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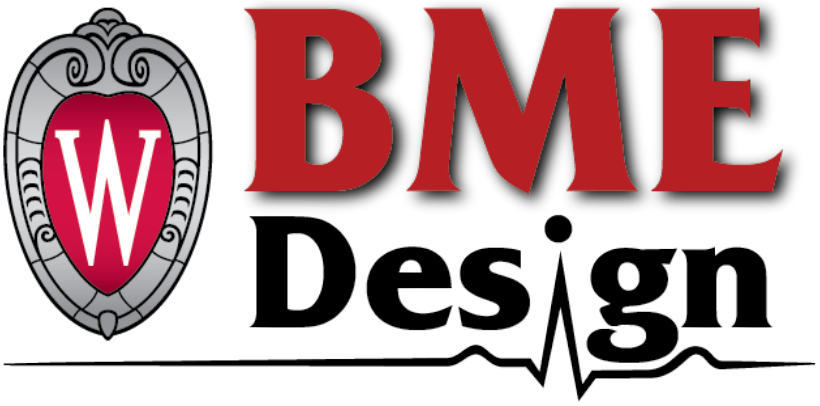

Langendorff Apparatus for Guinea Pig Cardiomyocyte Isolation

BME 200/300

12/12/2017

Client: Dr. Erick Ríos Pérez

Advisor: Dr. Wan-Ju Li

Team Members: Alyssa Walker (BSAC), Brittany Russell (Communicator) , Ethan York (Team Leader), Grace Restle (BWIG), Samuel Moss (BPAG)

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. The method to obtain these functional cells requires perfusion of the heart to preserve various ion channels of interest and retain the contractility function of the CMs. This project is aimed to design a Langendorff-like apparatus capable of retrograde perfusion with two solutions through the aorta of an excised Guinea pig heart. The purpose of the Langendorff apparatus is not to directly isolate the cells, but rather prepare the cardiomyocytes for functional isolation. The final design consists of a peristaltic pump to maintain a consistent flow through the coronary artery, a condenser utilized as water jacketed tubing to heat the perfusate, and a thermistor located in the bubble trap just before the cannula to ensure a final temperature of 37℃. The design is able to yield consistent results in maintaining the desired temperature and flow of the perfusate. Theoretically, this Langendorff-like apparatus should enable the researchers to isolate viable cardiomyocytes.

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I. Introduction

Motivation and Client Background

Our client, Dr. Erick Ríos Pérez, is a postdoctoral researcher in the Gail A. Robertson Lab at University of Wisconsin-Madison. The lab focuses on studying ion channels, such as the human Ether-à-go-go-Related Gene (hERG), and other elements of electrical excitability in nerve and heart cells. Dr. Robertson's lab has laid the foundation for tests on drugs that are now mandated by the Federal Drug Administration to ensure that these ion channels are not blocked[1]. Dr. Ríos Pérez's research is focused on further investigation of these channels in cardiomyocytes (CMs) through the use of a Guinea pig animal model. To perform these studies, primary cardiomyocytes must be isolated from the Guinea pig heart, while still retaining their contractility function, which ultimately prompts the need for a Langendorff-like apparatus.

The Langendorff Technique

While a variety of methods exist for obtaining populations of viable CMs, including the use of immortalized cell lines and differentiation via induced pluripotent stem cells, acutely isolated cells via the Langendorff technique are more physiologically relevant both structurally and functionally to the living organism [2]. Due to this technique's relevance in the study of heart physiology, there are many Langendorff apparatuses that are used today for academic and industry-based applications, all of which focus on preparing tissue for isolation through perfusion. Many literature explanations of the system exist, as the apparatus is commonly prepared on a per need basis. Existing consumer-ready products currently on the market cater to the largest client base through a robust system with expensive components which may be unnecessary depending on the specific application.

The Langendorff technique was originally developed by a German physician Dr. Oskar Langendorff nearly a century ago; his designs laid the groundwork for current systems, stemming from his work in isolation of a frog heart [3]. The basic function of a Langendorff apparatus is to keep consistent flow or pressure of a perfusate solution through an excised heart. This is done by cannulating the aorta; the aortic valve will close under pressure but the perfusate will flow through the coronary ostia. Perfusing the heart in this manner allows free drainage of the right atrium and the preparation can thus be done without any fluid filling the ventricular chambers [4]. These devices are used in biological application and often times must account for other factors such as maintaining constant temperature such that cardiomyocyte cell function is not compromised.

