Quantification of intramuscular pH to diagnose acute compartment syndrome (ACS) using ion-sensitive field-effect transistor technology

Biomedical Engineering Design: 400

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December 12th, 2018

Client: Christopher Doro, MD

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Abstract

Acute compartment syndrome (ACS) is a condition in which a traumatic injury causes the tissue pressure in a muscle compartment to increase. As a result, tissue pressure exceeds the blood perfusion pressure, leading to cell anoxia, muscle ischemia and muscle death. Current ACS diagnosis methods rely on subjective assessments such as clinical examinations and intracompartmental pressure readings that return a false-positive diagnosis in 35% of cases, resulting in unnecessary and highly invasive surgeries. Research has shown that pH is a more indicative biomarker of ACS than pressure. Our goal is to develop an invasive probe that accurately measures physiological pH in humans. Researchers will be able to use this probe to set a pH threshold below which doctors can diagnose ACS.

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Introduction

A. Motivation

The diagnosis of acute compartment syndrome (ACS) traditionally involves subjective clinical examinations of trauma patients [1]. In recent years, an effort has been made to replace such qualitative measures with a quantitative method that is both accurate and reproducible. Intracompartmental pressure readings have shown some promise; however, values may vary within the patients' compartments and a pressure threshold that defines ACS is not well defined [2, 3]. Most alarmingly, these readings result in false diagnoses 35% of the time, thus many patients undergo unnecessary fasciotomies to treat their perceived ailment [4]. It is clear that a more accurate and definitive diagnosis for ACS must be explored to prevent needless and highly invasive surgeries.

A. Current Methods

Currently, clinical examinations in combination with a pressure reading taken from the compartment are the standard for diagnosing patients with compartment syndrome (Fig. 1). This method is flawed as it has an unclear diagnosis threshold and is ultimately a subjective assessment performed by the medical professional. While this method attains 100% diagnostic sensitivity, it produces false-positive diagnoses 35% of the time [4]. Experimental methods are under investigation in the areas of near infrared oximetry, pH, glucose, and partial oxygen pressure measurements, some with more success than others [6]. One of the more promising alternatives for ACS diagnosis is interstitial pH quantification. The Orion™ 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 humans because it is infeasible to insert such a large device in a human patient [7]. Furthermore, Dr. Doro has shown that continuous glucose monitoring may unveil compartment syndrome in adult beagles [8]. 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 [9].

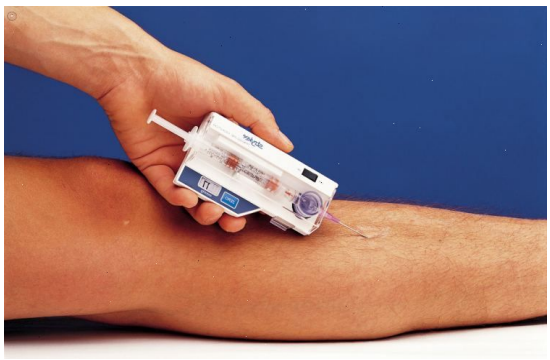

Fig. 1: Stryker pressure gauge for diagnosis of ACS. Pressure measurements are the current standard for diagnosing ACS, however, there is little consensus on the proper thresholds [5].

B. Problem Statement

Healthy patients diagnosed with ACS currently have a 35% chance of undergoing an unnecessary fasciotomy. However, if a patient with ACS is undiagnosed and therefore left untreated, they stand a near certain chance of their impacted muscle dying within eight hours. Due to the inaccuracy of diagnostic methods, surgeons frequently and incorrectly attempt to save dying limbs by performing highly invasive surgeries. It is therefore imperative that a more accurate, reliable, and novel diagnostic tool be developed to quantitatively and definitively decide the state of ACS in trauma patients.

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. An example is shown in Figure 2. 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 [11]. 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 and eliminates the perfusion gradient. Blood is no longer able to flow into the muscle because the pressure inside the compartment is higher than the arterial pressure. This syndrome leads to cell anoxia, muscle ischemia, and eventually muscle death if not treated, after which the only treatment is an amputation. 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]. Importantly, surgeons performing fasciotomies in ACS patients must ensure the entirety of muscular compartments are opened across the entire extremity. It is well understood that any revision of fasciotomy post-surgery

leads to a higher patient complications. One study found that revised-fasciotomy patients experienced a 1.5 and 3.33 times higher amputation and mortality rate, respectively [13].

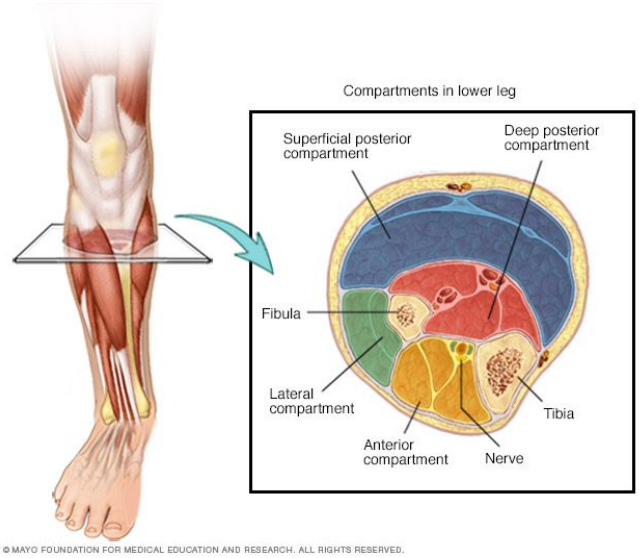

Fig. 2: The muscle compartments of the lower leg. Each muscle compartment consists of a group of muscles and the nerves and capillaries necessary to the muscles' function, surrounded by an inelastic fascia [10].

ACS affects up to 7.3/100,000 people in the general population, resulting from bone-fractures 69-75% of the time. ACS also occurs in men ten times more often than in women [14].

B. Design Research

Our client, Dr. Doro, has performed studies showing pH and glucose are effective biochemical markers for accurately diagnosing ACS. In Dr. Doro's experiments, a crude meat and cheese probe measured the intracompartmental pH in beagles with compartment syndrome. He found that the pH in an injured muscle can drop well below 7.1, a pH that would be fatal to a human [6, 15]. 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 [8]. Therefore, he highly encouraged that we try to build our probe measuring one of these biochemical markers. However, beyond Dr. Doro's initial research, he later informed us that potassium ions are a strong indicator of an increase in conductivity in the compartment. This is because dying muscle cells release potassium in mass quantities, which causes a major increase in conductivity. Therefore, potassium ions are also a potential biomarker to diagnose compartment syndrome.

Optical Fiber

In the spring semester of 2018, a previous team of BMEs working on this project decided to pursue the pH biomarker. Their literature searches showed that a glucose probe would have to be calibrated to each patient, which would increase both the length of the diagnostic procedure and leave more room for error. Unlike glucose, physiological pH is consistently 7.4 across all humans [13]. Therefore, a pH probe could be easily calibrated in a standard buffer solution.

