Langendorff Apparatus for Guinea Pig Cardiomyocyte Isolation

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UW BME Design

ABSTRACT

- Guinea pig primary cardiomyocytes (CMs) are used as a powerful tool to study heart function as a complement to in vivo animal models
- Primary CMs isolated via the Langendorff technique are more physiologically relevant both structurally and functionally to the living organism compared to other techniques[1]
- The purpose of the Langendorff apparatus is not to directly isolate the cells, but rather prepare the cardiomyocytes for functional isolation

BACKGROUND

Motivation

- With this device, the isolation of functional cardiomyocytes is made possible.
- Cardiomyocyte research can lead to invaluable advancements in drug delivery mechanisms and further knowledge of heart functionality[2]

Research

- The Langendorff technique has been used for over a century on various rodent hearts, from Guinea pigs to mice[3]
- Homeostasis throughout the cardiovascular system maintains circulatory pressures and flow

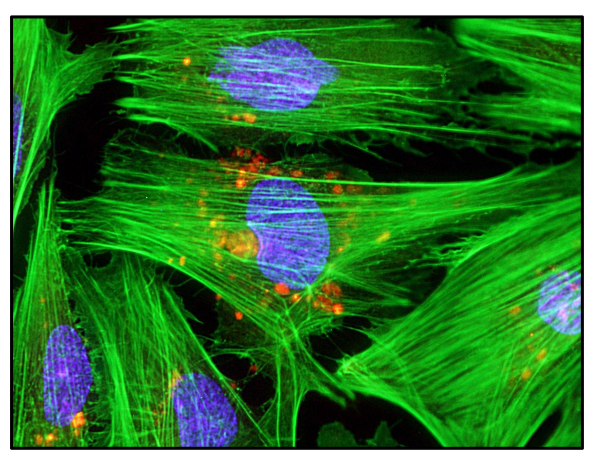


Figure 1: *Cardiomyocytes[5]*

- The Langendorff apparatus utilizes retrograde perfusion to prepare cardiomyocytes for functional isolation[1]
- Commercially available Langendorff systems offer many complex options, allowing the user to vary parameters such as temperature, method of perfusion, perfusion rate, and oxygenation of perfusate solutions with high precision.

DESIGN CRITERIA

Client Requirements

- System maintains either constant flow of 8.6 ±3.6mL/min or constant pressure of 60-80 mmHg[4]
- Perfusate solutions are 37°C upon reaching cannulated heart
- Device operates without significant error for a minimum of 10 minute
- Within budget of $200

Accuracy Requirements

- Temperature: accurate within 37°C ± 0.5°C
- Constant Flow: accurate within ±0.5 mL/min