Problem Statement

For the purposes of isolating functional cardiomyocytes, we must design a Langendorff apparatus capable of retrogradely perfusing solutions through the aorta of an excised Guinea pig heart. Criteria on design include achieving a desired flow rate catering to that of a Guinea pig heart. If a constant flow method is chosen, the perfusate flow rate must be kept in the range of 8.6 ± 3.6 ml/min [5]. Additionally, perfused solutions must reach a physiological temperature of 37℃ before entering the heart in order to avoid cell stress and death. The cost of all materials necessary for constructing the apparatus must remain within a budget of $200, with the exception of the cost of a peristaltic pump and a few other materials supplied by the client (see Appendix II). Furthermore, the finalized construction of the apparatus must fit in the provided lab space.

II. Background

Relevant Published Research

The isolation of functional cardiomyocytes has been done for at least a century, becoming largely possible through the perfusion of a frog heart and subsequent isolation in the 1890's [4]. Functional isolation through the Langendorff technique can be catered to the study of specific model species, ranging from mice to Guinea pigs. Regardless of the chosen animal subject, general principles of this system remain constant across studies as discussed above [6].

Homeostasis throughout the cardiovascular system maintains circulatory pressures and flows that vary across lumen diameter; most pressing for our application are these characteristics in the Guinea pig heart [5]. Being a functional isolation with a retrograde perfusion, limiting further stresses require that near physiological conditions are maintained [2]. This in mind, any interaction with the heart should consider physiological parameters (blood temperature, arterial pressure, and blood flow rate for example).

A large sum of our preliminary research revolved around understanding how the research-based variations of the original Langendorff system adapted in the 1890's [4] were constructed based on individual criteria. Upon literature review, it has been identified that there are existing Langendorff apparatuses with specific application to Guinea pig models [7]. Building a Langendorff apparatus for an individual situation allows for a specialized system with simplicity that is not seen commercially. Furthermore, the simplistic nature of the design allows for expandability.

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. For the purpose of this discussion however, we will focus on the basic underlying principles of the perfusion method. Published methods for constant pressure models focus on gravity, air, and mechanical devices.

Alternatively, models may maintain a specific flow rate through the use of a previously mentioned method which is ultimately done through relating effluent perfusate and contraction measured with a silk tie through the heart apex [4].

III. Preliminary Designs

Components of All Designs

The major varying component between each of the designs discussed relates to the ways in which the perfusate solutions are regulated by either constant flow, or constant pressure. Other components remain the same across each design. Each of the designs includes a 13 inch water jacketed condenser to which the primary tubing line is attached. The water jacketed condenser contributes to maintaining the desired temperature (37℃) of the perfusate solution by allowing the perfusate solution to flow through coiled tubing enclosed by heated water. A bubble trap will also be incorporated into each design to prevent any air bubbles from reaching the cannulated heart. The bubble trap is an essential component of the design, as the introduction of air bubbles into the heart could lead to blockages within the vasculature of the heart and would likely induce excessive stress resulting in reduced efficacy of the Langendorff apparatus. The bubble trap is attached in between the water jacketed condenser and cannula. The perfusate solutions flow into the cannula before finally reaching the excised Guinea pig heart.

Oxygen Constant Pressure

In order to provide constant pressure to the system, an oxygen tank was considered (*see Figure 1)*. Oxygen gas would flow out of the gas cylinder at a specified pressure (approximately 1-2 psi), creating a pressurized environment in the enclosed glassware containing the perfusate, which would ultimately drive the flow of perfusate out of the glassware and through the tubing to the cannulated heart. As noted, the perfusate solutions would be contained in glass media bottles (appx. 50mL), with the caps sealed. This model of a completely closed system allows for the pressure inside of the glassware to build-up to the pressure desired for modeling physiological pressures. Solutions would flow out of the glassware via tubing securely attached into the bottom of each bottle. The tubing would then be connected via a three-way stop cock, which would serve as the transition into a single, primary line of tubing. This primary line of tubing would follow the same design through the water jacketed condenser, bubble trap, and cannula as discussed above.

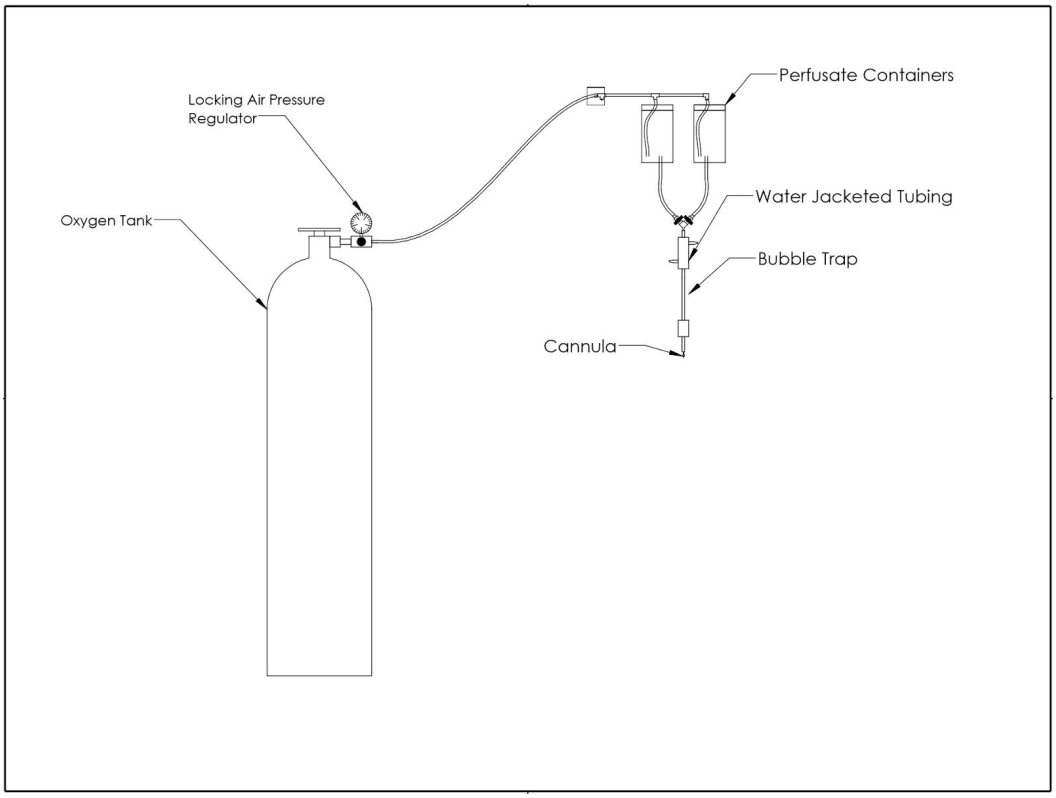

Figure 1. Design schematic of the Oxygen Regulated constant pressure system. Tubing from the oxygen tank is connected to closed containers holding the perfusate solution. The air pressure regulator can be adjusted to achieve the desired fluid pressure. Perfusate solution leaves the fluid reservoirs through tubing connected to a three-way valve. Tubing connected to the three way valve leads to a water jacketed condenser, in which the perfusate fluid is warmed to 37℃. After passing through the condenser, the warmed perfusate fluid enters the bubble trap before reaching the cannulated heart.

Pump Constant Flow

The hallmark characteristic of this design is the incorporation of a peristaltic pump, which would serve as the primary method by which perfusate solution is moved throughout the system. Utilizing a peristaltic pump allows for precise control of the flow rate at which the perfusate travels through the tubing by allowing the user to set the fluid propulsion rate to the desired value. In this design, each perfusate solution would be contained in 50 mL syringe, with a one-way stop cock valve attached the the bottom of the syringe. The stop-cock valve serves as the bridge between the syringe and the primary line of tubing through which the fluid flows. The tubing then fed through the peristaltic pump, before reaching the primary tubing as discussed above.

