing probes are disposable. The glucose probe was awarded the highest value

in the reusability category because the computing boxes can be reused, and the needles will be relatively cheap and disposable.

Cost: (5/100)

Refers to the ability of the device to be tested and fabricated under the restrictions of a reasonable budget. The disposable piece should be very cheap to buy, and the reusable part of the design should be of reasonable price. The glucose probe received the highest score since continuous glucose probes can be found for under $100. The disposable needles to be used with such a probe would also be quite cheap.

Criteria (Weight)	**pH Probe**		**Glucose Probe**		**Potassium Conductivity Technology**	
Accuracy and Precision (25)	5	25	4	20	5	25
Ease of Analysis (20)	4	16	5	20	2	8
Safety (20)	0	0	4	16	3	12
Ergonomics (15)	3	9	4	12	4	12
Ease of Fabrication (10)	4	8	4	8	3	6
Reusability (5)	4	4	4	4	4	4
Cost (5)	3	3	4	4	3	3
Total	65/100		84/100		70/100	

Table 1: Design matrix comparing potential devices including pH probe, glucose probe, and sodium conductivity technology. The total numbers are out of 100, and the highest number represents the most feasible option with regards to the criteria.

After evaluating the final design matrix, it was concluded that the relative measurements from the glucose probe would be the best option. It excelled in almost all of the categories and will offer the operator freedom with individual patient reading and continuous monitoring. It involves an easy application of two 18G needles, and similar technologies already exist in

medicine. Calibration should be negligible because the difference in the readings should offer all of the needed information to diagnose compartment syndrome. A downfall of this device could include if a patient came in with bilateral injuries where a reference measurement is not possible.

B. Proposed Final Design

The proposed final design was broken down into three main parts, the electrodes that would be placed within the compartment to detect glucose concentration, the signal processing unit that would control the reaction at the electrodes and analyze data coming from them, and the delivery model that would insert the electrodes into the compartment.

Electrodes

The final design will require three electrodes to serve as the input to the three-electrode potentiostat, discussed below. The three electrodes will consist of a working electrode, reference electrode, and counter electrode. The working electrode (*Fig. 4)* will be created by extending a silver wire with a commercially available Dexcom G5 Glucose Sensor. A possible flaw in this design is the chemistry of the Dexcom sensor is unknown - if problems arise during testing, it will be challenging to diagnose the sensor tip as the problem. Furthermore, the reference electrode will be created by chloriding a silver wire in a potassium chloride solution following standard protocol. Lastly, the counter electrode will be made by bare silver wire and serve as the third prong to the potentiostat.

Figure 4: A Dexcom G5 glucose sensor shown to scale next to a human hand [12].

Signal Processing

The signal processing unit will consist of a three-electrode potentiostat connected to both the electrodes and the Arduino Uno. The potentiostat must accomplish two tasks. Firstly, it needs to maintain a constant potential difference between the working electrode and the reference electrode. This potential difference will supply the energy that causes the redox reaction at the working electrode to occur. Secondly, the potentiostat needs to convert the current produced from the redox reaction into a readable voltage.

After reading in the voltage, the Arduino will then interact with a computer running Arduino IDE to analyze the data coming from the circuit and determine the glucose concentration of the solution the electrodes are in. A block diagram of this entire process can be seen below **(***Fig. 5***)**.

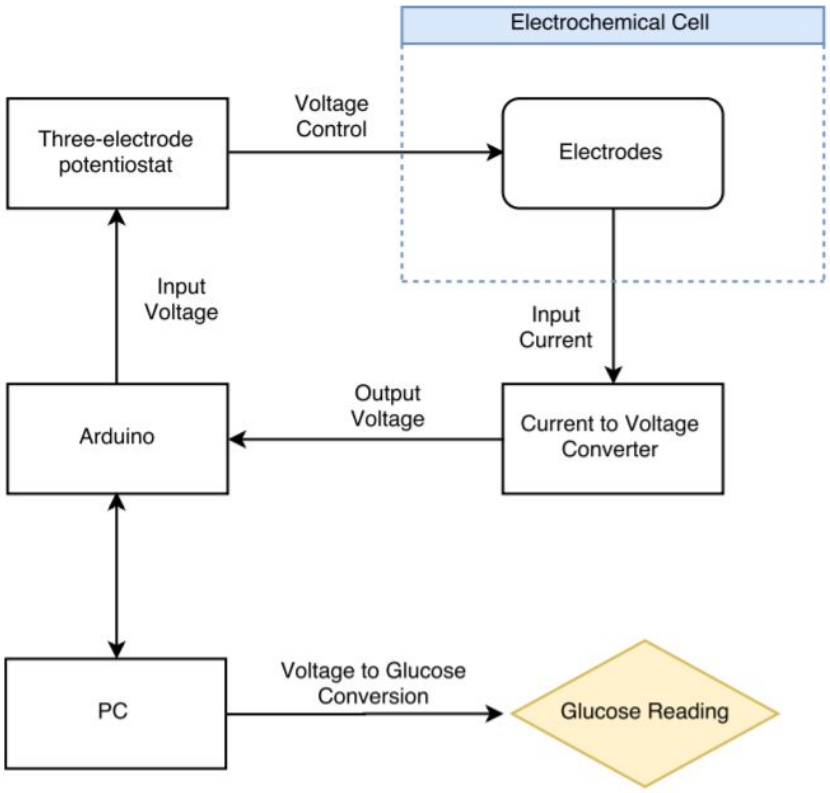

Figure 5: Block diagram of signal processing unit. This block diagram outlines how the Arduino, circuit, electrodes, and PC interact with one another to ultimately take the input voltage from the Arduino and turn it into a glucose reading. Arduino IDE is used to make the voltage to glucose conversion.

Delivery Model

A 10 cm, 18 gauge tuohy stylet needle, used for an epidural injection in the spine, is incorporated into the final design. These needles are commercially available and are frequently used in hospital settings, so no additional training would be required. This product is also cheap and disposable.