The previous team also steered us towards the use of optical fibers as a potential light source and collection method. In terms of invasiveness, optical fiber diameters are on the scale of microns. This means there would be no issue inserting several of them, if necessary, through a 16 gauge needle. In theory, only two fibers would be needed. One would act as a light source and another would measure the amount of light transmitted.

Transistors

Transistors are electrical components capable of acting as variable switches. An example can be seen in Figure 3. Transistors consist of three terminals: a source, drain and gate. The voltage between the source and drain terminals regulates the current flow within the gate channel. The current produced is proportional to the pH of the solution [17].

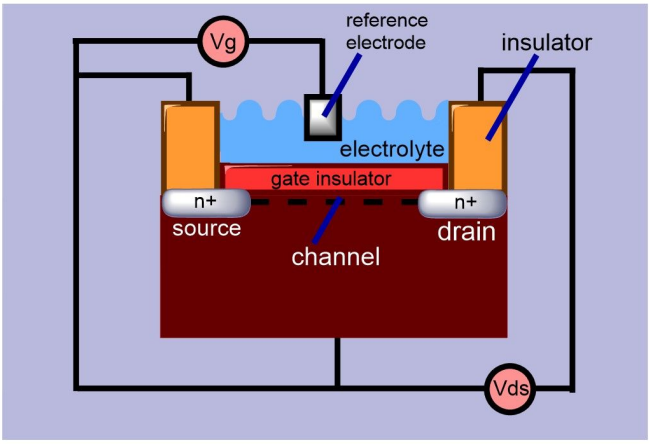

Fig. 3: A schematic of a field-effect transistor (FET). When the voltage at the gate insulator passes a threshold value, an electric current forms between the source and drain [16].

ISFET

Ion-selective field effect transistors (ISFETs) are a specific type of transistor in which the gate substrate is capable of developing a potential based on the proton concentration of its environment. Where a common transistor might have a metal-oxide semiconductor substrate at the gate, an ISFET tends to implement a SiOH layer, which creates a potential at the gate based on the acid-base reactions:

$$(1) \qquad SiOH + H_2O \leftrightarrow SiO^- + H_3O^+$$

(2) $SiOH + H_3O^+ \leftrightarrow SiOH_2^+ + H_2O$

This voltage stimulated at the gate now regulates the drain current output that will eventually be analyzed [18]. A more thorough explanation of ISFET technology will be given next semester as we begin to fabricate our own.

C. Client Information

Our client, Dr. Christopher Doro, is an Orthopedic Surgeon at the UW Hospital. He has conducted his own research on compartment syndrome, including the experimentation on canines to test the effectiveness of biochemical markers pH and glucose as standards to diagnose the condition. 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 is that the probe be able to improve the current 35% false positive diagnosis rate. This should be done using continuous and direct monitoring of intramuscular pH, with readings taken at least once every 10 minutes. The pH range of interest is 6 - 7.5. Additionally, the probe must be long and rigid enough to reach the muscle tissue (~1-5 cm) within a compartment in order to take measurements that are representative of the acidity in said compartment. The probe should also minimize discomfort for the patient and the 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 probe that is cheap and readily available, and a reusable data collection 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

We developed the following designs as possible diagnostic probes. All of the designs involve a component that punctures the muscle compartment, and will be applicable to any given compartment. Depending on the location requiring diagnosis, the probe will penetrate where the black lines in are drawn in Figure 4.

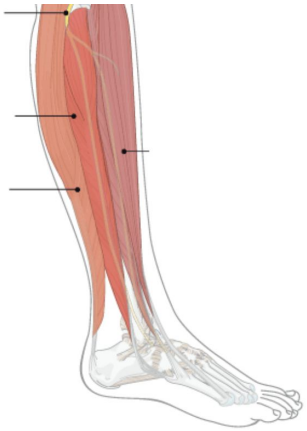

Fig. 4: Surgical reference of where the diagnostic device will be inserted into the patient [19].

A. Hydrogel-Dye Microenvironment:

This design is composed of 7 major components: the optical fibers, the epoxy glue, the hydrogel, the pH indicator, the hydrogen-permeable membrane, the control circuit, and the outer casing. The two optical fibers will extend into the compartment in a highly confined space. One optical fiber will be designated as the output optical fiber and shine light onto the pH indicator. The other optical fiber will be designated as the input optical fiber and will receive the light that is either reflected off or emitted from the pH indicator, depending on the exact indicator chemistry. Second, the epoxy glue will hold the optical fibers in place and maintain their structural stability. Third, a hydrogel will be located at the end of the optical fibers to allow light from the output optical fiber to pass through it relatively unimpeded and to immobilize the pH indicator. Fourth, the pH indicator will change the intensity of light reaching the input optical fiber depending on the pH of the compartment that it is placed inside. Fifth, the hydrogen-permeable membrane will allow protons to pass from the body to the pH indicator dye. This membrane will also serve as a biocompatible interface between the body and the indicator dye. Sixth the control circuit will regulate the light emitted from the output optical fiber, record the intensity of light from the input optical fiber, and perform calculations to correlate input light intensity to pH data. Finally, the outer casing will serve as a biocompatible barrier between the body and the contents of the probe, and it will hold the contents of the probe together. A complete diagram depicting the configuration of this design is shown below in Figure 5.

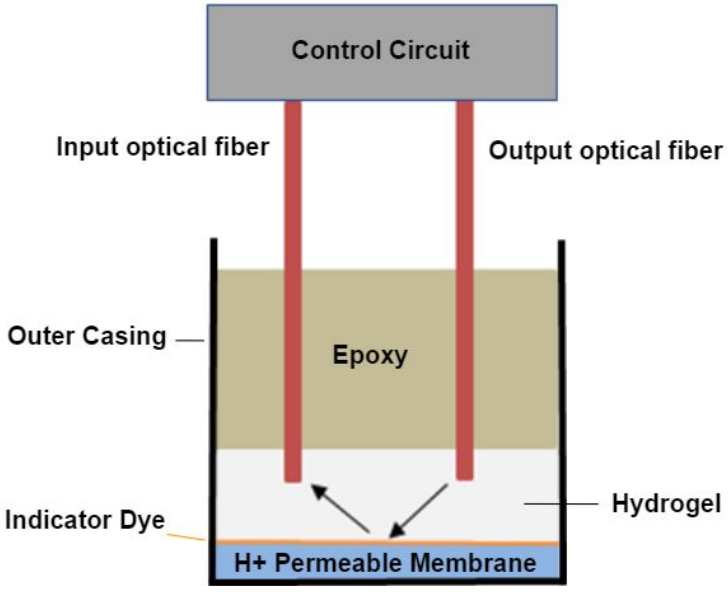

Fig. 5*:* Hydrogel-dye microenvironment design configuration

Overall, the compact design will allow for safe and easy measurements of pH. One potential drawback of this design could be immobilizing the pH indicator dye on a hydrogel, as this could prove to be quite difficult chemistry for us to perform with our resources.

