IPRO 308

Creating an Artificial Pancreas

Midterm Report

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Mentor	Raymond DeBoth
Sponsors	The IPRO office
Student Leader	Michael Morley
IPRO Team	Amanda Babicz Linda Goldstein Malgorzata Kochanek Rohan Mathews Walatta Mesquitta Devnaradev Narasimhan Bhavin Patel Amir Rahnavard Kirthi Reddy Maryum Riaz E-Faan Saung David Thomas

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1.0. Objectives

1. Advance extraction technique in order to extract a greater amount of interstitial fluid than achieved before.
2. Modify extraction technique to lessen time needed to extract required amount of interstitial fluid.
3. Research and design a technique of administering insulin into the body without needle injection.
4. Set up an experimental design to test glucose levels using the electrical current produced from oxidation reactions involving glucose oxidase*.
5. Set up an experimental design using Benedict's solution to test for glucose concentrations*.
6. Determine whether the glucose measuring techniques investigated function with an acceptable degree of accuracy over possible physiologic interstitial glucose levels.
7. Weigh the advantages and disadvantages of the different glucose measuring techniques investigated and select the most advantageous one.
8. Design a prototype small enough to be worn comfortably on the body.
9. Research and apply for various grant proposals to support the research*.

The objectives marked with an '*' identify it as a new or modified objective since the project plan. The most important modifications are due to the research into the different glucose measuring methods.

Initially in the Project Plan, objective 4 described the use of emission spectroscopy to determine the glucose concentrations; however, as is described more intricately in the following midterm report, it was found that the method could not be accurately administered at such low concentrations as those found in human interstitial fluid. Therefore the research committee identified many other possible glucose measuring techniques and the method provided in the objective 4 (glucose oxidation via glucose oxidase) of the midterm report was determined to be the most advantageous as it is currently being implemented in various glucose sensors on the market.

Additionally, the method of Impedance Spectroscopy (Project Plan Objective 6) was also altered because of resource setbacks. In the past the Artificial Pancreas Team used to visit Rush Medical to perform the experiments; however, our contact informed us recently that the instrumentation used in previous years is currently not operational. Therefore, the subteam which was focused on Impedance Spectroscopy is now pursuing another measuring method involving a reaction with Benedict's Solution followed by spectroscopy.

Finally, Objective 9 is an additional project that was added within the past month so that the human resources of the "research subgroup" can be optimized throughout the semester and secure more funding for our project.

2.0. Results to Date

Research Subcommittee:

At this point, the Research team was able to find three feasible patents for glucose measurement through the use of NMR spectroscopy. This information was required as a back-up solution, in case Emission and Impedence spectroscopy does not become a viable option. The team researched options for skin distributors and microscopes on campus through the help of Dr. Connie Hall of the BME Department also Dr. Zhang and Dr Stark of the BCPS Department. Most recently the Research team found three grants the team can apply for in order to supplement the current funding.

After looking through the grants, the Research team is deciding to focus on preparing for the NCIIA grant and the Ford grant. Since the NCIIA grant has an approaching deadline, the preliminary steps are being taken care of and the writing of the proposal will begin immediately afterwards. After writing the proposal for the NCIIA grant, the team will switch to the Ford grant which does not have a deadline.

The Research team assisted team leader Michael Morley in the writing of the Project Plan, and also authored the Ethics Paper document. The team will also be involved with the Final Presentation and the drafting of any remaining documents. The team also hopes to be providing a grant proposal for at least two foundations.

The results made by the Research team do not address the sponsor/customer directly. The team's focus was on supporting the experimental teams by providing information and supplemental funding. The Research team also took responsibility along with team leader Michael Morley on 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 the IPRO day and hopefully be successful in obtaining grant funding for the current and future teams.

Extraction Subcommittee:

After numerous sessions in the laboratory, the extraction group was able to analyze the working of the vacuum suction on the speaker, as well as the use of sonophoresis to open the pores of the pig skin. Preliminary studies show a presence of moisture on the speaker after the process though the source can not be proven to be

interstitial fluid as of yet. Analysis under a microscope showed marginal details as proof for enlargement of the pore size after the use of sonohphoresis (See Figures 1 and 2).

