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Improving Acute Compartment Syndrome Diagnostic Technology with an Ion-Sensitive Field Effect Transistor

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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. The focus of this project 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.

Keywords: ion sensitive field-effect transistors (ISFETs), acute compartment syndrome (ACS), muscle ischemia, buffer solution, microprocessor, in vivo, in vitro

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1. Introduction

Acute Compartment Syndrome (ACS) can occur when a serious injury, commonly a bone fracture or deep bruising, causes a nearby muscle compartment to swell. The fascia surrounding the compartment is unable to stretch, thus the pressure inside of the compartment rises and prevents blood from flowing out of the muscle [1]. The only treatment is a fasciotomy 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 [2]. At that point, the only treatment is an amputation.

Currently, clinical examinations in combination with a pressure reading taken from the compartment are the standard for diagnosing acute compartment syndrome. 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 [3]. Patients given this incorrect diagnosis therefore receive an unnecessary fasciotomy, a highly invasive procedure that results in increased risk of infection and delayed union of healing bone fractures [4]. Furthermore, these patients stay in the hospital an average of six extra days, and are charged an average of $25,900 more than if they had not received the needless treatment [5].

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 OrionTM 8133BNWP ROSSTM Combination Spear Tip pH Electrode, a glass bulb probe produced by Fisher Scientific, has been used to diagnose adult beagles with intentionally onset ACS. The tip of the probe is 3 mm wide, 40 mm long, and detects pH 0 - 14 $\mp$ 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].

Ion-sensitive field effect transistors (ISFETs) were identified as a viable replacement. ISFETs are transistors in which the gate substrate is capable of developing a potential based on the proton concentration of its environment. Where a common transistor would have a metal-oxide semiconductor substrate at the gate, an ISFET implements an SiOH layer, which creates a potential at the gate based on the acid-base reactions:

$$\text{(1)} \qquad SiOH + H_2O \leftrightarrow SiO^- + H_3O^+$$
$$\text{(2)} \qquad SiOH + H_3O^+ \leftrightarrow SiOH_2^+ + H_2O$$

This voltage stimulated at the gate regulates the current output of the drain, which can be correlated to the pH of the environment of the sensor [8]. Due to its ability to be used *in vivo*, ISFET technology has great potential for use in a tool to measure

muscle pH. Unlike glass bulb probes, ISFET sensors can be miniaturized and are currently being applied on the scale of millimeters and smaller [9]. Furthermore ISFET probes are composed of more durable materials than traditional glass bulb electrodes, which increases their practicality in a clinical setting [9].

To evaluate the capability of ISFET technology to measure physiological pH, a Sentron ISFET probe was tested against a DeltaTrak glass bulb probe, which had previously been used in studies correlating pH and ACS [6]. Additionally, a Sentron modular ISFET pH kit was calibrated and evaluated in physiologically relevant conditions. The current probe reports pH accurately and has an acceptable amount of drift over the client's desired diagnostic timeframe. This promising preliminary data supports funding the development of an ISFET probe that can be implemented in ACS patients. Although the future steps in developing this device may be infeasible for students to accomplish given the financial and temporal constraints of BME design, the team's characterization of ISFET sensors within pertinent conditions lay the foundation for outsourcing this production to well-equipped companies.

2. Materials and Methods

2.1. Reagent Preparation for DeltaTrak ISFET Testing

Sorensen's buffer solutions were prepared in 400 mL beakers by mixing 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 purchased from Sigma-Aldrich according to Table 1, filling the beaker to a total volume of 200 mL using diH_2O. Contents were stirred thoroughly.

Table 1. Mass of 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 required to prepare a physiologically relevant range of Sorenson's buffer solution pH values.

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
6.7	2.16	3.12
6.9	1.58	3.80
7.1	1.10	4.37

Buffer solutions of varying potassium concentration were prepared from 200 mL of Sorenson's buffer solutions of pH 6.9. KCl from Sigma-Aldrich was added to these buffer solutions according to Table 2 in 400 mL beakers. Contents were stirred thoroughly.

Table 2. Mass of KCl required to prepare a physiologically relevant range of Sorenson's buffer solution K^+ values.

[K^+] (mM)	KCl (g/0.2 L)
5	.0746
10	.1491
15	.2237

2.2. DeltaTrak ISFET vs Glass Bulb In Vitro Testing

Sirloin steak mini-cuts purchased from Trader Joe's were immersed in Sorenson's buffer solutions with pH values of 6.3, 6.5, 6.7, 6.9, and 7.1 for 3 hours. The ISFET pH reading for the steaks was acquired using the DeltaTrak Heavy Duty Piercing Probe Model 24312 and piercing into the interior of the samples. The glass bulb pH reading was acquired by using the Fisherbrand™ Accumet AB150 pH Benchtop Meter and pressing the probe against the exterior of the steak. Three trials were conducted at each pH value.

