MRI-Compatible Bioreactor for Cancer Cells

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Abstract

Our client, Dr. Sean Fain, is researching the variation 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 enables the breakdown of pyruvate to be tracked using magnetic resonance (MR). Our client asked us to design and fabricate a bioreactor to maintain and monitor high density cell cultures to aid in his research. Our final design incorporates a peristaltic pump, monitoring electrode probes with corresponding circuitry, and a cartridge to house the cells. The pump evenly disperses media through the culture cartridge in less than 11 min. The probes demonstrated R^2 values of 0.99977 (pH) and 0.9998 (temperature) when compared to corresponding laboratory instruments. Signal-to-noise ratio analysis confirmed that the system is compatible with an MR scanner. Water temperature dropped by 11.2 °C while traveling through the tubing and an additional 8.4 °C due to the cooler temperatures in the MR scanner bore. As a result, heated media, insulated tubing, and a hot air blower for the cartridge must be incorporated into the future design.

Introduction

- **Client: Dr. Sean Fain, UW-Madison & UW School of Medicine and Public Health**
  - Departments: Medical Physics, Biomedical Engineering, Radiology
  - Research: Differences in metabolism between healthy and cancerous cells
    - Technique: Track breakdown of hyperpolarized ^{13}C-tagged pyruvate with MR
  - Project proposal: bioreactor that can house and monitor high density cell culture
    - Additionally: compatible with MR scanner for data collection

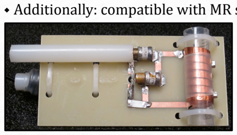

Figure 1: MR Solenoid Coil.
The bioreactor cartridge will be inserted into a solenoid similar to the one on the right side of the image. The outputs on the left allow the collected data to be transmitted to a computer for analysis.

Figure 2: Micro MR Scanner.
This scanner has a magnetic strength of 4.7 T. Items are placed in the central horizontal bore for scanning.

Figure 3: HyperSense® Hyperpolarization System.
Hyperpolarization is used to flip the atomic magnetization of carbon atoms. This technique increases the signal detected with MR scanning by ~10,000 fold. Researchers can track the metabolic breakdown of pyruvate with incorporated hyperpolarized ^{13}C using MR[1].

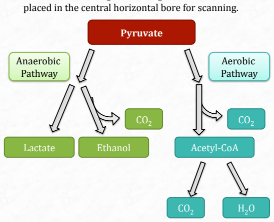

Figure 4: Metabolism of Pyruvate[2].
Since cancerous cells metabolize pyruvate more than benign cells, tracking pyruvate breakdown *in vivo* could help clinicians determine the extent and severity of a malignancy. This would aid in treatment planning and prognosis. Our bioreactor will be implemented in further *in vitro* analysis of pyruvate metabolism.

Design Criteria

- House tissue scaffolding and cell culture
- Monitor pH, dissolved oxygen, and temperature of cellular environment
  - Respective desired values for normal conditions: 7.4 ± 0.1, 0-20%, 37.0 ± 1.0 °C
- Pump media, oxygen, and other substances through bioreactor system
  - Desired rate: 0.25-4.00 mL/min
- Avoid interference with MR scanning and data acquisition
- Allow for injection of hyperpolarized ^{13}C
- Enable sterilization of tubing and cartridge

Testing

Cartridge Dispersion

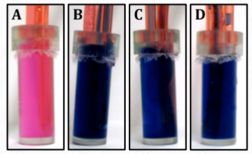

Figure 5: Images from Dispersion Tests.
A: Cartridge filled with media. **B**: Dyed media dispersion 20 min after initiating pumping. **C**: Dyed media dispersion 5 sec after simulating injection. **D**: Dyed media poured directly into cartridge for comparison.

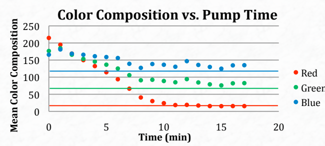

Figure 6: Rate of Fluid Dispersion in Bioreactor Cartridge.
Water was dyed blue with food coloring and pumped through the cartridge at a rate of 0.29 mL/min. A video was taken of the test and timestamps at every minute were analyzed with ImageJ. Solid lines indicate values of the standard for comparison. Sufficient dispersion was accomplished in less than 11 min. This rate meets the design criteria.

MR Compatibility

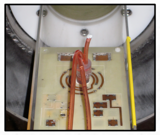

Figure 11: Test Setup.
The cartridge was filled with water, inserted into an MR coil, and placed inside the bore. A simple gradient-echo recalled pulse sequence was used to obtain images in both the axial plane (longitudinal axis) and coronal plane (transverse axis). Data was collected while the pump was off and again while it was on.

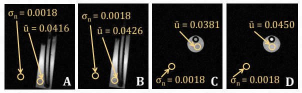

Figure 12: MR Images and SNR Calculations[3].
A: Axial slice with pump off. SNR = 23.1. **B:** Axial slice with pump on. SNR = 23.6. **C:** Coronal slice with pump off. SNR = 21.2. **D:** Coronal slice with pump on. SNR = 25.0. Measurements indicate the pump does not cause significant SNR degradation 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.

