MRI-Compatible Bioreactor for Cancer Cells

March 9th, 2010

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Abstract

Current research in the field of medical physics has offered insight into differences in metabolic rates between benign and cancerous cells *in vitro* using pyruvate tagged with a carbon-13 isotope (^{13}C). The presence of hyperpolarized ^{13}C allows researchers to track pyruvate and its metabolic products using magnetic resonance imaging (MRI). This research has significant potential to improve cancer staging techniques along with current treatment planning and prognosis methods. To test this technique, our client requires a bioreactor to house, maintain, and monitor high density cell cultures throughout the testing process. This bioreactor must be compatible with the horizontal bore, small animal MRI the lab currently uses for research. Additionally, the device requires a pumping mechanism to continuously distribute media and nutrients to the cells, a monitoring system to maintain ideal physiological conditions, a heater to maintain a constant temperature of 37 °C, and injection ports for gases and other substances. Currently available bioreactors do not provide the required sensing system and are often not compatible with a horizontal MRI scanner. Our group first addressed the pumping and monitoring mechanisms, and we will be creating the final design components to be integrated into the bioreactor system. Our group will use a peristaltic pump along with pH and pO_2 electrodes and manually constructed circuitry as the pumping and monitoring systems. Throughout the remainder of the semester our group will design and implement heating and injection mechanisms, then finish by testing our design.

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 using the technique of hyperpolarization to track pyruvate metabolism in cancerous cells (Figure 1). Hyperpolarization uses radiofrequency pulses to flip the atomic magnetization of carbon atoms into the transverse plane, which allows the atoms to be detected using magnetic resonance (MR) scanning[1]. This technique is necessary because 98.9% of naturally occurring carbon isotopes are carbon-12 (^{12}C), which have a neutral spin and are thus invisible to MR[1]. Hyperpolarization increases the occurrence of ^{13}C by more than ten thousand fold and allows researchers to track the metabolic breakdown of pyruvate using MR[1].

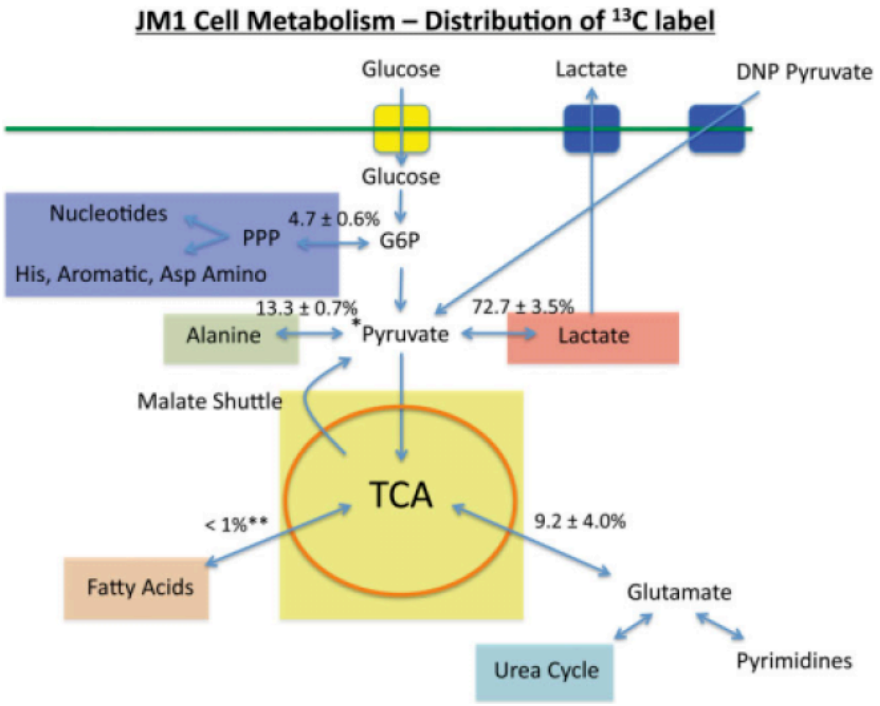

Figure 1: Map of pyruvate metabolism and final metabolites in JM1 cells. The metabolic rate within the pyruvate pathway differs between healthy and cancerous cells. Therefore, tracking pyruvate metabolism has the potential to help clinicians differentiate between benign and malignant cells *in vivo*[2].