B. Reflective pH-Reactive Tape:

Using a theory congruent to the optical fiber measurement in the hydrogel microenvironment design, this design would also measure the change in intensity of reflected light coming from an output optical fiber. Additionally, a control circuit similar to the hydrogel microenvironment design would be utilized since the same functions are desired from the control circuit. The main difference between these two designs is the way in which the optical probes and the indicator dye are connected to each other and the way in which the pH indicator dye is immobilized. For instance, instead of a hydrogel-dye microenvironment, the indicator dye will be immobilized on a reflective, adhesive surface that is secured directly to the end of the optical fiber probe. This means that the pH-reactive tape could be tested with a commercially available probe since it is independent of the probe.

Additionally, the pH-reactive tape will consist of four layers as outlined in Figure 6. The top and bottom adhesive layers accomplish two functions. The first function, specific to the bottom layer, is to adhere the pH-reactive tape to the probe, and the second function is to hold all four layers together. The second layer from the top is the gold reflective mesh layer, which acts as a biocompatible intermediary between the body and the pH indicator dye. This mesh allows diffusion of protons through it but is otherwise completely inert. Finally, the third layer from the top is the pH indicator dye. This dye will alter the intensity of the light reaching the input optical probe depending on the pH of the body.

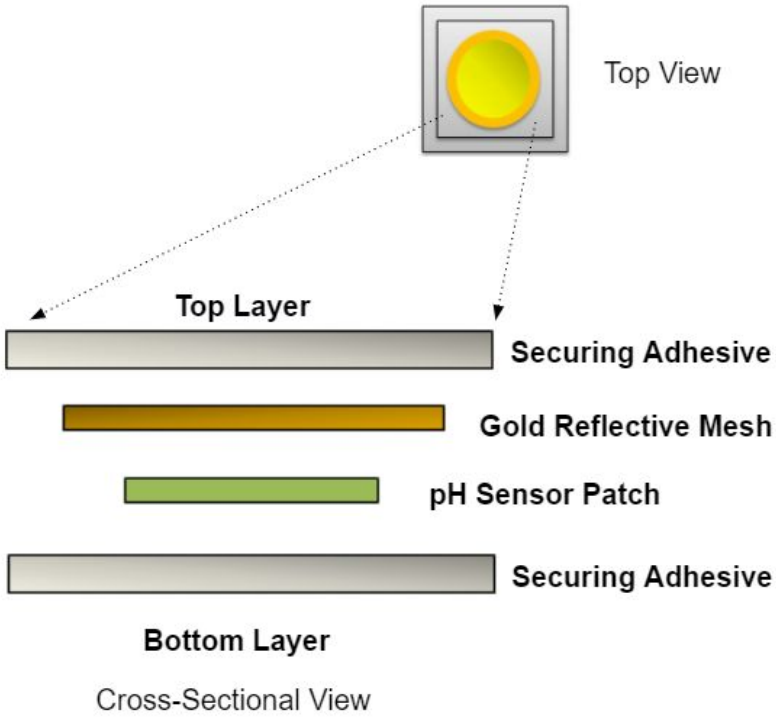

Fig. 6: Configuration of reflective, pH-reactive tape. The yellow circle represents a top view of the design. The four individual layers composing the tape can be seen in the cross-sectional view [20].

This design was based off of a current design utilized by Ocean Optics; however, their pH-reactive tape measures 0.50 inches in diameter making it far too large for our purposes [20]. Having said this, we can modify and miniaturize this design for our purposes. The greatest advantage of this design is that it allows for truly independent testing of the optical fiber portion and the biochemical portion of the design.

C. Microdialysis Chamber

This design is similar to the other two in that it relies on a pH indicator dye and spectrometry to determine pH; however, it differs from the other two in that the pH measurement will take place *ex vivo*. For this design, a minimally-invasive microdialysis probe will be inserted into the muscle compartment. This probe consists of three main parts: an inflow tube containing perfusate, a semipermeable membrane that allows for protons to be freely exchanged across it, and an outlet tube containing dialysate and the analyte. This configuration can be seen in Figure 7. First, the inflow tube continually provides perfusate, a solution that closely mimics the composition of healthy tissue fluid. Second, a semipermeable membrane within the microdialysis probe allows for the constant exchange of protons, so that a pseudo-equilibrium is achieved. Third, the outlet tube carries the pseudo-equilibrium solution out of the body and into a microdialysis chamber containing a known quantity of pH indicator dye. From here, standard absorbance spectrometry can be performed using any number of commercially available spectrometry devices.

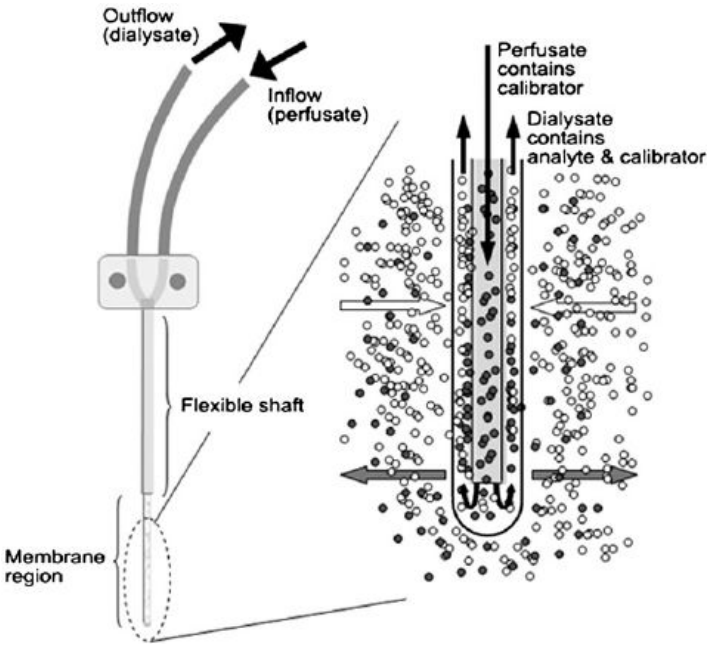

Fig. 7: Depiction of the microdialysis probe configuration as well as the microdialysis process [21].

This design is attractive because *ex vivo* measurement of pH would greatly simplify the spectroscopy portion of the measurement by removing the size limitations that come with *in vivo* measurement. Additionally, there would be no concerns of toxic pH indicator dyes interacting with the body. Conversely, calibration of this design could be quite difficult as a total equilibrium of protons is never reached within the body due to constant perfusate inflow. It also is not known whether the microenvironment within the compartment affects the pH drastically, thus measuring pH *ex vivo* could result in incorrect data.