Figure 1: Image of pig skin before experiment taken under an electron microscope with magnification 100x.

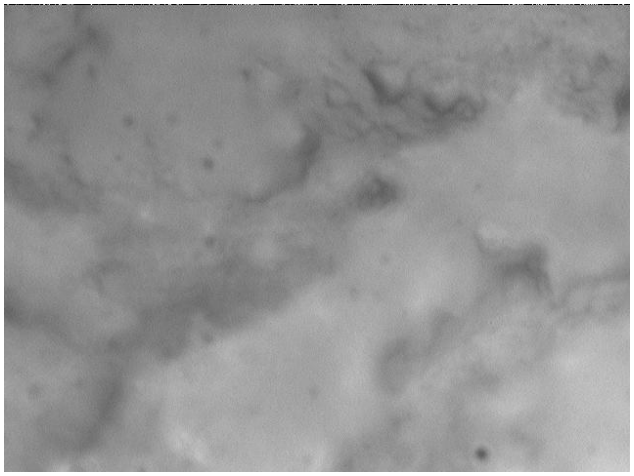

Figure 2: Image of pig skin after a experiment taken under an electron microscope with magnification 100x.

The mechanical section of the team analyzed the working of a data acquisition device that permitted the collection of information regarding the differential voltage when the device is connected to the vacuum/pump.

The voltage is measured and then converted into a pressure value. First it was necessary to determine the calibration curve before any pressure values could be obtained.

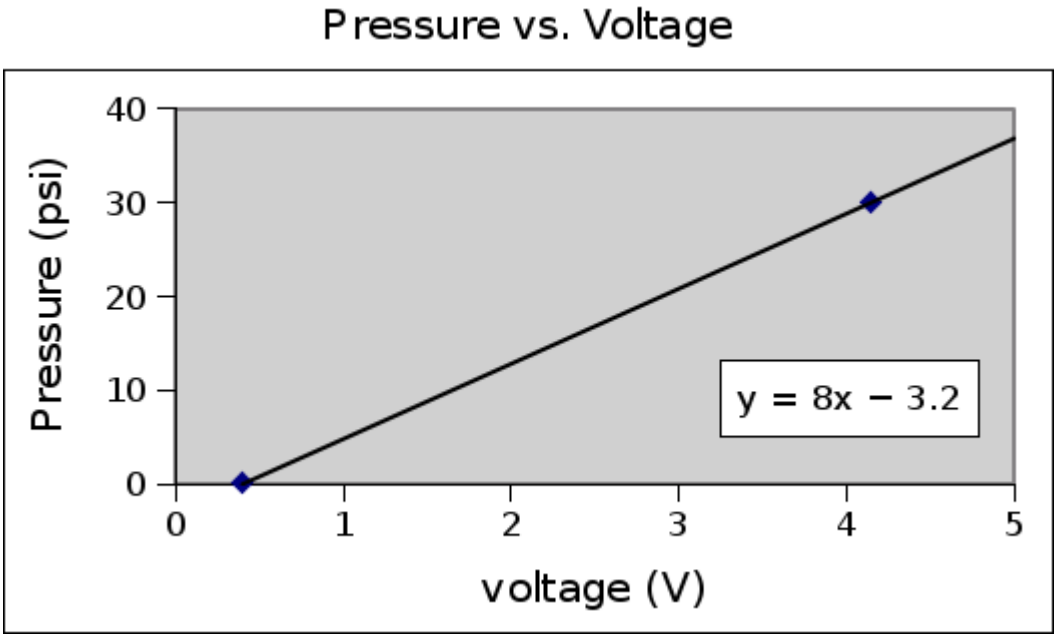

Figure 3: Calibration curve for vacuum pressure versus voltage used

A linear regression equation will be used in order to convert the voltage (V) to pressure (psi) values. Our first tests involved experimenting with the maximum allowable pressure. This was achieved by plugging the end of the vacuum tube against the skin. This ensures that no leakages were present. Figure 4 below shows the results of this test.