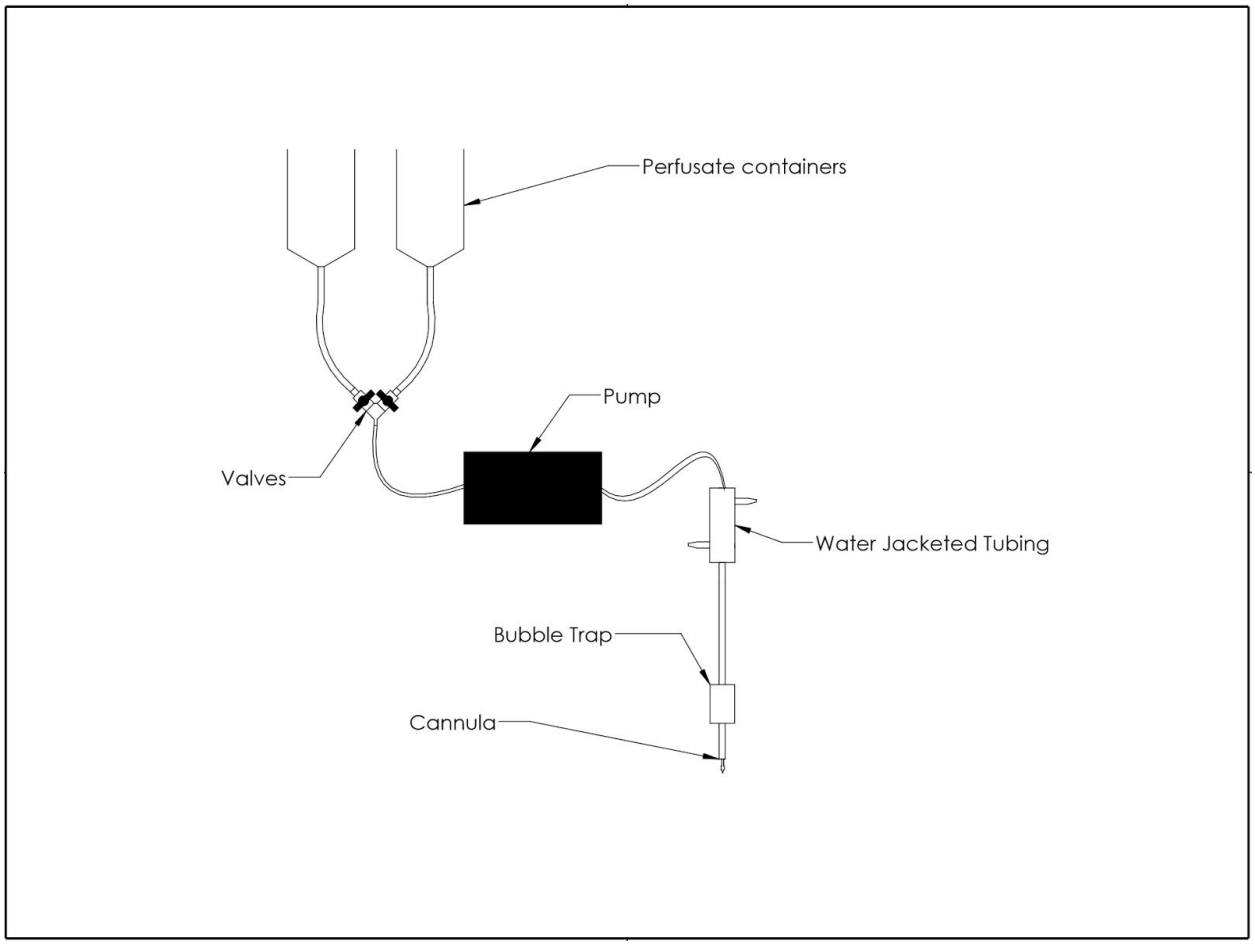

Figure 2. Design schematic of the peristaltic pump constant flow system. Perfusate solutions are contained in open 50 mL syringes. Perfusate solution leaves the fluid reservoirs through tubing connected to a three-way valve. Tubing connected to the three way valve leads to a peristaltic pump which propels the fluid at the desired flow rate. Tubing leaving the peristaltic pump is attached to a water jacketed condenser, in which the perfusate fluid is warmed to 37°C. After passing through the condenser, the warmed perfusate fluid enters the bubble trap before reaching the cannulated heart.

Gravity Fed

This design is the most minimalistic of the three proposed designs, utilizing only gravity to achieve the desired pressure to move the solutions through the tubing. The length of the primary tubing line would be measured and set to a specific calculated height (to be determined through testing), resulting in a 'constant' pressure flow. Similarly to the pump constant flow design, the perfusate solutions would be contained in 50 mL syringes. Tubing would attach to the bottom of each syringe, and then be connected to a three-way stop cock valve. The primary line of tubing would come off of the stop-cock valve and connect to the water jacketed condenser and bubble trap as previously described.