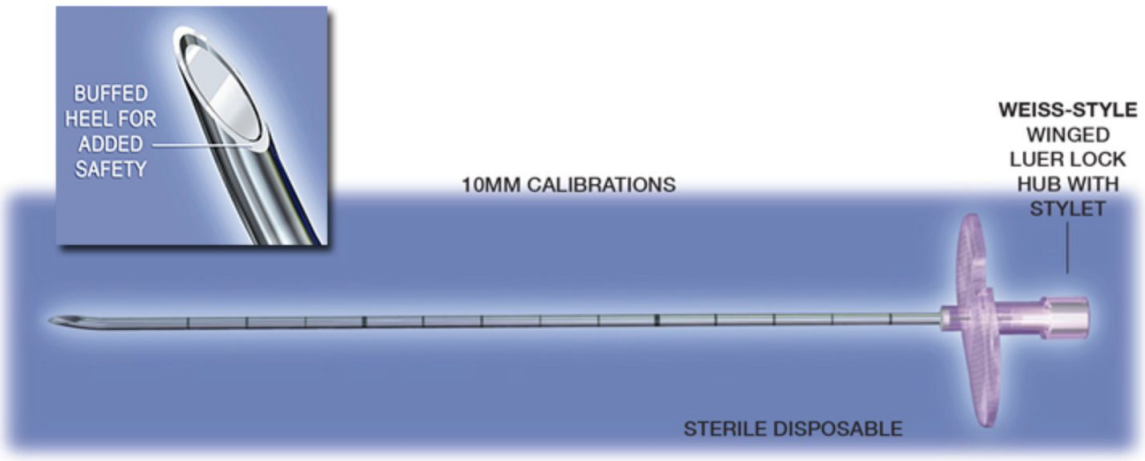

Figure 6: Above is a depiction of the touhy stylet needle. Features shown include the beveled tip for safety, and the ergonomic winged hold. [19]

The needle is inserted into the desired muscle compartment and the electrode is threaded into the hollow device. The stylet will house the electrodes separately in order to avoid damage and deformation of the wires from the surrounding environment. Signal noise will be limited since the needle ensures the electrodes will only read a glucose measurement from the desired muscle compartment that it is in contact with. This entry system also allows the potentiostat electrodes to penetrate the required 2-8 cm depth into the average muscle compartment.

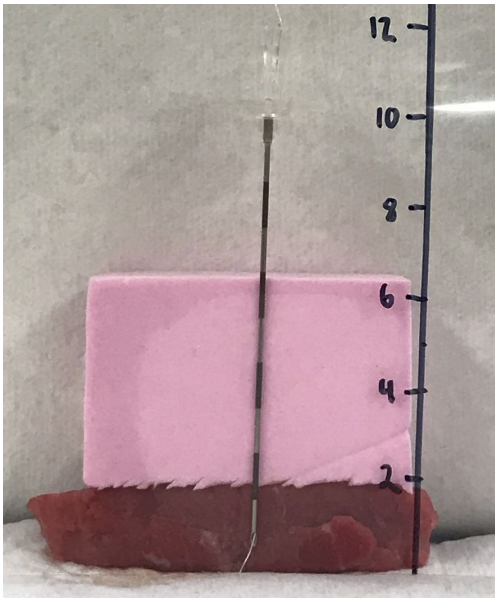

Figure 7: The above image shows a cross section of the intended system. The desired compartment is represented by a piece of meat, 4 cm below the surface. The pink foam piece serves as a barrier and represents any tissue prior to the compartment, including skin and surrounding compartments. The electrode is only in contact with the desired compartment.

Fabrication

A. Theory

Electrodes

The working electrode of the circuit will be made from a commercially available glucose sensor; although the exact chemical makeup of the probe is proprietary, recent publications allow speculation of the electrode's interfacial chemistry. To quantify glucose in an environment, glucose's chemical activity must be converted to an electrical signal. This is done by a series of redox-reactions localized on the interface of the electrode and environment. Specifically, a platinum-iridium wire is coated with glucose oxidase - an enzyme that reacts with glucose - metal mediators, and a variety of polymers used to stabilize these attachments and select specific ions associated with the electrochemical reaction. Glucose oxidase will first oxidize glucose to gluconolactone (*Fig. 8)*. As the enzyme and mediators typically exist within a polymer network, the electron is in close proximity to traverse through the system, where it will eventually reach a conductive wire. The transferred electron will then act as current and be interpreted by a circuit. It should be noted a +300 mV potential at the working electrode (vs. Ag/AgCl reference electrode) must be applied to provide sufficient activation energy for the glucose-glucose oxidase reaction. Additionally, this voltage is small enough to not oxidize other oxidizing species', such as ascorbic acid and acetaminophen; their electrons will therefore not be supplied to the system and noise will be reduced [14].

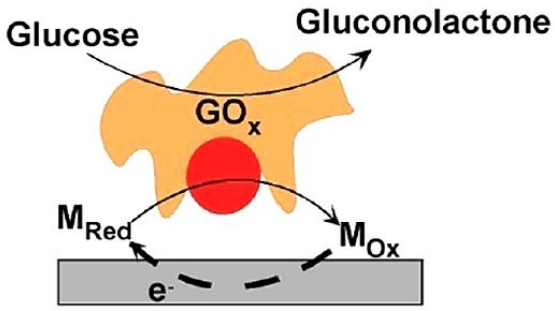

Figure 8: A redox reaction between glucose, glucose oxidase, and metal mediators. The electrons traverses through this system until it reaches a wire, where it will then act as a current [14].

Signal Processing

A three-electrode potentiostat was chosen to control the redox reaction taking place at the working electrode, as well as convert the current from the redox reaction into a voltage. The three electrodes within this potentiostat configuration are the working electrode, the reference electrode, and the counter electrode[15]. As described in the previous section, the working electrode serves as the reaction site for glucose and glucose oxidase. The reference electrode serves to create a voltage gradient between itself and the working electrode[15]. This gradient supplies the energy necessary for the reaction at the working electrode to occur.[15] A silver/silver chloride electrode was chosen for the reference electrode due its stability, ease of fabrication, wide commercial use, and low cost[16]. Finally, the counter electrode serves to complete the circuit between itself and the working electrode[15]. By doing so, it has the same current of 0-10 μamps flowing through it as the working electrode. A silver wire was used as the counter electrode since it is an inert, conductive, low cost metal[16].The addition of this counter electrode eliminates difficulties that arise with a two-electrode system where the potential at the reference electrode is being altered due to the current flowing through it[15]. Figure 9 below depicts a general three-electrode potentiostat set-up in a solution.