2.3. Influence of Potassium on pH

Three Sorenson's buffer solutions of pH 6.9 and potassium concentrations of 5 mM, 10 mM, and 15 mM were prepared as indicated in the reagent preparation section. The ISFET pH reading was acquired using the DeltaTrak Heavy Duty Piercing Probe Model 24312 and placing it into the stirred solution. The glass bulb pH reading was similarly acquired using the Fisherbrand™ Accumet AB150 pH Benchtop Meter. Three trials were conducted at each concentration.

2.4 Analog Front-end ISFET Module Calibration

The analog front-end module was purchased from Sentron, NL as part of their ISFET modular pH kit. This module was connected to Arduino Uno via breadboard utilizing the I/O pins as specified on the Sentron datasheet. The physical ISFET sensor and reference electrode were attached as directed on this same datasheet. Both probes were submersed in Sentron calibration buffers (pH 4, 7, 10), and the voltage output was read using Arduino standard ADC conversion equations; a calibration curve was developed as shown in Equation 1 using the Excel linear fit model (R^2 = 0.999).

$$(Voltage - 1.1393) / 0.0499 = pH \quad (1)$$

2.5. Reagent Preparation for Experimental ISFET Testing

A number of buffer solutions ranging in pH from 4.0 to 10.0 were prepared in 250 mL beakers according to the directions outlined in Table 3. Notably, all solution were filled to 200 mL in total volume using diH_2O after adding the appropriate reagents.

Table 3: Buffer Solution Preparation Table

Buffer A: pH 4.0	Buffer B: pH 5.0	Buffer C: pH 6.0 - 8.0	Buffer D: pH 9.0	Buffer E: pH 10.0
100 ml 0.1 M potassium hydrogen phthalate + **X** mls of 0.1 M HCl	100 ml 0.1 M potassium hydrogen phthalate + **X** mls of 0.1 M NaOH	100 ml of 0.1 M KH2PO4 + **X** mls of 0.1 M NaOH	100 ml of 0.1 M tris + **X** mls of 0.1 M HCl	Stock potassium carbonate buffer solution = pH 10.0
pH 4.0 → 0.2 m HC	pH 5.0 → 45.2 ml HCl	pH 6.0 → 11.2 ml NaOH	pH 9.0 → 11.4 ml HCl	N/A
N/A	N/A	pH 7.0 →58.2 ml NaOH	N/A	N/A
N/A	N/A	pH 8.0 → 93.4 ml NaOH	N/A	N/A

After adding the necessary reagents and filling to 200 mL in volume with diH_2O, the contents of each buffer solution were mixed thoroughly using a stir bar and stir plate.

2.6 Experimental ISFET Sensor pH Characterization

To characterize the experimental ISFET sensor, all of the pH solutions outlined in section 2.5 were utilized. In addition to these reagents, the Sentron ISFET kit pH sensor and Oakton WD-35619 pH 510 Benchtop Meter were used for making pH measurements at a room temperature of 22.8°C. To record a measurement, each sensor was placed in a pH buffer solution for 30 seconds and data was either recorded every 350 milliseconds using Arduino IDE for the experimental ISFET or read directly off the digital interface for the glass bulb benchtop meter. The data over this 30 second interval was averaged using Microsoft Excel to obtain a value for each pH buffer solution.

2.7 Experimental ISFET In Vitro *Drift Test*

To measure sensor drift, the Sentron ISFET kit pH sensor with digital interface was compared against the Oakton WD-35619 pH 510 pH Benchtop Meter at an ambient

temperature of 21.6°C. For this test, only the pH 7.0 buffer solution was used because it was close to the physiologically relevant pH of 7.4 [10]. To perform this experiment, both sensors were held in the buffer solution for one hour with measurements being recorded every 10 minutes. Once again Arduino IDE was used to average 30 seconds of data around each time point for the experimental ISFET sensor.

2.8 Degradation and Swelling Test for PEEK

Buffer solutions of pH 4.0, 7.0, and 10.0 were prepared as outlined in section 2.5 to test for degradation and/or swelling of PEEK tubing with a .125" outer diameter and .093" inner diameter. The PEEK tubing was cut into nine 1 cm long testing samples, and the mass and length of each sample was measured using a scale and caliper, respectively. Then, three PEEK samples each were immersed in the three buffer solution conditions at an ambient temperature of 22.8 °C for 2.5 hours. The time period of 2.5 hours was chosen because it well exceeds the duration that the sensor would be in a patient in a clinical setting. After 2.5 hours, the samples were removed from solution and dried using Kimwipes and a gas nozzle that blow-dried the samples with air. The dried samples were then remeasured using both the scale and caliper, and the final mass and length measurements were recorded.

2.9 Statistics

All statistical tests were performed using standard functions in MATLAB version R2018B. Data was checked for normal distribution using a Kolmogorov-Smirnov test. Data not found to be from normal distributions were compared using the parametric Mann-Whitney test. See Appendix A for the appropriate MATLAB code.

3. Results

3.1. DeltaTrak ISFET vs Glass bulb In Vitro Testing

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 as shown in Figure 1.

