MRI-Compatible Bioreactor for Cancer Cells

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Abstract

Current research in the field of medical physics has offered insight into metabolic differences between cancerous and benign cells in the pyruvate pathway. This research has significant potential to improve cancer staging and treatment planning techniques *in vivo*. For *in vitro* studies researchers use magnetic resonance imaging (MRI) to track the metabolic breakdown of carbon-13 (^{13}C) tagged pyruvate. These studies require an MRI-compatible bioreactor to house, maintain, and monitor high density cell cultures throughout the testing process. This bioreactor must include: a pumping mechanism to continuously distribute media and nutrients to the cells, a monitoring system to ensure ideal physiological conditions are sustained, a cartridge to house the cell culture, and a heater to maintain a temperature of 37 °C. Currently available bioreactors do not provide the required integrated sensing abilities and are not cost effective options for the lab. To address this problem, our group used a peristaltic pump to maintain media flow through a custom-designed cell canister and two sensing compartments to measure the dissolved oxygen (DO), pH, and temperature of the media. The complete monitoring system consists of a pH electrode probe and a DO electrode probe with an internal thermistor, all of which are interfaced with custom circuitry and LabVIEW for signal processing and display. The pH and temperature systems are accurate to ± 0.02 pH and ± 0.3 °C, respectively. Furthermore, dye dispersion testing confirmed that the peristaltic pumping system evenly distributes media and injected substances throughout the cell canister. Future work for the project consists of implementing and testing a heating mechanism, fabricating a final canister out of glass, and constructing a Faraday cage to house the sensing components.

Contents

Introduction

Background and Motivation

Our client, Dr. Sean Fain, is an associate professor in the Department of Medical Physics at the UW School of Medicine and Public Health. His research focuses on tracking pyruvate metabolism differences between benign and cancerous cells. To do this, his laboratory group initially exchanges the first carbons (C_1) of pyruvate molecules with the ^{13}C isotope. Prior to the substitution 98.9% of these carbon atoms are ^{12}C, which is spin neutral and therefore undetectable with a magnetic resonance (MR) scanner[1]. Replacement with ^{13}C enables the lab to obtain an MR signal from the sample. After the carbons are exchanged, the researchers use hyperpolarization to increase the signal of the ^{13}C atoms by approximately 10,000 fold. This type of hyperpolarization, known as dynamic nuclear polarization, transfers the spin polarization from electrons to the atomic nuclei to increase alignment, resulting in an amplified signal[1]. Tracking the breakdown of pyruvate is then accomplished by detecting its various metabolic products (Figure 1).

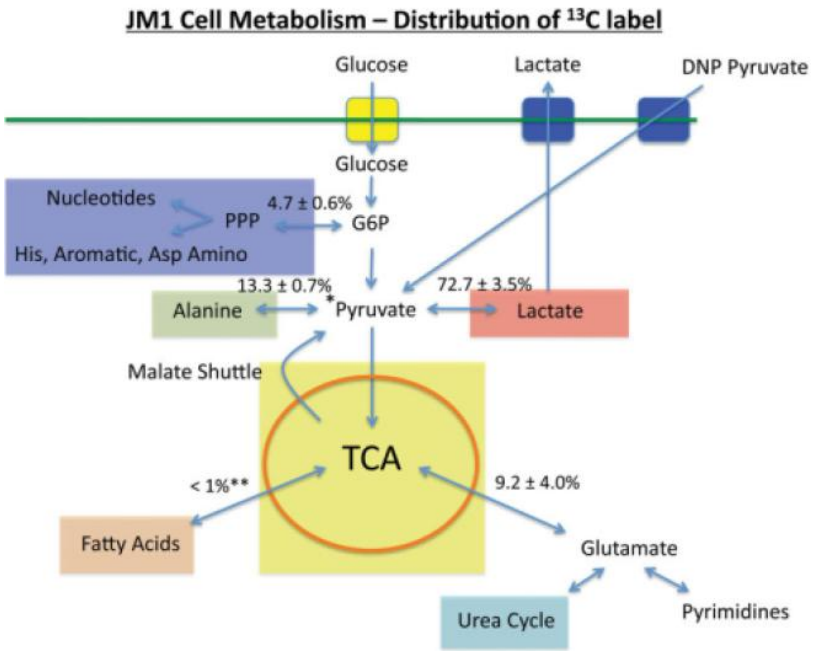

Figure 1: Map of pyruvate metabolism and final metabolites in JM1 cells. The metabolic rate within the pyruvate pathway differs between healthy and cancerous cells. Therefore, tracking pyruvate metabolism has the potential to help clinicians differentiate between benign and malignant cells *in vivo*[2].

Once the ^{13}C-tagged pyruvate sample is hyperpolarized, researchers quickly administer the sample to a cell culture and track its metabolic breakdown using a 4.7 T micro MR scanner (Figure 2). Though healthy cells convert pyruvate into Acetyl CoA in the presence of oxygen, malignant cells convert pyruvate to lactate regardless of oxygen levels. Researchers can therefore detect the rate of lactate formation, because lactate molecules will inherit the hyperpolarized ^{13}C from the original pyruvate molecule. The rate of lactate formation will be distinctive for different pyruvate metabolisms in cancerous versus benign cells[3].

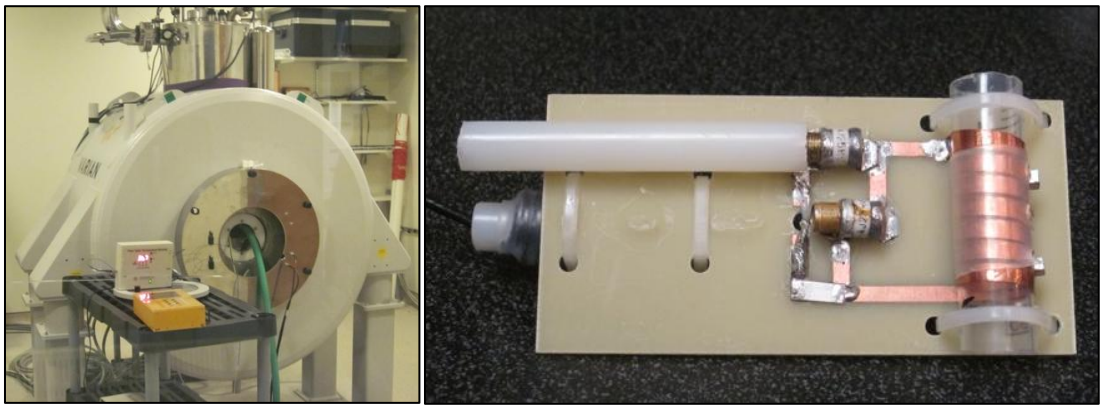

Figure 2: Horizontal bore micro MR scanner (left), with associated MR coil (right) used in pyruvate metabolism research. The horizontal configuration of the scanner restricts bioreactor designs by placing limits on the size and orientation of the cell canister.

Using this technique for *in vivo* cancer studies could enable clinicians to definitively identify cancerous cells. This would allow for an accurate determination of the extent and severity of a malignancy, and could provide a definitive guide during treatment planning and prognosis. However, before clinical research can begin, this technique must be verified *in vitro:* a process which requires a bioreactor to house and provide nutrients to high density cell cultures. This bioreactor must incorporate a pumping mechanism to continuously distribute media and nutrients to the cells, a monitoring system to ensure ideal physiological conditions are upheld, a canister to house the cell culture, a heater to maintain an ideal temperature, and injection ports for the hyperpolarized samples and other substances. In summary, this device should be compatible with a horizontal bore MR scanner and support high density cell cultures. Currently available bioreactors do not have the desired sensing mechanisms and are expensive. Furthermore, the dimensions,

materials, or orientation of many available bioreactors are not compatible with the small, horizontal MR bore used by our client.

Current Devices

Although some devices have been designed for similar applications in academic research, their major shortcomings include a lack of integrated sensing components and commercial availability. A horizontal bioreactor system exists commercially through FiberCell® Systems, but it also does not have the ability to monitor cellular conditions and its cost exceeds the budget for this project.

Harris et al. Bioreactor

Harris and coauthors utilize the bioreactor shown as a schematic in Figure 3 for their research with hyperpolarized ^{13}C and human breast cancer cells. In their experiments, cells are grown on microcarrier beads contained within the bioreactor cartridge. Continuous perfusion is employed to mimic physiological conditions[4]. The system has an injection port, which allows for the efficient delivery of the hyperpolarized mixture. A culture temperature of 36 ± 1 °C is maintained via a water jacket that encases the bioreactor. Along with the previously stated conflicts, this device needs to be disconnected from the perfusion system during data acquisition. This process consumes valuable time as the hyperpolarization signal rapidly decays[4].

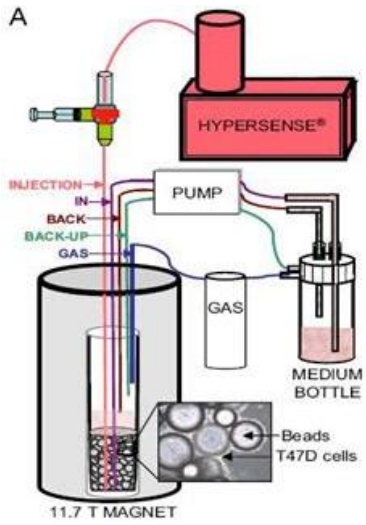

Figure 3: The bioreactor system used by Harris *et al.* allows for the injection of hyperpolarized ^{13}C as well as circulated flow of fluids and gases. However, it lacks sensing components and is unable to be connected to the pump during data collection[4].

Keshari et al. Bioreactor

Keshari and collaborators monitor metabolites in JM1 rat heptoma cells through hyperpolarized ^{13}C spectroscopy. They implement the bioreactor that is represented as a schematic in Figure 4 to maintain their cells during experimentation. The cell culture medium is circulated through the system by a pump that sustains a rate of 240 mL/hr. Water-jacketed lines are utilized to keep the medium at a constant temperature of 37 °C[2]. Although this system allows for the control of several factors that influence cellular conditions, there is no method for monitoring or verifying these conditions.