Preliminary Design Evaluation

A. Design Matrix Criteria (See Table 1 below)

Accuracy and Precision:

This ranking carries the most weight because of the current limitations of compartment syndrome diagnosis, which has a 35% false positive rate. Our new device must replace this current standard of diagnosis while still being 100% sensitive to true positives. The Hydrogel Microenvironment scored highest because the hydrogen-permeable barrier would expose the indicator to the pH within the compartment without influencing acidity.

Biocompatibility:

Biocompatibility refers to the inertness of the probe inside the body. It is ranked second-highest because the probe should not damage the surrounding tissues beyond the initial insertion. The Microdialysis Chamber scored the highest because the indicator would be outside the body, and it would involve simply removing fluid using an FDA-approved hypodermic needle.

Invasiveness:

Invasiveness refers to the degree of interaction between the probe and the inner body. Ideally, invasiveness would be minimized to decrease the amount of damage done to the patient's tissues. The nature of acute compartment syndrome and its diagnosis require an invasive procedure, so the device with the least invasiveness would score highest. All three probes, however, scored 9/15. Both the Hydrogel Microenvironment and the Reflective pH Tape would involve the insertion of a 16-gauge needle and an indicator container, a substance foreign to the body. The Microdialysis Chamber would not involve the insertion of an indicator, however, this method would require that fluid be removed from the body, which if done over a prolonged period could injure the patient.

Ease of Reuse:

Ease of Reuse refers to how easy it is to prepare the device for reuse. This category encompasses either the ease of sterilization for designs that do not have replaceable parts or the ease of replacement for designs that have replaceable parts. The Reflective pH Tape and Microdialysis Chamber tied for the highest score in this category. The Reflective pH Tape would simply need to be disposed of and then another pre-made piece of tape adhered to the end of the probe. For the Microdialysis Chamber the entire analysis chamber could be reused, and only new dialysate solution and needles would be needed for reuse.

Measurement Continuity:

Measurement continuity refers to how continuously we can receive pH measurements. The Hydrogel Microenvironment and Reflective pH Tape designs both received full marks in this category since they can be altered by the controlling circuit to be as continuous as needed.

Cost:

Cost refers to our ability to test and fabricate the probe within a reasonable budget. The disposable probe should cost less than $100 to purchase, and the reusable part of the design should be less than $2000. This project is funded by a grant through the surgery department at UW-Health, therefore the budget is large, but does not have an official ceiling. The Reflective pH Tape scored highest because its parts (optical fibers, reflective tape, etc.) would be the cheapest to purchase and assemble.

Table 1: Evaluation of Proposed Designs. Design matrix comparing potential devices including Hydrogel-Dye Microenvironment, Reflective pH-Reactive Tape, and the Microdialysis Chamber. Criteria are listed on the left next to their assigned weight. The total scores are out of 100, and the highest score represents the most feasible option with regards to the criteria.

Criteria (Weight)	**Hydrogel Microenvironment**		**Reflective pH Tape**		**Microdialysis chamber**	
Accuracy and precision (35)	5	35	4	28	3	21
Biocompatibility (25)	4	20	4	20	5	25
Invasiveness (15)	3	9	3	9	3	9
Ease of Reuse(10)	2	6	4	8	4	8
Measurement Continuity (10)	5	10	5	10	3	6
Cost (5)	3	3	4	4	3	3
Total	83/100		79/100		72/100	

B. Proposed Final Design

Based on our design matrix, we decided to move forward with the Hydrogel Microenvironment design. The Hydrogel Microenvironment design stood out for having superior accuracy and precision compared to the other designs. Additionally, it scored very well in biocompatibility and measurement continuity. The biggest drawback of this design was its low reusability score, as continuously replacing a dye-filled hydrogel could be cumbersome. Despite this, the Hydrogel Microenvironment design accomplished all the criteria outlined by our client, thus it served as an excellent blueprint to begin prototyping a probe.

Changing Direction

After encountering some seemingly insurmountable obstacles with the Hydrogel Microenvironment design, namely the inability to find a dye that is both biocompatible and capable of a pH-dependent fluorescence, we began consider other paths we might take to develop a pH probe. While consulting with faculty members of the UW-Madison Biomedical Engineering Department, we were steered towards ISFET technology. After extensive research into the technology, it became apparent that ISFET technology would give us the best chance of developing a miniaturizable probe to measure intracompartmental pH.

Fabricating ISFET sensors requires access to a clean room and the associated technology

[22]. As undergraduate students, we do not have enough expertise in this area to construct an ISFET prototype with confidence, therefore we will have to outsource fabrication to a company or lab that specializes in clean room manufacturing. This will likely be very expensive, so we decided to first build confidence in ISFET technology through our own proof-of-concept experimental testing [23].

Fabrication

A. Materials
    a. DeltaTrak Water resistant pH meter Model 24310
        - Processing CPU unit for purchased ISFET probe
    b. DeltaTrak Heavy Duty Piercing Probe Model 24312
        - Proof-of-concept ISFET technology
    c. Orion™ 8163BNWP ROSS™ Combination Spear Tip pH Electrode
        - Original glass bulb technology used in clinent's ACS study
    d. Fisherbrand™ accumet™ AB150 pH Benchtop Meters
        - Glass bulb probe used to compare to ISFET technology
    e. Sorensen's Buffer
        - Used to generate varying pH solutions
    f. Sirloin steak mini-cuts, Trader Joe's
        - Subjects for meat-piercing test
    g. Additional material details found in Appendix B
B. Methods
    a. Sorensen buffer creation
        - Five 200 mL buffer solutions were created
            - 6.3, 6.5, 6.7, 6.9, 7.1
        - *Details of this method can be found in Appendix C*
    b. ISFET vs. Glass Bulb Test
        - Five pH conditions were split into 3x 50 mL beakers
        - Sirloin steak was sliced into similarly sized pieces and submerged in pH conditions
            - 3 steaks' were a control variable and were left exposed to air
            - Submerged steaks beakers were covered in parafilm
        - After 3 hours, streaks were removed from beakers
        - All steaks' pH was measured using ISFET and Glass Bulb probe.
        - Results were recorded, averaged, and plotted in excel
        - *Details of this method can be found in Appendix C*
    c. Potassium's influence on pH
        - Three pH 6.9 solutions were created in 500 mL beakers using Sorensen buffer
        - A 5 mM, 10 mM, and 15 mM K^+ solution was made using KCL
        - pH of each concentration was measured using ISFET and glass bulb sensor
        - Repeat measurements 3x
        - *Details of this method can be found in Appendix C*

C. Final Prototype

Before we build our own ISFET prototype, it is imperative that we develop confidence in the technology itself. DeltaTrak's piercing ISFET pH sensor is shown in Figure 8. This probe was used in comparison to current glass bulb pH technology during various tests. Using ImageJ, we determined that the sensing portion of the ISFET probe is ~0.5 mm in diameter, which is far smaller than a 16-gauge needle. Therefore, it is reasonable to assume this technology may be miniaturized.

