IPRO 308

Creating an Artificial Pancreas

Midterm Report

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Sponsors	The IPRO office
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	Michael Tishler
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1.0 Objectives

Our original objectives:

1. Modify the extraction technique in order to increase the volume of interstitial fluid obtained in previous semesters.
2. Modify the extraction technique to decrease time needed to extract required amount of interstitial fluid for glucose measurement.
3. Research and design a device to obtain interstitial fluid and administer insulin into the user in a non-invasive manner.
4. Determine optimal wavelength to analyze glucose concentrations with emission spectroscopy.
5. Investigate the glucose-oxidation reaction as a means of measuring the amount of glucose present within the interstitial fluid.
6. Determine whether the glucose measuring techniques investigated function with an acceptable degree of accuracy over possible physiologic interstitial glucose levels.
7. Determine an optimal voltage level that will not harm the skin while performing impedance spectroscopy.
8. Analyze the data obtained from (4) and (5) in order to establish a correlation between them.
9. Weigh the advantages and disadvantages of the different glucose measuring techniques investigated for integration into a prototype.
10. Design a prototype small enough to be worn on the body.

The Research Team has not changed their objectives of gaining funding from external sources through applying for a grant, successfully submitting the deliverables on time, and data mining through information for the other teams.

The Closed-Loop Team objective has largely remained the same. Although administration of insulin through iontophoresis and reversing the electrical gradient is still a priority, until the team is able to extract interstitial fluid, the rest of the design is void. As such, the closed-loop subgroup is focusing all its resources to the extraction a microvolume of interstitial fluid by the end of the semester and determining the optimal values for the variables associated with the sonophoresis.

The objective of the Measurement Subcommittee remains to attempt to reproduce the results from the last IPRO term. Currently, no changes have been made in the procedure. Adaptions made in the last IPRO term ensured that these results could be recreated without the introduction of additional interfering variables. A linear range was defined and experiments were conducted within this established limit.

2.0. Results to Date

Research Subcommittee:

The Research Team has completed writing and submitting the animal protocol. The team is currently waiting on the approval of this protocol that will allow the Closed-Loop subgroup to start experimenting with rat skin. In addition, they have also completed the writing and submission of the Project Plan, and also authored the Code of Ethics.

The team will now be focused with the Final Presentation, looking for grants and writing a grant proposal. The team hopes to submit a grant proposal to at least two foundations. The research team is also responsible for providing the necessary documents for IPRO, leaving the other groups to keep their primary focus in the laboratory.

The results obtained by the Research Team will provide IPRO 308 with a presentation for IPRO day and hopefully be successful in obtaining grant funding for the current and future teams.

Closed Loop Subcommittee:

Results to date are as follows:

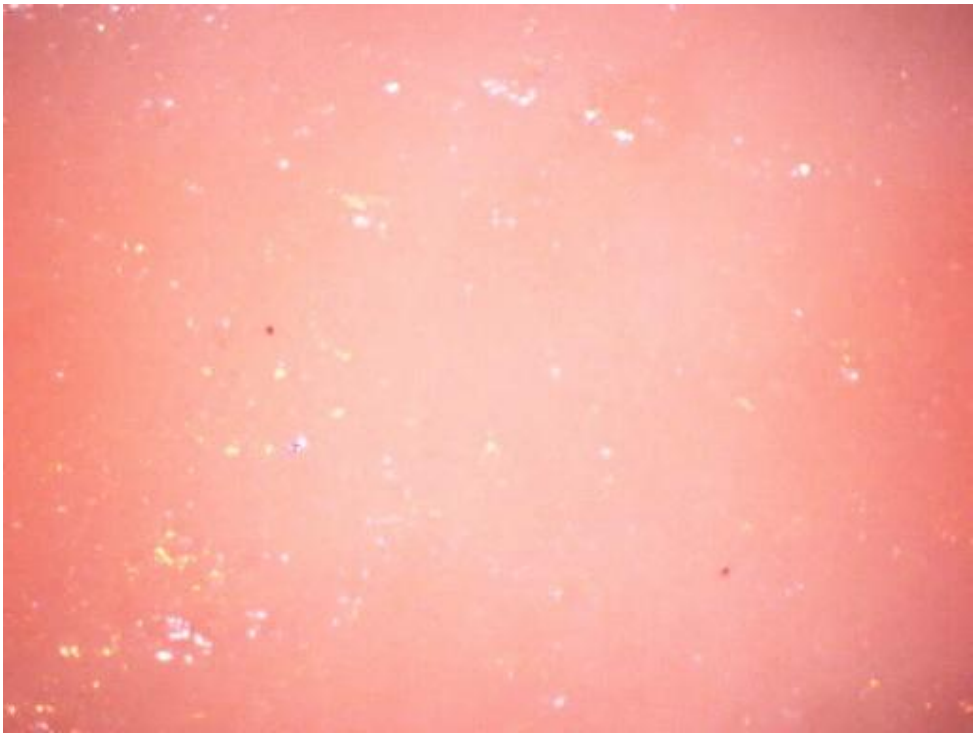


Fig. 1: We have observed what we believe are skin pores on porcine skin under 200 times magnification.