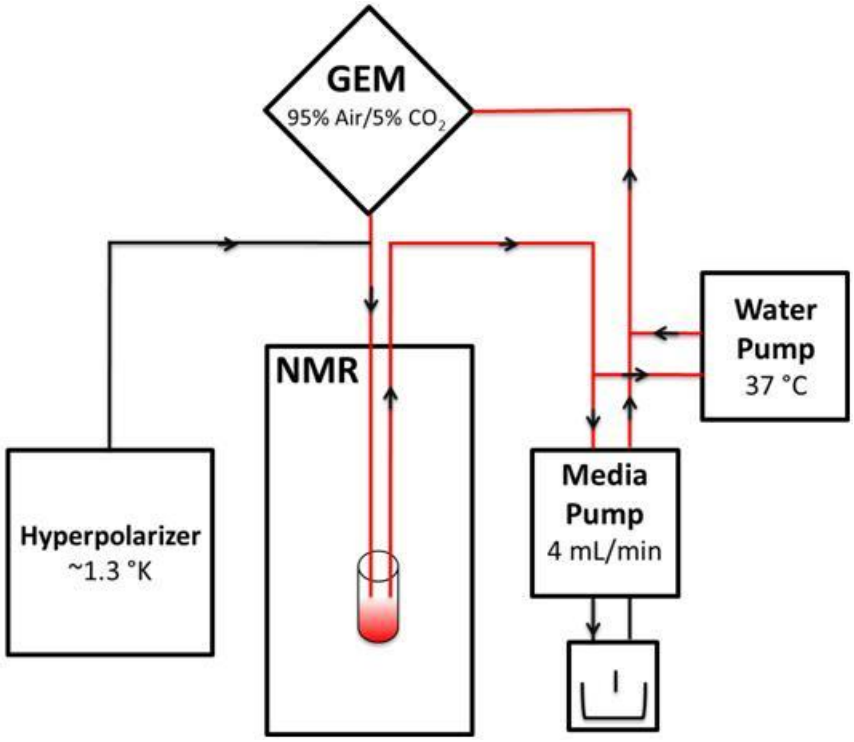

Figure 4: The bioreactor system used by Keshari *et al.* allows for the control of gas exchange, temperature, and flow rate. It also enables hyperpolarized ^{13}C to be delivered to the cell culture. Similar to the Harris *et al.* bioreactor, its inability to measure cellular conditions poses a major hindrance for its use in this project. Abbreviations – GEM: gas exchange modulator. NMR: nuclear magnetic resonance[2].

FiberCell® Systems Bioreactor

The bioreactor system developed by FiberCell® Systems Inc. is shown as a schematic in Figure 5 and incorporates a hollow fiber bioreactor cartridge. The fibers act like filters that can transport media through the cartridge and allow for the exchange of nutrients and waste with the cells. The pumping system enables continuous flow that can be varied between 60 and 8,400 mL/hr[5]. The manufacturers designed the system to operate within

a standard CO_2 incubator, so the device is dependent on the temperature and gas exchange features of the incubator and has no means of independently controlling cellular conditions. MR scanning is not listed as an intended application for this system, so testing would need to be done to confirm that the materials are non-ferromagnetic, radiolucent and do not hinder data acquisition[5].

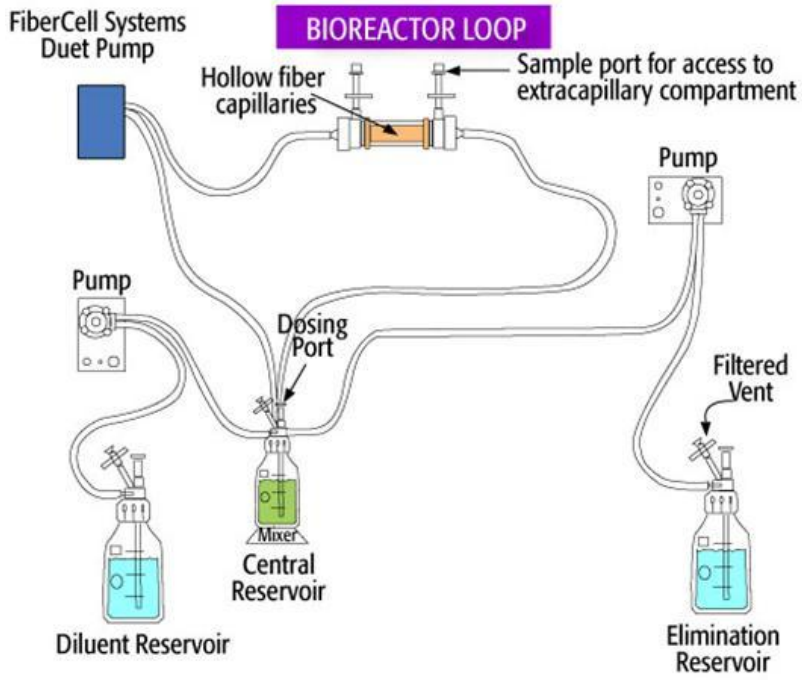

Figure 5: The FiberCell® Systems bioreactor is a commercially available system that has the ability to support continuous and variable flow of substances. Unfortunately, its cost would entirely consume the funds for the project and prevent the purchase of sensors to monitor conditions[5].

Design Requirements

In developing any product, it is first necessary to establish the client's needs and product requirements. The Product Design Specifications document reviews these necessities in detail and can be found in the Appendix.

The purpose of the device is to house a tissue scaffold structure while delivering fluids and gases required to maintain cell viability or establish various experimental conditions. This delivery must be accomplished through an even dispersal to all cells at a rate of 0.25-4.00 mL/min. The device must also remove metabolic waste products from the cellular environment at this same rate. Furthermore, this system must have integrated

sensing components to allow technicians to monitor dissolved oxygen (DO), pH, and temperature and adjust them if necessary. These anticipated values under normal conditions are 0-20%, 7.4 ± 0.1 pH, and 37.0 ± 1.0 °C, respectively. As MR will be used during experimentation, all components in the vicinity of the magnetic field must be non-ferromagnetic and should not interfere with data collection.

The bioreactor system cannot harm or damage the culture it contains, its operators, or the MR components and other testing devices. Substances injected into the device must be evenly and accurately dispersed to the cells within 5 sec without causing cell damage. The device must be capable of maintaining an ideal cellular temperature of 37.0 ± 1.0 °C.

To verify that tracking cellular metabolism is an effective method for staging cancer, researchers will use the device to complete *in vitro* studies, which will take approximately two years. The bioreactor must retain its functionality throughout this time and have a two-year shelf life in which it can be stored between trials. The device will operate in a standard 20 °C environment at a pressure of 100 kPa. Sterility must be easily achieved and once set up is complete, the system should operate without constant supervision.

Substances must be injected into the bioreactor system within 2-5 sec, from an injection port laying 45-60 cm outside of the MR bore. Although the sizes of components that will remain outside of the MR scanner are not a concern for the development of the system, any component that will be scanned can be no greater than 10.8 cm in diameter or height. This will ensure that the culture vessel can be inserted into a solenoid coil and still fit easily into the bore opening. Weight is another aspect that is negligible for external elements but can be no greater than 2.0 kg for any part entering the MR scanner. Functionality is emphasized over aesthetics, so although the device is not required to have a commercial appearance, components must be MR compatible. This entails that it is made of non-ferromagnetic and radiolucent materials. Additionally, components must be easy to sterilize or dispose of and non-cytotoxic.

The client only requires one device for his experiments and testing. The total budget for the project is $3,000, so the cost for production must not exceed this value. Although

there are no federal regulations that will govern the use of the product, it must conform to basic cell culture guidelines to be effectively implemented for its intention.

Overview of Design Alternatives

Sensing Systems

Sensing systems allow researchers to maintain ideal physiological conditions for the cells by monitoring DO, pH, and temperature. Measuring these parameters ensures that the cultured cells are in their optimal growing environment and alerts researchers when the cell medium should be changed. Furthermore, a monitoring system ensures that experimental conditions can be controlled precisely and reproduced accurately.

Flow Through Monitor

The flow through monitor is a convenient cell culture monitoring system from Harvard Apparatus (Figure 6). The system contains four probes for measuring pH, pO_2, pCO_2, and temperature, and is designed specifically for cell culture, since it is capable of being autoclaved[6]. The device also comes equipped with a data acquisition interface, which allows researchers to collect and store data using a laptop computer.

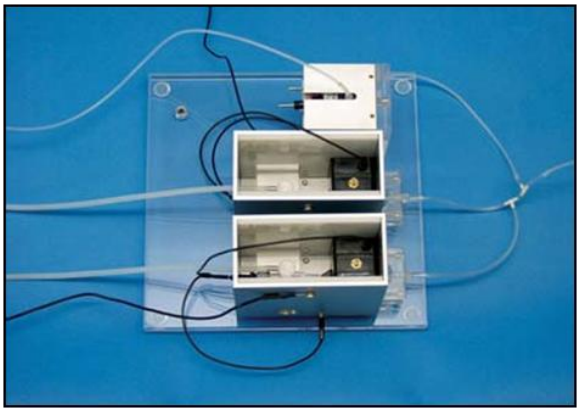

Figure 6: Harvard Apparatus flow through monitor senses pH, pO_2, pCO_2, and temperature. The device can be autoclaved and comes with a data acquisition interface, which makes it convenient and easy to use. However, the price is outside of our proposed budget[6].

To integrate the system into the bioreactor design, we would divert a small amount of medium from the cell cartridge and pass it through the flow through monitor using the tubing provided by Harvard Apparatus. The system has a response time of approximately

90 sec[6]. After the media flows through the sensor, it can be redirected into the cycle, forming a closed loop. The device is highly accurate (± 0.2%) and precise (± 0.2%); however, the price for the flow through monitor ranges from $15,000 to $21,000 and is outside of our proposed budget[6].