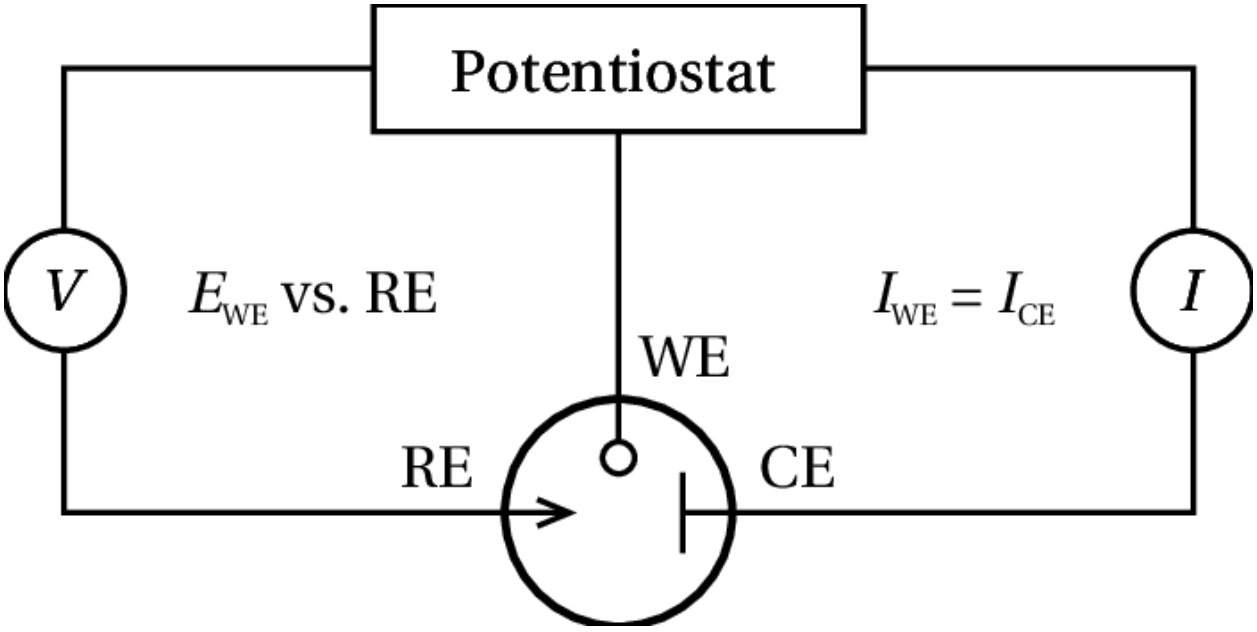

Figure 9: The general configuration of a three-electrode potentiostat. The working, counter, and reference electrodes are all placed in the same solution. The voltage difference between the working and counter electrode (E_{WE} vs. RE) powers the redox reaction. Current flow between the counter and working electrode completes the circuit.[17]

B. Materials

1. Electrodes
   - ❑ .25mm PTFE coated silver wire that is 2m long: Used for the counter electrode, acted as the extension of the Dexcom Sensor, and used to create the reference electrode. All insulation will be stripped away where connections to circuit are necessary.
   - ❑ Silver Epoxy Adhesive: Used to cement the Dexcom sensing wire and silver wire together. Silver Epoxy provides a strong, conductive connection between the two wires.
   - ❑ Dexcom-G4 Platinum Sensors: This is necessary to create the glucose redox reaction and sense output the current into the circuit.
   - ❑ Potassium Chloride: Used to make the solution for the silver chloride reaction which is then used to fabricate the reference electrode.
   - ❑ 9V Transistor Battery: Used to create the reference electrode by providing voltage to the silver-chloride reaction.

2. Signal Processing
   - ❑ SparkFun Resistor Kit: Supplied resistors for circuit.
   - ❑ Jumper Wires: Connected circuitry components.
   - ❑ Breadboard: Served as platform to connect all circuitry components.
   - ❑ Digital Multimeter: Used to test functionality of circuit.
   - ❑ Alligator Test Leads: Connected electrodes to circuit. Also connected DC power supply to circuit.
   - ❑ LM324n: Quad operational amplifier used for potentiostat control circuit and transimpedance amplifier.
   - ❑ Arduino Uno: Powered circuit and received raw voltage data from circuit.
   - ❑ PC: Ran Arduino IDE code to convert raw voltage to glucose concentration.
   - ❑ DC power supply: Supplied power to the LM324n operational amplifier.

3. Testing Materials

   - ❑ Deionized water, NaCl, KCl, NaH_2PO_4: Used to create the PBS solution for testing.
   - ❑ D-Glucose: Used in combination with the PBS solution to create the different concentrations of glucose solutions for testing.
   - ❑ Scoopula: Used to scoop solid materials out of containers to be weighed for the solutions.
   - ❑ Weigh boats: Used to hold solid materials to be weighed for the solutions.
   - ❑ Balance: Used to record the mass of the solid materials used for testing.
   - ❑ Beakers: Used to contain and mix the glucose/PBS solutions for testing.
   - ❑ Stir bar and stir plate: Used to stir and create well-mixed solutions for testing.
   - ❑ Contour Next ONE Blood Glucose Monitor: Used to verify the glucose concentrations of the created solutions.
   - ❑ Contour Next Test Strips: Used with the glucose monitor to verify the glucose

concentrations of the created solutions.

- ❏ Sponges: Used to represent the tissue leading up to the muscle compartment in the delivery model.
- ❏ Steak: Used to represent the muscle tissue within the compartment in the delivery model.
- ❏ Stylet: Used to enter the body and deliver the electrode to the muscle tissue and also used to show the cross section of the delivery model.
- ❏ Wire: Used to represent the theoretical glucose sensor in the delivery model.
- ❏ Clear plastic sheets: Used to place a scale on the images of the delivery model.

A. Methods

Working, Counter, Reference Electrode

The reference electrode was made first using the silver wire, KCL, and 9V battery and following the protocol given by Warner Instruments [12]. A 1.23M KCL solution was first prepared in a 300mL beaker of deionized water. Two pieces of the silver wire were then cut to 15 cm, and 7.5 cm were stripped on each. There was also 1 cm stripped on the opposite end of each wire. The 7.5cm stripped end of each wire was then coiled around the screwdriver to ensure the stipped part of the wires were fully emerged and did not touch each other. The side with only 1 cm stripped was folded over the beaker, so the entire 1 cm was exposed to attach an alligator clip to. The folded part of the wires were then taped on opposite of each other on the beaker to ensure they did not move during the chloriding. One alligator clip was attached to each of the wires and to the 9V battery, ensuring the cathode was attached first (*Fig. 10)*. The reaction ran for 8 minutes, the wires were switched to the opposite end of the battery, and then ran for another 8 minutes. The wire connected to the cathode first is the reference electrode. The working electrode was fabricated next using the Dexcom sensor and silver wire. The sensing wire was extracted from the Dexcom, and 1.5 cm of the silver wire was stripped. The epoxy was made and used to attach the sensing wire and silver wire by being placed on the end of the silver wire and joining the non-sensing end. This was let sit for several days with sufficient pressure on top. The counter electrode was fabricated last by simply stripping 1 cm off of one end, and 7.5 cm off of the other end.