Once metabolic substrates have been hyperpolarized, the research technicians quickly administer the ^{13}C-tagged pyruvate into a cell culture to track its metabolic breakdown using a 4.7 T micro MRI scanner, as shown in Figure 2. The horizontal bore of the scanner is incompatible with many currently available bioreactors and imposes some restrictions on our design by limiting the size and materials of the cell culture vessel.

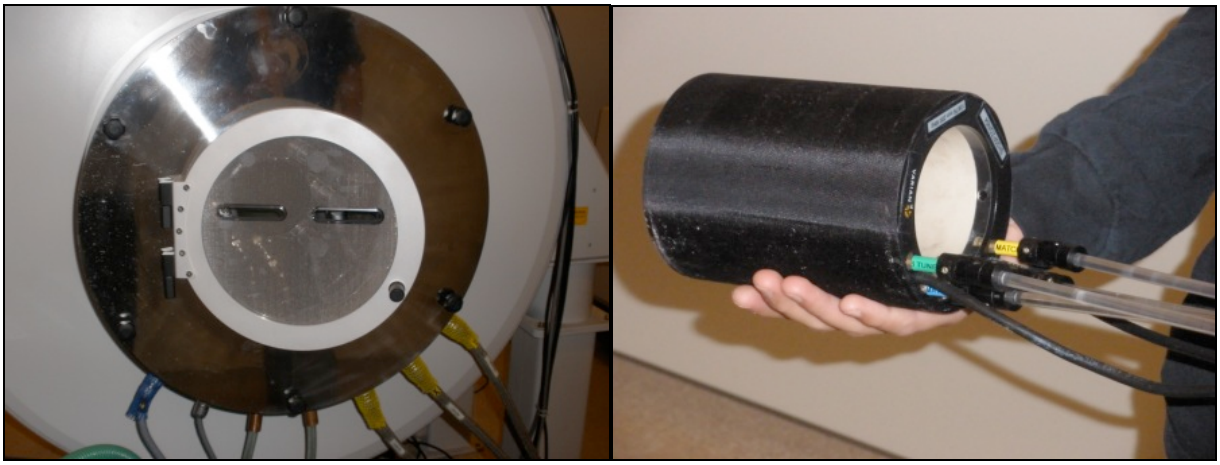

Figure 2: Horizontal bore micro MRI scanner (left), with associated MR coil (right) used in pyruvate metabolism research. The horizontal configuration of the scanner imposes restrictions on bioreactor designs[3].

Usage of this technique for *in vivo* cancer studies could help clinicians determine the extent and severity of a malignancy and aid in 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 maintain ideal physiological conditions, a heater to maintain an ideal temperature, and injection ports for gases and other substances. In summary, this device should be compatible with a horizontal bore MRI scanner and support high density cell cultures. Currently available bioreactors do not have the desired sensing mechanisms, are expensive, and are typically not compatible with MRI devices containing a horizontal scanning bore. To begin this project, our group focused on designing the sensing system and pumping mechanism.

Current Devices

Although some devices have been designed for similar applications in academic

research, their major shortcomings include vertical bioreactor orientations and lack of integrated sensing components. A horizontal bioreactor system exists commercially, but it also lacks the ability to monitor cellular conditions and its cost exceeds the budget for this project.

Harris et al. Bioreactor

Harris and coauthors utilized the bioreactor shown as a schematic in Figure 3 for their research with hyperpolarized ^{13}C and human breast cancer cells. In experimentation, cells are grown on microcarrier beads contained within the bioreactor cartridge and 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 is unable to maintain flow during data acquisition, so the bioreactor must be disconnected prior to injection and remain this way until scanning is completed. This results in a 2-4 min gap in which nutrients and wastes are not pumped into or out of the cartridge. This can destabilize cellular conditions and reduce accuracy of results[4].

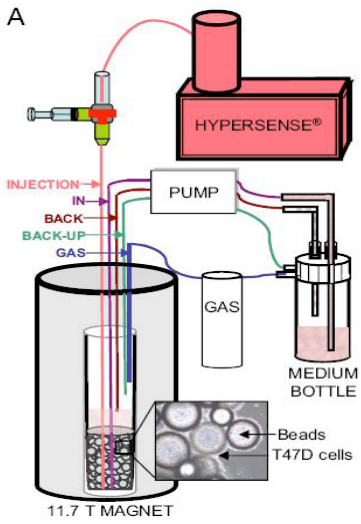

Figure 3: The bioreactor system used by Harris et al. allows for the injection of hyperpolarized ^{13}C as well as circulated flow of fluids and gases. However, it has a vertical cartridge orientation, lacks sensing components, and is unable to pump substances during data collection[4].