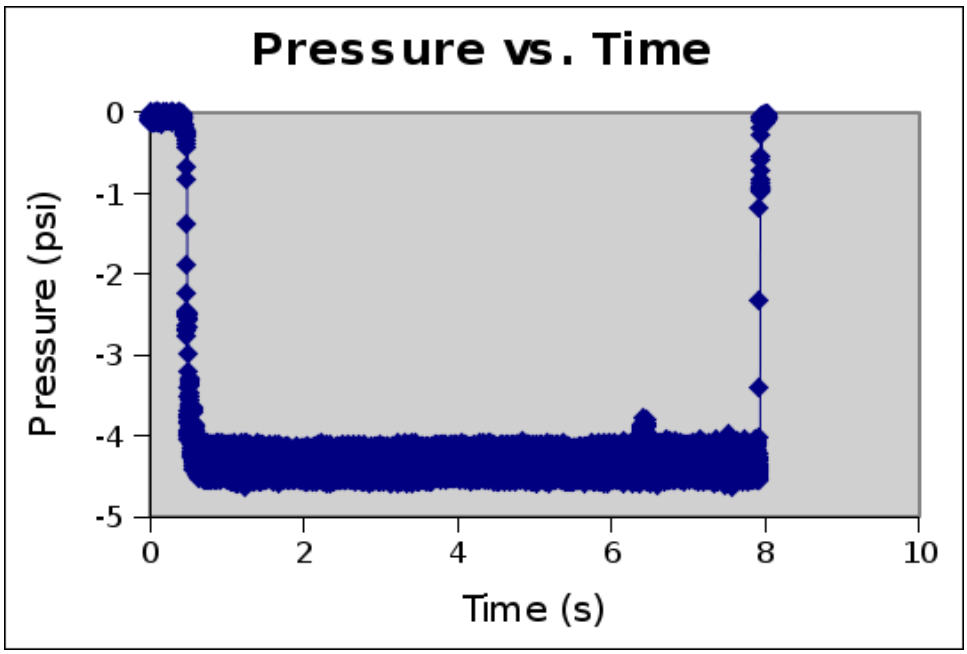

Figure 4: Pressure of vacuum apparatus versus time

The maximum vacuum pressure is found to be -4.64 psi. This plot has been reduced to show the maximum pressure achieved. Due to leakages in the speaker system itself, the tests with the speaker have been delayed until a better testing device can be achieved to ensure no leakages are present.

Different gasket materials have been acquired and will be tested for optimum suction. Every gasket has a different durometer setting. The durometer setting is a measure of softness, and each gasket will be tested to determine the best fit for our suction device.

From the research and outputs of the extraction group, the team is exploring the possibility of the pulling a vacuum through multiple points on the gasket at the base of the speaker rather than drilling a hole through the magnetic material at the base of the speaker. The conductive epoxy used on the previous model is to be replaced with a metal base plate as well as conductive Aluminum foil attached to the speaker's voice coil. This should provide a better control system for the current being applied to the skin as well as hopefully improve the quality of the suction being caused by the vacuum. Even though this model is larger in dimensions than its predecessor, the team believes size is a problem that can be dealt with at a later time should the results prove successful.

The vacuum experiments will also be of primary concern. An optimum vacuum pressure will need to be achieved to prevent any injuries to the user or patient. A pressure that is too large may increase the amount of fluid extracted and even retain suction better, however, the utmost importance will always be given to the patient. A compromise must be made to the maximum amount of available vacuum pressure as to not harm the individual using the device. Research about suction against the skin will help determine at what pressures to operate.

The extraction group aims to have a working model of a speaker, able to apply a high frequency to the skin, open the pores using the process of sonophoresis, as well as pass current through the skin to extract interstitial fluid through the opened pores to be collected externally for analysis and measurement.

Additionally, an experimental test device solely to measure the vacuum pressure will be available. This device will make it much easier for following groups to continue testing in this area.