The light-colored spots in the illustration above are assumed to be skin pores. They are the proper diameter and are of a reasonable concentration to be skin pores.

We submerged the skin just below the surface in a bath of warm water w/ green coloring. After applying vacuum for 1.5 hours we infer the green spots in fig. 2 are representative of skin pores saturated with the green solution in which the skin was submerged.

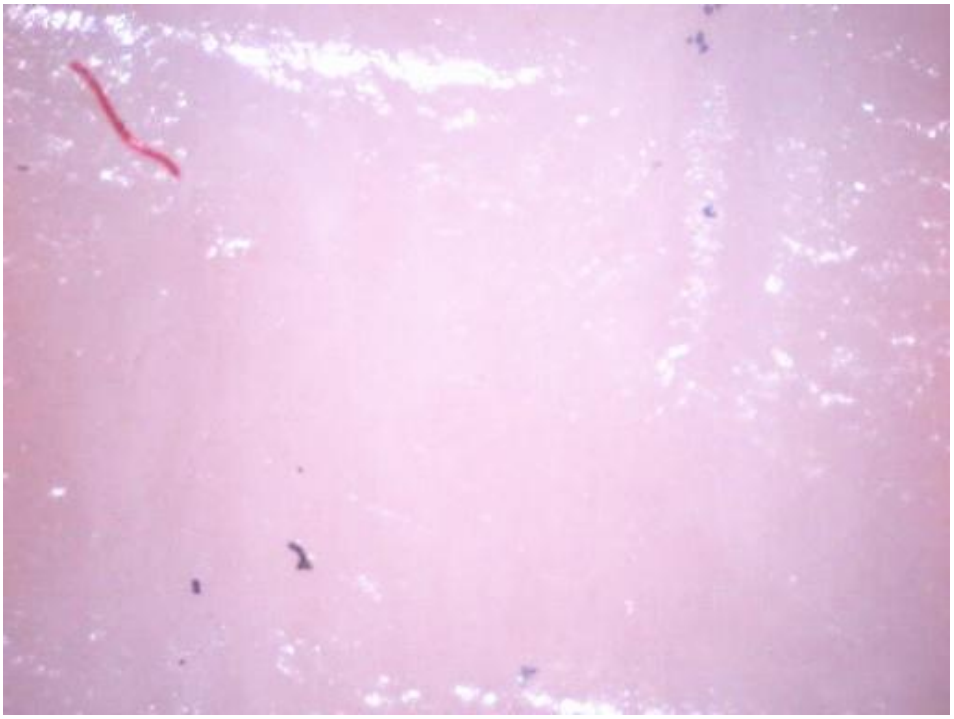


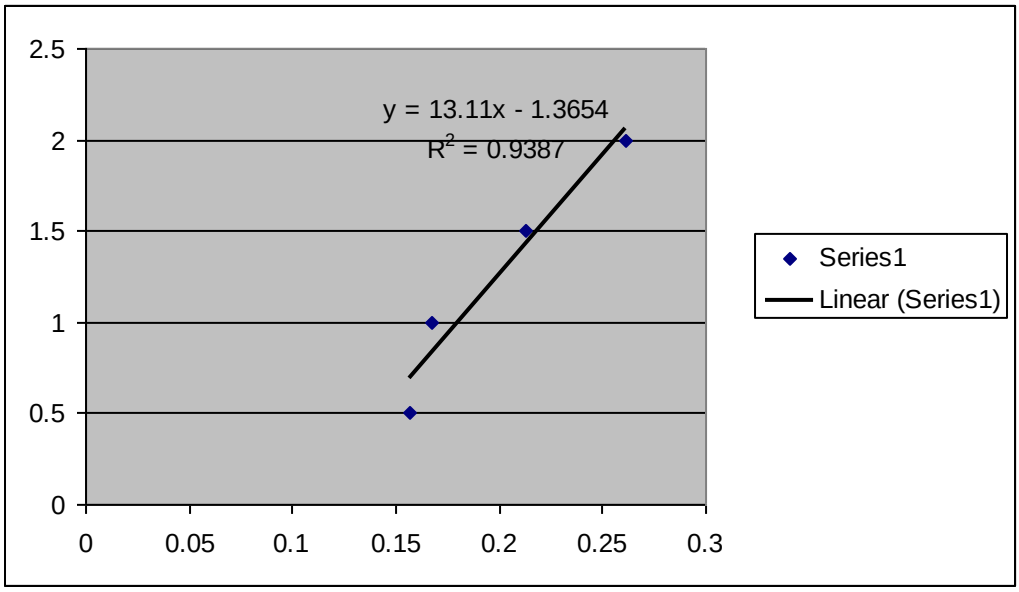
Fig. 2: Surface of porcine skin after submersion in green dyed water and application of vacuum for a period of 1.5 hours.