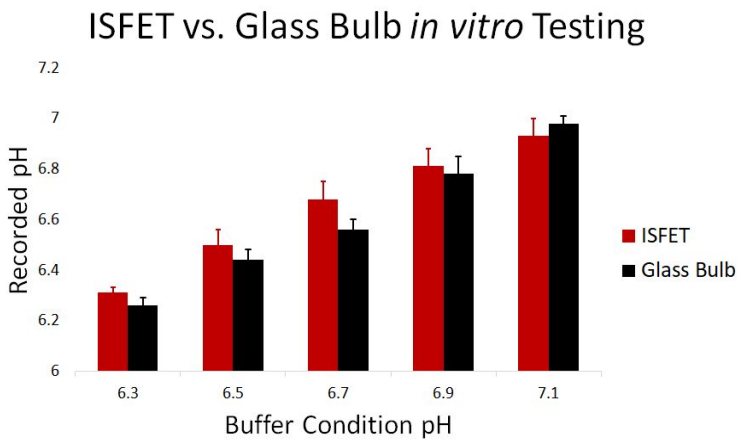

Figure 1. Results of ISFET vs. glass bulb pH recordings in steak models. Internal pH was measured for the ISFET probe due to its ability to pierce the meat. External pH was measured for the glass bulb probe since it is not capable of piercing. There was no significant difference found between the measurements.

3.2. Potassium's Influence on pH

After collecting data from all three samples using both ISFET and glass bulb probes, the results were once again averaged and analyzed using a program in Excel. There was no significant difference between the probes as shown in Figure 2.

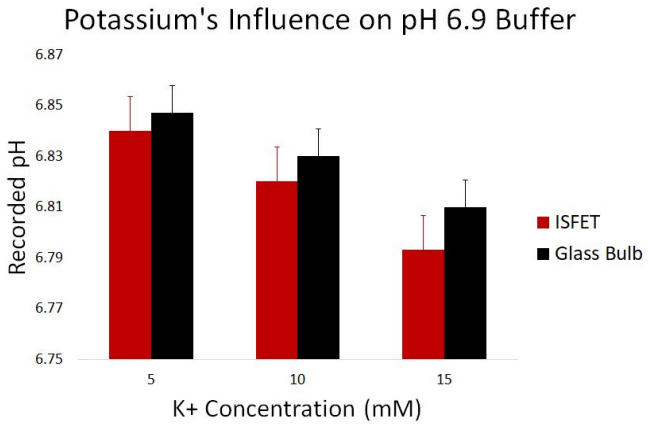

Figure 2. Variations in pH due to potassium concentration were recorded with both ISFET and glass bulb technology. Tested potassium concentrations covered both homeostatic and ischemic conditions.

3.3 Experimental ISFET Sensor pH Characterization

As seen in Figure 3, the experimental ISFET sensor displayed a linear relation between its reported measurements and the actual pH as recorded by the benchtop pH meter.

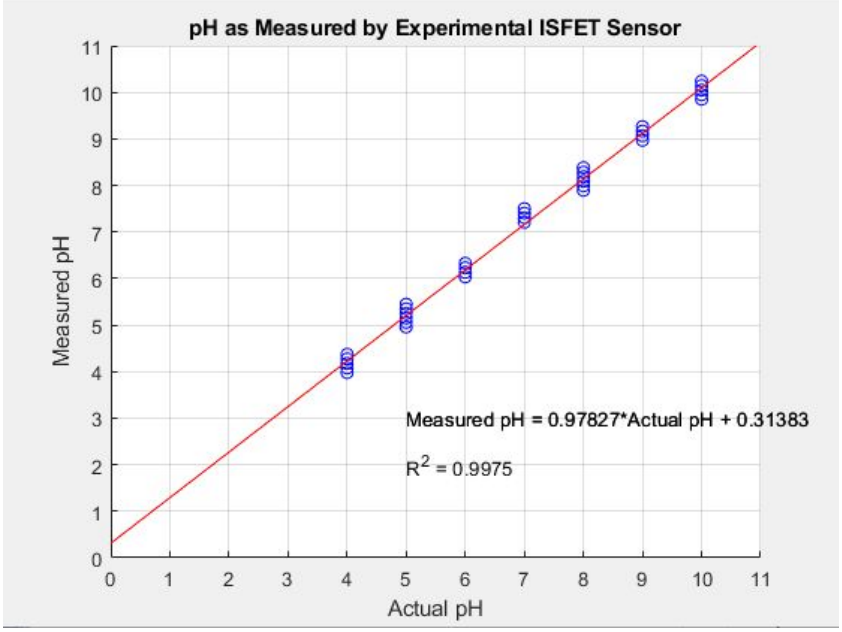

Figure 3. Agreement between pH measurements as reported by the standard glass bulb probe and experimental ISFET sensor. (Measured pH = 0.97827*(Actual pH) + 0.31383, R^2 = 0.9975)

See Appendix A for the MATLAB code used to create the graph. The experimental measurements were consistently 0.1 pH units higher than the actual pH, suggesting that, while the probe was precise, its accuracy could be improved by adjusting the voltage output equation in the Arduino code. This was further supported by the results of a Mann-Whitney parametric test, which found that the pH measurements reported by the experimental sensor and the glass bulb standard were significantly different ($p < 0.05$). See Appendix A for the MATLAB code used to analyze the results of the characterization data.