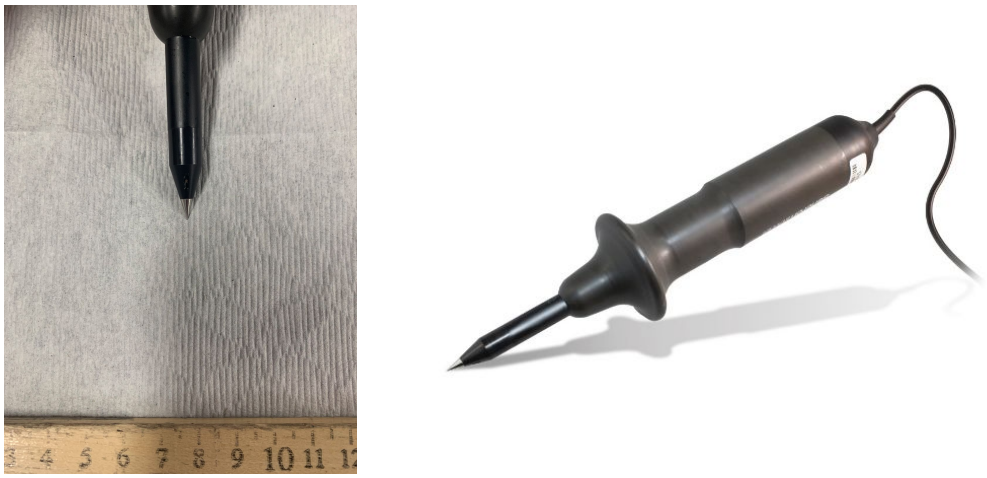

Fig. 8: Left: T DeltaTrak ISFET probe in relation to a ruler. Right: The DeltaTrak ISFET probe [24].

In preparation of building our own signal collection circuit, a preliminary circuit was breadboarded to simulate signals that would be collected using the ISFET probe. A voltage follower was used to incorporate the transistor and control the current input, while a bandpass filter was used to create corner frequencies capable of attenuating signals with frequency components outside of the desired range for measurement. Using the corner frequency equation,

$$f_c = 1/(2 * pi * R * C) \tag{3}$$

we constructed corner frequencies of 0.5 Hz (high pass filter) and 10 Hz (low pass filter). This means that at 0.5 Hz and 10 Hz, the signal will be attenuated to a gain of roughly -3 dB (70.7%) of the maximum signal amplitude. This circuit was tested experimentally and a corresponding Bode plot was constructed, which can be seen in Figure 9. The corner frequencies found in practice were approximately 0.5 Hz (as expected) and 6.9 Hz (likely due to an inherent added impedance in the transistor itself).

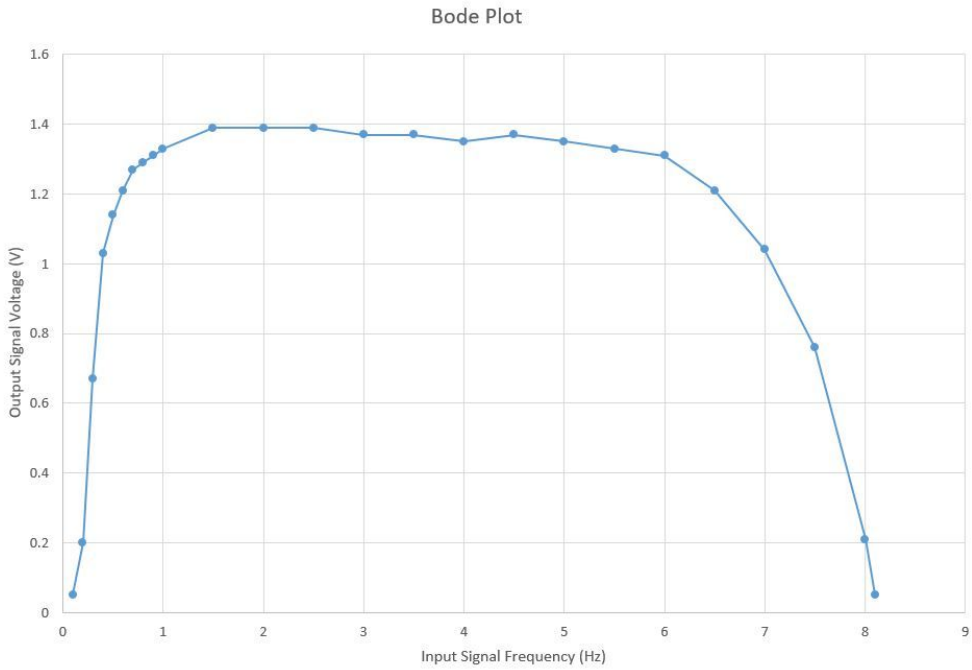

Fig. 9: Bode plot acquired by running signals of varying frequencies through the circuit. Note that $f_{C\text{-}HPF}$ = 0.5 Hz and $f_{C\text{-}LPF}$ = 6.9 Hz.

D. Testing

a. ISFET vs. Glass Bulb in a Steak Model

The purpose of this test was to compare the *in vitro* pH readings of the ISFET probe and glass bulb probe. Dr. Doro used glass bulb technology to diagnose ACS in canine models, therefore it is crucial that the ISFET probe make a comparable reading. If the probes provide similar readings, it would confirm that ISFET technology is a good direction to take. This is especially true because ISFET probes can be miniaturized to fit in 16-gauge needles, while this process would be more difficult with glass probes [22]. Store-bought steak was used as a model of the muscle compartment. The meat samples were submerged for 3 hours in buffers ranging in pH from 6.3 to 7.1. As shown in Figure 10, the two probes were inserted into different meat samples to measure the resulting pH. Figure 11 shows the meat samples submerged in the buffer solutions.

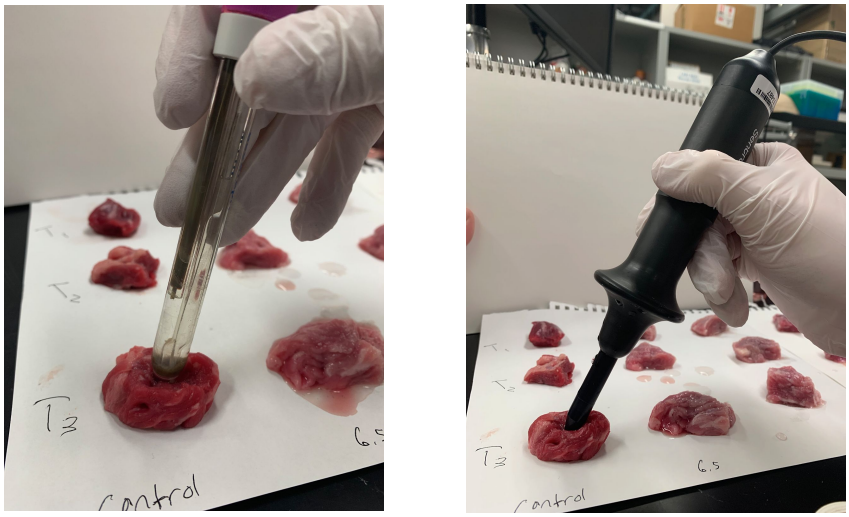

Fig. 10*:* Left: Insertion glass bulb pH probe into a buffer-soaked meat sample. Right: Insertion of the ISFET probe into a buffer-soaked meat sample.