The current results address the problem of non-invasive glucose measurement and insulin administration, in essence acting as an artificial pancreas for those with insulin and blood sugar related ailments. The first steps to this process involve the extraction of interstitial fluid instead of blood to make our design non-invasive. The mechanism of sonophoresis and iontophoresis can perform this action and provide enough fluid to analyze, test and determine the patient's blood sugar levels.

The results obtained will be incorporated into the new design that will allow for a better functioning and an easier method of analysis of the effects of the sonophoresis and the iontophoresis on the skin, leading to a better and more functional model for the eventual prototype to be developed.

Measurement Subcommittee:

Our measurement group has run several experiments to determine glucose concentration at physiologically significant levels within faux interstitial fluid i.e. Krebs Ringer Buffer. Concentrations of glucose from six serial dilutions were measured repeatedly at 1000nm using a photoemissions spectrophotometer. In extracting the absorbance values for our standard calibration curve, we found that the concentrations of glucose in these dilutions didn't allow for reliable readings. Because the wavelength of absorbance (1000nm) is below that required for a truly linear Beer Lambert's law curve, and we are working under significant time constraints, we have decided to begin transforming our procedure to allow measurement at a lower more reliable wavelength. This has required the introduction of Benedict's reagent solution to the initial faux interstitial stock.

By measuring glucose concentration of faux interstitial fluid using photoemissions spectroscopy, we seek to assess the reliability and efficacy of this particular method in measuring actual blood glucose concentrations. The current method is one of several being examined as a means of measuring blood glucose concentration within the developing prototype. If we succeed in obtaining reliable measurements, through this method, then we could work towards miniaturization of the technology involved to allow incorporation of ES as the standard measurement component for the final product.

Because our experimental runs have not yet produced results that allow for quantification and analysis, and our initial method has proved unreliable, these results will not be a component of any deliverables produced towards the construction of a final working prototype. By transforming our experiment in the method previously described, we hope to obtain results that will be strong enough to allow development of photoemissions spectroscopy as the method of choice for measuring the blood glucose concentration of diabetic individuals.

The current results don't yet address the problem, which is obtaining reliable recordings of the blood glucose levels of diabetic individuals for monitoring and insulin administration. Upon experimentation, we encountered difficulties in achieving reliable, representative readings at the initially selected wavelength (1000nm).

The current results will not be incorporated into the proposed solution framework, because they don't address the problem. After we have obtained sufficient accurate and precise glucose concentration readings with the revamped procedure, we hope to incorporate these results to allow for selection of a final measurement method for the developing Artificial Pancreas prototype.

3.0. Revised Task / Event Schedule

Extraction Subcommittee:

The extraction team has decided to incorporate the ideas behind the current model and develop a new and more functional model for analysis and testing the laboratory. This model requires further engineering and testing to compare efficiency with the previous model, but is perceived to be the better solution for the long run, as well as for immediate implementation in the laboratory.

Date	**Task**
10/23	In-class presentation of results to date
10/25	Final testeing and assembly of new model
10/26	Experimentation with both pervious and new models in the laboratory
10/30	No Class. Meet with members of group to discuss progress
11/01	Analysis of results so far
11/02	Further Experimentation
11/09	Further Experimentation

Secretary for extraction subgroup: Rohan Mathews

Assembly of new mode : Rohan, Bhavin, E-Fann, Prof De Both

Experimentation : Entire subgroup

Due to complications in obtaining the laboratory space needed of our experimentation and the associated delays, the experimentation was set back. However, with the use of two workable models, we can now look to finishing the proposed tasks in the originally decided time frame.

Research Subcommittee:

The focus has adjusted away from patent research for the individual subteams at the beginning of the semester, to investigating more grant proposals such as the Ford Grant and the NCIIA Grant.

