Automation of a Sensor Driven Continuous Flow Bioreactor for Cartilage Tissue Engineering

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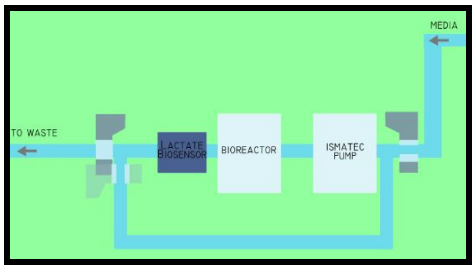
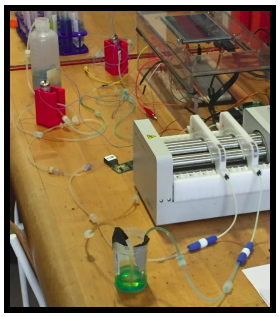
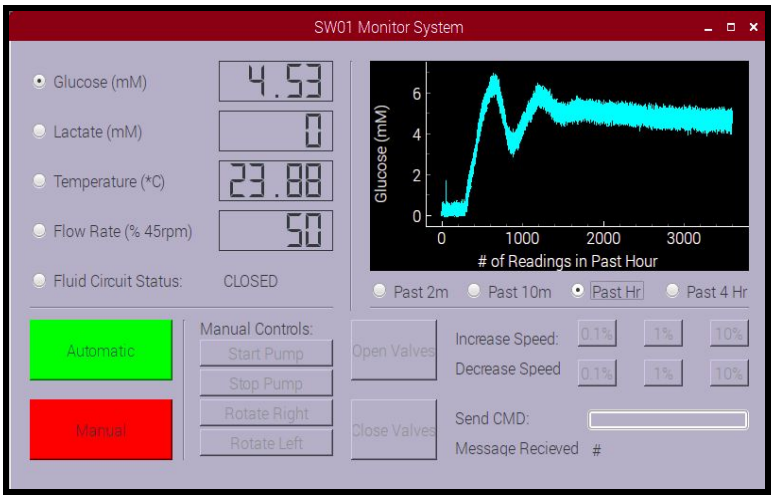
Problem Description: One of the major research initiatives undertaken by scientists at the iBEST-Biomedical zone is the *in vitro* growth of cartilage tissue for nasal reconstruction, resurfacing joints, or replacing ear/trachea tissue. One such researcher, Dr. Stephen Waldman, focuses on cartilage tissue engineering using environmentally controlled growth vessels referred to as "bioreactors" by seeding cartilage cells from a developed and living source. Dr. Waldman and other Canadian researchers [1] are looking towards studying glucose and lactate concentrations in bioreactor media solutions to better understand the growth of cartilage cells. Glucose serves as the cell culture's energy source, and will need to be held at desirable levels for ideal culture growth. [1] Due to cartilage tissue metabolism, lactate will build up in the cell culture fluid as per the tissue's natural respiration process. The buildup of lactate can be detrimental to the cell culture growth in large amounts. [1] One way to avoid the buildup of unwanted lactate would be to have a continuous flow of culture media running from the bioreactor influent glucose source to the effluent waste pathway. This process is effective at maintaining ideal environmental conditions, but is wasteful, and may be an expensive waste of cell culture media for the experimenter. Our tissue engineering project serves to prevent the waste of viable cell culture media, while also providing scientists with an easily understood method of monitoring *in vitro* concentrations of glucose and lactate.





Design Solution: A simple fluid circuit (right) was created to direct cell culture media to and from a given bioreactor vessel. Three solenoid valves were used to close the media tubing forming either an open or closed fluid circuit. The open fluid circuit pumps fluid from a culture media source, through the bioreactor, and then towards waste collection. The closed fluid circuit sends effluent culture fluid back to the pump for recycling purposes, eliminating unnecessary waste.