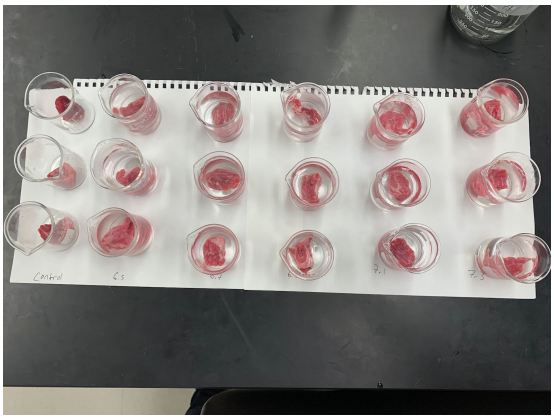

Fig. 11: Array of meat submerged in buffer solutions. Each slice is exposed to one of 6 conditions. Control, pH 6.3, 6.5, 6.7, 6.9, 7.1.

Error in the results of this test may arise from the spatial heterogeneity of a meat sample's pH. The exact pH may therefore be inconsistent across samples; therefore, the test was repeated three times. A more detailed testing protocol is available in Appendix C.

b. Influence of Potassium Ions on pH

The goal of this test was to quantify the effects of varying potassium levels on the ISFET sensor's pH reading. The interstitial concentration of potassium ions are known to rise during muscle ischemia [25]. Potassium is also a monocation similar to the hydrogen ion, therefore it is crucial to understand the effect of the ion's concentration, if any, on the pH measurements reported by ISFET technology. We compared the pH reading of

ISFET to the glass bulb probe; as stated before, our client currently uses glass bulb technology to measure muscular pH for ACS diagnosis. Therefore, if the pH reported by the ISFET is consistent with that of the glass bulb, it is likely that the ISFET will make comparable readings *in vivo*. Based on our previous comparison test, we did not expect to see drastic variances between the two devices, although we did expect that both technologies would report decreasing pH readings as the concentration of potassium increased [25]. It should be noted this test did not mimic *in vivo* ionic concentrations perfectly. In the body, potassium levels may vary drastically across local microenvironments. Therefore, while this test could confirm or deny the similarity of ISFET to glass bulb technology, it may not accurately mimic an actual muscular compartment's ionic signature. A full description of this protocol may be found in Appendix C.

Results

a. ISFET vs Glass bulb in a Steak Model

After collecting data from all three samples using both ISFET and glass bulb probes, the results were averaged and analyzed using a program in Excel. There was no significant difference between the probes. Standard error bars are included on the graph in Figure 12.

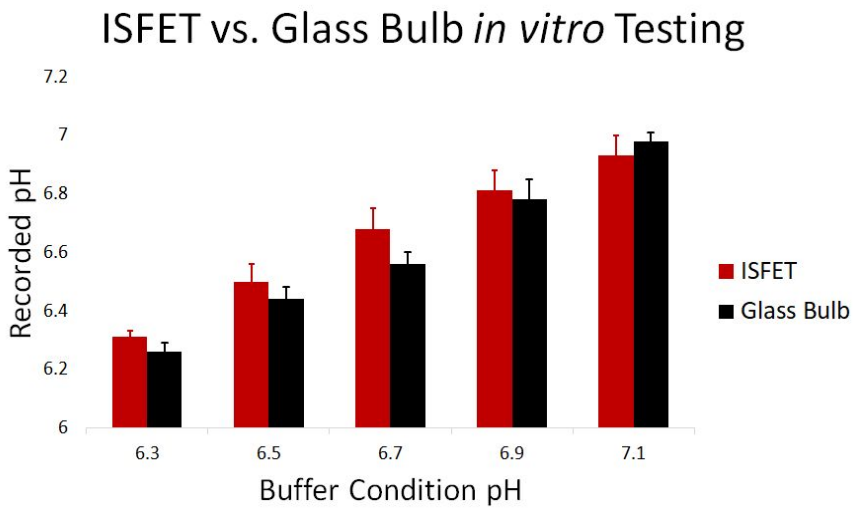

Fig. 12*:* Results of ISFET vs. glass bulb pH recordings in steak models. There was no significant difference found between the measurements.

b. The Influence of Potassium on pH

After collecting data using the ISFET and glass bulb probes, the results were once again averaged and analyzed using a program in Excel. The results can be seen in Figure 13. Potassium concentration was found to have no significant effect on the pH readings of either probe.

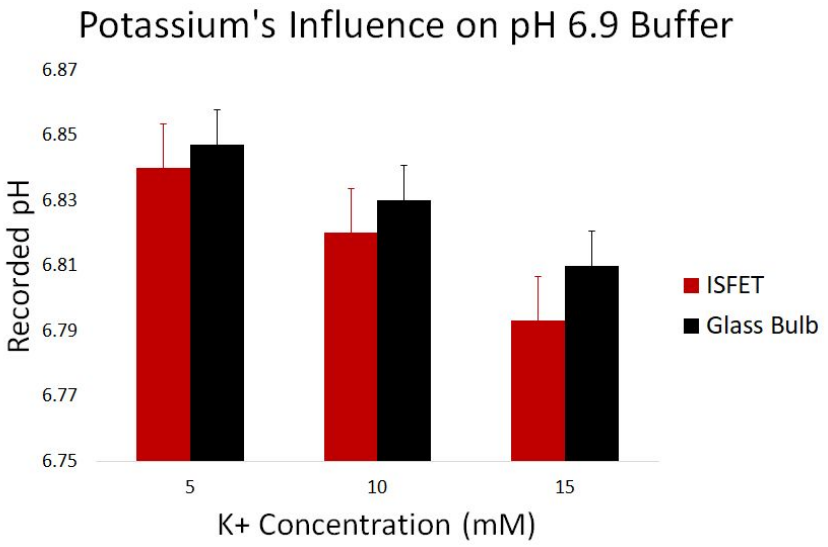

Fig. 13: Variations in pH due to potassium concentration were recorded with both ISFET and glass bulb technology.

Discussion

Based on the results of the steak model testing, it is clear that ISFET and the glass bulb probe produce similar pH measurements across the entire range of healthy and ischemic muscle pH (6.3 - 7.1). While this result was expected since ISFET probes are known to work within a pH range of 1.0 - 14.0, confirming its accuracy within a physiologically relevant model provided confidence in continuing with ISFET technology [18]. Another notable result from this experiment was the pH of the meat converged to the pH of the surrounding buffer solution. This is important because it reduces any potential error stemming from the individual meat samples beginning at different pH values. Finally, it should be noted that while both probes produced comparable measurements, there was still some error between values. The most likely explanation for this variation is that the ISFET probe was capable of actually piercing the meat and measuring the interior pH, while the glass bulb probe was unable to enter the meat and therefore had to measure the pH at the surface.