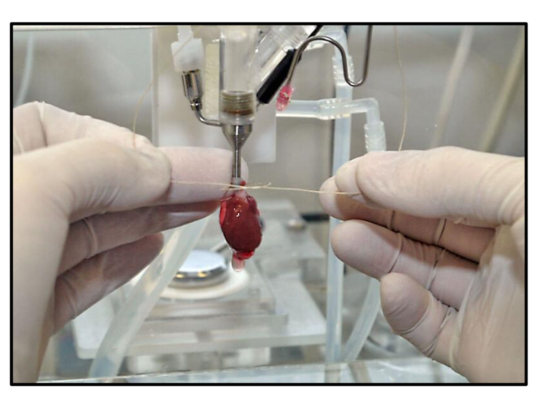
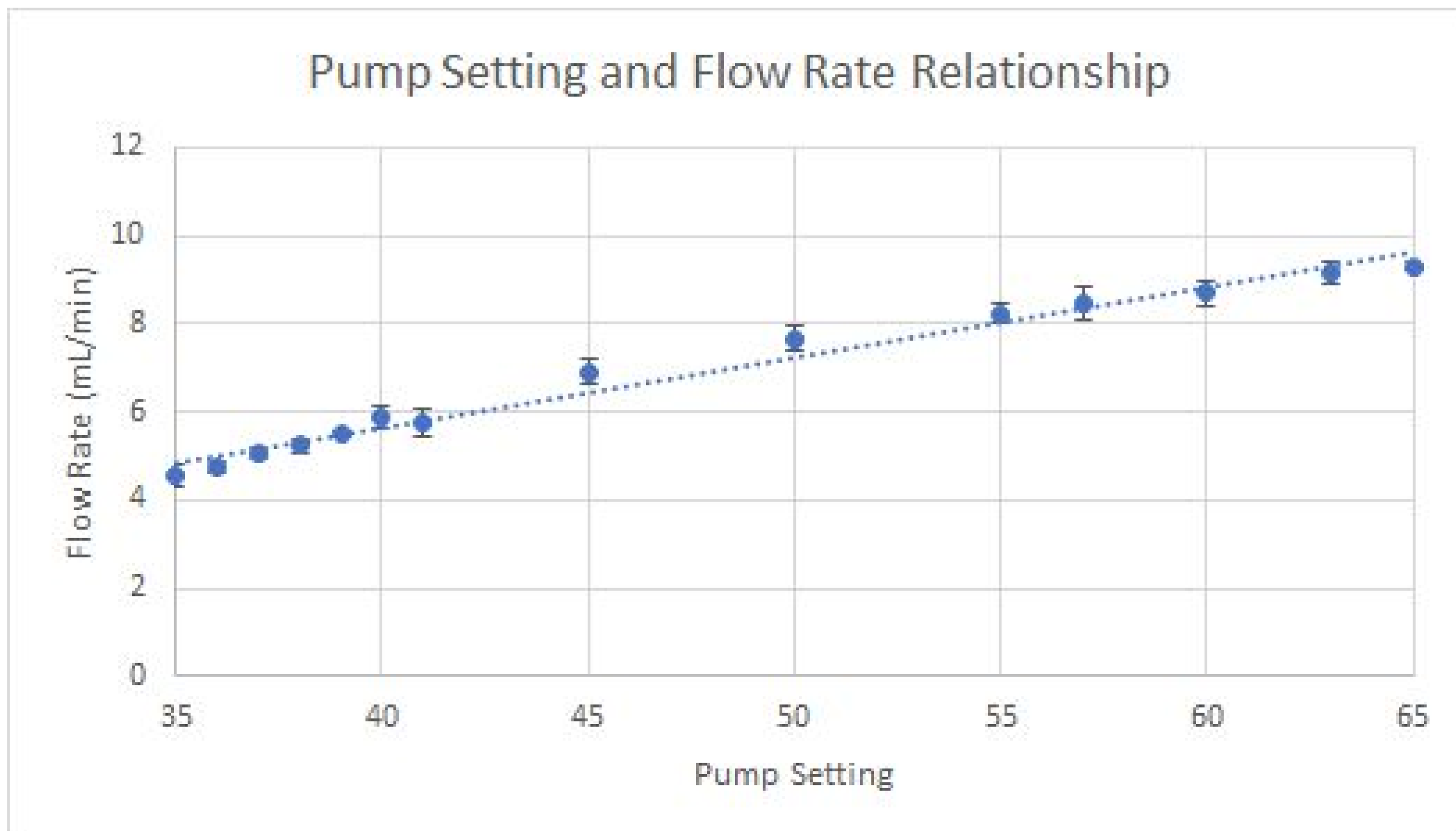


Figure 2. *Small Mammal Cannulated Heart[6]*

TESTING AND RESULTS

Flow Rate



$N_{pump} = FlowRate * 6.2814 + 4.53077$ $\quad R^2 = 0.9785$

Figure 3. *Numerical Pump setting correlation with flow rate, linear model of the measured flow rate and the corresponding pump setting equation, and R^2 statistic*

- Each of the pump settings shown was tested a total of three times and the time to fill 5mL was measured and the flow rate was calculated