Probe Accuracy

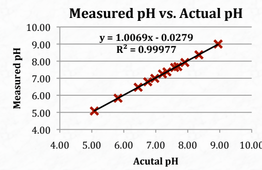

Figure 7: Accuracy of pH Probe.
pH solutions were made by adding HCl or NaOH to a 7.00 pH buffer. The solutions were read using a laboratory pH meter (actual) and the pH probe with corresponding circuitry and LabVIEW program (measured).

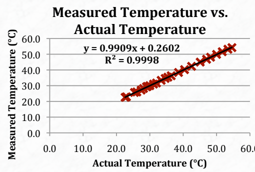

Figure 8: Accuracy of Temperature Probe.
A beaker of water was heated using a hot plate. The solutions were read using a digital thermometer (actual) and the temperature probe with corresponding circuitry and LabVIEW program (measured).

Heat Dissipation

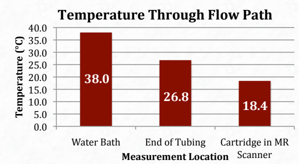

Figure 13: Heat Dissipation Through Flow Path.
Water was heated to 38.0 °C in a water bath. After being pumped through 15 ft of tubing the temperature dropped to 26.8 °C. When the temperature was measured in the cartridge while it was in the MR scanner, it dropped even lower to 18.4 °C.

- Test Setup
  - Water heated to 38.0 °C in water bath
  - Heated water pumped through the bioreactor system
  - Temperature measurements taken at indicated locations
- Results
  - Water bath to end of 15 ft of tubing: 11.2 °C drop
  - Water bath (through 15 ft of tubing) to cartridge inside MR scanner: 19.6 °C drop

Final Design

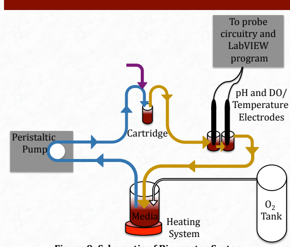

Figure 9: Schematic of Bioreactor System.
Components and direction of flow for integrated system. Blue lines indicate active pumping. The purple arrow represents the injection port. Yellow lines indicate passive flow. The white arrow is for the active pumping of O_2 into the system.

Components:

- Peristaltic pump: maintains media flow
- Sensing system: monitors pH, DO, and temperature conditions
- Cartridge: houses cell culture and scaffold
- Injection port: enables efficient injection of hyperpolarized ^{13}C

Accomplishments:

- Confirmed pH and temperature probe accuracy
- Verified MR compatibility
- Even dispersal of media in cartridge

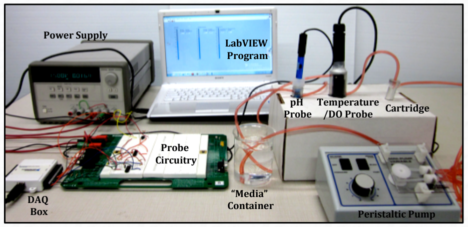

Figure 10: Integrated Bioreactor System Prototype.
The peristaltic pump drives the media in a closed loop through the cartridge and sensing containers with the probes. The output of the probes is transmitted and manipulated by the circuitry and then fed into the LabVIEW program for display. Abbreviations – DO: dissolved oxygen, DAQ: data acquisition.

Future Work

- Testing
  - Confirm DO probe accuracy
  - Confirm biocompatibility with cell culture
- System enhancements
  - Create Faraday cage for sensing components
  - Blown glass cartridge with rubber stopper
  - Cartridge filter for microspheres
- Reduce heat dissipation
  - Heat media container
  - Insulate tubing
  - Hot air blower for cartridge

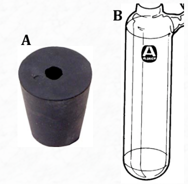

Figure 14: Examples of a Rubber Stopper (A)[4] and Glass Cartridge (B)[5].
A: Rubber enables the stopper to create an air-tight seal when fitted into the cartridge. The hole in the center of the stopper will be used for the output tubing. **B:** Glass is MR compatible and can be autoclaved. The rubber stopper will fit into the top hole. The side port will be used for the input tubing.

Acknowledgements

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References

1. Rowland, I. J., Peterson, E. T., Gordon, J. W., & Fain, S. B. "Hyperpolarized ^{13}Carbon MRI." Curr Pharm Biotechno 11 (2010): 709-19.
2. Nelson, D. L., Lehninger, A. L., & Cox, M. M. *Lehninger Principles of Biochemistry*. New York: W.H. Freeman, 2008.
3. Images and data courtesy of Matt Smith, Department of Medical Physics, UW School of Medicine and Public Health.
4. *Home Science Tools*. (2011). Retrieved April 26, 2011, from Home Training Tools, Ltd. website: http://www.hometrainingtools.com/rubber-stopper--2-1-hole/p/CE-STOP02A/
5. *Reaction and Storage Tube with Glass Stopcock on Side-Arm*. (2011). Retrieved April 26, 2011, from Sigma Aldrich Co website: http://www.sigmaaldrich.com/catalog/ProductDetail.do?lang=en&N4=Z173126|ALDRICH&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPECC