EIROF Electrode-Facilitated Solution to Compartment Syndrome Diagnosis Using pH

Jonah Mudge, Hunter Huth, Lucas Ratajczyk, Syafiqah Saidin

Department of Biomedical Engineering, University of Wisconsin - Madison, Madison, WI, U.S.
E-mail: jmudge@wisc.edu

Received xxxxxx
Accepted for publication xxxxxx
Published xxxxxx

Abstract

Acute compartment syndrome (ACS) is a complex and difficult-to-diagnose condition in which trauma causes increased pressure in a muscle compartment, which can subsequently lead to muscle ischemia and death. Current methods of ACS diagnosis are often inaccurate, with pressure-based diagnosis reaching a rate of 35% false-positive. False-positive ACS diagnosis results in unnecessary fasciotomies, which are incredibly invasive and expensive procedures that often leave the patient with permanently impaired limb function. More recent methods of ACS diagnosis continue to suffer from inaccuracy and a lack of supporting literature, indicating the necessity of a new, more effective method to reduce misdiagnosis of ACS. The previous team that worked on this project presented a solution involving Ion-Sensitive Field-Effect Transistor (ISFET) pH sensors to detect acidic intramuscular environments indicative of muscle ischemia. We will be miniaturizing and implementing this design in a 16-gauge needle for eventual use in humans after testing in animals with an 11-gauge needle. Additionally, we have fabricated and tested iridium oxide (IrOx) electrodes as a possible miniaturized replacement for ISFETs.

Keywords: biomedical engineering, bioinstrumentation, acute compartment syndrome, EIROF, iridium oxide

1. Introduction

Acute compartment syndrome (ACS) is a difficult-to-diagnose and complex condition caused by an increase in muscle compartment pressure resulting in insufficient blood flow to the muscle tissue, causing muscle ischemia and possible tissue death [1]. ACS is uncommon, with an annual incidence of approximately 7.3 per 100,000 men and 0.7 per 100,000 women [2]. Because of this, there is limited information available from literature, and no gold standard for clinical diagnosis [3]. Furthermore, current methods of ACS diagnosis are flawed, with false-positive rates for direct pressure-based diagnosis reaching up to 35% [4]. The treatment for ACS is usually a fasciotomy of the affected muscle compartment, which can involve complications such as uncontrolled bleeding and infection [3]. Even if initially successful, subsequent operations are needed to remove dead muscle tissue and prevent sepsis, sometimes requiring limb amputation [3]. Fasciotomy survivors suffer from diminished range of motion, pain, and emotional trauma, making false-positive ACS diagnosis a serious issue, especially with the high cost of a fasciotomy operation [3]. Seeing that ACS misdiagnosis is a fixable problem in modern medicine, our client, Dr. Christopher Doro (an orthopedic surgeon with UW Health Orthopedics and Rehabilitation in Madison, WI), submitted this project to the BME department in an effort to minimize false-positives and reduce the number of ACS patients that suffer as a result.

1.1 Physiological Background

ACS is a limb- and life-threatening condition in which blood is prevented from leaving a muscle compartment. The most common cause of this blockage is bone fracture, which puts athletes at higher risk of developing this condition. The inability for blood to leave causes intracompartmental pressure to increase which eventually exceeds arterial pressure, preventing blood from entering the compartment. The lack of fresh blood causes buildup of tissue metabolites like CO2 that can cause tissue death and necrosis [5]. These conditions can damage the patient's whole body but will at least cause permanent damage to the muscles in the compartment [5]. If 6 hours have passed since the injury or blockage began but the physician is unsure if the compartment has ACS, a fasciotomy is performed. A fasciotomy, as seen in figure 2, is a procedure where the muscle compartment is sliced opent to reduce intracompartmental pressure and allow blood flow to return to normal [5].