Mass Flow Controller

The mass flow controller, produced by Lambda Laboratory Instruments, regulates gas exchange within the bioreactor to maintain pO_2 and pH (Figure 7). The controller is connected to oxygen and carbon dioxide tanks along with pH, pO_2, and pCO_2 electrodes, which must be purchased separately from Lambda Instruments. These electrodes allow the controller to monitor cell culture conditions, specifically pH and pO_2. Once the desired pH and pO_2 are programmed into the device, the controller maintains these conditions by adjusting the amount of oxygen and carbon dioxide distributed to the cells[7]. Adding or removing carbon dioxide from the system maintains the culture pH through Equation 1.

Figure 7: The lambda mass flow controller regulates cellular pO_2 and pH by monitoring the flow of oxygen and carbon dioxide through the culture. The system requires the purchase of additional electrodes to sense the O_2 and pH levels[7].

$$CO_2 + HOH \longleftrightarrow H_2CO_3 \longleftrightarrow H^+HCO_3^-$$

Equation 1: pH balance equation between carbon dioxide and bicarbonate. This equation is used by the mass flow controller to maintain a constant pH within the cell culture by altering the amount of carbon dioxide delivered to the system[8].

This system has an accuracy of ± 3.0% and flow rates can range between 0 to 3,000 mL/hr. Since the system controls air flow, adding a connection to a nitrogen tank would

provide a convenient method for accurately inducing hypoxic conditions for further studies[7]. The device does not contain components that could contaminate the culture, since all gases would pass through sterilized tubing. Our group could easily integrate this monitoring system into the bioreactor because it would not require additional air ports into the cell culture cassette. Additionally, the device decreases the need for human monitoring, as it both senses and regulates the pO_2, pCO_2, and pH of the culture. The price of the controller is $1,490 excluding the cost of the supplementary electrodes[7]. The entire system is expected to cost between $1,600 and $1,700, and would consume the majority of our budget.

Electrode Probe Sensors

Another potential sensing system consists of electrode probe sensors, which are widely available for both pH and DO sensing (Figure 8). Both electrodes have a Bayonet Neill-Concelman (BNC) output connector that requires external amplification, filtration, and a digital output display system, such as LabVIEW.

Clark electrodes, used in sensing DO, are constructed from an electrochemical cell with a glass covered platinum cathode and silver-silver chloride anode[9]. Current produced within the electrode is proportional to electron transfer in the reduction-oxidation reactions that occur at the cathode and anode. The rate of electron transfer is in turn proportional to the concentration of oxygen present in the test solution[9]. The amount of current produced in the electrode is thus proportional to DO, which allows sensing to be conducted with proper calibration. The DO electrode most viable for our system is the Oakton WD-E5642-50 probe. This probe costs $256.50 and can sense oxygen concentrations ranging from 0 to 50 mg/L. The probe is accurate to ± 0.5% with a resolution of 0.01 mg/L[10]. Furthermore, this device can be sterilized with an ethanol wash and is constructed of glass and epoxy, both of which are non-cytotoxic[10].

Electrode probes that sense pH are constructed from ion specific membranes. Within a pH probe, a highly selective H^+-permeable glass membrane separates the test solution from a reference solution[9]. When placed in a test solution of a different pH, a potential develops across the selective membrane proportional to H^+ concentration. The

membrane potential of the electrode is sensed as a voltage difference, which is amplified and processed into a corresponding pH value. At $39.90, the Oakton WD-35801-00 pH probe is cost effective and meets the specified design requirements[11]. This sensor has a pH range of 0 to 14, an accuracy of ± 0.01, and a resolution of 0.01[11].

Figure 8: Oakton WD-E5642-50 DO probe[9] (left) and Oakton WD-35801-00 pH probe[10] (right). These electrodes sense DO and pH, respectively, and must be interfaced with a digital output source to convert data to relevant values.

Pumping Systems

Our bioreactor cartridge will be perfused with cell culture media using a pumping mechanism. Flow of media must continuously supply cells with nutrients and be adjustable to prevent dislodging the cells from the tissue scaffold. The various pumping systems that encompass the necessary design requirements of our bioreactor system are the diaphragm, peristaltic, duet, and syringe pumps.

Diaphragm Pump

The diaphragm pump employs both suction and pressurization to drive fluid flow. The pump contains a diaphragm coated with polytetrafluoroethylene (PTFE) and two one-way check valves constructed from perfluoroelastomer (FFKM)[12]. Both of these materials are chemically inert and biocompatible, meaning they will not damage the cell culture. To operate a diaphragm pump, a 12 V DC power supply is needed to drive a solenoid-operated

diaphragm to cycle upwards and downwards in the pump cavity. As the diaphragm is pulled upward, the input check valve is opened and fluid is suctioned into the pump cavity. The diaphragm then pushes downward while the input check valve closes and the output valve simultaneously opens. The downward motion pressurizes the cavity and expels its contents through the output check valve into the tubing. The flow from the pulsatile pumping mechanism has a low shear stress and would support a continuous, closed loop perfusion system. Although the rate is predetermined and not adjustable, diaphragm micropumps are sold with a wide variety of flow rates. The most relevant rates for our design are 288, 360, and 432 mL/hr[12]. Diaphragm pumps are readily available through BioChem Fluidics at a cost of $318.25[12]. The most relevant model for the bioreactor perfusion system is the 130SP1250-ITP, shown in Figure 9.

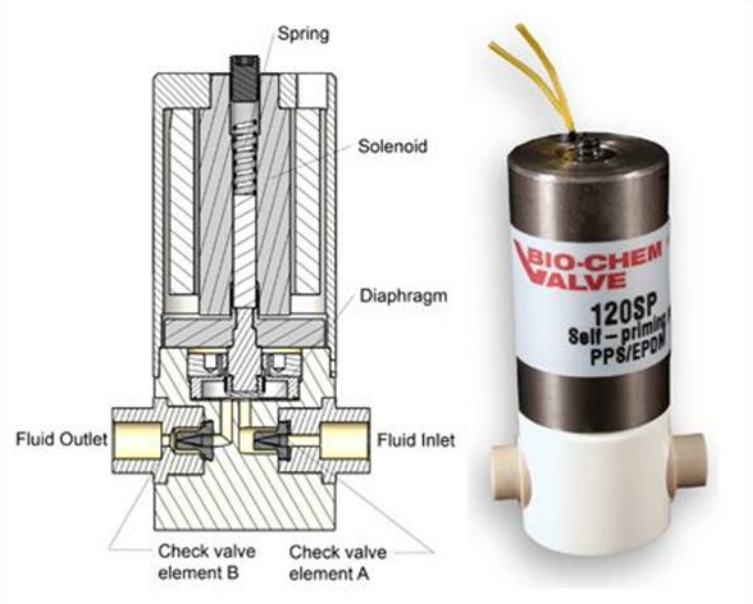

Figure 9: Diaphragm micropump from BioChem Fluidics. This particular model has a 360 mL/hr flow rate[12].

Peristaltic Pump

Peristaltic pumps use rollers to compress elastic tubing, which creates a positive pressure to direct the flow of gases and fluids. Each revolution of the rollers pumps through a specific volume that is dependent on the diameter of the tubing used and speed of the motor[13]. Although pulsation previously hindered the use of peristaltic pumps, most modern versions include multiple rollers, as in Figure 10, or have replaced the rollers with

glass bead bearings to greatly reduce this effect (Figure 11). Since the motor and pumping mechanisms are enclosed, typical operating temperature and humidity values for the pump can range safely from 0-40 °C and 0-90%, respectively[14].

An advantage of these pumps in regards to sterility is that fluids and gases only contact their transport tubing. The most commonly used tubing is silicone, which can be autoclaved and comes in a vast range of diameters and thicknesses. The pumps themselves are typically encased in hard plastic that can also be sterilized using ethanol[13]. Flow rates for substances can range from 0.01 to 3,000 mL/hr, can be adjusted via a control dial or programming, and may be able to change over time depending on the device[14].

Figure 10: Peristaltic pump with multiple rollers to direct flow. Having multiple rollers reduces the pulsations created and increase the device's functionality[13].

Figure 11: Peristaltic pump with glass bead bearings to compress the tubing and direct flow by the same means as a standard peristaltic pump, but with a reduced pulsation effect[13].

Duet Pump

The duet pump from FiberCell® Systems Inc. is a hybrid of the diaphragm and peristaltic alternatives. It accomplishes positive pressure displacement pumping by systematically squeezing the tubing and directing flow using two one-way check valves. This results in a nearly frictionless pumping action[5]. Users can vary the rate of flow from 60 to 8,400 mL/hr by turning a control dial on the pump. This rate is also dependent on the sizes of the tubing and bioreactor cartridge that are integrated into the system[5].

The tubing, usually made of silicone, can be run through the pump and thus sterility is independent of most pump components. The pump valves can be sterilized using ethanol or through autoclaving, while the pump casting can be wiped with ethanol. The system, which can be seen in Figure 12, is designed to operate in a standard CO_2 incubator, and can therefore function in temperatures up to 60 °C and over 95% humidity[5]. The prominent advantage of the duet pump is its ability to provide a continuous flow, which results from the combined pumping mechanism[5].

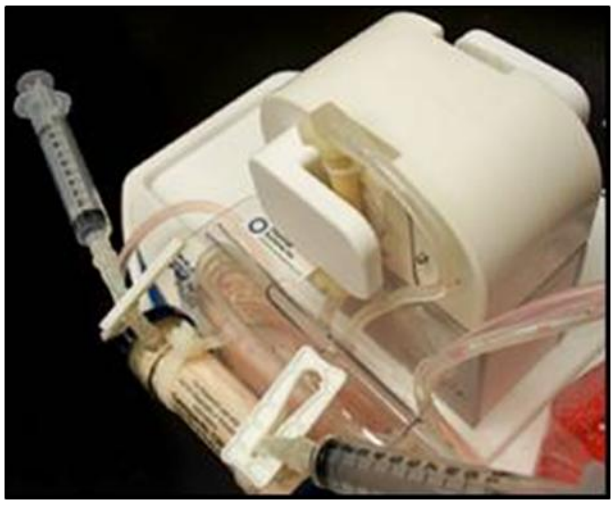

Figure 12: Duet pump used with hollow fiber bioreactors. This positive pressure displacement pump squeezes the inserted tubing, while using integrated one-way check valves to determine the direction of flow[5].