Temperature

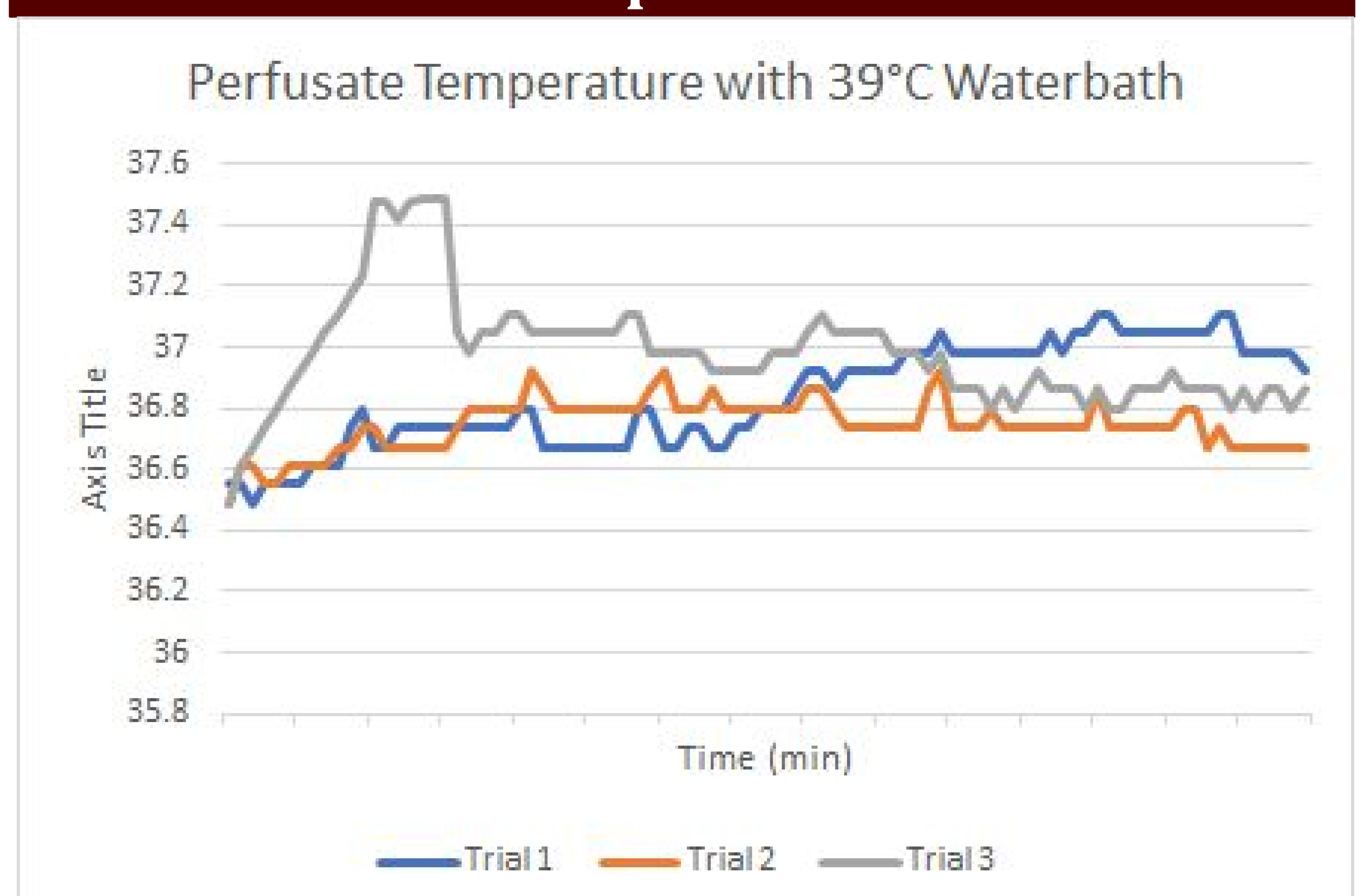


Figure 4. Temperature of perfusate measured over a 15 minute time period. Three different trials with a constant water bath temperature of 39°C

- In all three trials the variability of the temperature was within 37°C ± 0.5°C
- Measurements were taken from thermistor in bubble trap immediately before the cannula

FINAL DESIGN

- Multiple perfusate solutions are pumped through system by a peristaltic pump
- Water condenser warms perfusate solution as it travels from perfusate reservoir to cannulated heart
- Bubble trap connected at the bottom of the condenser restricts bubbles from entering cannula
- Temperature of perfusate is measured with a thermistor at nearest point to cannula.
- All tubing and bubble trap are chemically inert and biologically compatible.