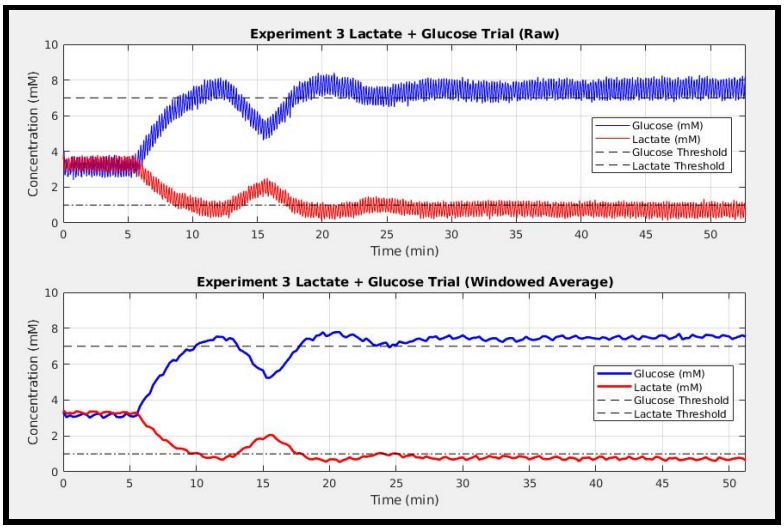
The B.LV5 biosensor by Innovative Sensor Technologies-AG was used to measure glucose and lactate concentrations. The sensor was connected to the effluent pathway of the bioreactor, and returned environmental information every one second to the system's single board computer. The single board computer in question is the Raspberry Pi 3B+: a popular computational device for undergraduate students due to its inexpensive cost, and extensive list of features. The computer system was responsible for controlling pump speed, as well as changing the fluid circuit status based on sensor readings and the user's input regarding ideal minimum glucose/maximum lactate concentrations. The sensor, pump, and valve conditions were recorded by the computer software into a non-volatile .csv format for later analyses through whichever software the user wishes to use. All live information was displayed on a basic graphical user interface hosted by a mounted 7 inch touchscreen (left, data shows a simple glucose-only experiment, no P control). The computer program also allowed for manual control of the system by the user through the touch screen.



The pump used in this project was the IPC-16 peristaltic pump by Ismatec®. The pump output flow rate is automated by the computer system. Flow rate was moderated through proportional control with respect to the error margin between the bioreactors set glucose/lactate values and the actual environment measurements. Simple speed, start/stop, and rotation direction commands could be given to the pump with buttons featured on the system touchscreen, but all additional pump commands could be given by connecting a keyboard to the computer.

The automated computer program runs immediately from startup. The computer program was created entirely with free and open source API toolkits in the python programming language. Programming toolkits include PyQT5, PyQTGraph, and Sci-Py's NumPy.

Application: A simple experiment using known concentrations of sodium bicarbonate buffered glucose (10mM source concentration) and lactate solutions was conducted to test the efficacy of the system. Undesirable concentrations of glucose and lactate (3.3mM of each) were placed into the bioreactor at the start of the experiment. The program was set to have a maximum concentration of 1 mM lactate, and a minimum concentration of 7 mM glucose. The automated program was then ran, and was to receive no additional assistance from the user.



The results of the experiment (right) show that the system was successfully able to reach and then hold specified values for both glucose and lactate. The system held 7 mM of glucose as instructed (blue in plot), instead of flooding the system with its 10 mM of source glucose. No more than 1 mM of lactate (red in plot) was held within the bioreactor. The fluid within the bioreactor was then left in recycle mode. The plot above was created in MATLAB using the .csv files exported by the Raspberry Pi after the experiment ended.

Implementation: While successful at balancing glucose and lactate concentrations which can be used as indicators for cartilage tissue growth, there exists additional necessary installations before our system can be used in real cartilage tissue experiments. The previous experiment serves as a proof of theory only, and is not yet ready for live cultures. For starters, our system does not incorporate filters for the cell culture debris that will inevitably be produced by the cartilage tissue sample during its growth cycles. This cellular debris may render the sensor ineffective if not dealt with appropriately. There is also much more than just glucose and lactate when it comes to environmental control within a cartilage tissue bioreactor. Dissolved gasses, pH, and available amino acids, are all major components that would also need to be considered in a full-scale project.

If we were to continue to improve upon this project, we would begin by accommodating the factors mentioned. It may also prove useful to experiment with some simple glucose-lactate fermenting bacteria to test the system's legitimacy.

References: [1] - S. Hossain, D. J. Bergstrom, and X. B. Chen, "Modelling and simulation of the chondrocyte cell growth, glucose consumption and lactate production within a porous tissue scaffold inside a perfusion bioreactor," *Biotechnology Reports*, vol. 5, pp. 55–62, Dec. 2014.