Syringe Pump

This pumping mechanism can infuse and withdraw substances through a syringe. Fluid is pumped or withdrawn using controlled mechanical displacement of the plunger component of the syringe, which allows for consistent flow (Figure 13). Syringes can come in different sizes, and pumps can infuse multiple syringes at once depending on the user's

needs. The flow rates for these devices range from 0.001 µL to 8,400 mL/hr and are easily programmed[15]. Additionally, since the syringes are disposable, cytotoxicity is not a major concern. One downfall to implementing this device is the cost, which can be as much as $3,500[15]. Another fault of this pump is that it does not incorporate continuous flow; that is, recycled material cannot be pumped back into the system without loading a new syringe.

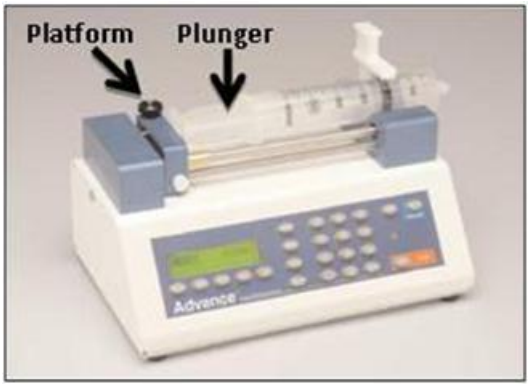

Figure 13: Advance Fusion Series 1200 from SouthPointe Surgical outlining the platform and plunger[15].

Design Matrices and Evaluation of Design Alternatives

To determine which design alternatives were best suited for our final design prototype, our group conducted comparative examinations for both sensing and pumping systems using design matrices (Tables 1 and 2). The matrices provided a quantitative analysis of how well each design alternative adhered to the proposed design requirements. The five categories assessed in the sensing design matrix were cost, cytotoxicity/sterilizability, accuracy, precision, and feasibility. The pumping matrix also evaluated cost and cytotoxicity/sterilizability, but included ease of use, flow (continuity and rate), and physiologic stress. Based on the point allotments shown in the matrices below, the electrode probe sensing systems and peristaltic pump system were pursued as components of our final design prototype.

Table 1: Sensing Design Matrix: The maximum possible point values are indicated in parentheses in the row headings. The electrode probes scored highest and were used in prototype construction.

	Electrode Probes	Mass Flow Control	Flow Through System
Cost (30)	30	12	0
Accuracy (20)	18	17	15
Precision (20)	16	17	18
Cytotoxicity/ Sterilizability (20)	13	15	19
Feasibility (10)	7	4	9
Total	**84**	65	61

Table 2: Pumping Design Matrix: The maximum point values are indicated in parentheses in the row headings. The peristaltic pump was used in prototype construction.

	Diaphragm Pump	Peristaltic Pump	Duet Pump	Syringe Pump
Cost (25)	20	22	12	16
Cytotoxicity/ Sterilizability (25)	20	23	25	25
Ease of Use (10)	7	9	9	7
Flow (Continuity & Rate) (20)	12	15	18	8
Physiologic Stress (20)	12	17	17	8
Total	71	**86**	81	64

Cost

Cost was a major limiting factor in selecting both sensing and pumping systems, and as a result, represented 30 and 25 points of a total 100 points for the sensing and pumping matrices, respectively.

In the sensing design matrix the electrode probes received a perfect 30 points, as they were by far the most cost effective option. The mass flow control system would consume the majority of our budget so it was allotted 12 out of 30 possible points. The final option, the flow through system, received 0 points as its price significantly exceeded our budget.

For the pumping systems, the peristaltic pump was most cost effective and was allotted 22 of 25 possible points. No perfect scores were allocated, as all of the design alternatives were significantly expensive. The diaphragm pump was slightly more expensive than the peristaltic pump, and received a score of 20 points. The syringe pump and duet pump were the most expensive options, and received 16 and 12 points, respectively. All of the pumping systems were feasibly within our budget, so no scores below 12 were allotted.

Cytotoxicity/Sterilizability

The second category common to both matrices, cytotoxicity/sterilizability, assessed the biocompatibility of the device with cell culture as well as its potential to be sterilized effectively. For our application, a simple ethanol wash is sufficient for cleaning the devices; however, more reliable forms of sterilization, such as autoclaving, are beneficial. Though vital to growing and monitoring cells, this category represented only 20 and 25 points in the sensing and pumping systems, respectively. This low value was because all of the systems are sufficiently biocompatible.

In the sensing matrix, the flow through system received the highest score of 19 points out of a possible 20 because it is entirely autoclave compatible. The mass flow and electrode probes were given slightly lower values as they cannot be autoclaved.

The point allotment in the pumping matrix was very similar to that of the sensing matrix. All of the alternatives received very similar values, however the duet, syringe, and peristaltic pump (25, 25, and 23 points, respectively) received slightly higher values as the media only contacts the tubing rather than the pump itself. The diaphragm pump, scoring 20 out of 25 points, was not far behind as all of the components contacting cell media are made from chemically inert fluoropolymers.

Accuracy

The first category unique to the sensing matrix was accuracy. The sensing system must accurately monitor cell conditions to ensure that cultures are healthy. Though less limiting than cost, accuracy is a defining design characteristic and was allotted 20 possible points. The electrode probes received the highest score (18 points) as they are more accurate than both the mass flow and flow through systems, which received 17 and 15 points, respectively.

Precision

Precision of the sensing system was equally as important as accuracy, and also received 20 possible points. The flow through system had the most repeatable measurements and was allotted the highest score of 18 points. The electrode probes (16 points) and mass flow system (17 points) were slightly less precise; however, all are sufficiently precise for our application.

Feasibility

The feasibility of integrating each sensing mechanism into a bioreactor system may affect the overall functionality of the prototype and this category was allotted 10 possible points. The flow through system would be most feasible as it has simple input and output tubing ports and requires no additional circuitry. This alternative received the highest score of 9 points. The electrode probes, allotted 7 points, would be slightly less feasible as they require additional circuitry and must be interfaced with a digital display system. The mass flow system received the lowest score of 4 points because it requires external electrodes and tubing connected to input and output ports.

Ease of Use

The ease of use category was used in assessing the pumping systems and represents how easily the user can operate the pump and manipulate the flow rate. This category was allotted 10 points because, although significant, it is less of a determining design characteristic because technicians can be trained if necessary. The peristaltic and duet systems received 9 points, as their flow rates are adjusted with a simple knob and a single switch powers the devices. The diaphragm and syringe pumps were given slightly lower values of 7 points. The diaphragm pump does not have an adjustable rate. The syringe

pump has a controllable rate programmable through a keypad, but is slightly more complicated than the simple dial of the peristaltic and duet pumps.

Flow (Rate and Continuity)

Another important category was the flow rate and continuity. This category received a possible 20 points because a continuous, accurate flow rate is essential for proper cell nourishment. The syringe pump received the lowest value of 8 points as it does not have continuous flow, but rather must be reloaded at regular intervals. The diaphragm pump does have a continuous flow with both suction and pumping action; however, it does not have a controllable flow rate. Predetermined flow rates depend on the pump model, so this option was allotted 12 points. The peristaltic pump (15 points) has an easily controlled flow rate; however, backflow may be present in the pump. The final option, the duet pump, was allotted 18 points as it contains an adjustable flow rate and valve system to prevent backflow.

Physiologic Stress

This final category assessed the ability of each pumping system to minimize physiologic stress and was allotted 20 possible points. The duet and peristaltic pump each received 17 points as they are specifically designed to simulate the pulsatile pumping experienced *in vivo* by the heart beating. Neither received a perfect score, as they only model physiologic stress and still contain larger shear stress than body systems. The diaphragm pump also has low shear stress, and received a score of 12 points. The syringe pump is not designed to model physiologic stress and thus was allotted a mere 8 points.

Final Design

Based on the results of the design matrices, we chose to use electrodes for the sensing system and a peristaltic pump for the pumping mechanism. These two components were integrated into the system seen as a schematic in Figure 14 and image in Figure 15.

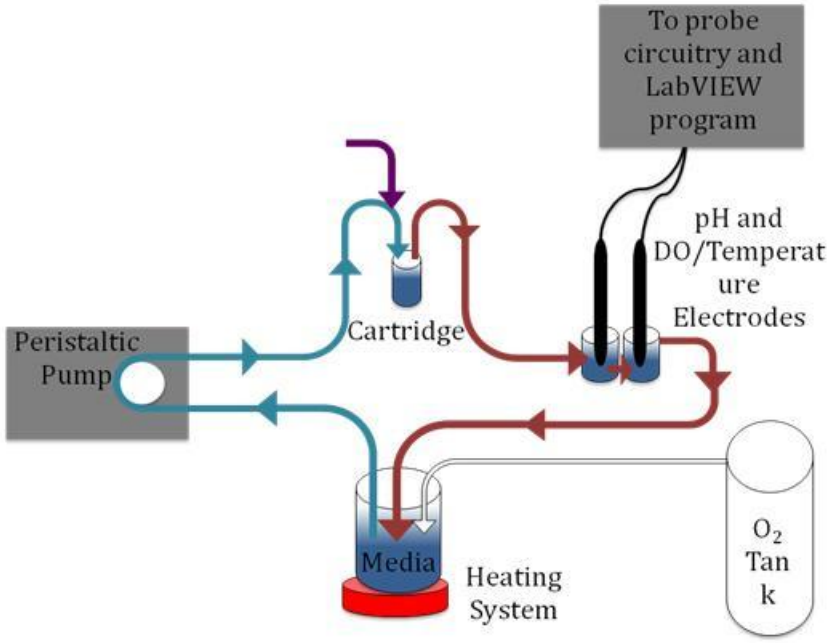

Figure 14: Schematic of components and direction of flow for integrated system. Teal lines indicate active pumping. The purple arrow represents the injection port. Red lines indicate passive flow. The white arrow is for the active pumping of O_2 into the system.

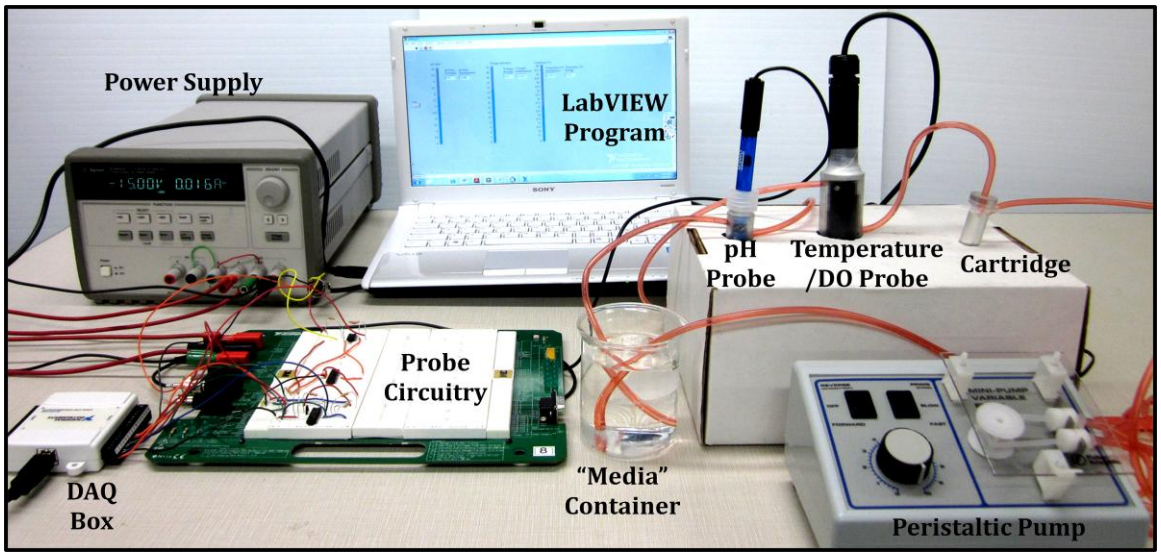

Figure 15: The peristaltic pump drives the media in a closed loop through the cartridge and sensing containers with the probes. The output of the probes is transmitted and manipulated by the circuitry and then fed into the LabVIEW program for display. Abbreviations – DO: dissolved oxygen, DAQ: data acquisition.

The peristaltic pump is connected to both a media container and the bioreactor canister, forming a closed loop perfusion system. As shown above, the final design will also include an oxygen perfusion system to facilitate cellular respiration as well as a gas emissions port to remove carbon dioxide. Finally, our design will include a simple syringe

injection port for hyperpolarized pyruvate samples. All of the tubing used to transport media between components will be medical grade low density polyethylene (LDPE), as it is biocompatable, non-cytotoxic, and can be sterilized with ethanol.

Both the oxygen and pH electrodes were integrated into circuits constructed with an NI elvis breadboard, common resistors, and Texas Instruments JFET operational amplifiers. The output of the pH electrode, as seen in Figure 16, is passed through a summation amplifier to allow for simultaneous amplification and voltage offset.

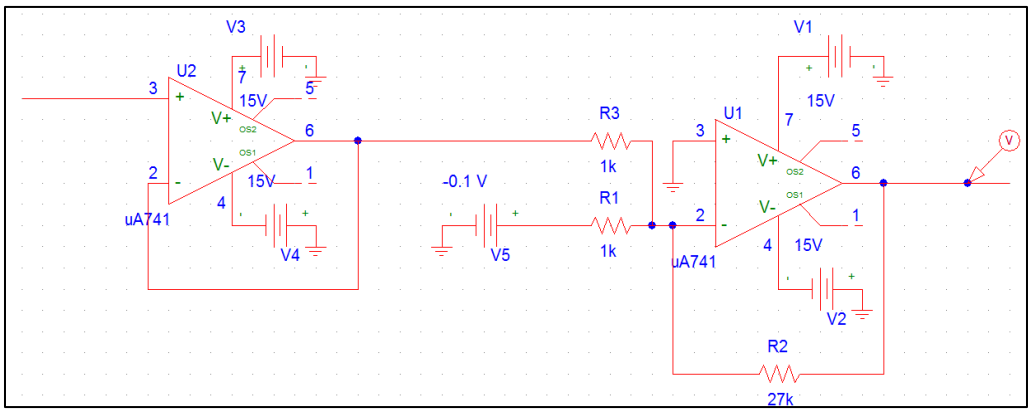

Figure 16: The pH PSpice circuit design includes a voltage divider and a summation amplifier.

The thermistor output was amplified using a simple non-inverting amplifier with the negative type thermistor in a voltage divider configuration at the positive amplifier input. This circuit, shown in Figure 17, produced a voltage output that changed with surrounding temperature. The final sensing component, DO sensing, was not amplified as the voltage output appeared stable and the resolution was sufficient. If it is discovered that amplification is in fact required in the future, it would be accomplished with an applied voltage to the probe and an ammeter at the output.

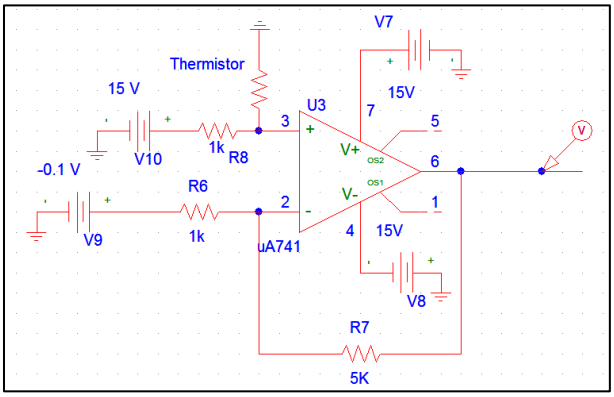

Figure 17: PSpice schematic of accompanying thermistor circuitry for monitoring temperature.

All three sensing components were interfaced with LabVIEW. The LabVIEW program, shown in Figure 18, pulls input from three channels of the data acquisition device (NI USB-6008) and processes the data using calibration equations. Calibration for the pH electrode was achieved by using three standard solutions, and yielded the relationship: $pH = 0.6498V_o + 4.4646$, where V_o is the output voltage of the circuit. The DO calibration was also accomplished by using three standards, and adhered to the equation: $\%\ Saturation\ O_2 = 3.77.74V_o - 0.6028$. Finally, the temperature sensing system was calibrated with 23 temperature values, and follows the relationship: $Temperature = -\ln(V_0/13.293)/0.018$. The final display in the LabVIEW program averages 10 successive readings produced by each of the three equations in a numerical indicator.

The electrode probe sensors are each individually housed in containers made from acrylonitril butadiene styrene (ABS) with a 3D stereolithography printer. They receive input from the perfusion line exiting the bioreactor canister. Separate housings were integrated to reduce the total housing volume, which in turn induces more rapid media exhange in these stations and increases sensor response time.

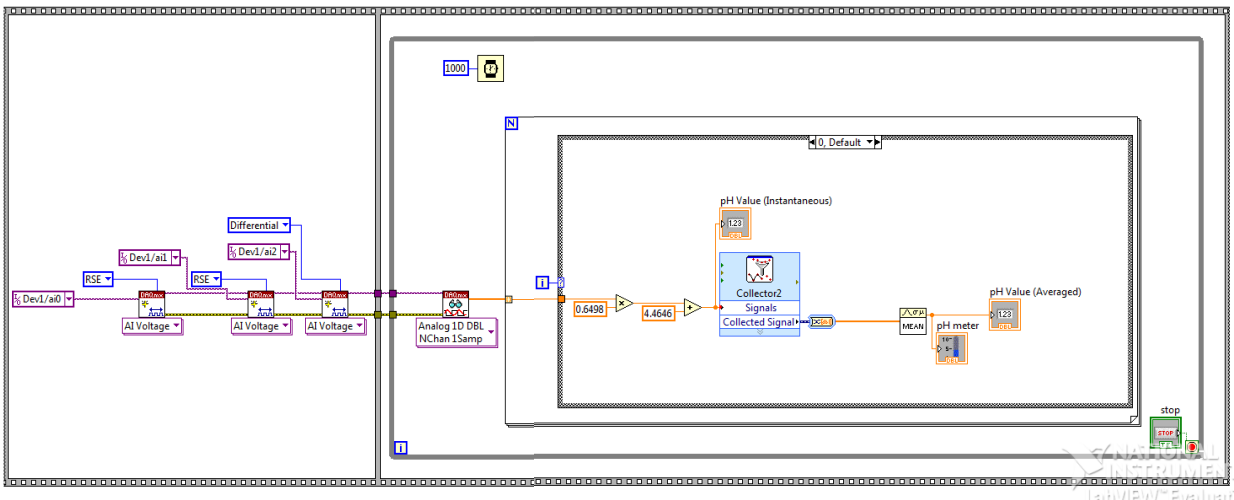

Figure 18: LabVIEW program used to manipulate the data output from the electrode probes and their corresponding circuitry for display as meaningful values.

The central bioreactor cartridge was designed using SolidWorks software and printed using a 3D stereolithography printer (Figure 19). The internal volume of the canister is approximately 5 mL, which is sufficient to house both the the cell culture scaffold as well as the input and output ports for the perfusion system. The final canister will be constructed from blown glass, which will be entirely transparent and autoclave-compatible.

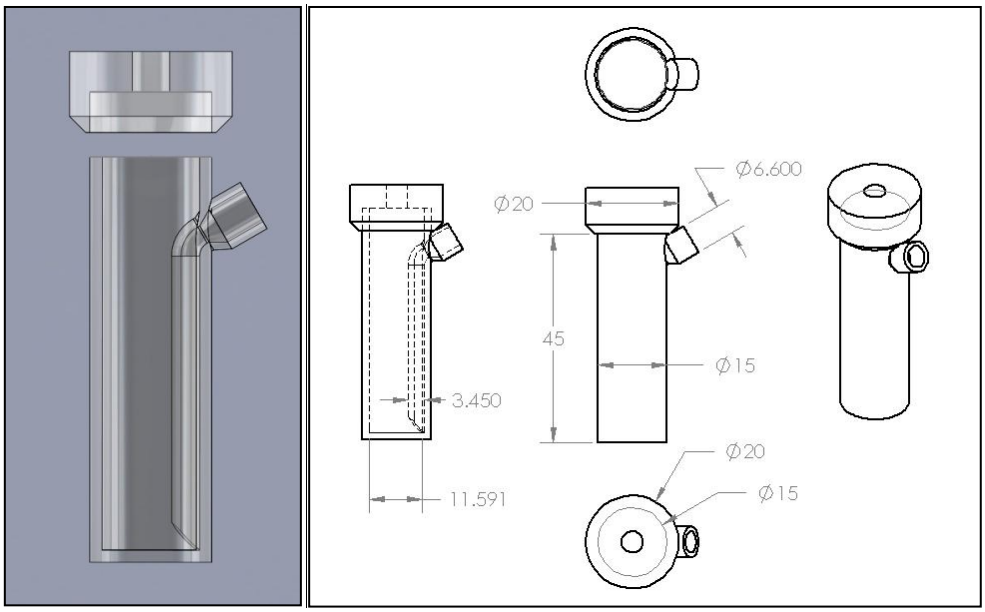

Figure 19: Bioreactor cartridge design (left) rendered in SolidWorks. Incoming media is pumped in through the side port. The hollow cylinder on the inside ensures that media is directed to the bottom of the cartridge for even dispersal. Waste fluids are directed out the hole in the center of the cap and subsequently transferred to the sensing containers to monitor cellular conditions. The schematic (right) shows cartridge dimensions in mm.

Ergonomics

The primary ergonomic concerns for the bioreactor involved initial setup, pyruvate injection, and intuitive use and maintenance. The components of the device are not too heavy as to inhibit or strain researchers during setup or when moving the device. Additionally, the bioreactor is easy to assemble; the tubing connections fit cleanly with their connecting parts. The system is simple to use and maintain during a study. Parameters such as temperature, media flow rate, DO, and pH can be regulated and sterilization of the final device will be possible. The data displayed in the LabVIEW program is easy to read and interpret. Finally, the injection ports, particularly for pyruvate, will be easily accessible and comfortable for the researcher, as the pyruvate must be injected within a 2-5 sec time period.

Ethical Concerns

Since our device will be used for testing techniques *in vitro* the primary ethical concern deals with the delivery of consistent and accurate data. The device will precisely

and accurately monitor cellular conditions and allow for maintenance to ensure the integrity of the studies performed. Additionally, to preserve the safety of the researchers, the device is not excessively heavy, unwieldy, or complicated to use.

Testing

Testing the final prototype design was centered on the accuracy and reliability of sensing along with the dispersion of fluid at injection ports and heat dissipation along the media lines and within the canister.

Flow Dispersion

To assess the dispersion dynamics within the cartridge, several tests were completed using both water and cell media. First, the cartridge was connected to the peristaltic pump and water was driven in to fill the entire cartridge and tubing connections. Next, water that was dyed with blue food coloring was pumped through the system at a rate of 0.29 mL/min. This rate is equivalent to the rate that would be used in formal studies. To prevent dispersion of the dye as it traveled through the tubing, a small air bubble was introduced between the blue and colorless water by pumping for few seconds with no water supply.

The cartridge used for this testing had an internal volume of 3.14 mL, which meant the pump would send an equivalent volume of water through the canister in 10 min and 50 sec. Our group observed the dye diffusion for 20 min to ensure we had sufficient data. A digital camera recorded the dispersion of dyed water as it was pumped into the canister.

In the first experiment, the input tube was placed slightly above the bottom of the cartridge, and after 20 min the dyed water had not completely dispersed throughout the entire volume of the cartridge (Figure 20A). The experiment was repeated with the tube inserted all the way to the bottom of the cartridge, which resulted in an even dispersion throughout the cartridge (Figure 20B). Finally, this experiment was repeated using dyed media, which, again, resulted in an even dispersion pattern (Figure 20C).

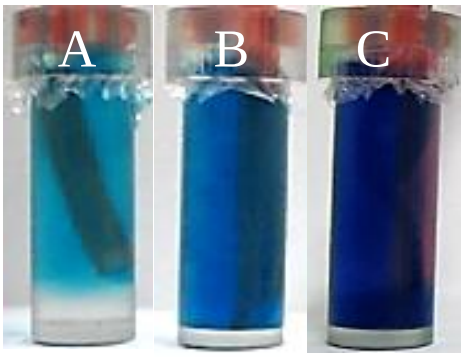

Figure 20: A: Incorrect placement of tubing leads to incomplete dispersion. B: Dispersion of dyed water 20 min after initiating pumping. C: Dispersion of dyed cell media 20 min after initiating pumping.

To quantify the visual data, still images were taken at one-minute intervals during dispersion trials and assessed with ImageJ®. Using the analysis tools in ImageJ® we determined the volume of dyed water and the mean amount of red, blue, and green pixels at each recorded minute. The results depicting the percentage of volume that contained dyed water or media over time are displayed in Figures 21 and 22. As is shown, both the water and media trials achieved complete replacement by their respectively dyed solutions in less than 20 min. This time frame is sufficient for completely replacing the media in order to maintain nutrient concentrations, remove cell waste, and maintain cell viability. The color composition at each time stamp can be found in Figure 23. The horizontal lines represent the standard color composition of the dyed water in the cartridge. The color composition at the beginning of the experiment contained an approximately equal number of red, blue and green pixels, indicative of the clear water that initially filled the cartridge. The color compositions asymptotically approached the standards in less than 20 min, which is further proof that full dispersion occurred.

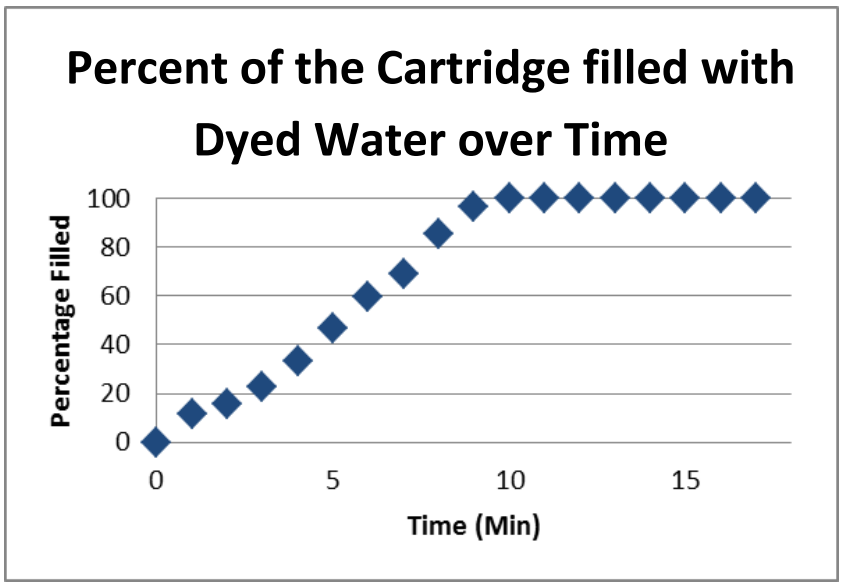

Figure 21: Percentage of cartridge volume that consisted of dyed water at each minute of the experiment.

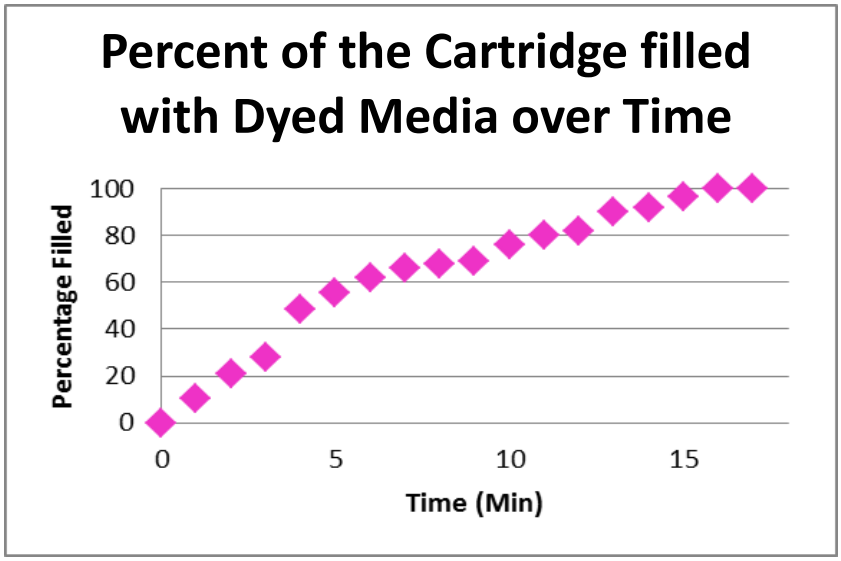

Figure 22: Percentage of cartridge volume that consisted of dyed media at each minute of the experiment.