Week of	**Task**
09/17/07	Write rough draft of Project Plan
09/24/07	Assist team members in compiling and editing Project Plan
10/01/07	Set up meeting with expert on grant submittal procedures, become aware of best avenues for securing external financial sponsor, and be aware of all deadlines for grant proposal submissions
10/08/07	Write rough draft of Code of Ethics
10/15/07	Finalize Code of Ethics and write draft of Mid-Term Report
10/22/07	Finalize Mid-Term Report and begin writing grant proposal
10/29/07	Continue writing grant proposal
11/05/07	Rough Draft of Grant Proposal due
11/12/07	Final draft of grant proposal due and assist in creating an exhibit/poster and abstract/brochure.
11/19/07	Assist in preparation for IPRO Day
11/26/07	Compose final IPRO Day presentation on semester's work

IPRO Document Writer: Linda Goldstein

Patents Researcher: Malgorzata Kochanek

Grant Proposal Leader: David Thomas

The Timeline has not varied in any way from the projected schedule in the project plan.

Measurement Subcommittee:

Our team has decided to adapt our initial protocol to allow for measurements at a more reliable (500nm) wavelength, permitted by the addition of Benedict's Reagent Solution to the experimental stock. Benedict's is a deep-blue alkaline reagent solution containing cupric sulfate, sodium citrate, and sodium carbonate, and used as a test for the presence of reducing sugars such as glucose. The test is based on the reduction of cupric

ion (complexed with the citrate ions) to cuprous ion by the aldehyde group of the sugar in solution. The cupric ion reduction is accompanied by changes in color, dependant on the amount of reducing sugar present. Addition of Benedict Reagant solution to our initial stock solution of Ringer buffer and glucose will allow us to obtain a more linear Beer-Lambert's plot, but at a lower wavelength (500nm), that would resolve an issue from the last IPRO term. The wavelength of our present absorbance readings, 1000nm, was a problem in the last IPRO term, because it was too low to allow for a truly linear curve, which is necessary for reliable measurement of blood glucose concentration. Immediate transformation of our present procedure to allow for incorporation of this reagant test would be efficient and productive, giving the time we are working with, and the not entirely linear Beer-Lambert Law plots obtained in the last IPRO period.

Our goal continues to be the assessment of photoemissions spectroscopy as a reliable and effective method for measuring the blood glucose concentrations of diabetic individuals. In order to adequately assess this, we need a sufficiently linear Beer-Lambert's plot. The linear equation of this plot would allow for extraction of glucose concentrations from solutions and eventually blood of unknown concentration. Our projected goal can best be achieved by adapting our initial procedure to enable production of a standard calibration curve that truly meets this linear requirement.

Additionally, the Impedance Spectroscopy team as described previously has instead decided to focus on measuring the glucose concentration through the current produced during the oxidation of glucose via glucose oxidase. It is a standard technique used in many blood glucose sensors on the market but has not been tested to see whether it can be fine tuned to measure glucose concentrations in the interstitial fluid.

Date	***Task***
10/26/07	*In Class presentation of sub-group's work and progress to date & Submission of Midterm Report to igroups*
11/02/07	*Continue experimentation*
11/09/07	*Continue experimentation*
11/16/07	*Perform Data Analysis to determine statistical significance of findings*
11/22/07	*Final Presentation to class on goals met and prospects for future semesters*
11/23/07	*Compose final IPRO Day presentation on semester's work*

Benedict's Solution Team: Walatta Mesquitta, Amir Rahnavard

Glucose Oxidation Team: Kirthi Reedy, Michael Morley

Although the measuring methods have changed, our generic timeline is still more or less the same as approximated in the project plan.

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

Research Subcommittee:

Changes from Project Plan

In the project plan, we had anticipated that it would take us two weeks to meet with an expert on Grant Proposals, choose foundations to which we should apply for funding, and to become familiar with the application processes and due dates for those foundations. However, we were able to accomplish all those tasks in one week.

Another change to the project plan includes the due dates of the rough draft and final draft of the grant proposal. We had originally decided to have the rough draft due 10/25/07 and the final draft to be due 11/01/07. However, that was before we knew that our subgroup would be in charge of writing the Code of Ethics and before we knew that the due date for the Mid-Term report was pushed back a week. As a result of these changes, we had to push back the due dates for our grant proposal. This is not a problem, as the foundation to which we are applying has a due date of 12/03/2007. We plan on having our final version of the grant proposal completed by 11/12/07.