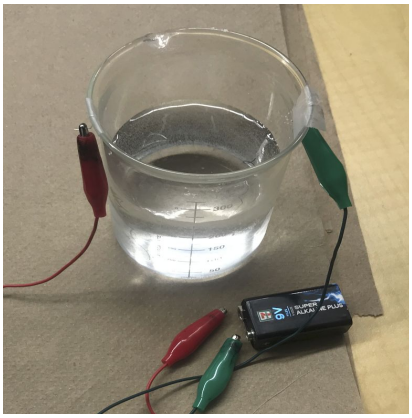

Figure 10: Chloriding the silver wire in a 1.23 M potassium chloride solution. The reference electrode, shown in green, is connected to the cathode of the 9V battery.

PBS/Glucose Solution

In a one liter beaker with 900mL of deionized water, 7.2g Nacl, 0.18g KCL, and 0.195g NaH_2PO_4 were added and dissolved into the solvent. 100 mL of the PBS solution was poured into 9 containers and labeled with 0, 25, 50, 60, 70, 100, 300, 500, and 1000 mg/dL. The correct glucose measurements were weighed out (0, 0.025, 0.05, 0.06, 0.07, 0.1, 0.3, 0.5, and .1 g of glucose respectively) and inserted into the PBS solutions. The solutions were then mixed.

Signal Processing

The Arduino Uno served as both the input voltage supply and output voltage input for the overall circuit. Immediately connected to the Arduino was the three-electrode potentiostat, which consisted of two parts. The first part was the potentiostat control circuit. This circuit's primary function was to establish a -300 mV potential at the reference electrode with respect to ground. Additionally, this circuit supplied an undefined, variable voltage to the counter electrode. The purpose of this undefined voltage was to keep up with the reaction at the working electrode and complete the circuit with the working electrode. The second part of the potentiostat was the transimpedance amplifier circuit. This circuit also had two functions. Its first function was to ground the working electrode, thus establishing the desired +300 mV difference between the working and reference electrodes. It's second function was to convert the current generated from the redox reaction at the working electrode into an output voltage. This output voltage was read into an analog input of the Arduino. Finally, the output voltage was converted into a glucose concentration using an experimentally determined voltage-glucose calibration curve (Arduino Code in *Appendix C*). The Arduino Uno, three-electrode potentiostat, and computer were connected according to the block diagram shown (*Fig. 11*).

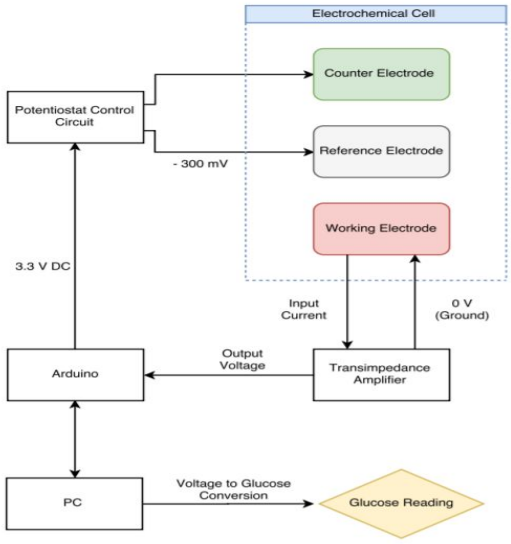

Figure 11: Block diagram of three-electrode potentiostat circuit. This block diagram outlines how the Arduino, circuit, electrodes, and PC interact with one another to ultimately take the input voltage from the Arduino and turn it into a glucose reading. Arduino IDE is used to make the voltage to glucose conversion.

The three-electrode potentiostat was created on a breadboard using a combination of wires, resistors, and operational amplifiers. The full circuit diagram for the potentiostat can be seen below (*Fig. 12)*.

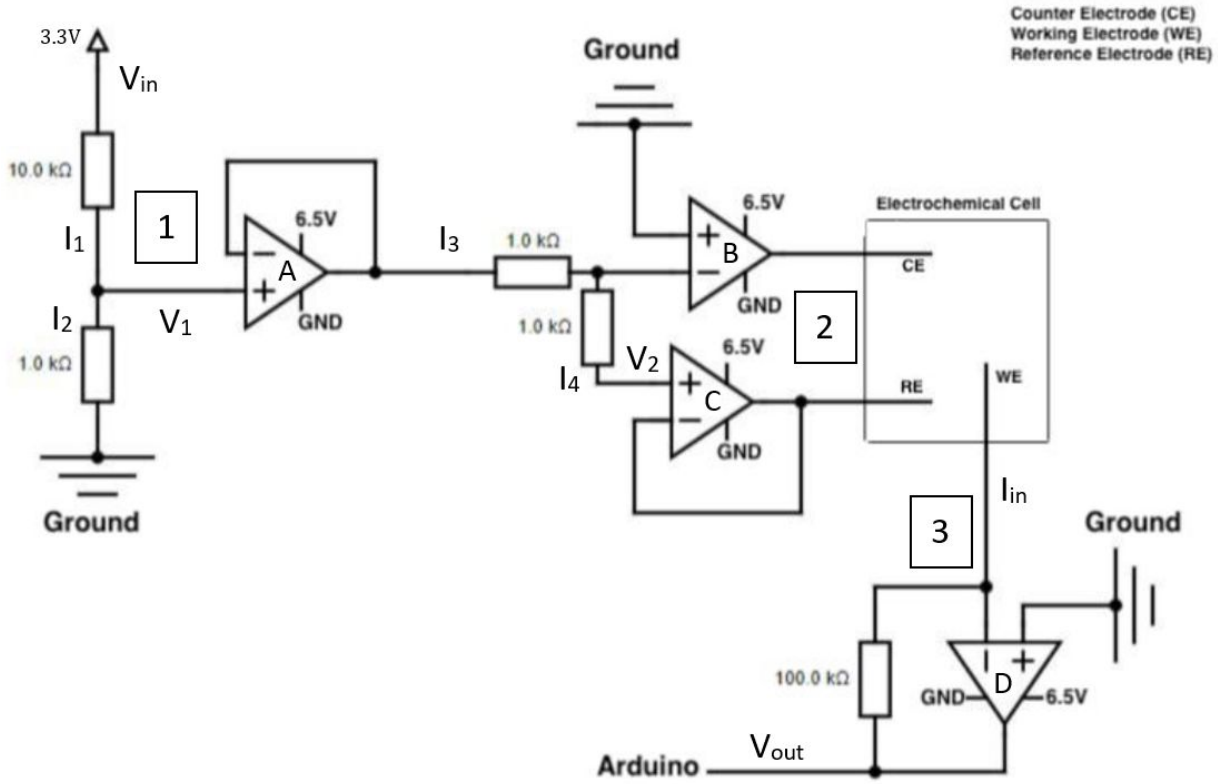

Figure 12: Three-electrode potentiostat circuit diagram. This circuit diagram depicts the overall configuration of the Arduino controlled potentiostat that was used for testing. There are three main components of this circuit – the voltage divider (1), the potentiostat control circuit (2), and the transimpedance amplifier (3). The LM324 quad op-amp was the only op-amp used, and it was supplied with +6.5 V and 0.0 V using a DC power supply.

The Arduino Uno supplies the initial V_{in} for the system of 3.3 V. This input voltage immediately passes through a voltage divider and voltage buffer circuit labeled as (1) on the circuit diagram above. Equations (1), (2), and (3), derived from Kirchoff's Laws, describe the change in voltage through these systems.

$$3.3V - (10.0\ k\Omega) * (I_1) = V_1 \qquad (1)$$

$$3.3V - (10.0\ k\Omega) * (I_1) - (1.0\ k\Omega) * (I_2) = 0\ V \qquad (2)$$

Since operational amplifiers have such high input impedance, they draw essentially no current. This means $I_1 = I_2$. Applying this assumption and solving for I_1 gives the following current.

$$I_1 = 3.3 \text{ milliamps} \qquad (3)$$

This value is plugged into equation (1) to solve for V_1.

$$V_1 = 0.30\ V \qquad (4)$$

V_1 next travels through a simple voltage follower that maintains the voltage at +300 mV, and serves to buffer the voltage divider from the rest of the circuit. From this point, V_1 runs through the second portion of the potentiostat control circuit labeled (2). This part of the circuit will connect to the counter and reference electrodes. To determine the voltage reaching the reference electrode, equations (4) and (5) were utilized. Note that the voltage at the inverting input of op-amp B is equal to the voltage at the non-inverting input. Thus, the voltage at the inverting input is 0 V.

$$V_1 - (1.0\ k\Omega) * (I_3) = 0\ V \qquad (4)$$

$$V_1 - (1.0\ k\Omega) * (I_3) - (1.0\ k\Omega) * (I_4) = V_2 \qquad (5)$$

Solving equation (4) for I_3 give the following current.

$$I_3 = 300\ \mu amps \qquad (6)$$

Since no current is drawn to the inverting input of op-amp B due to its high input impedance, $I_3 = I_4$. Applying this assumption and solving equation (5) for V_2 gives the following value.

$$V_2 = -300\ mV \qquad (7)$$

Since the inverting and non-inverting inputs of op-amp C must be equal, this means the voltage supplied to the reference electrode is - 300 mV. With respect to op-amp B and the counter electrode, there is no defined voltage or current being supplied to the counter electrode, as indicated by its open loop op-amp configuration. Instead, the voltage/current at the counter electrode will depend upon the redox reaction occurring at the working electrode, as the counter electrode is simply completing the circuit within the electrochemical cell.

The final part of the potentiostat circuit is the transimpedance amplifier (3), which grounds the working electrode and converts the current at the working electrode into a voltage. The current at the working electrode is directly caused by the redox reaction occurring on its surface. A higher glucose concentration within the electrochemical cell results in a higher current input into the transimpedance amplifier. Equation (8) describes how this input current is converted into a voltage value to be read by the Arduino.

$$(I_{in}) * (100\ k\Omega) = V_{out} \qquad (8)$$

Equation (8) holds true because the voltage reaching the 100 kΩ resistor of op-amp D is negligible since it is grounded at the non-inverting input. Realizing that our expected range of input currents is 0 - 10 μamps, equation (8) gives an expected voltage range of 0 - 1.0 V.

B. Final Prototype

The final prototype consists of three electrodes submerged in a known glucose solution. The electrodes lead to a potentiostat where the current input is converted to a voltage. The voltage will then be read by an arduino and processed by simple code **(***Fig. 13, Fig. 14***).**

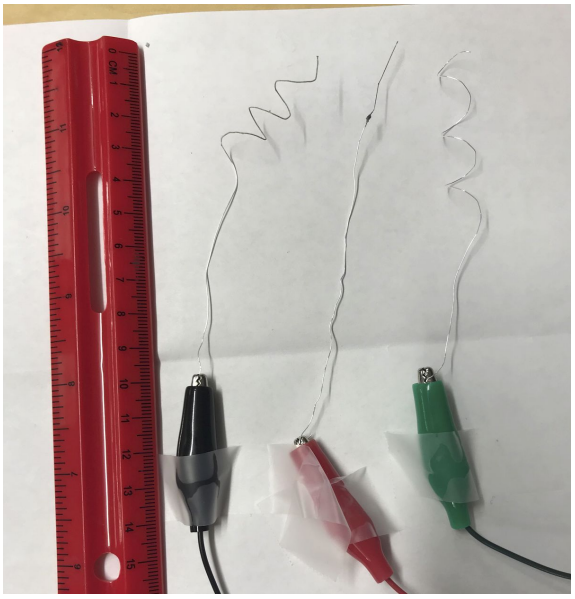

Figure 13: Three electrodes shown connected to the wires leading to the circuit. From left to right; reference, working, and counter electrode. The working electrode has been extended using silver Epoxy.

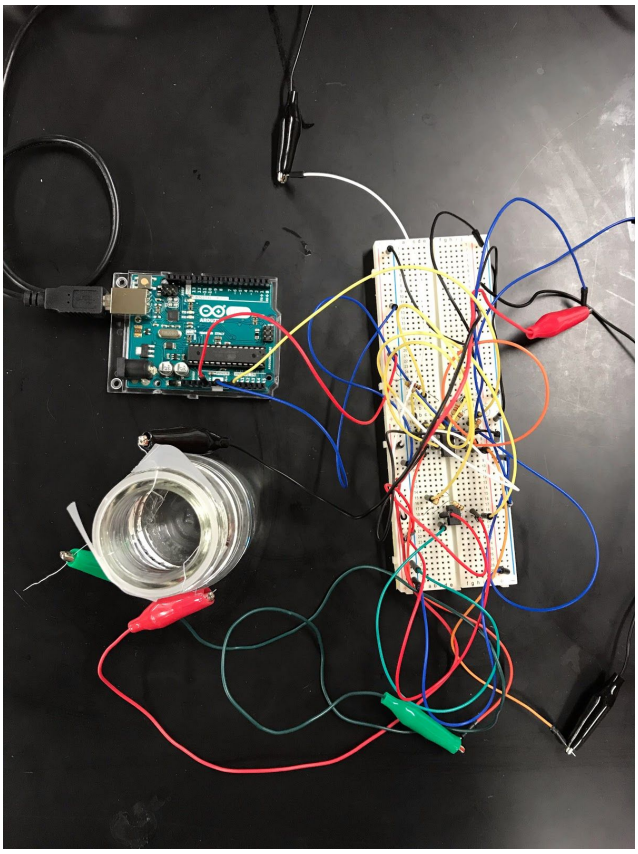

Figure 14: The final prototype consists of the three electrodes submerged in a known glucose concentration. The signal is then processed by the circuit and given an output value by the code written for the arduino.