Keshari et al. Bioreactor

Keshari and collaborators monitored metabolites in JM1 rat heptoma cells through

hyperpolarized ^{13}C spectroscopy. They implemented 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[5]. Although this system allows for the control of several factors that influence cellular conditions, there is no method for monitoring or verifying these conditions.

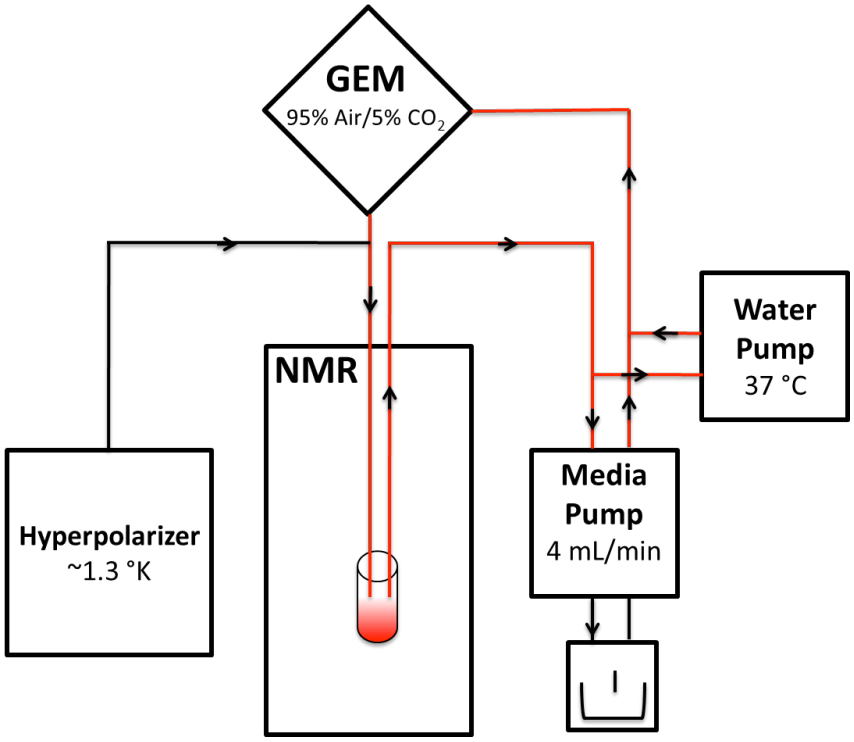

Figure 4: The bioreactor system used by Keshari et al. allows for the control of gas exchange, temperature, and flow rate. It also enables hyperpolarized ^{13}C to be delivered to the cell culture. Similar to the Harris et al. bioreactor, its orientation and inability to measure cellular conditions pose major hindrances for its use in this project. GEM: gas exchange modulator. NMR: nuclear magnetic resonance[5].

FiberCell Systems Bioreactor

The bioreactor system developed by FiberCell Systems 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[6]. 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 cell conditions.

MRI scanning is not listed as an intended application for this system, so testing would need to be done to ensure that the materials are non-ferromagnetic, radiolucent and do not hinder data acquisition[6].