3.4 Experimental ISFET In Vitro *Drift Test*

While sitting in a buffer solution of pH 7.0, the experimental ISFET sensor drifted by 0.04 pH units over the course of one hour, as can be seen in Figure 4.

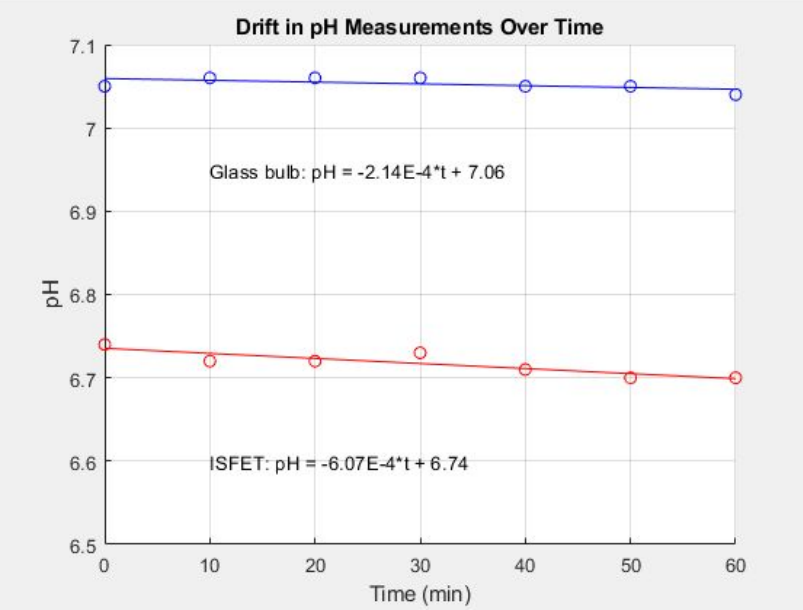

Figure 4. pH drift measurements for glass bulb and ISFET pH sensors over a one hour time period in a ph 7.0 buffer solution. Glass bulb standard: pH = (-2.14E-4)*t + 7.06. ISFET experimental: pH = (-6.07E-4)*t + 6.74

See Appendix A for the MATLAB code used to obtain the graph in Figure 4. The ISFET sensor also reported measurements approximately 0.3 pH units below that of the glass bulb sensor. The change in measurement accuracy between the characterization test and the drift test is likely due to physical shifts of the circuitry in the probe or the sensor itself during transportation, as some of the connections in the device were fragile and could be easily moved when bumped.

3.5 Degradation and Swelling Test for PEEK

After soaking in buffer solutions of different pH for 2.5 hours, there was no significant difference in the the mass or length of the PEEK samples (data not shown). Refer to Appendix A for the MATLAB code used to analyze the data in this experiment.

4. Discussion

When comparing the DeltaTrak ISFET probe and Fisherbrand™ Accumet AB150 pH Benchtop Meter, there was no significant difference between the pH measurements taken in different buffer solutions. Additionally, the pH variance between the homeostatic potassium concentration of 5 mM and the max ischemic concentration of 15 mM was also insignificant [11]. Both of these results were in line with initial hypotheses. Notably, concerns about increasing ionic concentrations appreciably affecting pH were quelled. As shown in Figure 2, pH decreased by only approximately 0.04 pH units as ionic concentrations increased. This phenomenon is explained by increasing ionic

concentrations effectively lowering the activity coefficient of hydrogen ions [12]. 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 [12]. 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 would concurrently decrease in the ECF and maintain consistent overall ion concentration within the ECF [13]. In summary, both tests provided encouraging results that validated the continued use of ISFET technology for this project.

Following this validation of ISFET technology, a modular ISFET pH probe from Sentron was purchased and constructed. While this probe cannot be used for *in vivo* applications (the ISFET/Reference casing is approximately 3 mm wide and too large for human subjects), it allowed the team to better understand the sensing element of ISFET technology and create Arduino code that can be translated to a future sensor.

Using the current configuration, the experimental ISFET probe can measure pH ranging from at least 4.0 to 10.0, which completely encompasses the range that would be found in a patient with ACS [10]. The measured pH and actual pH are strongly linearly related, and the experimental sensor consistently reports measurements 0.1 pH units higher than the actual pH. While there is currently no set pH threshold below which ACS is diagnosed, it is possible that a difference of 0.1 pH units could affect a clinician's decision to perform a fasciotomy on the afflicted patient. It is the goal of this project to prevent such an occurrence from happening, so in future iterations, it will be important to decrease this difference using a more accurate voltage-pH calibration curve.