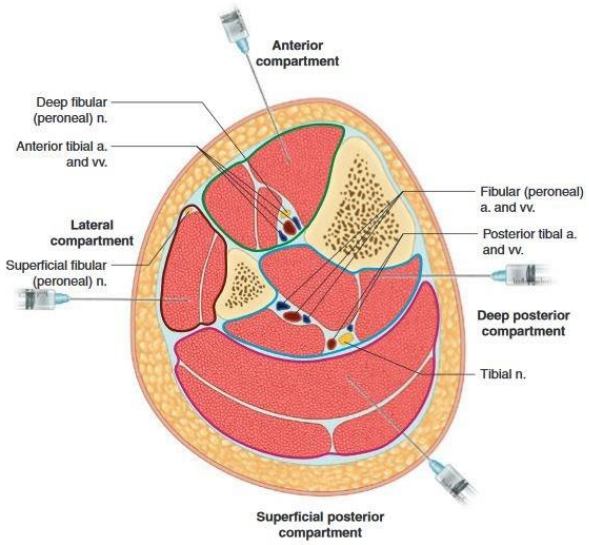


Figure 1: Muscle compartments in the lower left leg [5]

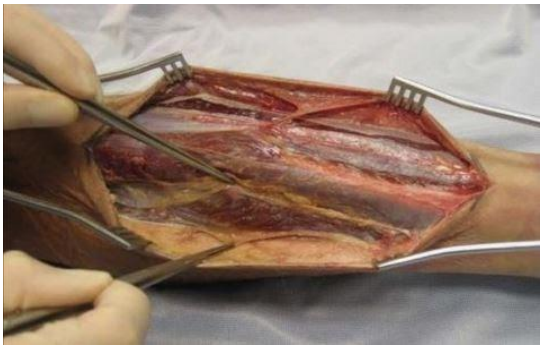


Figure 2: Fasciotomy of right forearm [3]

1.2 Modern ACS Diagnostic Alternatives

Common methods of ACS diagnosis today involve first testing compartment pressure directly using a catheter-enclosed pressure monitor [5]; however, this is often error prone and can result in misdiagnosis. More recent advancements have resulted in the formation of companies such as Odin Technologies and their near-infrared spectroscopy (NIRS)-based Valkyrie, which estimates blood oxygenation and is completely non-invasive [6]. However, NIRS has yet to demonstrate great accuracy in diagnosing ACS, and given that the technology has been in existence for several decades, it may not be the best solution [3]. Another alternative developed by NASA looked into the possibility of using ultrasound to estimate compartmental pressure, but this also has yet to be proven effective [7].

1.3 Current ACS Diagnostic Methods

Currently used methods of ACS diagnosis (such as direct pressure measurement) are highly unreliable and result in a very large percentage of false positive diagnoses (35%) [8]. A more accurate diagnostic method measures the partial pressure of oxygen in the blood of the compartment to detect the decrease associated with ACS. This method, while very reliable, is also far more expensive than pressure measurements, with the cost of single-use probes reaching upwards of $2,000 on top of multiple-use equipment that is even more expensive [3] [9]. Recent research has indicated that pH is also a reliable method of diagnosing ACS and can theoretically be implemented in a more cost-effective manner than partial pressure of oxygen diagnostic methods [10]. Because pH measurement in muscle compartments requires direct contact with the extracellular fluid in each compartment, restrictions require that the pH measuring device be able to at least fit in a 16 gauge needle (the largest permitted in human patients), with tip inner diameter of 1.19 mm [11].

1.4 Iridium Oxide Electrode Background

Iridium Oxide (IrOx) electrodes can be used to detect pH [12]. When deposited on a substrate such as platinum (Pt) or platinum-iridium (Pt-Ir) wiring, IrOx reacts with hydrogen ions in solution and can be used in combination with a reference electrode (such as Ag/AgCl) to get an output voltage that has a Nernstian relationship with pH [13]. The interaction between the IrOx layer and hydrogen ions in solution can be seen in equation 1 below.

$$2[IrO_2(OH)_2 \cdot 2H_2O]^{2-} + 3H^+ + 2e^- \leftrightarrows [Ir_2O_3 \cdot 2H_2O]^{3-} + 3H_2O$$

Equation 1: *Redox reaction between IrOx and solution*

Repeated cycling of current can create an Electrodeposited Iridium Oxide Film (EIROF), which is far more stable than a single-layered electrode. The number of electrons transferred for each hydrogen ion present can be used to determine the pH solution via a voltage measurement and the Nernst equation [13]. Consisting of merely two wires (the IrOx pH sensing electrode and the Ag/AgCl reference electrode), this pH sensing solution is extremely small in diameter and would fit easily within a 16 gauge needle for use in testing. However, no commercial solutions exist for this technology, and the methods required to fabricate it are complicated [13]. In order to pursue IrOx as a solution, our group fabricated each electrode ourselves. The same is true of the Ag/AgCl wire reference electrodes; both types of electrode were fabricated using a potentiostat to create layered films on each substrate for maximum electrode stability.