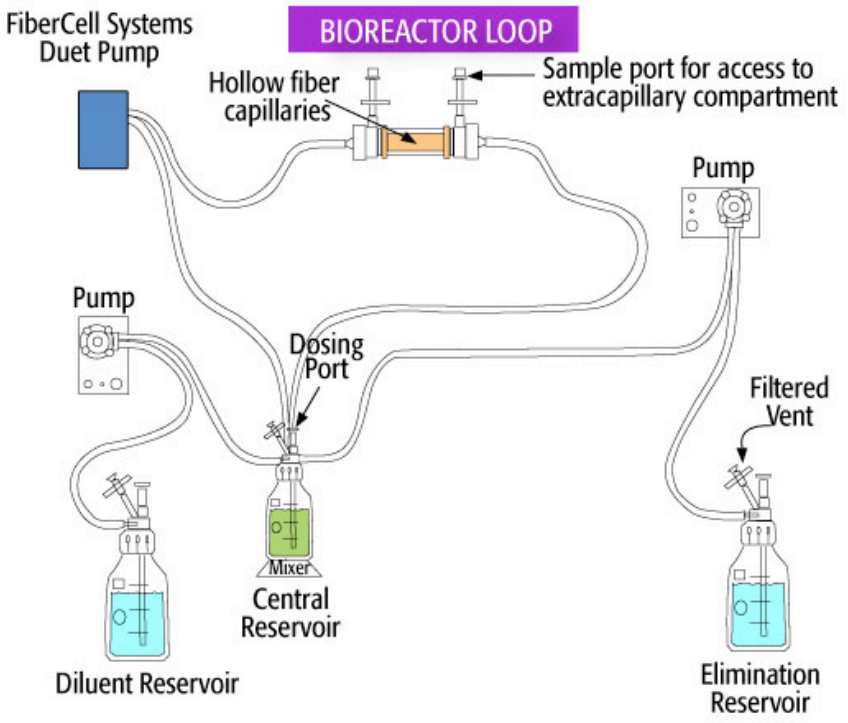

Figure 5: The FiberCell Systems bioreactor is a commercially available system that has the proper cartridge orientation and the ability to support continuous and variable flow of substances. Unfortunately, its cost would entirely consume the funds for the project and prevent the purchase of sensors to monitor conditions[6].

Design Requirements

In developing any product, it is first necessary to establish client needs and product requirements. The Product Design Specifications document reviews these requirements 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. The device must also remove metabolic waste products from the cellular environment. Furthermore, this system must have integrated sensing components to allow technicians to monitor pO_2, pCO_2, pH, and temperature and adjust them if necessary. As MRI 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 MRI components and other testing devices. Substances injected into the device must be evenly and accurately dispersed to the cells at a rate of 240 mL/hr. The device must be capable of maintaining a 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 s. Although the sizes of components that will remain outside of the MRI 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. 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 kg for any part entering the MRI scanner. Functionality is emphasized over aesthetics, so although the device is not required to have a commercial appearance, components must be MRI compatible, which entails that it is made of non-ferromagnetic and radiolucent materials. Additionally, components must be easy to sterilize or dispose of and not 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 pO_2, pCO_2, 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[7]. The device also comes equipped with a data acquisition interface, which allows researchers to collect and store data using a laptop computer.

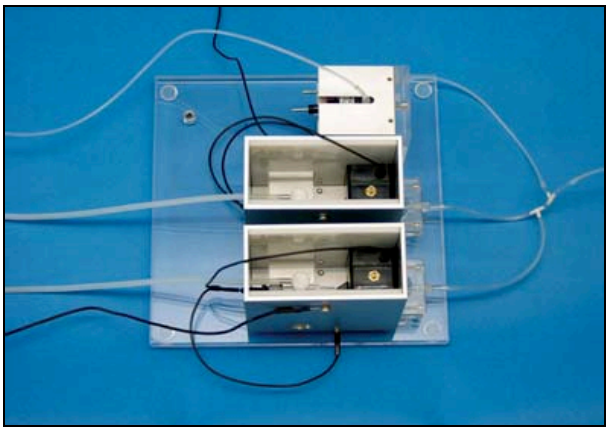

Figure 6: Harvard Apparatus flow through monitor senses pH, pO_2, pCO_2, and temperature. The device can be completely autoclaved and comes with a data acquisition interface, which makes it convenient and easy to use. However, the price is outside of our proposed budget[7].

To integrate the system into the bioreactor design, we would simply 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 s[7]. 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[7].

Mass Flow Controller

The flow through monitor, 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[8]. 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[8].

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

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[9].

The 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[8]. 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[8]. 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 pO_2 sensing. 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 pO_2, are constructed from an electrochemical cell with a glass covered platinum cathode and silver-silver chloride anode[10]. 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[10]. The amount of current produced in the electrode is thus proportional to the pO_2, which allows sensing to be conducted with proper calibration. The pO_2 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[11]. Furthermore, this device can be sterilized with an ethanol wash and is constructed of glass and epoxy, both of which are non-cytotoxic[11].

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[10]. 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[12]. This sensor has a pH range of 0 to 14, an accuracy of ± 0.01, and a resolution of 0.01[12].

Figure 8: Oakton WD-E5642-50 pO_2 probe[10] (left) and Oakton WD-35801-00 pH probe[11] (right). These electrodes sense pO_2 and pH, respectively, and must be interfaced with a digital output source to convert data to relevant values.