C. Testing

The delivery model, explained in the Proposed Final Design section (*Fig. 7)*, was used to demonstrate the device meets the delivery and invasive requirements for this project. This testing model shows the stylet is able to deliver the electrode to varying depths of muscle tissue, anywhere from 2-8 cm, and prevents the sensing wire from deformation.

Nine different concentrations of PBS and glucose solutions were created in the BME Design Lab in order to test the accuracy of the design by comparing measured values of glucose

concentration to the known glucose concentrations, and then analyzing the results to determine the sources of error and variance. This design needs to be able to accurately detect low levels of glucose concentrations in order to improve the diagnosis of ACS, so the testing ranges from low concentrations of glucose to high concentrations. However, an overall functioning system was not able to be created in time to allow this test to be performed. Once it is determined how to get the electrodes properly communicating with the functioning circuitry, these solutions will be able to be used to get results that communicate the accuracy of the design, and will give direction for the next steps required. In the meantime, a store bought glucose monitor, the Contour Next™ ONE Blood Glucose Monitor, was used to measure the glucose concentrations of the PBS and glucose solutions created for testing. These measurements would be used as the known glucose concentrations when testing the accuracy of the device. These measurements were obtained by placing a testing strip into the glucose monitor, and then inserting the strip into the solution until a reading was outputted. These measurements were not able to be repeated due to repeated error codes being given by the glucose monitor.

Results

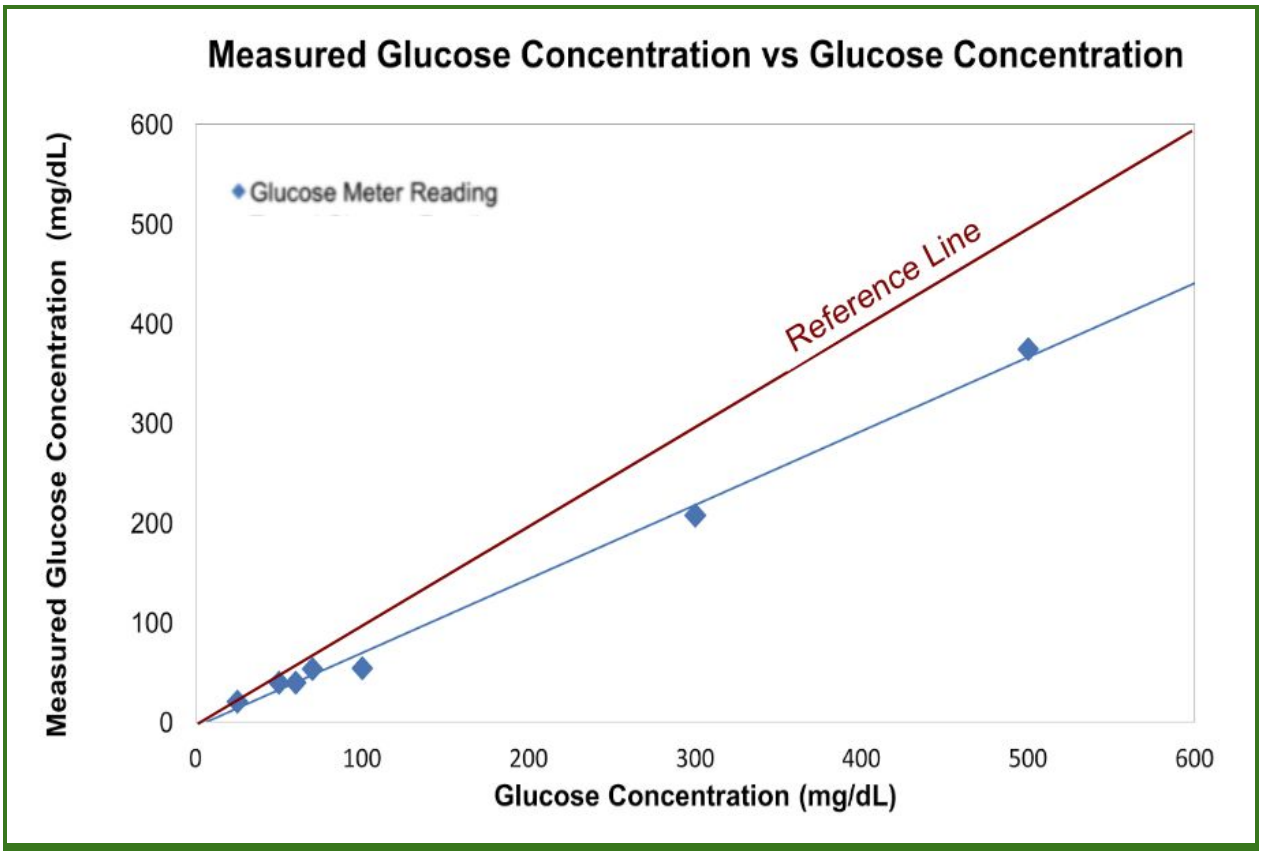

Figure 15: Graphical representation of the glucose solutions verified by store-bought glucose meter. Glucose meter shows strong linear relationship and is roughly 29% deviant from the reference line. Verifies the glucose concentrations were in fact increasing and somewhat predictable.

Glucose Solution Concentration (mg/dL)	Glucose Monitor Reading (mg/dL)	% Deviation from Reference
25.0	20	20%
50.0	39	22%
60.0	39	35%
70.0	53	25%
100	53.5	46.5%
300	207	31%
500	374	25.1%
	Average	29%

Table 2: Numerical representation of store-bought glucose meter compared to reference line average deviation from known glucose is 29%.

Discussion

Electrode

This semester, the working electrode was re-engineered from a commercially available product to a sensing electrode that would potentially work in the circuit. However, because the chemistry and manufacturing of the Dexcom sensor was unknown, difficulties arose in transmitting output values that were precise and accurate. Also, because of the unknown fabrication of the sensing wire, it became difficult to diagnose issues between the working electrode and the reference/counter electrodes.

3-Electrode Potentiostat

The Potentiostat control circuit and transimpedance amplifier were designed and built this semester. The voltage differences between electrodes were verified after each stage of fabrication and gave the expected values detailed in the methods section of signal processing. The circuitry system is functional, and is ready to be miniaturized.