Measurement Subcommittee:

Because of the time delay involved in obtaining new supplies of the requisite reagents, and our limited lab times, we have not acquired enough data to confirm our prior results.

After carrying out emission spectroscopy, the following results were obtained. A graph is also displayed, showing absorption in nanometers as a function of glucose concentration. There are five data points representing five different concentrations in faux interstitial fluid/ glucose. The final data point was removed due to deviation likely introduced by procedural error. Higher R^2 values are required.

Absorbance (760 Nm)	**Concentartion**
0.105	0
0.157	0.5
0.167	1.0
0.213	1.5
0.261	2.0



In the final analysis, we will be examining ways in which this technology could be incorporated into the prototype. A simple battery connection can be attached to the fluid extraction compartment of the current prototype to heat the extracted ISF solution and therefore initiate the requisite coloremetric reaction. Since the amount of interstitial fluid extracted is very small, this heating can possibly be done quite efficiently. The compartment containing the reacted Benedicts and Interstitial fluid solution could have a simple LED, and a photodiode (which would act as the photometer). This light system would act as the 'spectrophotometer' and be set at the optimum wavelength for glucose measurement. It would also be attached to a simple circuit to relay the percent absorption, which would be used with a representative linear regression model to establish the concentration of glucose in the extracted interstitial fluid.

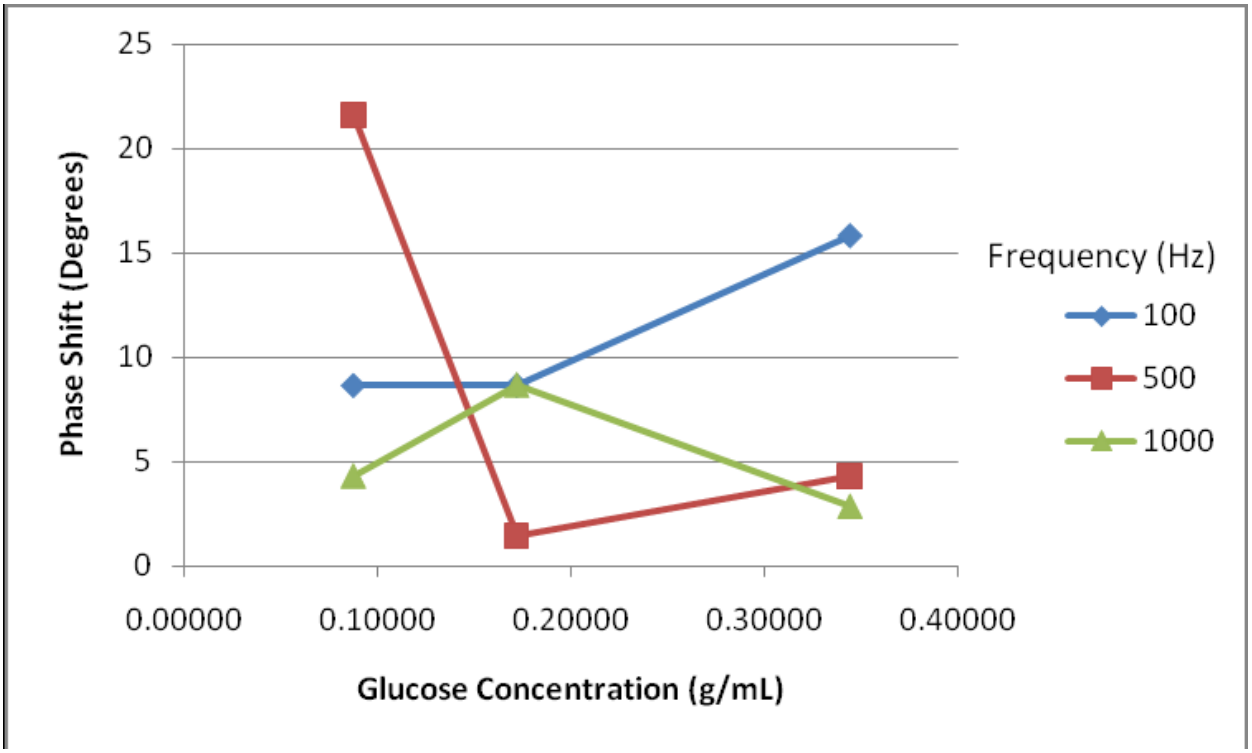
For the glucose – oxidase experiment, the group started with 30mL of distilled water and 2.63g of Glucose. The capacitance was measured with air as the dielectric and it came out to 0.041nF. Sticking the capacitor into the initial mixture, a capacitance of .02μF was measured. We then put the capacitor in series with a 10kΩ Resistor. We put a sine wave of 100Hz across the capacitor. We then used the digital oscilloscope to measure the difference from the peaks of the input and output wave. We recorded the time delay and then calculated phase shift from that.

Using the formula for phase shift in degrees: $\varphi^\circ = 360^\circ \cdot f \cdot \Delta t$

The data gathered is plotted and shown below:

	Frequency	100	500	1000
Concentration				
0.087666667		8.64	21.6	4.32
0.172002		8.64	1.44	8.64
0.344004		15.8	4.32	2.88

The values in figure 1 are phase delays in degrees.



The data is largely inconclusive because water was used as the solvent and it slowly ionized as the experiment went on. The capacitance in the solution decreased while the capacitor was left in the solution. Finding a better solution is required.

In the final analysis, impedance spectroscopy, we will be examining ways in which this technology could be incorporated into the prototype. A simple battery connection can be attached to the fluid extraction compartment of the current prototype to heat the extracted ISF solution and therefore initiate the requisite coloremetric reaction. Since the amount of interstitial fluid extracted is very small, this heating can possibly be done quite efficiently. The compartment containing the reacted Benedicts and Interstitial fluid solution could have a simple LED, and a photodiode (which would act as the photometer). This light system would act as the 'spectrophotometer' and be set at the optimum wavelength for glucose measurement. It would also be attached to a simple circuit to relay the percent absorption, which would be used with a representative linear regression model to establish the concentration of glucose in the extracted interstitial fluid.

3.0. Revised Task / Event Schedule

Closed-Loop Group:

In preparation for the final prototype, the closed-loop group has decided to design and build a new prototype using a speaker without its magnet in the middle so as to optimize suction from the vacuum, but also permit better functioning of the speaker coil for sonophoresis. This design will incorporate all the necessary features for the closed-loop process, namely extraction using all three components and an extra vacuum pump so as to permit reversing the action for eventual administration of insulin into the patient's body.

Date	**Task**
2/18/08-3/15/08	Interstitial Fluid Extraction
3/17/08-3/22/08	Spring Break
3/24/08-4/19/08	Interstitial Fluid Extraction (cont'd.)
4/21/08-05/01/08	Prepare Presentations

Subgroup Leader: William Wakeman

Procurement of porcine skin: Joon Park

Experimentation: Entire subgroup

Research Subcommittee:

The research subcommittee has been responsible for data mining through various information sources for the other two subgroups. In addition the group has also formulated a protocol for animal use; a first for this particular IPRO. All deliverables thus far have been compiled, edited, and submitted by the research group. All tasks have largely remained the same, however the grant application process has been pushed forward.

Date	**Task**
3/14/08	Midterm Report Due
3/25/08	Meet with professor about Grant Application Process
4/11/08	Rough Draft of Grant Proposal
5/02/08	Final Draft of Grant Proposal Due

IPRO Document Writer: Shezami Khalil & Kyle Laster

Grant Proposal Leader: Walatta Mesquitta

Measurement Subcommittee:

One revision has been made in the task or event schedule of the measurement subcommittee. Due to technical restraints, the glucose-oxidase measurements will begin March 24th. This will give the team an opportunity to compare and optimize their technique in order to ensure a quick and reliable measurement.