Pumping Systems

Our bioreactor device 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 bioreactor 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)[13]. 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 pulsatile pumping mechanism has a low shear stress in flow and would support a continuous, closed loop perfusion system. Although the rate is predetermined and unadjustible, 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[13]. Diaphragm pumps are readily available through BioChem Fluidics and cost $318.25[13]. The most relevant model for the bioreactor perfusion system is the 130SP1250-ITP.

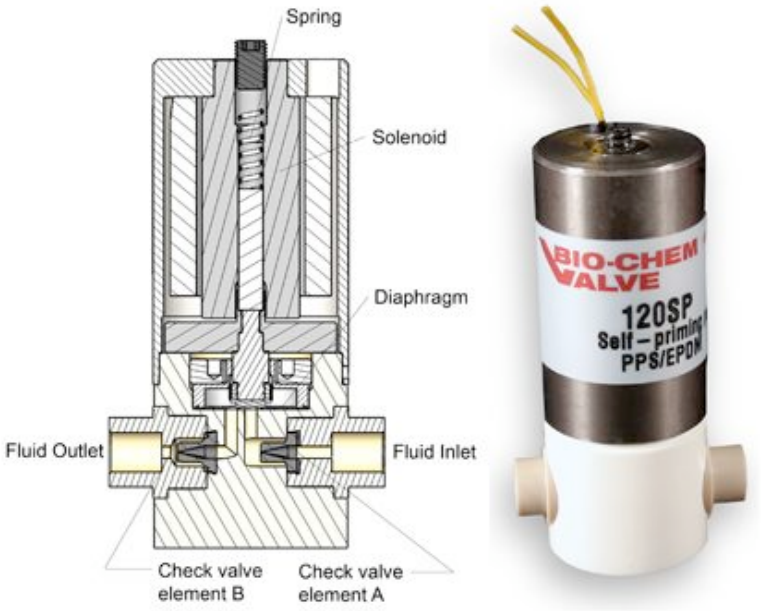

Figure 9: Diaphragm micropump from BioChem Fluidics. This particular model has a 360 mL/hr flow rate[13].

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[14]. 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). Typical operating temperature

and humidity values are a range of at least 0-40 °C and 0-90%, respectively[15].

A major advantage of these pumps in regards to sterility is that fluids or gases only come into contact with 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[14]. 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[15].

Figure 10: Peristaltic pump with multiple rollers to direct flow. Having multiple rollers reduces the pulsations created to increase the device's functionality[14].

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[14].

Duet Pump

The duet pump 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[6]. Users can vary 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[6].

The tubing, usually made of silicone, can be run through the pump and thus sterility is independent of pump components. However, the pump casing can be wiped with ethanol if sterility beyond autoclaving the tubing is desired. 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[6]. The prominent advantage of the duet pump is its ability to provide a continuous flow, which results from the combined pumping mechanism[6].

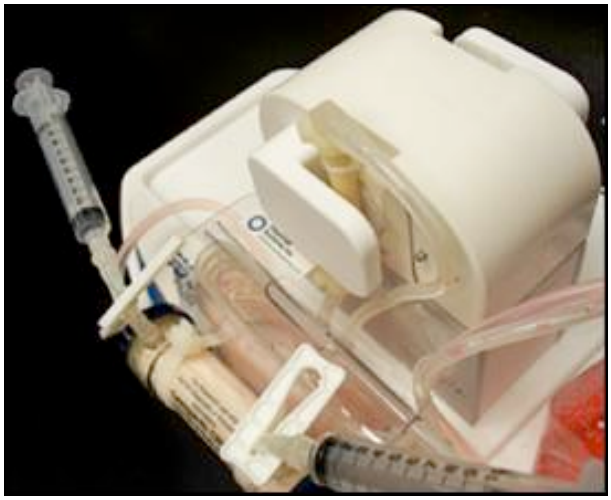

Figure 12: Duet pump used with hollow fiber bioreactors. This positive pressure displacement pump squeezes the inserted tubing, while using integrated one-way check valves to determine the direction of flow[6].

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. Flow rates of these devices range from 0.001 µL to 8,400 mL/hr and are easily programmable[16]. Additionally, since syringes with these devices can be easily replaced, cytotoxicity is not a major concern. One downfall to integrating these devices into the

bioreactor is their cost, which can be as much as $3,500[16]. 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.