Ethical considerations

One of the biggest considerations of the project is patient and nurse/doctor care when using the device. The delivery model chosen is the safest and most efficient way for the probe to be inserted into a compartment. The 18 gauge stylet is readily available, and can effectively penetrate into any compartment in any body due to the the ease of reaching different depths. The stylet also provides a housing unit for the glucose sensor that will protect from cross contamination and breaking of the electrode inside the patient's body. Any nurse or doctor who uses this device will be able to operate it themselves and be comfortable doing so.

Contour Next™ ONE Glucose Monitor

The glucose monitor gave consistently low glucose readings of the PBS/Glucose solutions, and the most relevant contributing factor can be attributed to the environment it was testing in. The monitor was made for detecting levels of glucose in the blood, whereas in this case, it was used in a solution of water with other dissolved molecules. Nonetheless, the monitor's prediction fit a linear trend and consistently verified our glucose concentrations were increasing.

Future Work

There are a number of alterations to the prototype design that are required to reach the health and safety standards for medical equipment.

The system will need to be shrunken down to fit into one stylet needle. The three electrodes cannot touch and currently, all electrodes need to be in the tissue to get an accurate reading. This would require three 18 gauge needles be inserted into the body. This would be very uncomfortable for the patient as they are potentially 8 cm deep and continuously monitor the muscle health for up to eight hours. Any movement would irritate the tissue and it would be best to minimize the discomfort of the needle.

Commercially available glucose monitors are required to have an accuracy within 20% for glucose concentrations greater than 75 mg/dl, and usually reach a maximum at around 500 mg/dl. Therefore, continuous glucose monitors are most accurate between ranges of 100-500 mg/dl. In hypoglycemic ranges (< 75 mg/dl), the accuracy is only required to be within 15 mg/dl, meaning a measurement of 60 mg/dl, may actually be anywhere from 45-75 mg/dl [18]. The final prototype needs to be accurate within these low glucose concentrations as a concentration below 60 mg/dl is indicative of compartment syndrome. Therefore, there are concerns with the potential accuracy of using an over the counter meter. For the future, it is expected that a glucose oxidase hand-coated platinum wire can eliminate the concerns with using an unknown chemistry of the commercial monitor.

Further testing with liquid and muscle models should also be implemented as part of the testing procedure. As current glucose readings are either taken directly from a blood sample, or measure from a subcutaneous fat layer, there is concern whether a change in the surroundings of the electrode may alter the accuracy and measurement. Comparing different models of testing will either emphasize this issue or prove insignificant.

To reach the standard of the client requirements, the device will need to be calibrated prior to use. The clinician should not have to translate the reading from an intermediate number. The potentiostat outputs a voltage and with a voltage to glucose concentration calibration curve, ideally the interface will read the concentration.

Conclusion

The aim of this design project was to explore alternate biomarkers that are indicative of acute compartment syndrome and to build a prototype that can quantify a selected marker. We chose to build a probe that quantifies intramuscular glucose considering its direct relation to compartment syndrome and continuous glucose monitoring technology is widely available. Other biomarker candidates, pH and sodium conductivity, proved too invasive and imprecise to build a working prototype. The probe was designed with a commercially available glucose sensor which generated current that led into a three electrode potentiostat. Various PBS/glucose solutions were also made to test and calibrate the circuit, yet due to complications among the working, reference, and counter electrode, the sensor was unable to detect changes in glucose concentrations. However, we validated the solutions contained predicted amounts of glucose via store-bought glucose meters. An invasive delivery model was also made to demonstrate the various depths the electrode may reach. In the future, it may be necessary to design a custom coating on the working electrode to minimize the amount of unknown variables in the circuit. Additionally, the potentiostat must be re-designed and tested thoroughly to ensure all signal processing portions are functioning properly. Long term, a properly constructed invasive glucose probe may be used efficiently in an Emergency Room to quickly and precisely diagnose patients with acute compartment syndrome. An accurate diagnosis may prevent patients from unnecessarily entering surgery to receive a fasciotomy.

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Appendix

A. Product Design Specifications

Function:

Acute compartment syndrome (ACS) impacts many trauma patients and presents medical providers with perplexing dilemmas regarding the diagnosis and treatment of this condition. ACS diagnosis is most frequently based on clinical examination findings, but, traditional measurements of intracompartmental (IC) pressure are unreliable and therefore commonly lead to misdiagnosis and unnecessarily invasive procedures. The goal of this project is to create a diagnostic tool that accurately, continuously, and easily quantifies biochemical marker associated with ACS. These markers – pH, glucose, or pyruvate – may expedite ACS diagnosis and prevent patients from misdiagnosis or the trauma of compartment syndrome.

Client Requirements:

- Design a probe to that can continuously measure and quantify specific biomarkers associated with acute compartment syndrome.
- The probe must be long enough to invade various muscular depths (1 cm - 5 cm)
- Probe must be cheap and autoclavable before use
- The probe should be continuously analyzed by a main analyzer
- The probe must be ergonomic for clinicians to operate

Physical and Operational Characteristics:

a. Performance Requirements:

- The probe must be able to measure a chosen biomarker that directly relates to the presence of compartment syndrome in a patient
- The probe must be able to continuously monitor the biomarker
- The probe must be precise, so there is a lower incidence of false positives (<34%) than the currently used pressure gauge detector.

b. Safety:

- In order for the probe to be up to the current standard of care for detecting compartment syndrome, the probe, if being inserted to the patient, must be smaller than an eighteen gauge needle.
- Cannot cause an increase in discomfort for the patient.
- Cannot increase the risk of infection in the already wounded limb of the patient.

c. Accuracy and Reliability:

- *The detector must accurately measure the specified biomarker/signal to avoid falsely diagnosing the patient.*

d. Life in Service:

- The disposable probe should be used once per patient. This means from the time the patient enters the hospital until the patient is discharged.
- The main analyzer should be able to be reused for many patients, lasting six months.

e. Shelf Life:

- The main analyzer should have a shelf life of approximately 3 years
- The disposable probe should have a shelf life of 1 year.

f. Operating Environment:

- The probe should be continually monitoring the compartment in all situations.
    - The ER immediately following the patient's arrival into the hospital.
    - The second is the patient' hospital room.
    - Another possibility is into an operating room for possible surgery.

g. Ergonomics:

- Physicians must easily probe the patient with one hand while securing their limb with the other. Will be similar to administering a shot.

h. Size:

- The probe to detect compartment syndrome has to be small enough so a nurse can bring it into the ER and collect a reading efficiently within a crowded area surrounding a patient.
- Also, the client does not want it to "scare" the patient as the probe is getting data.
- There are no specific specifications for the size.

i. Power Source:

- The main analyzer will utilize standard wall outlets as a power source.

j. Weight:

- The probe will be roughly 5 ounces. The main analyzer will be roughly 1 pound, subject to change.

k. Materials:

- Invasive probe, spectrometer/pH meter, glucose monitor, plastic box to house analyzer equipment

l. Aesthetics, Appearance, and Finish:

- The overall finish of the probe should not include any abrasive edges or jagged surfaces, which could injure the patient or doctor.
- The probe color will likely consist of neutral colors such as white, black, or grey.