The experimental probe displayed promising long-term behavior. Over the course of an hour, its measurements drifted by 0.04 pH units. While it will ideally be eliminated in future iterations, the current drift rate is low enough for the sensor to be practical in the timeframe in which ACS patients are likely to be diagnosed; in a study performed on canines with ACS, muscular pH reached dangerous levels within 15 minutes, and decreased by approximately 0.5 units over the course of an hour [6]. This is a more than 10-fold larger change in pH than the drift that occurred in the experimental ISFET probe. Therefore, it is unlikely a drift rate of 0.04 pH units/hour would result in an incorrect diagnosis of acute compartment syndrome.

While there are many criteria that must be met by this ISFET probe, three are of particular importance: optimal ISFET sensor geometry, optimal ISFET-microprocessor interface, and optimal probe configuration. First, in choosing the ISFET chip, it was critical to consider the surface area of the sensor in relation to the entire ISFET probe. Because the voltage recorded by the ISFET is proportional to the total charge accumulated on the sensor itself, the transistor geometry had to be fine-tuned to obtain a signal of usable magnitude while remaining small enough for use in human patients. The current goal is to fit the probe inside a 16-gauge needle, which has an inner diameter of 1.194 mm.

As stated above, with the 3 mm-wide casing, the current iteration of the ISFET probe is too large for human use. However, the ISFET chip inside the probe is 1 mm

wide, which, once the outer casing is modified, will be small enough to fit inside a 16-gauge needle. Therefore, the team is confident that the results gained from the current iteration will be replicated in a future, smaller probe, as an ISFET sensor with the same surface area will be used.

The current iteration was constructed using a 1 x 3 mm ISFET die. The printed circuit board (PCB), which contains the components used to acquire, filter and amplify the signal generated across the ISFET, is exposed, and at 20 mm wide is also too large to fit inside a 16-gauge needle. The current PCB also performs a number of unnecessary steps when analyzing the acquired signal, including and analog-to-digital conversion for data transmission via USB connection.

The future circuit will eliminate some of these unnecessary functions. Using 25 μm gold/copper wire, the ISFET die will be wire-bound to a 6-pin, 0.5 mm pitch FFC-type ribbon cable of a width no greater than 1mm. This will permit the ISFET to transmit its acquired signal over a length of greater than 10 cm, allowing it to connect to a PCB completely exterior to the insertion site of the sensor. This PCB will filter electromyographic and 60 Hz noise using a series of bandpass and integer filters and amplify the remaining signal to a notable measurement within the range of 0.5 - 3.3V for implementation with the Arduino code.

Lastly, placing a reference electrode within the confines of the 16-gauge probe along with the actual ISFET sensor is vital for obtaining a clean signal from the body. Using SolidWorks, the team designed a custom ISFET probe that meets the specified criteria and will help with generating a full-scale production protocol. This protocol will ideally be followed by a group with access to a cleanroom workspace and microinjection technology, as accuracy on a micrometer scale will be crucial in fabricating an accurate probe.

To be used safely *in vivo*, the probe will need to be encapsulated in a protective polymer. Based on the results of the degradation and swelling test and a thorough search of the literature, PEEK was chosen as the ideal encapsulant due to its historical use as an ISFET encapsulant, as well as its excellent biocompatibility, high resistance to wear and degradation, high strength, and electrical and thermal stability [14]. Furthermore, PEEK can be steam sterilized and processed into a suitable shape using microinjection molding [14]. This information was incorporated into the SolidWorks CAD model to showcase a final product design that could be used for *in vivo* testing. Refer to Appendix C for an image of the design.

5. Conclusions

Using the Sentron modular pH kit, an ISFET probe was made that can quickly and consistently measure pH in the physiological range. The 1 mm-wide ISFET chip in the probe showed promising results in accuracy and drift, which will be improved upon with future changes to the voltage-pH calibration curve and encapsulation. PEEK was selected as the encapsulant material due to its past use in *in vivo* medical technology and ISFET probes and its resistance to change in environments of varying pH. The future probe will consist of an ISFET sensor encapsulated with its circuitry in microinjected PEEK and connected to a computing element to report the pH of

muscular compartments.

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Appendix A

MATLAB code used for linear regression of *Experimental ISFET Sensor Characterization* data:

```
%% Characterization of Sensor in Buffer Solutions

% Load data from Buffer_characterization_data.xlsx using Import Data
% Creates table with two columns: Actual pH and Measured pH

% Create vectors from table:

ActualpH = BuffercharacterizationdataS1{:,1};
MeasuredpH = BuffercharacterizationdataS1{:,2};
model = fitlm(ActualpH, MeasuredpH);  % Reports R-squared value

ApH = [ones(length(ActualpH),1) ActualpH];   % Calculates linear regression
model
    Relation = ApH\MeasuredpH;
    m = Relation(2,1);   % Slope
    b = Relation(1,1);   % Y intercept
x = linspace(0, 11, 100);

pHline = m*x + b;   % Linear regression model

scatter(ActualpH, MeasuredpH, 'b');
axis([0 11 0 11]);
hold on
plot(x, pHline, 'r')
xlabel('Actual pH');
ylabel('Measured pH');
title('pH as Measured by Experimental ISFET Sensor');
grid on

text(5,3, ['Measured pH = ', num2str(m), '*Actual pH + ', num2str(b)])
text(5,2, ['R^2 = 0.9975'])
```