2. Methods

2.1 Preparation of Electrodepositing Solution for IrOx Electrode

The fabrication of the IrOx electrode utilizes electrodeposited iridium oxide films (EIROF) technique in alkaline solution. The electrodepositing solution was prepared according to methods by Yamanaka, using iridium tetrachloride, oxalic acid dihydrate, hydrogen peroxide, and anhydrous potassium carbonate [14]. The oxides from iridium, which is a group VII metal, are insoluble in alkaline solution. Precipitation of iridium oxide (IrO_2) will occur once a strong base such as potassium carbonate is added. Therefore, oxalic acid can be used to stabilize and form an iridium oxide complex even in a basic solution [12].

2.2 Electrodeposition of Iridium Oxide Electrode

The three-electrode cell setup includes a thin Pt/Ir wire as the working electrode, thicker Pt/Ir wire as the counter electrode, an Ag/AgCl as the reference and the electrodepositing solution. By using the Autolab Potentiostat, potential cycling of triangular waveform was applied from 0 to 0.55V at 50mV/s for 50 cycles, followed by pulse of square wave at 0.5s intervals for 1600 cycles. The triangular waveform was used to improve the EIROF adhesion of the oxide layer on the substrate [15].

2.3. Electrodeposition of Layered Ag/AgCl Reference Electrode

The Ag/AgCl reference electrode was fabricated using two Ag/AgCl wires that act as the working and counter electrode, respectively. Potential cycling of square wave was performed to improve the layering of the Ag/AgCl to ensure the functionality of the electrodes for 48 hours.

2.4 48-hour Drift Test

To test the clinical scenario of diagnosing patients with compartment syndrome, 48-hour drift tests were conducted to compare the sensitivity, reliability, and accuracy of different combination of electrodes. The electrodes used includes a glass bulb pH probe as the control, ISFET with Ag/AgCl reference, and IrOx with Ag/AgCl reference electrode. These electrodes were put inside a neutral pH buffer solution and the voltages were recorded for 48 hours. We hypothesized that the ISFET combination will maintain voltage longer than IrOx electrode due to flaking issues.

2.5 Cadaver Testing

The IrOx electrode was tested inside the muscle compartment of a cadaver to test its efficacy and functionality in a semi-solid substrate. A glass bulb pH probe was also used to compare the pH readings obtained. The pH measure should be the same for both probes.

2.6 In-Vivo Animal Testing

ACS was be induced in live pig legs via injection of saline directly into the muscle compartment. The IrOx electrode with the Ag/AgCl reference was inserted into the compartment periodically to confirm expected pH decline as the induced ACS progressed in the patient. The glass bulb pH probe was not used to validate the electrode results as the results of the cadaver testing did this already.

3. Results

3.1 48-Hour Drift Test of the Ag/AgCl Reference Electrode

The results of the 48-hour drift test are shown in Figure 3. The values recorded in the first hour were very consistent averaging 7.57 pH with a standard deviation of only 0.162. However, the values recorded during the following 1.5 hours were extremely erratic with a *standard deviation* of 8.36 pH and were therefore excluded from figure 3 to provided a clearer view of the overall pH trends. For the first 20 hours, the pH steadily increases until it reached its peak value of 8.92 pH where it remained constant for 5 hours. Following this, the pH declined in the same fashion to the original readings of 7.67 pH before dropping nearly instantaneously from 6.92 to 3.42 at 39 hours in solution. The pH remained at this low level for the rest of the drift test (10 hours). We believe this steep pH drop is due to the final layer of AgCl flaking off the reference electrode.

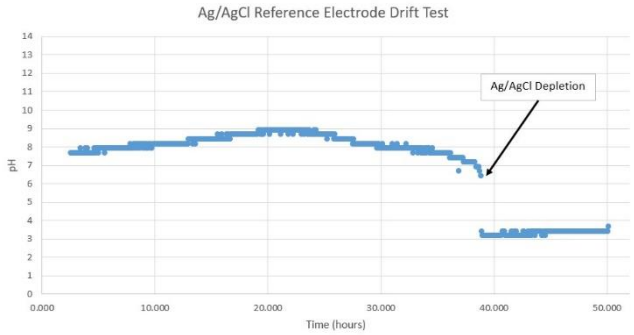


Figure 3: Ag/AgCl 48-Hour Drift Test. The pH recorded automatically every 5 minutes for 48 hours. Measured using a Winsense ISFET and our electrodeposited Ag/AgCl reference electrode. Erratic data from 1.0 – 2.5 hours has been removed for clarity. Steep drop in pH at 39th hour is believed to be caused by Ag/AgCl depletion.

Discussion

The current diagnostic method for ACS involves intracompartmental pressure readings, but the current accepted criteria for a diagnosis has a false positive rate of 35%. [8] A diagnosis of compartment syndrome requires a fasciotomy, which is an invasive procedure that has a substantially higher infection rate than patients not requiring a fasciotomy. Along with the risk of infection, a fasciotomy has a risk of non-union for the injury. [16] To protect patients from wasteful fasciotomies, there is a need for a new diagnostic method that has a more universal diagnostic criteria that will reduce false-positive diagnosis of ACS. Due to the physiology of muscle activity increasing carbon dioxide and lactic acid, pH has shown to have a significant decrease with an increase in muscle pressure [10]. Previous limitations for measuring pH inside the muscle compartment are due to the cumbersome size of pH electrodes.

ISFET technology has made improvements towards miniaturizing pH detection instrumentation, but it still suffers limitations due to impurities, instability and size. [17] This design mitigates these limitations by using only two wire electrodes of diameter 127 µm. The working electrode is a Pt-Ir with IrOx which has high corrosion resistance and biocompatibility. A counter electrode of Ag/AgCl has slight toxicity, but it has been used previously in short-term biomedical applications. [18] The results from testing this design showed *INSERT RESULTS HERE!!!!!!!!!!*

These two electrodes could make a significant impact on diagnosing compartment syndrome; therefore, improving patient health and satisfaction after certain cases of trauma. With improvements to longevity of the reference electrode, this design could be used for long term monitoring in cases of extreme trauma. The delivery method could be improved to reduce invasiveness by using a catheter-like method that uses a smaller gauge needle that retracts after electrode placement in the muscle compartment for continuous monitoring.

Conclusion

A two-electrode system that employs a platinum and iridium alloy wire electrodeposited with Iridium Oxide as a working electrode along with a reference electrode made from silver chloride coated silver wire provides a viable replacement for the current diagnostic methods of ACS. These electrodes were able to measure compartmental pH inside the muscle compartment with a statistically significant correlation to artificially induce ACS.

Acknowledgements

The authors would like to extend their gratitude towards the UW-Madison Biomedical Engineering Department for their support of this project. Additionally, they would like to thank their advisor, Dr. Amit Nimunkar, and client, Dr. Christopher Doro, for their guidance and support throughout the design process.

Ethical Statement

All procedures involving live animals were conducted with approval from IACUC.

References

[1] S. Olson and R. Glasgow, "Acute compartment syndrome in lower extremity musculoskeletal trauma," *The Journal of the American Academy of Orthopaedic Surgeons*, vol. 13, no. 7, pp. 436-444, 2005.

[2] M. M. McQueen, P. Gaston and C. M. Court-Brown, "Acute compartment syndrome. Who is at risk?," *The Journal of Bone and Joint Surgery*, vol. 82, no. 2, pp. 200-203, 2000.

[3] C. Doro, "Private Communication," Madison, 2019.

[4] A. Whitney, R. V. O'Toole, E. Hui, M. F. Sciadini, A. N. Pollak, T. T. Manson, W. A. Eglseder, R. C. Andersen, C. Lebrun, C. Doro and a. J. W. Nascone, "Do one-time intracompartmental pressure measurements have a high false-positive rate in diagnosing compartment syndrome?," *Journal of Trauma and Acute Care Surgery*, vol. 76, no. 2, pp. 479-483, 2014.

[5] D. Purcell, B. A. Terry and B. R. Sharp, "Acute Compartment Syndrome," *Emergency Orthopedics Handbook,* pp. 79-85, 2019.

[6] "The Valkyrie: A non-invasive diagnostic device for monitoring blood flow in trauma patients," Odin Technologies, [Online]. Available: https://www.odinhealthtech.com. [Accessed 8 October 2019].

[7] T. Ueno, A. R. Hargens and W. T. Yost, "Method and apparatus to assess compartment syndrome". United States of America Patent US7381186B2, 2 October 2004.

[8] A. Whitney, R. V. O'Toole, E. Hui, M. F. Sciandini, A. N. Pollak, T. T. Manson, W. A. Eglseder, R. C. Anderson, C. Lebrun, J. W. Nascone and C. Doro, "Do one-time intracompartmental pressure measurements have a high false-positive rate in diagnosing compartment syndrome?," *The journal of trauma and acute care surgery,* vol. 76, no. 2, pp. 479-483, 2014.

[9] J. W. Weick, "Direct Measurement of Tissue Oxygenation as a Method of Diagnosis of Acute Compartment Syndrome," *Journal of Orthopedic Trauma,* vol. 30, no. 11, pp. 585-591, 2016.

[10] S. T. Challa, A. R. Hargens, A. Uzosike and B. R. Macias, "Muscle Microvascular Blood Flow, Oxygenation, pH, and Perfusion Pressure Decrease in Simulated Acute Compartment Syndrome," *The Journal of Bone and Joint Surgery,* vol. 99, no. 17, pp. 1453-1459, 2017.

[11] Sigma-Aldrich, "Syringe Needle Gauge Chart," Sigma-Aldrich, [Online]. Available: https://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/needle-gauge-chart.html. [Accessed 11 Dec 2019].

[12] S. A. M. Marzouk, S. Ufer, R. P. Buck, T. A. Johnson, L. A. Dunlap and W. E. Cascio, "Electrodeposited Iridium Oxide pH Electrode for," *Analytical Chemistry,* vol. 70, no. 23, pp. 5054-5061, 1998.

[13] M. Khalil, S. Wang, J. Yu, R. L. Lee and N. Liu, "Electrodeposition of Iridium Oxide Nanoparticles for pH Sensing Electrodes," *Journal of The Electrochemical Society,* vol. 163, no. 9, 2016.

[14] K. Yamanaka, "Anodically Electrodeposited Iridium Oxide Films," *Japanese Journal of Applied,* no. 28, p. 632, 1989.

[15] R. D. Meyer, S. F. Cogan, T. H. Nguyen and D. R. Rauh, "Electrodeposited iridium oxide for neural stimulation and recording electrodes," *IEEE Transactions on Neural Systems and Rehabilitation Engineering,* vol. 9, no. 1, pp. 2-11, 2001.

[16] J. A. Blair, T. K. Stoops, M. C. Doarn, D. Kemper, M. Erdogan, R. Griffing and H. C. Sage, "Infection and Nonunion After Fasciotomy for Compartment Syndrome Associated With Tibia Fractures: A Matched Cohort Comparison," *Journal of Orthopaedic Trauma,* vol. 30, no. 7, pp. 392-396, 2016.

[17] C.-S. Lee, S. K. Kim and M. Kim, "Ion-Sensitive Field-Effect Transistor for Biological Sensing," *Sensors,* vol. 9, no. 9, pp. 7111-7131, 2009.

[18] B. D. Ratner, S. A. Hoffman, J. M. Yaszemski, E. J. Lemons and J. F. Schoen, Biomaterials Science: An Introduction to Materials in Medicine, Elsevier Science & Technology, 2012.

[19] J. Clark, M. Neuman, W. Olson and J. Webster, "Medical instrumentation," New York, Wiley, 2010, pp. 7-10.

[20] S. A. M. Marzouk, S. Ufer, R. P. Buck, T. A. Johnso, L. A. Dunlap and W. E. Cascio, "Electrodeposited Iridium Oxide pH Electrode for Measurement of Extracellular Myocardial Acidosis during Acute Ischemia," *Analytical Chemistry,* vol. 70, no. 23, pp. 5054-5061, 1998.

[21] S. T. B. Challa, A. R. Hargens, A. Uzosike and B. R. Macias, "Muscle Microvascular Blood Flow, Oxygenation, pH, and Perfusion Pressure Decrease in Simulated Acute Compartment Syndrome," *Journal of Bone,* vol. 99, no. 17, pp. 1453-1459, 2019.

Appendix A: Product Design Specification

pH Probes to Diagnose Compartment Syndrome

Date:	26 September 2019
Team Members:	Jonah Mudge, Lucas Ratajczyk, Hunter Huth, Nur Saidin
Advisor:	Dr. Amit Nimunkar
Client:	Dr. Christopher Doro

Function:

The pH probe interface must reliably display the pH level read by an ISFET sensor, and record it on a screen that is easily usable by a surgeon. It must be capable of recording pH data for a clinically relevant time period, while ensuring that no data or timestamps are lost. The device must also feature buttons (on a touch screen or otherwise) that are simple and reliable for a surgeon to interact with quickly, especially in high-stress situations in an OR. Along with probe interface is the ISFET probe casing, which must be able to fit into a 16 gauge needle while allowing accurate pH readings.

Problem Statement:

Compartment syndrome is a difficult-to-diagnose condition that occurs when tissue pressure in a muscle compartment rises enough to cause ischemia and possible muscle death. False-positive diagnosis of compartment syndrome can lead to expensive, invasive surgeries, and unnecessary surgeries. Our goal is to design and test a device for clinical use capable interfacing with an ISFET probe and measuring the pH within a muscle compartment, and using the pH as a reliable indicator of whether compartment syndrome is actually occurring.

Client requirements:

Create a device capable of measuring intramuscular pH in vivo

The device must be able to record at least 48 hours of pH measurements

The device should be minimally invasive

Design requirements:

1. Physical and Operational Characteristics

a. Performance requirements:

The probe should accurately measure the pH that relates to compartment syndrome that is within the range of 5 to 7.

The probe must continuously record the pH inside the compartment up to 48 hours.

b. Safety:

The electronics should not cause electrical shock to the user or patient.

The device should not cause any infection to the muscle compartment.

The device should not dissociate or fragment during compartmental insertion.

The device must not release toxic materials into the patient. The device must be sanitizable to prevent transfer of infectious material.

c. Accuracy and Reliability:

The device must be able to acquire the signal from the ISFET probe without noise

pH read from the probe must be accurate within a range of 5 - 7

Accuracy must be within 0.5 to ensure accurate readings and diagnosis

d. Life in Service:

The probe must maintain its structure and function over many daily uses.

The probe is disposable for a single use but the electronics of the pH sensor should last at least 5 years.

The electronic systems must be resilient for repeated use without breakdown.

e. Operating Environment:

The probe must survive insertion into a muscle compartment without shattering

The probe casing must not degrade or otherwise allow any leakage into the muscle compartment during insertion and monitoring

The main analyzer/probe interface must be able to survive falls in the case of an accidental drop

The main analyzer/probe interface must be able to weather small spills of bodily fluids or chemicals that might occur during an OR situation

f. Ergonomics:

The handheld probe interface should be shaped in a form that is easy to hold and does not pose any risks of injury from dropping

g. Size:

The probe must fit through the hole of a 16 gauge needle and 11 gauge needle for human application and canine testing, respectively.

The handheld portion of the device must not exceed a prism of the size 8”x8”x3”

h. Weight:

The probe must not exceed 2 ounces in weight

The handheld portion of the device must not weigh more than 16 ounces

i. Materials:

Semiconductor for the probe

Metal for the wiring to and within the handheld device

Hard plastic for the housing of the handheld portion of the device

j. Aesthetics, Appearance, and Finish:

Skin safe coating and material for use inside the body (muscle compartment)

The device should be intuitive and simple to understand and operate

The coating of the handheld portion of the device should have a rough texture to allow for better grip in time-sensitive situations

2. Production Characteristics

a. Quantity: 1 (prototype)

b. The budget is dependent upon grants received by the client with minimum immediately available funds exceeding $1,000

3. Miscellaneous

a. Standards and Specifications:

The size of the needle is limited to a 16-Gauge needle to align with standards for use in trauma patients.

b. Customer:

Customers (practicing trauma doctors) would desire a pH sensor that is placed inside a 16-gauge needle, which can read the real-time pH inside the muscle compartment of a patient who is at risk for compartment syndrome.

c. Patient-related concerns:

The device must have a detachable and replaceable needle/sensor. The display and electronics casing should be sterilizable with an alcohol.