Product Characteristics:

a. Quantity:

- One main analyzer compartment and many (20) reproducible probes to test on various subjects.

b. Target Product Cost:

- We have not been given a strict budget, the technology will be paid for through grants from the client.

Miscellaneous:

Standards and Specification:

- The probe will be no bigger than an 18 gauge needle and able to penetrate roughly 5 cm into muscular tissue.
- The main analyzer should withstand small drops and falls

Patient-Related Concerns:

- The patient does not want a large needle or series of tubes coming from their injured limb.
- The probe itself should also not be large or complex enough to frighten the injured patient.
- The patient is not under anesthesia so the insertion of the probe should not be lengthy.

Competition:

- Currently the only way to detect compartment syndrome is by pressure. This is very inaccurate and has led to a lot of false fasciotomies.
- There is also a new market using infrared detection to detect oxygen levels. This is also quite inaccurate.

Customer:

- Dr. Doro is an orthopedic surgeon at the UW Health Orthopedics and Rehabilitation center in Madison, Wisconsin. His research primarily focuses on diagnosing trauma patients with acute compartment syndrome.

B. Materials Table

Silver Epoxy Adhesive	MG Chemicals	8331S	1	$42.95	$42.95	https://www.amazon.com/MG-Chemicals-Silver-Epoxy-Adhesive/dp/B003BDMJSY/ref=pd_sim_328_2?_encoding=UTF8&psc=1&refRID=TSEARHG2QWSBF80KXWC8
0.25 mm PTFE coated silver wire (2 m)	Harvard Apparatus	64-1321	1	$50.82	$50.82	https://www.harvardapparatus.com/ag-agcl-pellet-disc-and-wire-electrodes-silver-wire.html
High Density Sponge	Wotesport	699907495752	2	$8.01	$16.02	https://www.amazon.com/Wotesport-Bath-Sponge-Density-Adult/dp/B074X1Y6P6/ref=sr_1_13_a_it?ie=UTF8&qid=1508722320&sr=8-13&keywords=high+density+sponge
AmazonBasics 9 Volt Everyday Alkaline Batteries (8-Pack)	Amazon	6LR16-8 PK	1	$9.49	$9.49	https://www.amazon.com/AmazonBasics-Everyday-Alkaline-Batteries-8-Pack/dp/B00MH4QM1S/ref=sr_1_1_sspa?ie=UTF8&qid=1509147263&sr=8-1-spons&keywords=9+volt+batteries&psc=1
Dexcom-G4 Platinum Sensors	DEXCOM INC	EDSTSGL041	2	$0.00	$0.00	https://www.edgepark.com/diabetes/cgm-continuous-glucose-monitors-and-supporting-supplies/sensors/dexcom-g4-platinum-sensor-kit/p/edstsgl041
Deionized water	N/A	N/A	900 mL	$0.00	$0.00	BME Design Lab
NaCl	N/A	N/A	~7 g	$0.00	$0.00	BME Design Lab
KCl	N/A	N/A	~65 g	$0.00	$0.00	BME Design Lab
NaH2PO4	N/A	N/A	~1 g	$0.00	$0.00	BME Design Lab
D-Glucose	N/A	N/A	~15 g	$0.00	$0.00	BME Design Lab
Scoopula	N/A	N/A	1	$0.00	$0.00	BME Design Lab
Weigh Boat	N/A	N/A	6	$0.00	$0.00	BME Design Lab
Balance	N/A	N/A	1	$0.00	$0.00	BME Design Lab
Beaker	N/A	N/A	10	$0.00	$0.00	BME Design Lab
Stir Bar and Stir Plate	N/A	N/A	1	$0.00	$0.00	BME Design Lab
Contour Next ONE Blood Glucose Monitor	Contour Next	30193781801	1	$19.99	$19.99	https://www.walgreens.com/store/c/contour-next-one-blood-glucose-meter/ID=prod6352499-product?ext=gooPLA_-_Home_Medical&pla&adtype=pla_with_promotion&kpid=sku6288304&sst=9191a044-be55-4a4c-828a-e8d6e8e311fc&reactjs=true
Contour Next Test Strips	Contour Next	30193731025	1	$29.99	$29.99	https://www.walgreens.com/store/c/contour-next-blood-glucose-test-strips/ID=prod6144656-product?reactjs=true
Steak	Fresh Grocery Store	N/A	1	$3.94	3.94	Fresh Grocery Store
Stylet	N/A	N/A	3	$0.00	$0.00	UW Hospital

Clear Plastic Sheet	Badger Bookstore	N/A	4	$0.20	$0.80	Badger Bookstore
Resistor Kit- 1/4W (500 total)	N/A	N/A	1	$0.00	$0.00	https://www.sparkfun.com/products/10969
Jumper Wires Standard 7" M/M - 30 AWG (30 Pack)	N/A	N/A	25	$0.00	$0.00	https://www.sparkfun.com/products/11026
Breadboard - Self-Adhesive (White)	N/A	N/A	1	$0.00	$0.00	https://www.sparkfun.com/products/12002
Digital Multimeter	N/A	N/A	1	$0.00	$0.00	BME Design Lab
Alligator Test Leads - Multicolored (10 Pack)	N/A	N/A	6	$0.00	$0.00	https://www.sparkfun.com/products/12978
LM324N Quadruple Operational Amplifier	Texas Instruments	5962-01-238-5501	1	$4.68	$4.68	https://www.amazon.com/gp/product/B01E910MGW/ref=oh_aui_detailpage_o01_s00?ie=UTF8&psc=1
Arduino Uno - R3	N/A		1	$0.00	$0.00	https://www.sparkfun.com/products/11021
DC power supply	N/A	N/A	1	$0.00	$0.00	BME Design Lab

C. Arduino IDE code

```
double analogIn=A0; // initializes analog input 0 to receive the Vout from the thermistor

void setup() {
  // put your setup code here, to run once:
Serial.begin(9600)
}

void loop() {
  // put your main code here, to run repeatedly:
int count = 0;
double Reading = 0;

while count < 50 {
  double adcIn = analogRead(analogIn); // Saves analog value of 0 - 1023 as adcIn
  double voltage = adcIn*(5.0/1023.0); // Converts adcIn value to voltage in volts
  Reading = Reading + voltage;
  count = count + 1;
  Serial.println( voltage);
  Serial.println(Reading);
  delay (1000); // wait 1 second
}
double average = Reading/50;
Serial.println(average);
}
```