MATLAB code used to compare the pH measurements of the experimental sensor and the glass bulb standard:

```
%% Comparison of Experimental ISFET to Glass Bulb

% Load data from Buffer_characterization_data.xlsx using Import Data
% Creates table with two columns: Actual pH and Measured pH

% Create vectors from table:

ActualpH = BuffercharacterizationdataS1{:,1};
MeasuredpH = BuffercharacterizationdataS1{:,2};

% Check for normal distribution using Kolmogorov-Smirnov test
% Null hypothesis: Variable follows a normal distribution
% Therefore, we want p > 0.05 to get a normal distribution
% Need to test that each sample set for each pH is normal:

pH4 = MeasuredpH(1:56, 1);
pH5 = MeasuredpH(57:112, 1);
pH6 = MeasuredpH(113:168, 1);
pH7 = MeasuredpH(169:224, 1);
pH8 = MeasuredpH(225:280, 1);
pH9 = MeasuredpH(281:336, 1);
pH10 = MeasuredpH(337:392, 1);

h4 = kstest(pH4);
h5 = kstest(pH5);
h6 = kstest(pH6);
h7 = kstest(pH7);
h8 = kstest(pH8);
h9 = kstest(pH9);
h10 = kstest(pH10);

% h = 1 for each sample set, therefore we reject the null hypothesis that
% the data is normal at the 5% significance level. Therefore, we need to
% perform a Mann-Whitney test to compare the median measurements of the
% experimental ISFET sensor and glass bulb probe.

GBpH4 = ActualpH(1:56, 1);
GBpH5 = ActualpH(57:112, 1);
GBpH6 = ActualpH(113:168, 1);
GBpH7 = ActualpH(169:224, 1);
GBpH8 = ActualpH(225:280, 1);
GBpH9 = ActualpH(281:336, 1);
GBpH10 = ActualpH(337:392, 1);

[PpH4, HpH4] = ranksum(GBpH4, pH4);
[PpH5, HpH5] = ranksum(GBpH5, pH5);
[PpH6, HpH6] = ranksum(GBpH6, pH6);
[PpH7, HpH7] = ranksum(GBpH7, pH7);
[PpH8, HpH8] = ranksum(GBpH8, pH8);
[PpH9, HpH9] = ranksum(GBpH9, pH9);
[PpH10, HpH10] = ranksum(GBpH10, pH10);

% Each of the logical decisions were returned as H = 1, meaning we reject
% the null hypothesis that the glass bulb probe and the experimental ISFET
% probe have the same medians for each sample. Therefore, our experimental
% ISFET is not producing the same values as the glass bulb probe, meaning
% it needs to be improved in accuracy.
```

MATLAB code used to analyze the drift of the pH sensors:

```
%% Sensor Drift Testing

% Turn data into vectors

t = [0 10 20 30 40 50 60];
ISFET = [6.74 6.72 6.72 6.73 6.71 6.70 6.70];
GBulb = [7.05 7.06 7.06 7.06 7.05 7.05 7.04];

ISFET_model = fitlm(t, ISFET)
Glass_model = fitlm(t, GBulb)

scatter(t, ISFET, 'r');
hold on
scatter(t, GBulb, 'b');
grid on

% From linear regression models, we get the following linear relations:

glass = -0.00021429*t + 7.0593;
isfet = -0.00060714*t + 6.7354;

plot(t, isfet, 'r', t, glass, 'b')

axis([0 60 6.50 7.10]);
xlabel('Time (min)');
ylabel('pH')
title('Drift in pH Measurements Over Time');

text(10, 6.95, ['Glass bulb: pH = -2.14E-4*t + 7.06']);
text(10, 6.6, ['ISFET: pH = -6.07E-4*t + 6.74']);
```

MATLAB code used to analyze the change in mass and length of PEEK samples:

```
%% PEEK Mass Change Testing

pre_length = [10.53 10.36 10.54 10.73 9.71 10.22 9.96 10.63 9.51];
pre_mass = [0.05 0.04 0.05 0.04 0.04 0.04 0.05 0.05 0.04];

post_length = [10.28 10.53 10.08 10.50 10.36 10.17 10.19 10.53 9.74];
post_mass = [0.05 0.05 0.04 0.05 0.04 0.04 0.05 0.04 0.04];

% Check to see if data in each vector is normal

h_pl = kstest(pre_length);
h_pm = kstest(pre_mass);
h_pol = kstest(post_length);
h_pom = kstest(post_mass);

% Each KS test reported that each sample is not normal. Therefore, use the
% Wilcoxon parametric test to compare the medians of each data set.

% Length:

[P_length, H_length] = ranksum(pre_length, post_length)
[P_mass, H_mass] = ranksum(pre_mass, post_mass)

% For both, H came back as 0, meaning we fail to reject the null hypothesis
% that the medians are different. Therefore, we can conclude that soaking
% PEEK in the buffers did nothing to change the length or mass.
```