For the potassium test, there was once again no significant difference between the pH measurements for the ISFET and glass bulb probes. Additionally, the pH variance between the homeostatic potassium concentration of 5 mM and the max ischemic concentration of 15 mM was also insignificant [25]. Both of these results were in line with what we expected. Notably, concerns about increasing ionic concentrations appreciably affecting pH were quelled. As shown in the results section, pH decreased by only about 0.04 pH units as ionic concentrations increased. This phenomenon is explained by increasing ionic concentrations effectively lowering the activity coefficient of hydrogen ions [26]. Since it is the activity of hydrogen ions that is actually being measured by both probes, a lower activity coefficient results in a lower pH measurement [26]. While this effect is important to keep in mind for *in vitro* tests with varying

ion concentrations, *in vivo* tests would likely not be prone to this type of measurement error because the extracellular fluid (ECF) is a dynamic system in regards to ion concentration. As potassium levels rise during ischemia, both sodium and chlorine levels decrease in the ECF, which maintain proper ion concentration within the ECF [27].

In summary, both tests provided encouraging results that validate the continued use of ISFET technology for this project. Having said this, both tests also have some level of error associated with them. This error could either be a factor of the probe itself or the test conditions. The latter is the most likely case due to the highly variable nature of store-bought steaks both between and within the samples. Additionally, the Sentron ISFET probe claims to be accurate within +/- 0.01 pH units, and it was properly calibrated according to the meter that accompanied the probe.

Future Work

As previously stated, although there was no significant difference between the ISFET and glass bulb sensors, there was still error in the measurements. This was likely due to the variation between meat samples; however, it could also result from the sensor instrumentation. The goal of this project is to develop a probe that accurately measures compartmental pH to improve the specificity of ACS diagnosis. Therefore, we must minimize the error in the instrumentation as much as possible. An effective method for evaluating the sensitivity and specificity of diagnostic tests is the receiver operating curve (ROC) [28]. In the coming semester, we will design a test to produce the ROC curve of the ISFET probe. This will help us differentiate between the error resulting from variations in samples and the error coming from the probe itself. Based on the results, we will modify our design to minimize any error in the probe.

In conjunction with this test, we will also develop a CAD model of the miniaturized ISFET probe. This model must fit inside a 16-gauge needle and connect to the circuitry necessary to collect and process the signal. The signal processing circuit developed this semester will be updated so as to be incorporated into this CAD model. We will also select a new probe casing material to make it biocompatible; the current model uses acrylonitrile butadiene styrene (ABS), which is cytotoxic when placed in an *in vivo* environment [29]. The other materials used in this probe, stainless steel and polyether ether ketone (PEEK), are already approved by the FDA for long-term implants, and will therefore not have to be changed [30], [31].

While the CAD model is under development, we will also contact companies and labs with the ability to fabricate ISFET sensors. We are currently in contact with Sentron, a company that manufactures ISFET sensors to other organizations [32]. We will request quotes from them and any other prospective groups we find. During this process it will be important to work in close collaboration with Dr. Doro, as it will likely be very expensive to outsource ISFET fabrication.

Due to the time constraints placed on us as a team (14 weeks in a semester), this is as far as we are likely to get before graduation. However, we recommend that this project be continued by another design team starting Fall 2019. Assuming they receive the ISFET prototype by then, we will recommend that they begin testing to ensure that the probe provides accurate pH readings. For example, they should replicate the tests we performed this semester to ensure that the prototype is properly constructed and calibrated. They should also perform a drift test to ensure that the probe can continuously and accurately measure pH for up to 8 hours. Finally, they

should collaborate with Dr. Doro to begin *in vivo* testing with canines.

Conclusions

The goal of this project is to develop a probe that monitors the intracompartmental levels of a biomarker to diagnose acute compartment syndrome. Based on our own research and work performed by a past design team, we decided to use pH as the biomarker because of its consistency across humans and its ease of *ex vivo* calibration. Our proposed design was the Hydrogel Microenvironment, which used optical fibers and a pH sensitive dye. After receiving feedback from faculty in the Biomedical Engineering Department, we decided to forgo the use of optical fibers and pursue ISFET sensor technology. In a series of tests intended to quantify the accuracy of the pH sensor, we concluded that ISFET technology is a good direction to take this project. Starting next semester, we will incorporate our findings into a CAD model of a miniaturized sensor and collaborate with our client to outsource the fabrication to a company equipped with cleanroom technology.

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Appendix A

Product Design Specifications

Function:

Acute compartment syndrome (ACS), a dangerous condition in which the increased intracompartmental pressure (ICP) of a muscle prevents blood flow to the region, 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 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 receiving a false diagnosis and undergoing the trauma of a fasciotomy, the standard treatment for 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 - 5 cm)
- The probe must be cheap and preferably autoclavable before use (<$100 final prototype)
- The probe should provide up to 8 hours of continuous measurements.
- The probe must be ergonomic for clinicians to operate (setup time 5 minutes)

Physical and Operational Characteristics:

a. Performance Requirements:

- The probe must be able to measure pH that directly relates to the presence of compartment syndrome in a patient (pH 6-7.5)
- The probe must be able to continuously monitor the biomarker (1 sample/10 minutes, 8 hours in total)
- The probe must be precise, so that there is a lower incidence of false positives (<35% of diagnoses) than the currently used pressure gauge detector while still ensuring that no cases of ACS are missed.

b. Safety:

- In order for the probe to be up to the current standard of care, the probe, if being inserted into the patient, must be smaller than a 16-gauge needle.
- Minimize patient discomfort.
- Minimize the risk of infection for the already wounded patient.

c. Accuracy and Reliability:

- The probe must accurately measure the specified biomarker/signal to avoid falsely diagnosing the patient. (pH 6-7.5, high sensitivity +/ - .01 pH)

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 at least 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 patient's hospital room.
    - An operating room in the case of 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 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, our client does not want it to "scare" the patient as the probe is getting data.
- Must be able to reach at least 4-5 cm into the body
- Must fit within an 16-gauge needle.

i. Power Source:

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

j. Weight:

- The probe will weigh roughly 5 ounces. The main analyzer will be roughly 1 pound.

k. Materials:

- Invasive probe, ISFET sensor, instrumentation connecting probe to analyzer, 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. Final prototype should be $100.

Miscellaneous:

Standards and Specification:

- The probe will be invasive, and will therefore require FDA approval to be used in the United States.
- Before the device can be tested *in vivo* on animal models, the study will have to be approved by an internal review board (IRB).

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 may not be under anesthesia so the insertion of the probe should be as quick as possible.