Figure 5. *Final Langendorff Design*

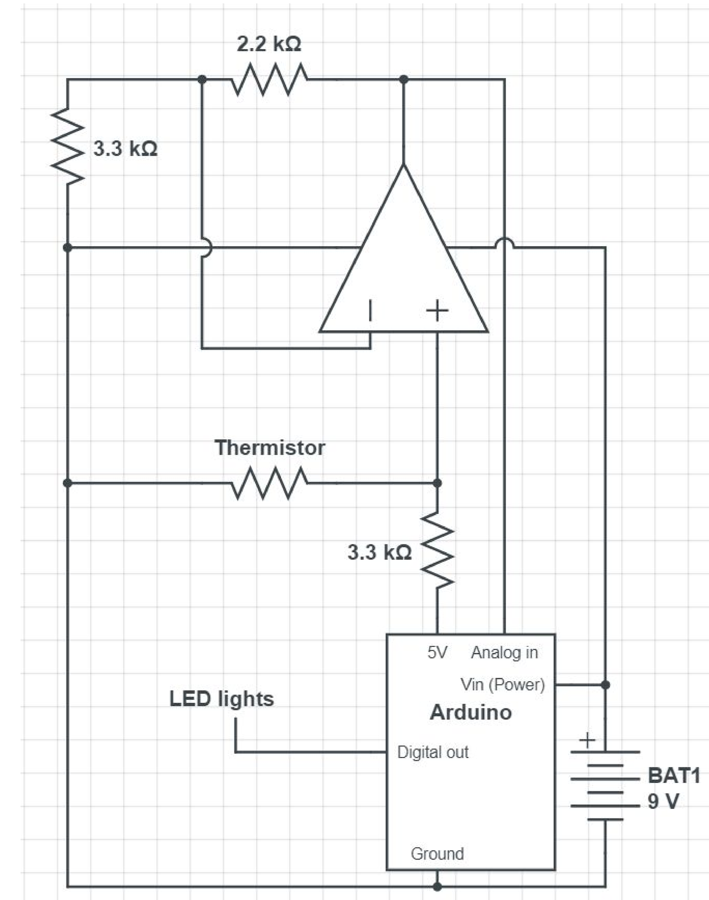


Figure 6. *Circuit Schematic*

FUTURE WORK

- Implement digital temperature display
- Compare viability of CMs against various flow rates
- Implement mechanism for oxygenation of perfusate solution
- Implement pressure transducer, to allow for monitoring of pressure
- Implement mechanism for recycling perfusate solution back to reservoir
- Commercialize the product for mass distribution

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[2] Robertson, G. (2013). Gail Robertson, PhD - Helping Hearts Keep the Beat. UW School of Medicine and Public Health
[3] Bell, R. Mocanu, M. Yellon, D. “Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion,” J. Mol. and Cell. Cardio., vol. 50, issue 6, pp. 940 - 950, June, 2011.
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[5] D. B. Cowan and J. D. McCully, *Micrograph of laboratory-grown rat heart muscle cells. Fluorescent labeling shows mitochondria (red), cytoskeleton (green), and nuclei (blue).* NIH.
[6] Isolated heart perfusion systems. Measuring Development Engineering. http://mdegmbh.eu/images/BIOLOGICAL_RESEARCH_ENG/Complete_systems/Isolated_heart_perfusion_systems/Compact/Compact_pump-controlled_heart_perfusion_system_10.jpg