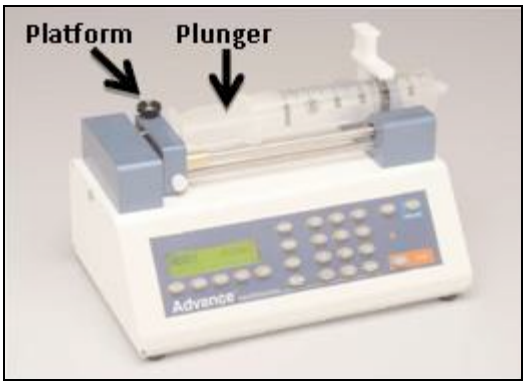

Figure 13: Advance Fusion Series 1200 from SouthPointe Surgical outlining the platform and plunger[16].

Design Matrices and Evaluation of Design Alternatives

To determine which design alternatives are 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 design matrices provide a quantitative analysis of how well each design alternative adheres 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 will be 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 will be 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 will be 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 allocated 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 is 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

Although not as important as previous categories, the feasibility of integrating each sensing system into a horizontal bore bioreactor system may affect the overall functionality of the prototype and 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 thus 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 allotted10 points because, although significant, it is less of a determining design characteristic. The peristaltic and duet system received 9 points, as their flow rates are adjusted with a simple knob and the devices are powered by a single switch. 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

The 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 model the pumping action of body systems. 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

Using the design matrices, we decided to use electrodes for the sensing system and a peristaltic pump for the pumping mechanism. These two components would be integrated into the system seen in Figure 14. The central bioreactor cartridge will be a commercially produced cylindrical canister, which contains both space for the the cell culture scaffold and input/output ports for the perfusion and sensing systems. The peristaltic pump will be

connected to both a cell culture media tank and the bioreactor canister, forming a closed loop perfusion system. As shown below, 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. All of the tubing used within the design will be medical grade silicone, as it is both biocompatable and non-cytotoxic.