Appendix B

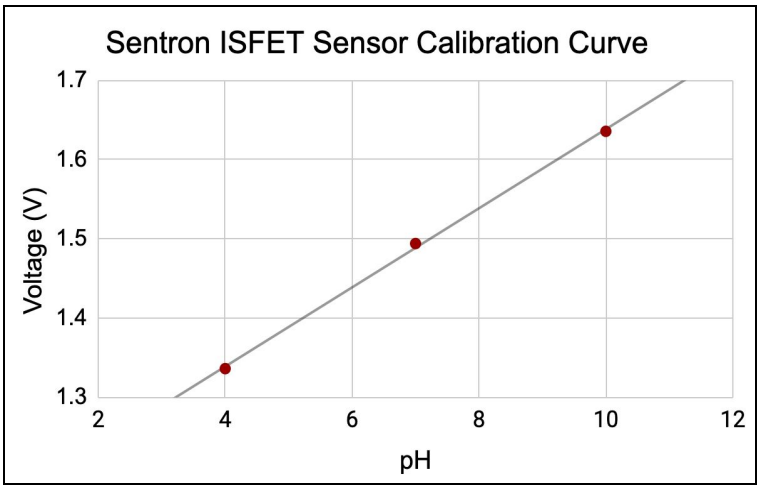

Figure B1. Experimental ISFET calibration curve. Measurements at pH 4, 7, 10 were taken three times. Conversion equation: (Voltage - 1.1393) / 0.0499 = pH $R^2 = 0.999$

```
int analogVphIn = A1;
float adc = 0.0048828125;
float phVoltageIn = 0.0;
int phAnalogIn = 0;
float phOut = 0;
float pH = 0.0;
// y = 0.0499x + 1.1393;

void setup() {
  // declare the ledPin as an OUTPUT:
  pinMode(analogVphIn, INPUT);
  Serial.begin(115200);
}

void loop() {


  phAnalogIn = analogRead(analogVphIn);
  phVoltageIn = phAnalogIn*adc;
  pH = (phVoltageIn - 1.1393)/0.0499;
  //Serial.print("pH: ");
  Serial.println(pH, 4);


  delay(350);
}
```

Figure B2. Arduino IDE code used to read-in voltage values from Sentron sensor and convert to pH output on serial plotter.

Appendix C

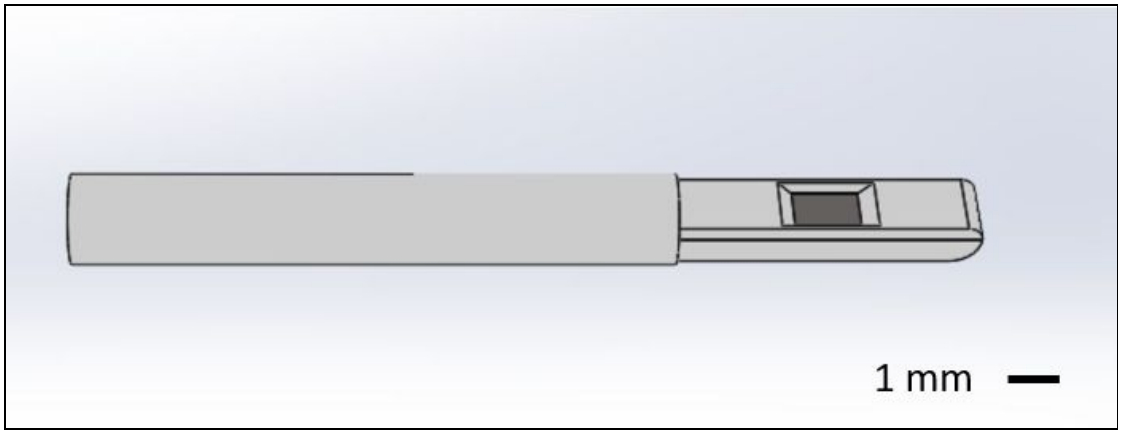

Figure C1. Solidworks model of encapsulated ISFET design using PEEK. Ion-sensitive membrane of the bare die (~1.1 mm in length) is exposed to the surface to detect pH.