Projected Time line for Emission Spectroscopy:

Date	**Task**
3/3-3/7	Conduct initial evaluation/ Report on progress to be sent in for Midterm presentation
3/10-3/14	Continuing experimentation + Midterm presentation/ Final reports for Midterm Paper
3/24-3/28	Correlation analysis

Projected Timeline for Impedance Spectroscopy:

Date	**Task**
3/24-4/16	Start experimentation/ optimize technique
4/16-4/20	Final Presentation to class on goals met and prospects for future semesters
4/21-4/25	Compose final Ipro Day presentation material on semester's work
5/2/2008	IPRO Day

Projected Timeline for Glucose-Oxidase Reaction:

Date	**Task**
3/10-3/14	Make the test effective with the electrochemical sensory electrode by determining the lowest volume of solution required for use + Midterm presentation/ Final reports for Midterm Paper
3/24-3/28	Continuing experimentation
3/31-4/4	Move to more physiologically significant glucose concentrations and test for a linear relationship
4/7-4/11	Continuing experimentation
4/12-4/16	Begin using faux interstitial fluid/(Krebs/glucose admixture)
4/16-4/20	Final Presentation to class on goals met and prospects for future semesters
4/21-4/25	Compose final Ipro Day presentation material on semester's work
5/2/2008	IPRO Day

Benedict's Solution Team: Walatta Mesquitta

Glucose Oxidation/ Impedence Spectroscopy Team: Richard Hanley, Zachary Estrada

4.0. Changes in Task Assignments and Designation of Roles and Team Organization

Task Assignments have not deviated from those listed within the project plan. Role designation is as follows:

Research Subcommittee

Name	**Role**	**Concentration**
William Wakeman	Closed-Loop Team Leader	Vacuum, Sonophoresis, and Iontophoresis
Rohan Mathews		Vacuum, Sonophoresis, and Iontophoresis
Michael Tishler		Vacuum, Sonophoresis, and Iontophoresis
AnjuSassendran		Vacuum, Sonophoresis, and Iontophoresis
Joon S Park		Vacuum, Sonophoresis, and Iontophoresis

Measurement Subcommittee

Name	**Role**	**Concentration**
Walatta Mesquitta	Measurement Team Leader	Emission Spectroscopy
Richard Hanley		Impedance Spectroscopy, Glucose-oxidase
Zachery Estrada		Impedance Spectroscopy, Glucose-oxidase

Extraction Subcommittee

Name	**Role**	**Concentration**
Shezami Khalil	Research Team Leader	Grant Proposals, Patent

		Research, Deliverables
Kyle Laster		Grant Proposals, Patent Research, Deliverables
Walatta Mesquitta		Deliverables

5.0. Barriers and Obstacles

Research Subcommittee:

We have been fortunate to have not encountered any obstacles. However, we feel that we may miss deadlines for grant applications or be unable to find appropriate companies to apply for a grant due to a break within the work schedule.

Closed Loop Subcommittee:

We feel our largest barrier to date is the limitations of the sonophoresis portion of the extraction process. We are still uncertain of the operating intensity. Also, the limitations involved with skin type have been variable. Porcine skin is not close to human skin in thickness and must be scathed down before it can be tested. This poses a problem with uniform skin thickness. Also the time from harvest to testing we feel is still to great in that in directly affects the permeability of the porcine skin.

In order to solve the intensity issue, we have obtained an intensity equation that our research sub-group is currently looking into. Per reports that we have read on the subject of sonophoresis, intensity over frequency is the deciding factor in the expansion of skin pores. This could be what is limiting our expansion and ultimately our extraction of fluid.

We have gained a contact in the IITRI that will be able to supply us with rat skin once the rat has been deceased. He also may be able to obtain rabbit skin for our use, which is closest to human skin in thickness and permeability. Also, we have submitted an animal protocol that we hope will enable us to test the apparatus on live rats. This will not only solve the skin thickness problem but at the same time will solve any previous issues with harvest to test time.

Measurement Subcommittee:

There have been many obstacles in attaining laboratory space and equipment needed to do the research that is required in developing our prototype. Additionally, the Emission Spectroscopy technique was not achieving significant results at interstitial fluid glucose concentrations and the necessary instrumentation to perform impedance spectroscopy was unavailable. Since different individuals from last semester are carrying out the experiment, new solutions were produced for testing and there may be some initial deviation from linearity. Our barriers are trying to find a way to prevent the glucose solution from ionizing as well as making

precise measurements on the scope. An obstacle for our current measurement in the glucose oxidase reaction is the price of glucose oxidase.

The results of each lab period and our goals must be set and agreed upon before entry in the lab each week. This will forestall loss of additional time in constructing experiments to resolve difficulties as these arise. Several independent tests will be run per period to make up for the reduced lab availability.