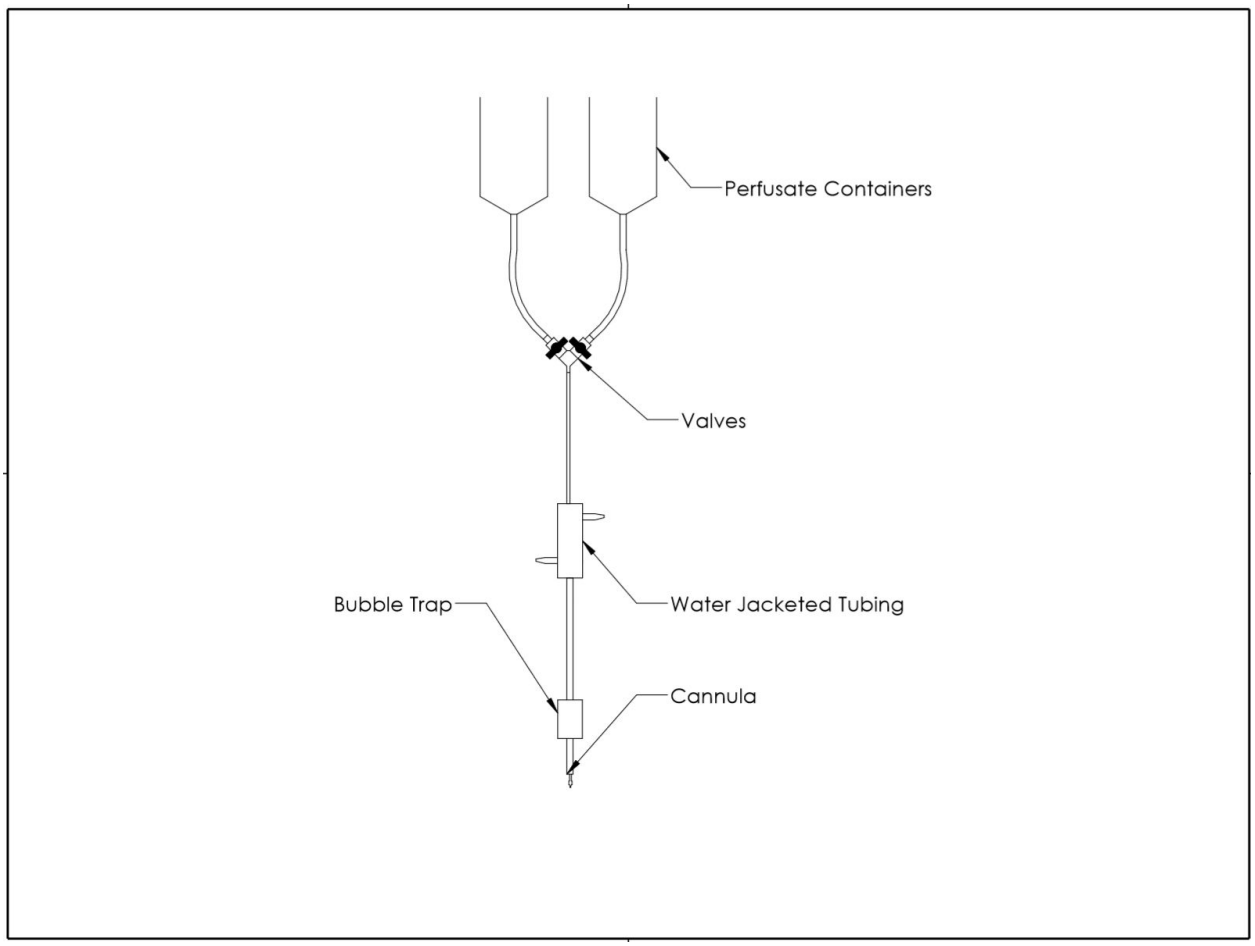

Figure 3. Design schematic of the gravity fed design. Perfusate solutions are held in 50 mL syringes that are open to the environment. Fluid flows from the fluid reservoirs into tubing that is connected to a three way valve. The desired fluid flow rate is achieved by adjusting the height at which the fluid reservoirs are positioned. Tubing from the output valve is attached to the water jacketed condenser. Within the condenser the fluid is warmed to physiological temperatures. The warmed fluid exits the condenser and enters the bubble trap before reaching the cannulated heart.

IV. Preliminary Design Evaluation

Design Matrix

Following the design matrix in a sequential matter from highest weight criteria to lowest, we reach our preliminary conclusion on suitability of the designs. Ease of use leads with a weight of 30% as this project is to be used in a research lab where alteration of methodology/use of the device may be important. Furthermore, our client would potentially be using this device on daily basis; thus ease of operation is essential. For example, recycling of the perfusate solutions would contribute to greater ease of use. This aids in explaining the highest weight on the gravity fed design as there is little to no interaction necessary past switching the perfusate solution with the stopcock valve and recycling the solutions to the unclosed 50mL syringes.