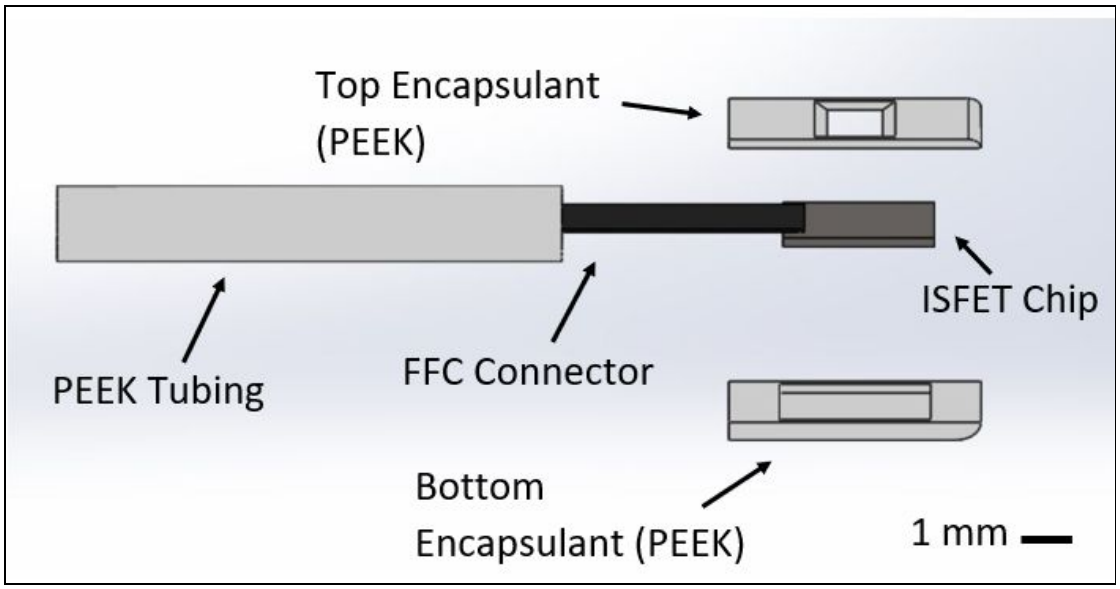

Figure C2. Solidworks model showing exploded view of encapsulated ISFET design. ISFET chip is held in place with epoxy and connected to a main circuit via an FFC cable. PEEK tubing protects the FFC cable.

Appendix D

pH Probe for Diagnosing Acute Compartment Syndrome: Product Design Specifications

Client: Dr. Christopher Doro, UW Health Orthopedics and Rehabilitation
Advisor: Dr. Jeremy Rogers
Team Leader: Alex Goodman
Communicator: Kelsey Murphy
BSAC: Mark Austin
BPAG/BWIG: Will Bacon

September 20, 2018

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 in - 5 in)
- Probe must be cheap and preferably autoclavable before use (<$100 final prototype)
- The probe should be continuously analyzed by a main analyzer (8 hours of readings)
- 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)
- The probe must be able to continuously monitor the biomarker (1 sample/15 minutes, 8 hours in total)
- The probe must be precise, so that there is a lower incidence of false positives (<34% 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 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. (pH 6-7, 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 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, 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 inches into the body
- Must fit within an 18-gauge needle.

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, pH meter, optical fibers, plastic box to house analyzer equipment, hydrogel, chlorophenol red indicator, indicator immobilization substrate

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 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.

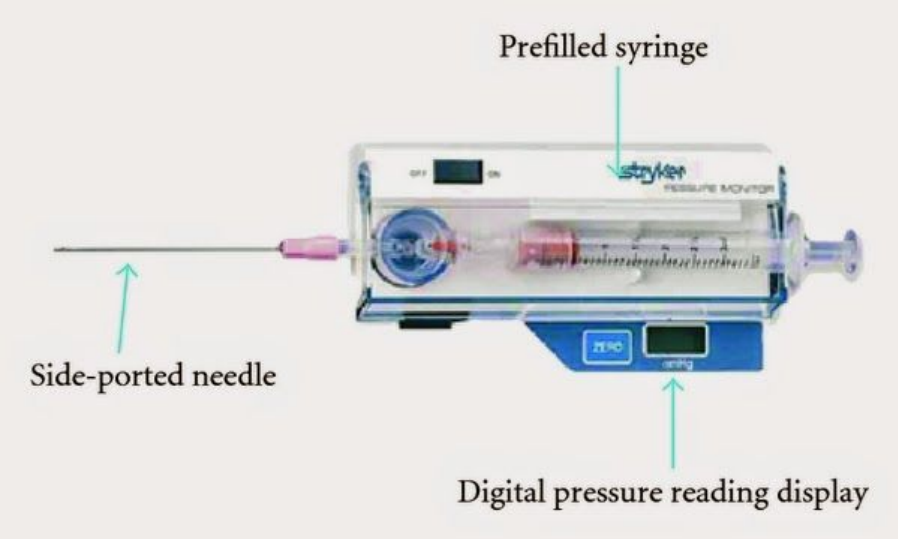

Figure 1: A Stryker Needle, a common instrument for monitoring pressure in a muscle compartment. The side-ported needle is inserted into the affected compartment, leading to a digital pressure reading that the clinician then compares to established threshold values for diagnosis.

- 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.