Material of the device doesn't cause an inflammatory response, which could further increase pressure in the limb.

d. Competition:

The Valkyrie by Odin Technologies uses Near-infrared spectroscopy to estimate the blood oxygenation. This device has a benefit of being completely non-invasive, but this technology has been around for decades without success in accurately diagnosing compartment syndrome.

Appendix B: Design Matrix

To evaluate the three pH electrode options, ISFET, Platinum and Iridium wires, and Iridium-Coated Needle, we generated 5 criteria: size, fabrication complexity, ease of use, durability, and cost. Size and fabrication complexity are tied for the greatest importance given that a low score in either of these categories severely limits our ability to make a working prototype. Ease of use relates to the electrode's pH sensitivity - with greater sensitivity meaning a larger voltage change is produced from the same change in pH - and the difficulty of integrating it into our prototype's circuitry. This criterion also heavily impacts the likelihood that we can make a working prototype. Durability is an important consideration as it affects the sensor's fitness for our application environment. If the sensor has a low durability score, it is much more likely to fail during use, with potentially life-threatening consequences. Additionally, this criterion evaluates the expected lifetime of the device. In the case of the two options manufactured by us, this relates to the time before the electrolyte buffer begins to flake off the sensor or to become depleted. Last is cost, which, while important in all design situations, is less of a consideration for this project due to the low cost of the sensor and materials relative to our budget. Table 1 summarizes the scores of each design in each of these categories.

Table 1: Summary of the pH sensor design evaluation.

Criteria	ISFET	Pt-Ir	Ir-Needle
Size (25)	15	20	25
Fabrication Complexity (25)	10	20	20
Ease of Use (20)	20	15	15
Durability (20)	5	20	15
Cost (10)	10	5	7
Total (100)	**60**	**80**	**82**

Size

The ISFET design ranked lowest in this category due to the fact that the bare die's width of 1.44 mm has a small margin of fit within the inner diameter of a 11-gauge needle (2.388 mm) [11]. The iridium-platinum wire design was ranked lower than the iridium-coated needle because it requires a second wire to be inserted into the needle where the iridium-coated needle does not.

Fabrication Complexity

The iridium-platinum wire and the iridium-coated needle both follow the same fabrication protocol and thus, were ranked equally in this category. They also ranked higher than the ISFET because fabrication of the ISFET design requires the use of a cleanroom to handle circuitry on such a small scale, making the process much more difficult.

Ease of Use

Here, the ISFET proved better than the other two designs because the fact that it is purchased means that it also comes with an analog front-end system that greatly eases the integration of the ISFET into any prototype circuitry. In this category again, the platinum-iridium wire is not significantly different from the iridium-coated needle. Both acquire their pH-dependent in the same way and thus, require the same noise reduction and signal amplification prior to integration with the prototype circuitry.

Durability

The ISFET also ranked lowest in this category due to the delicate nature of the electrical connections formed at the micro-scale in the cleanroom. The iridium-coated needle was lower in rank than the platinum-iridium wire because the iridium-coated needle has less protection from the shear stresses on the exterior of the needle during injection and removal. However, since the coating of iridium is chemically

bonded to the needle, this is much less likely to impact the sensor performance than the weak electrical connections in the ISFET design.

Cost

The materials prices for the two iridium-based design options were all very similar, hence their very similar scores. The iridium-coated needle design is cheaper than the platinum-iridium wire design because it doesn't require a platinum-iridium wire. However, the ISFET design would actually be cheaper than either of the iridium-based designs, hence its high score in this category.

Total

The ISFET's low ranking in almost every category leads to the unsurprising conclusion that it is the worst of the three designs. However, the similarities in design and ranking of the other two designs led to a correspondingly close score gap between the iridium-coated needle and the platinum-iridium wire. The iridium-coated needle's advantages in size and cost overcame the platinum-iridium wire's minor advantage in durability, resulting in the proposal that the final prototype follow the iridium-coated needle design.