Competition:

- Currently the only way to detect compartment syndrome is by using pressure. There is little agreement in the literature and amongst surgeons on the proper pressure threshold for diagnosing ACS; therefore, this is very inaccurate and has led to a lot of unneeded fasciotomies.

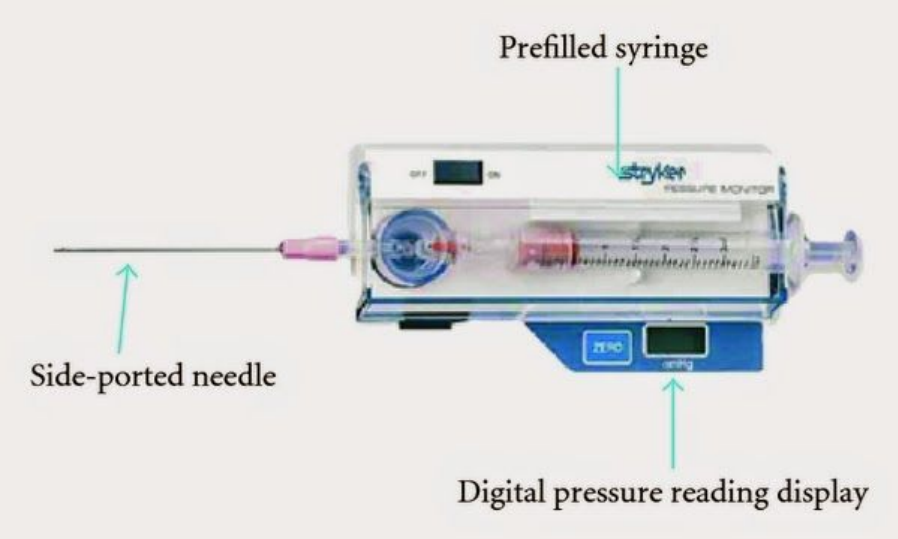

Figure 1: A Stryker Needle, a common instrument for monitoring pressure in a muscle compartment. The side-ported needle is inserted into the affected compartment, leading to a digital pressure reading that the clinician then compares to established threshold values for diagnosis.

- There is also research surrounding the use of near-infrared (NIR) spectroscopy to detect oxygen levels. While accurate in a lab setting, it has been difficult to adapt to a clinical setting.

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.

Appendix B

Materials list

1. DeltaTrak Water resistant pH meter Model 24310
2. DeltaTrak Heavy Duty Piercing Probe Model 24312
3. Orion™ 8163BNWP ROSS™ Combination Spear Tip pH Electrode
4. Fisherbrand™ accumet™ AB150 pH Benchtop Meters,
5. Sorensen's Buffer
    a. Disodium hydrogen phosphate, Millipore Sigma, NIST2186II
    b. Potassium dihydrogen phosphate, Millipore Sigma, NIST200B
6. Potassium chloride, Millipore Sigma, P9541-500G
7. Sirloin steak mini-cuts, Trader Joe's
8. 15x 50mL beakers
9. 3X 500 mL beakers
10. Parafilm
11. Magnetic stir rod
12. DATAPLATE® Digital Hot Plate/Stirrer

Order Form

Item	Description	Price	Link
Control pH probe	Used in original ACS diagnostic test - "meat and cheese"	$607.50	https://www.thermofisher.com/order/catalog/product/8163BNWP
Water resistant pH Meter w/AC adapter (24310)	Meter of test pH probe	$760.00	https://www.deltatrak.com/isfet-ph-meters/water-resistant-ph-meter-model-24310#accessories
Heavy Duty Piercing Probe (24312)	Insertion pH probe	$650.00	https://www.deltatrak.com/isfet-ph-meters/water-resistant-ph-meter-model-24310#accessories
Deltatrak handling fee	Actual shipping and taxes aren't listed	$14.10	
Thermometer	Measure temperature of solutions	$10.54	https://www.amazon.com/Weber-6750-Instant-Read-Thermometer/dp/B01MCWS5C9/ref=asc_df_B01MCWS5C9/?tag=hyprod-20&linkCode=df0&hvadid=167145798705&hvpos=1o3&hvnetw=g&hvrand=7847434038678928270&hvpone=&hvptwo=&hvqmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=9018953&hvtargid=pla-312518056556&psc=1
Large Tea Bags	To encase meat model during drift/test	$11.89	https://www.amazon.com/Disposable-Infuser-Drawstring-Natural-Unbleached/dp/B01GJ1VY7Q/ref=sr_1_5?s=home-garden&ie=UTF8&qid=1541371179&sr=1-5&keywords=large+tea+bags
		$2,054.03	

Appendix C

Methods

1. Sorensen Buffer creation
    a. Protocol used to generate 5 containers of Sorensen buffer for pH ranges 6.3 - 7.1 in 200 mL of deionized water
    b. Based off mixing 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 according to predetermined values of Sorensen buffer
        i. MW of NaH_2PO_4 = 119.98 g/mol
        ii. MW of Na_2HPO_4 = 141.96 g/mol
    c.

pH	NaH_2PO_4 (g/0.2 L)	Na_2HPO_4 (g/0.2 L)
6.3	3.28	1.79
6.5	2.71	2.47
7.1	2.16	3.12
7.2	1.58	3.80
7.5	1.10	4.37

    d. Each pH solution was created in 200 mL of DI water by adding the proper grams of Sorensen buffer components into the solution.
    e. Stir bar was placed in the beaker while chemicals were being mixed to ensure proper mixing
2. ISFET vs. Glass Bulb Test
    a. Each Sorensen solution was split into 3 separate 50mL small beakers
    b. Sirloin steak was sliced into similarly sized pieces
        i. 18 pieces of steak in total
    c. 3 steaks' pH was measured as a control variable
        i. 15 other steak pieces were submerged in 50 mL beakers for 3 hours
    d. Beakers were wrapped in parafilm during meat submersion to eliminate exposure to air
    e. After 3 hours, parafilm was removed and steak was placed on table
    f. All steaks' internal pH was measured using both ISFET and Glass Bulb probe
    g. Results were recorded, averaged, and plotted in excel
3. Potassium's influence on pH
    a. Create 3 pH buffers at pH 6.9 using the Sorensen pH buffer protocol in 3x 500 mL beakers
    b. Add KCl to each buffer solution to acquire a 5 mmol/L, 10 mmol/L, and 15 mmol/L K+
        i. Add 0.19 g KCl to 500 mL solution to create a 5 mmol/L K+ solution (homeostatic condition)

ii. Add 0.38 g KCl to 500 mL solution to create a 10 mmol/L K+ solution (Potential muscle ischemia)
iii. Add 0.56 g KCl to 500 mL solution to create a 15 mmol/L K+ solution (Typical plateau concentration for ischemia)

c. Measure pH of each solution using ISFET sensor
d. Measure pH of each solution using glass bulb pH sensor
e. Repeat experiment 3 times to reduce variation errors
    i. Record data and graph in excel