Measurement Subcommittee:

The team organization is still consistent with what was proposed in the project plan. Research is more efficiently conducted in two separate groups pursuing two separate glucose measuring methods. The data produced will then be compared at the end of the semester to identify which glucose measuring technique might be most advantageous to incorporate into the prototype. However, the subteam tasks have changed since emission spectroscopy was determined not to be accurate at such dilute glucose concentrations and impedance spectroscopy is not possible as certain instruments are needed which are currently unavailable. One subteam is focusing on measuring the glucose concentrations using Benedicts Solution, followed by spectroscopy while the other subteam is pursuing a method currently used for blood glucose sensors by measuring the current produced when glucose is reacted with glucose oxidase.

Extraction Subcommittee:

The overall team organization is still consistent with what was proposed in the project plan and the individual tasks. The problems that were encountered have been addressed in an organized manner and the results provided by the extraction committee

should be evidence for the progress that is being made through our research. The team structure and tasks have not changed because the subcommittee has currently been working as efficiently as could be hoped.

5.0. Barriers and Obstacles

Research Subcommittee:

Obstacles:
1. The Research team was asked to identify possible patents of NMR spectroscopy that may be useful to the project; however, the team was not experienced in this field.
2. The team was searching for possible grants or other means of financial backing for the project.
3. At the request of the Extraction team, the team searched for possible distributors of artificial or animal skins, however many options were out of our price range
4. Extraction team required specialized microscopes.

Resolution:
1. Professor DeBoth provided background information and specifications of what the team should look for and where to find it.
2. A meeting was set up with Dr. Opara on the issue, and Dr. Opara directed the team to stress the NCIIA grant.
3. Dr. Connie Hall of the BME department and Dr. Zhang of the BCPS department were contacted and information about possible distributors was passed to Bhavin.
4. Dr. Stark of the BCPS department was contacted by the research team and information was passed to the Extraction team.

The Impedence Spectroscopy team asked the Research team to locate the wavelength for the uptake of glucose. The team consulted prior IPRO 308 information, online references and patents, but was unable to find the information. However, the Impedence Spectroscopy team has been suspended for the time being and the members are helping the Emission Spectroscopy team; so the search for the answer has also been suspended.

The Research team is now pressed for time to make the deadline for the NCIS grant. The team decided to abandon other projects (i.e. the search for NMR spectroscopy and artificial skin suppliers) and shifted the entire goal of the team to finish the grant proposal.

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.

After performing a mandatory laboratory safety and protocol course and communicating with the BME and BCPS departments we secured consistent lab space. As described above, in order to readjust our project focus and optimize our human resources the initial two measuring techniques were dropped and two more, Glucose measurement via glucose oxidase reactions and measurement via Benedict's solution, are being explored.

The remaining barriers will be to acquire the instrumentation necessary to perform the glucose oxidase experimentation and the Benedict's solution tests; however, our teams have already been communicating with Dr. Bishnoi from the BCPS Department to setup the experimental design for this next method and acquire the necessary chemicals to perform it. Additionally, we may run into the potential barrier that these new methods might also not be sensitive enough to measure glucose accurately at physiologic levels that we need. In this scenario additional research would be performed to identify other measurement methods to explore.

Extraction Subcommittee:

As with the measurement subcommittee, the first obstacle was simply to acquire lab space to perform the experiments and also to obtain all of the necessary equipment to set up the vacuum and speaker devices. After laboratory safety training and help from the BME faculty, lab space was acquired for up to 3 days a week of experimentation. Additionally, after clarifying with the IPRO office how to order necessary materials offline, the needed suction tubes and gaskets were ordered to start experimentation.

Obstacles still remaining are to increase the effectiveness of sonophoresis to open up the pores of the pig skin and to increase the efficiency of the suction formed. These are currently being addressed by creating a new metallic plate to fit around the gasket in order to increase the connective surface with the skin and also by developing new ways to acquire thinner pig skin or trimming down the samples that we currently are provided with.