Appendix C : Fabrication Protocol

Ag/AgCl Layered Electrodeposition

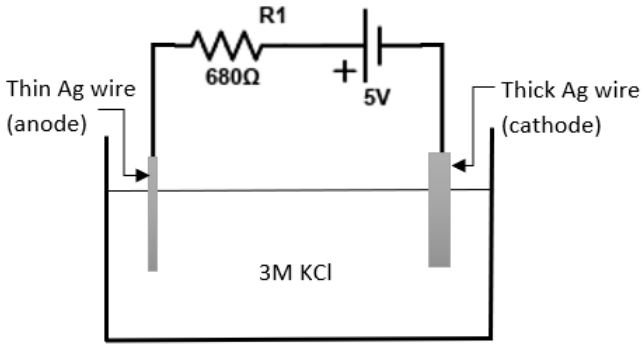
The steps of fabricating the Ag/AgCl reference electrode through layered electrodeposition are described as below, modified from the procedure described in *Medical Instrumentation* [19]:

1. A thin and thick Ag wire are prepared and rinsed with ethanol to remove finger oils
2. 3M of KCl (22.3 g) solution is prepared with 100 ml of deionized water and stirred until dissolved.
3. Both wires are immersed in the KCl solution. The thin Ag wire is connected to 680 **Ω** resistor and the positive terminal (act as anode) and the thick Ag wire is connected to the negative terminal (act as cathode).
4. The wires are connected in a two-electrode setup to their respective terminals (Working Electrode/Sensing Electrode, Reference Electrode/Counter Electrode) on an Autolab potentiostat
5. 1.5 V is passed through the circuit using a square wave (one second on, one second off) for 1000 cycles.
6. The fabricated Ag/AgCl electrode is then wiped and stored.

Figure 10: Setup of Ag/AgCl electrodeposition

IrOx Electrodeposition Solution

The fabrication protocol that we followed is as follows [20]:



1. Dissolve 75mg iridium tetrachloride in 50mL water
2. Stir 30 min
3. Add 0.5mL 30% hydrogen peroxide (aq) and stir 10 min
4. Add 250mg oxalic acid dihydrate and stir 10 min
5. Adjust pH slowly to 10.5 by adding small portions of anhydrous potassium carbonate
6. Leave at room temperature for 2 days to stabilize

The solution turns from greenish-yellow to blue-black after 48 hours.

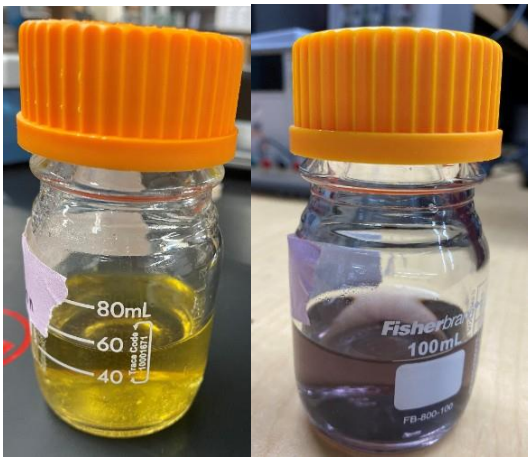


Figure 11: Electrodeposition solution before (left) and after (right) two days

IrOx Layered Electrodeposition

The steps for the electrodeposition of IrOx electrode are as follows [15]:

1. Set up Pt-Ir working electrode with Pt-Ir counter electrode and Ag/AgCl reference electrode in electrodeposition solution
2. Acquire wave generator and oscilloscope

3. Vary triangular waveform from 0 to 0.55V at 50mV/s for 50 cycles
    a. This is to improve EIROF adhesion to substrate
4. Pulse 0 to 0.55V square wave at 0.5s intervals for up to 1600 cycles

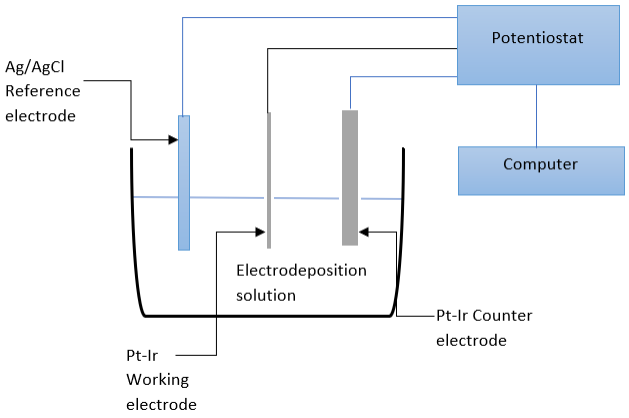


Figure 12: Three-electrode cell setup for IrOx electrodeposition