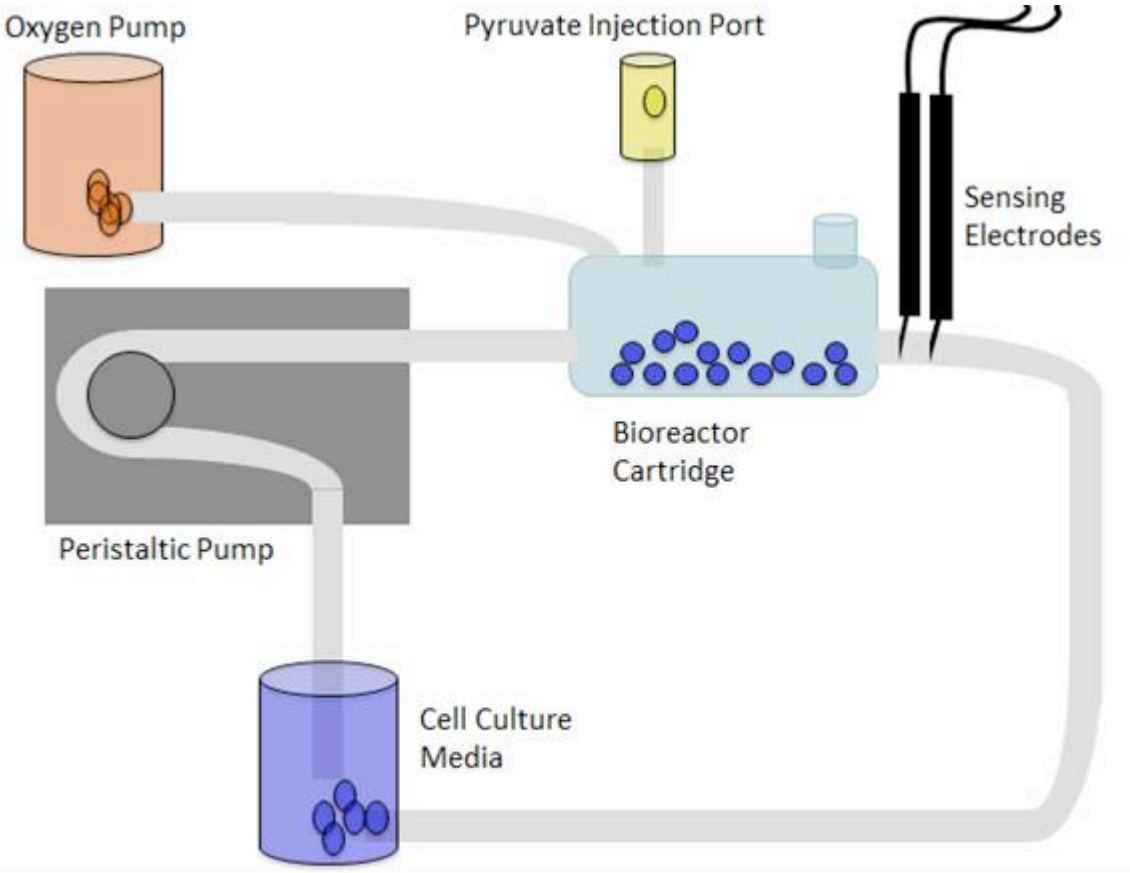

Figure 14: Layout of bioreactor in horizontal configuration. Components include oxygen pump, pyruvate injection port, sensing electrodes, peristaltic pump, cell culture media, and bioreactor cartridge.

Ergonomics

The primary ergonomic concerns for the bioreactor involve initial setup, pyruvate and gas injection, and intuitive use and maintenance. The components of the device should not be too heavy as to inhibit or strain researchers during setup or when moving the device. Additionally, the bioreactor should be easy to assemble. This means tubing

connections should be clearly labeled and should fit cleanly with their connecting parts. The bioreactor should also be easy to use and maintain during a study. This includes regulating parameters such as temperature, media flow rate, and pH along with regular sterilization of the device. Though adjustment of flow rates and temperature depends on the purchased equipment, our group must ensure that researchers can easily disassemble and sterilize bioreactor components. Furthermore, when designing the data acquisition system, our group should make certain that the data display is easy to read and interpret. Finally, the injection ports, particularly for pyruvate, should be easily accessible and comfortable for the researcher as the pyruvate must be injected within a 2-5s 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 should precisely and accurately maintain cellular conditions to ensure the integrity of the study at hand. Additionally, to preserve the safety of the researchers, the device should not be excessively heavy, unwieldy, or complicated to use.

Future Work

Testing

Testing the final design will be centered on the accuracy and reliability of sensing along with the dispersion of fluid at injection ports. In order to analyze sensing accuracy, we will compare the output of our sensing system with other available sensing methods using standard solutions of known pH. Some possible modes of comparative measure include litmus paper and additional pH electrodes available in campus labs. The diffusion characteristic of injection ports will be tested with simple observation. Dispersion will be observed by incorporating a dye into a mock injection fluid prior to insertion into the bioreactor canister.

Cost Analysis

The total projected cost of our prototype, approximately $1,200, is sufficiently lower than the client's proposed budget ($3,000). The majority of the projected costs are exhausted by the pumping and sensing systems alone. The peristaltic pumping mechanism has a cost of approximately $375 [15]. Electrode probe sensors have a slightly lower cost of approximately $325, but also require additional circuit components and a data acquisition server[11,12]. Other significant costs are outlined in Table 3.

Table 3: Projected costs for materials needed to assemble bioreactor.

Item	Cost
Electrodes	$310.00
Electrode Circuitry	$125.00
Peristaltic Pump	$350.00
Bioreactor Cartridge	$250.00
Tubing and Connectors	$100.00
Total	$1,135.00

Fabrication

Fabrication of our final design will include the assembly and integration of individual design components, construction of electrode circuitry, and the incorporation of a digital interface such as LabVIEW. Assembly and integration of individual design components consists of the simple attachment of silicone tubing between each element to form a closed loop perfusion system. Electrode circuitry will be constructed using common resistors, capacitors, and operational amplifiers, and will be directly interfaced into LabVIEW.

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Appendix

Product Design Specifications

Product Design Specifications – March 9th, 2011
MRI-Compatible Bioreactor for Cancer Cells (MRI Bioreactor)

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

Advisor
Professor 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 pO_2, pCO_2, 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 (approximately 240 mL/hr). Device must maintain a cellular environment of 37.0 ± 1.0 °C.
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 client is expecting to utilize this device as soon as it is completed so shelf life is not applicable.
f. *Operating Environment:* The expected environment for use is at standard room temperature and pressure, 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 materials 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.