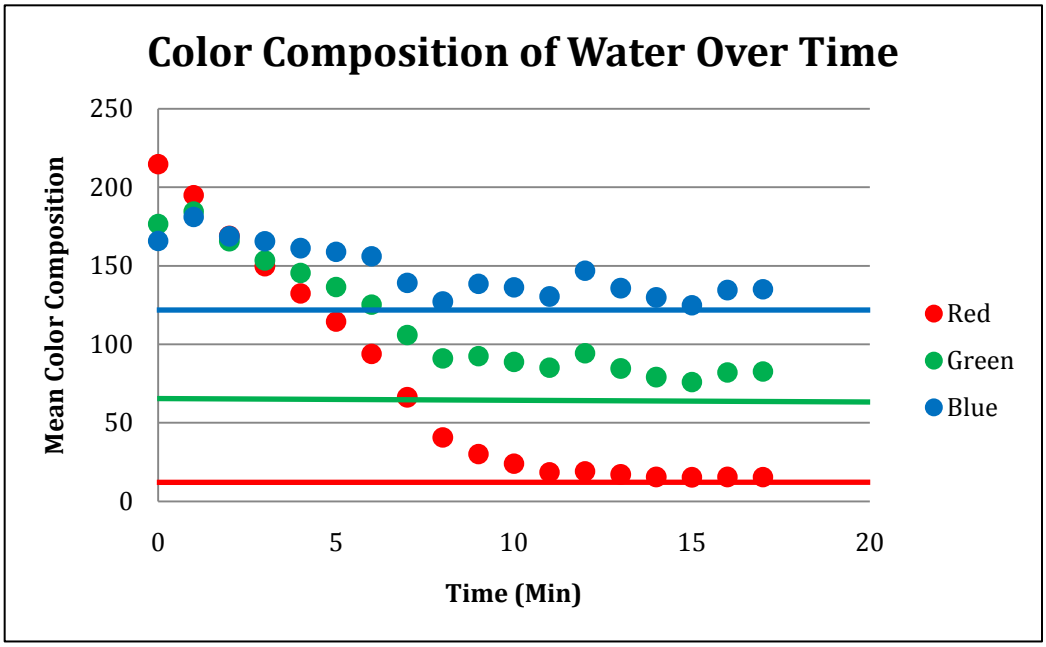

Figure 23: Color composition of dyed water over time. Horizontal lines represent standard solution of dyed water placed into cartridge.

A second set of experiments was conducted to observe the dispersion characteristics of injecting fluids. A syringe was used to manually inject dyed water or dyed media into the cartridge that had been prefilled with colorless respective liquid. In both experiments, complete dispersion was observed after 2 sec (Figure 24). This rapid dispersion will be critical for when hyperpolarized ^{13}C is injected so that the cells can be imaged before the signal decays too extensively.

Figure 24: The image on the left shows the dispersion of dyed water after injection into the cartridge. The image on the right shows the dispersion of dyed media after injection into the cartridge.

Probe Accuracy

We tested the accuracy of both the temperature and pH systems using comparisons with lab grade instruments. To analyze the pH electrode output we prepared 12 sample

solutions within a range of five to nine pH. Following preparation, we tested each sample solution using a lab-grade meter as well as our constructed system, and plotted the results in Figure 25. The linear trend-line fit to the data shows a highly linear response with an R^2 value of 0.99977, suggesting that the constructed system is extremely accurate in comparison with the lab-grade meter. Furthermore, average discrepancy between the constructed and lab grade systems was a mere 0.02 pH, much smaller than the 0.1 pH resolution desired by the client.

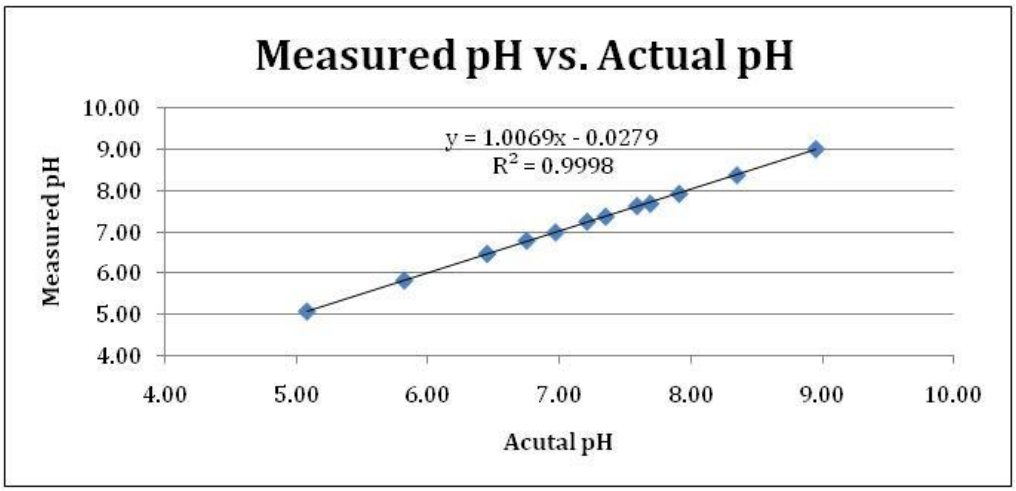

Figure 25: pH solutions were made by adding HCl or NaOH to a 7.00 pH buffer. The solutions were read using a laboratory pH meter (actual) and the pH probe with corresponding circuitry and LabVIEW program (measured).

To conduct temperature testing we placed both our designed system and a lab-grade digital thermometer in a beaker of water heated to 60 °C. As the beaker cooled, we recorded the measured temperature from both systems at approximately one degree intervals down to room temperature. Plotting the measured values from our system against the digital thermometer readings produced a linear response with an R^2 value of 0.9998 (Figure 26). Much like we observed with the pH system, the temperature sensing system adheres to the actual values accurately, with an average difference of 0.3 °C.

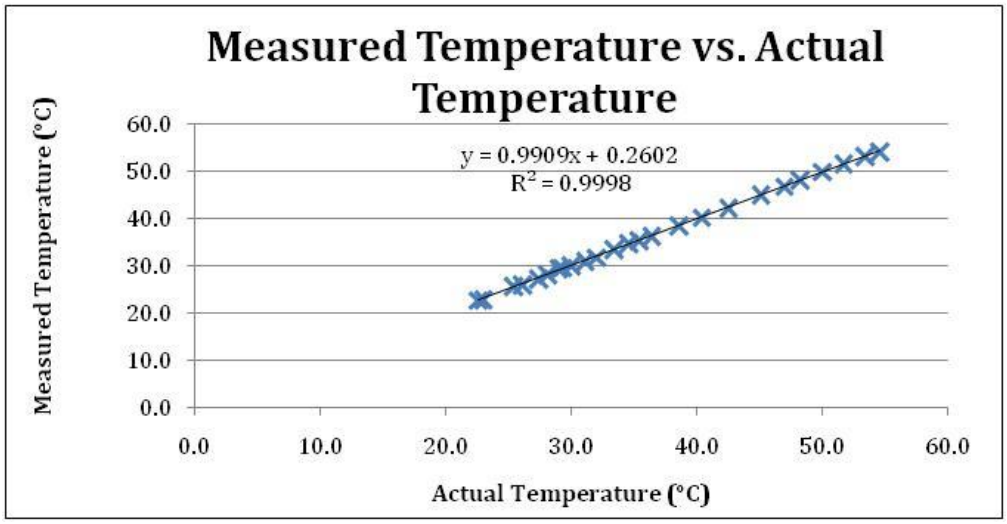

Figure 26: A beaker of water was heated to 60 °C using a hot plate and allowed to cool to room temperature. The solutions were read using a digital thermometer (actual) and the temperature probe with corresponding circuitry and LabVIEW program (measured).

Accuracy testing with the dissolved oxygen system was not conducted because we were not able to obtain testing standards and a comparative instrument. This test will be conducted in the future when these items become available.

MR Compatibility

We performed an MR compatibility test to verify that our design will not interfere with data acquisition during experimentation using the MRI scanner. We filled the bioreactor cartridge with water, connected the tubing, inserted it into an MR coil, and placed this apparatus in the MR bore for scanning (Figure 27). A gradient-echo recalled pulse sequence, which concentrates the MR signal, was used for imaging. To compensate for the electron spin dephasing that occurs as a result of the gradients, these gradients are only switched on intermittently in an MR pulse sequence. This ensures that when the echo occurs the electron spins are in phase and yield a signal. The polarity of the gradient field is then inverted to obtain the recorded MR signal[16].

Images were obtained in both the axial (longitudinal axis) and coronal (transverse axis) planes of the cartridge. Scans were taken while the peristaltic pump was off, and then again while it was on. The resulting images are shown in Figure 28. Digital analysis was used to determine the values of the signal means and standard deviations of noise. Signal-to-noise ratios (SNR) were found by dividing the mean of the signal by the standard

deviation of the noise. The SNRs for the axial plane were 23.1 and 23.6 while those for the coronal plane were 21.2 and 25.0, for when the pump was off and on, respectively[17]. Only having two values for comparison for each plane prevented statistical analysis, but our client stated that the observed differences are not significant enough to impact accurate data collection and analysis. Flow artifacts were seen due to the pulsation of the pump, but these can be suppressed during data collection by synchronizing the acquisition to the peristaltic pulses, as is currently done for *in vivo* scans to compensate for blood flow[18]. Therefore, it can be concluded that our device is sufficiently MR compatible.

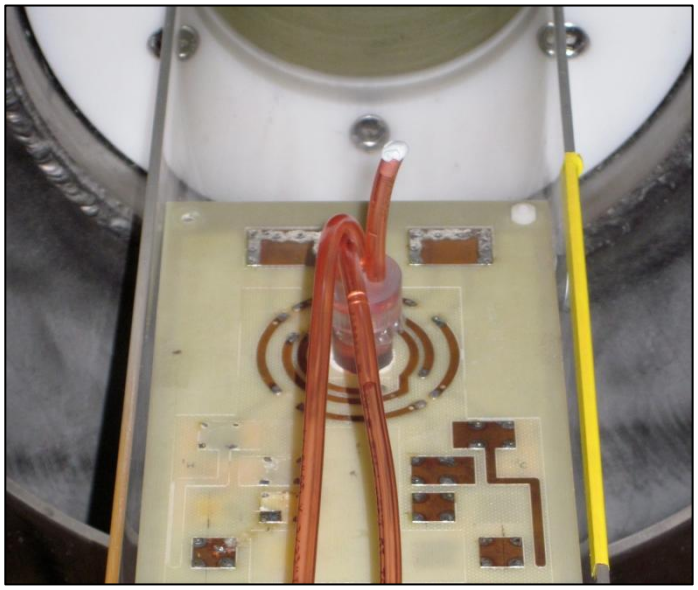

Figure 23: Bioreactor cartridge filled with water and fitted into MR coil as it is being moved into MR bore for testing.

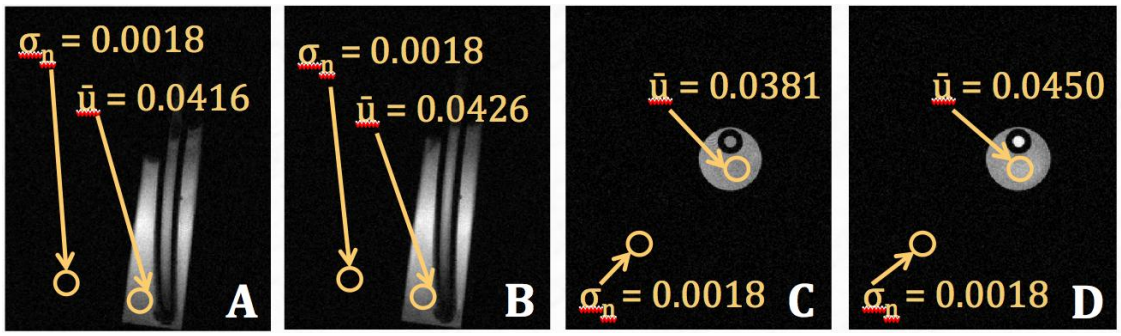

Figure 24: A: Axial slice with pump off. SNR = 23.1. B: Axial slice with pump on. SNR = 23.6. C: Coronal slice with pump off. SNR = 21.2. D: Coronal slice with pump on. SNR = 25.0. Measurements indicate the pump does not cause significant SNR degradation during MR acquisition. Flow artifacts do appear, but this phenomenon is frequently dealt with *in vivo*. Abbreviations - ū: mean of signal, σ_n: standard deviation of noise, SNR: signal-to-noise ratio.

Heat Dissipation

Our final test measured the amount of heat that dissipated from the system between the pump and cartridge. The purpose of this assessment was to determine if only heating the media would be adequate enough to deliver 37.0 ± 1.0 °C fluid to the cells in the cartridge. We heated a water bath to 38.0 °C and then pumped this water through the bioreactor system. After 15 ft of tubing the measured temperature was 26.8 °C, a 11.2 °C drop. When this tubing was inserted into the cartridge and placed in the MR bore, the temperature was measured to be 18.4 °C, a total of 19.6 °C lower than the heated bath. The additional decrease in temperature seen in the scanner was due to the fact that the inside of the bore is significantly cooler than room temperature. Although the media could be heated to a higher temperature so that the heat loss yielded a temperature of 37.0 °C by the time it reached the cells, the proteins in the media would become denatured and therefore ineffective. As a result, insulated tubing and a warm air blower for the cartridge will be implemented in addition to heating the media, as described in the future work.

Cost Analysis

The prices of materials purchased for this project are summarized in Table 3. The expenditures totaled $1,091.57, only about a third of the proposed budget of $3,000. One of the main reasons for this was that the items that were 3D-printed at the Wisconsin Institutes for Discovery were free of charge. If these components had been made through other means they would have likely cost at least a few hundred dollars.

Table 3: Itemized list of costs. The total amount spent was less than the project budget of $3,000. Symbols – ‘: courtesy of the UW-Madison Bioinstrumentation Lab, ‘’: courtesy of the Wisconsin Institutes for Discovery.

Item	Cost
Peristaltic Pump	$267.81
pH Probe	$42.00
DO Probe	$369.15
BNC Breakout Board	$4.99
Breadboard	$18.53
Power Supply	$94.90
Banana to Banana Cables	$9.90
Resistors, operational amplifiers, wires	$0.00’
DAQ Box	$169.00
3D-Printed Cartridges and Sensing Containers	$0.00’’
Tubing	$71.54
Poster	$43.75
Total	**$1,091.57**

Time Management

Our predictions and actual accomplishments for the semester were slightly different. Figure 29 displays the Gantt chart created at the beginning of the semester, while Figure 30 shows what our group achieved. The biggest difference between the two charts is the amount of time allotted to designing and fabricating the prototype. Receiving our initial 3D-printed cartridge earlier than expected allowed for testing to be completed sooner, and eventually lead to modifications of the designed cartridge. Additionally, the fabrication and integration of the pH and DO and temperature probe were extremely time consuming due to several unforeseen complications. However, these components were still completed before the presentation deadline. The surplus of client meetings was needed to effectively coordinate with our client’s other design team, which is creating the cell scaffold for our device. Despite the various delays, efficient time management allowed us to complete the required components and produce a working prototype before the end of the semester.

Tasks	January		February			March					April				
	21	28	4	11	18	25	4	11	18	25	1	8	15	22	29
Meetings															
Advisor															
Client															
Team															
Product Development															
Research															
Brainstorming															
Design Matrix															
Design Prototype															
Fabricate Prototype															
Testing															
Deliverables															
Progress Report															
PDS															
Mid Semester PPT															
Mid Semester Report															
Final Report															
Final Poster															
Website Updates															

Figure 29: Gantt chart created at the beginning of the semester.

Tasks	January		February			March					April				
	21	28	4	11	18	25	4	11	18	25	1	8	15	22	29
Meetings															
Advisor															
Client															
Team															
Product Development															
Research															
Brainstorming															
Design Matrix															
Design Prototype															
Fabricate Prototype															
Testing															
Deliverables															
Progress Report															
PDS															
Mid Semester PPT															
Mid Semester Report															
Final Report															
Final Poster															
Website Updates															

Figure 30: Gantt chart detailing what was accomplished each week over the course of the semester

Future Work

In the future our group would like to confirm accuracy of the DO probe against oxygen standards, as well as assess system biocompatibility with the cell culture.

Furthermore, we would like to create a Faraday cage for the sensing components to ensure that the magnet from the MR and other electronic equipment does not interfere with accurate monitoring of cellular conditions and vice versa. We would also like to have the cartridge made of blown glass as shown in Figure 31. This would ensure that it can be autoclaved, has an air-tight seal when fitted with a rubber stopper, and is MR compatible. We would also like to incorporate a filter into the canister to ensure that the micro-carriers of the tissue scaffold do not get pumped out of the cartridge during perfusion.

Figure 31: A rubber stopper like the one of the left enables the creation an air-tight seal when fitted into the cartridge. The hole in the center of the stopper will be used for the output tubing[19]. A glass cartridge like the one of the right will be MR compatible and can be autoclaved. The rubber stopper will fit into the top hole. The side port will be used for the input tubing[20].

Since a significant amount of heat is lost as media flows through the tubing, we would like to add insulation to the tubing between the pump and canister. Additionally, integrating an air blowing mechanism to warm the canister while it is in the MR bore will be beneficial. This will hopefully eliminate heat dissipation as the media is pumped to the canister and allow maintenance of the proper temperature.

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Appendix

Product Design Specifications

Product Design Specifications – May 4th, 2011
MRI-Compatible Bioreactor for Cancer Cells (MRI Bioreactor)

Members
Jeff Hlinka – Team Leader
Samantha Paulsen – Communicator
John Byce – BSAC
Sarah Reichert – BWIG

Advisor
Dr. Brenda Ogle

Function

The purpose of staging cancer is to describe the severity and extent of the malignancy. Stage is one of the most important factors that oncologists consider when determining treatment plans and prognoses for their patients. There is a potential to use information about the metabolic state of cancer cells to characterize their stage. One way to follow this metabolism noninvasively is to implement magnetic resonance imaging (MRI) to track hyperpolarized carbon-13 labeled pyruvate as the cells break it down. We aspire to create a bioreactor that will permit a cell scaffold containing malignant cells to be imaged by an MRI scanner. This system will be supplemented with equipment capable of flowing the necessary gases and substances through it for testing.

Client Requirements

- Device needs to house tissue scaffolding and other components needed to sustain cell viability.
- Device must have sensors to monitor dissolved oxygen (DO), pH, temperature, and other conditions to maintain an optimal environment for the cells.
- Device must have the ability to inject substances into the cellular environment and ensure even dispersal.
- Device must be MRI compatible.

Design Requirements

1. **Physical and Operational Characteristics**
   a. *Performance Requirements:* The product must be able to house, monitor, and deliver nutrients to a tissue scaffold and all the cells maintained within it.
   b. *Safety:* The product cannot cause any harm to the operators, MRI components, or data acquisition.

c. *Accuracy and Reliability:* Any substances injected into the cellular environment must be dispersed evenly at an accurate rate (between 0.25 and 4.00 mL/min). Device must maintain a cellular environment of 37.0 ± 1.0 °C, 7.4 ± 0.1 pH, and 0-20% DO.
d. *Life in Service:* The device will be used throughout the time period required to determine if monitoring cell metabolism is a viable method for staging cancer. This is expected to be around two years.
e. *Shelf Life:* The device should be able to maintain a shelf life of approximately two years for storage between experiments.
f. *Operating Environment:* The expected environment for use is at standard room temperature (20 °C) and pressure (100 kPa), in an isolated MRI scanner.
g. *Ergonomics:* The device must be easily sterilized and operate without continuous user supervision. The operator should be able to inject substances in 2-5 seconds.
h. *Size:* Any components not entering the MRI scanner can be of any size, however, the portion that houses the cellular environment must be less than 10.8 cm in diameter.
i. *Weight:* Weight is negligible for any components outside of the MRI scanner. Components within the scanner should be no more than 2.0 kg.
j. *Materials:* The device should be made of materials that are MRI compatible, non-ferromagnetic, and either easily sterilized or disposable. The canister that houses the cellular environment cannot be cytotoxic.
k. *Aesthetics, Appearance, and Finish:* For this project the client emphasized functionality over appearance and therefore this category is not applicable to our design.

2. Production Characteristics

a. *Quantity:* One device is required.
b. *Target Product Cost:* The entire device should not exceed the cost of a commercially available bioreactor, which is typically around $3,000.

3. Miscellaneous

a. *Standards and Specifications:* There are no federal regulations that need to be met for this device; however, basic cell culture guidelines must be followed.
b. *Customer:* The final product is intended for use by our client; however, it has the potential to be integrated into other research protocols involving imaging of *in vitro* cell cultures.
c. *Patient-related Concerns:* The device is intended for use on *in vitro* cell cultures and not actual patients. However, it must be able to maintain cell viability throughout its use.
d. *Competition:* FiberCell® Systems currently makes a hollow fiber bioreactor system with a horizontal cartridge. No known testing for MRI compatibility has been performed. Additionally, the system lacks sensing components and all together costs around $3,000.