Output consistency refers to the ability to maintain either a constant flow rate of the perfusate solutions through the heart or a constant pressure within the system. The oxygen constant pressure system outperforms the neighboring designs as the closed system would be able to propel fluid by applying pressure while also applying a negative pressure to hold back fluid against gravity. Following closely behind is the peristaltic pump design which is limited by the fact that the pump would be less efficient at holding the fluid against gravity, especially as an open system. The gravity fed design ranks the lowest in this criteria, because changes in fluid volume would result in a change in fluid flow rate in combination with the some of the previous issues listed. For example, the gravity fed design would not have negative pressure to aid in slowing flow rate.

The next two criteria considered were fabrication and safety. Fabrication is relevant as we have a timeline to consider, and the team's current skill set will be beneficial to bypass time spent learning the processes necessary to fabricate the design. Safety is considered largely due to the disparity presented when considering the integration of pressurized containers, particularly an oxygen gas cylinder, as is presented in one of the designs. In this category, the oxygen fed design system is an obvious negative outlier, due to the highly flammable nature of pure oxygen gas and the closed pressurized system. Conversation around the fabrication criterion followed the idea that as the number of components comprising the system increases, complexity of fabrication is likely to increase as well. Additionally, it was considered that incorporating mechanistic devices (ie. peristaltic pump) would likely add another layer of fabrication complexity.

The "deviation from closed system" criterion was considered because with an increasingly open system increases the likelihood of contamination. Designs with the least connection points and closed system were favored due to the decreased risk of contamination. The gravity fed design marks as the lowest in this category because the open system design,

which inherently leads to greater exposure of the perfusate solutions to air contamination. The oxygen constant pressure design outperformed the rest largely due to the closed system.

Lastly, because our client is a postdoctoral researcher working under a granted budget, cost was another factor considered. It was not weighted very heavily, however, because some of the more expensive components of each of the designs (ie. peristaltic pump, gas tank) could be provided by the client, without implication on our allotted budget for the project. Leading in this category is the gravity pump design as this design includes materials that would be incorporated into other designs. The following two designs ranked lower in this category due to some of our findings during preliminary material searches (see *Appendix C* for details).

	Weight	Peristaltic Pump Constant Flow		Oxygen Constant Pressure		Gravity Fed	
Ease of use	30	3/5	18	3/5	18	4/5	24
Output Consistency	30	4/5	24	4/5	24	1/5	6
Safety	15	4/5	12	2/5	6	5/5	15
Fabrication	10	2/5	4	3/5	6	5/5	10
Deviation from Closed System	10	4/5	8	5/5	10	2/5	4
Cost	5	3/5	3	2/5	2	5/5	5
TOTAL	100	**69**		**66**		**64**	

Figure 4. Design Matrix of the three designs discussed above. Criteria is outlined on the left, evaluations of that criteria for each design is highlighted in grey.

Proposed Final Design

Based on the criteria discussed in the former section, our construction of the design matrix showed that the peristaltic pump design scores the highest. Upon presenting the three designs to our client, we determined that the client would be most comfortable with using the peristaltic pump design. Our client had concerns with the gravity fed design due to its low score in output consistency. Our client was also concerned with the oxygen constant pressure design due to the possibility of oversaturating the perfusate solutions with oxygen, which would ultimately jeopardize cell health. Another downfall of the oxygen constant pressure design was that it would be difficult to ensure that the fluid pressure was at the desired value without the incorporation of additional flow control valves.

V. Fabrication/Development Process

Materials

The pump chosen for use in the design is a Cole-Parmer variable speed peristaltic pump. The variable speed characteristic of the pump was of critical importance, as it allowed for the calculation of actual flow rates comparatively to the pump output "speed". This ultimately allowed us to report on which pump output "speeds" must be used to achieve the specific desired flow rate. A Laboy glass water condenser is another material included in the apparatus, as it allows the flow of perfusate solution through glass tubing surrounded by warm water, ultimately serving to maintain the desired temperature range of the perfusate solution around 37ºC. Silicone rubber tubing (inner diameter 0.8 mm, outer diameter 2.4 mm) serves as the pump and mainline tubing throughout the apparatus. Additionally, a variety of polypropylene fittings are used throughout the system with dimensions catering to the individual joint to be connected (tubing to the bubble trap and water condenser for example). Two two-way valves are also incorporated in the apparatus, serving as a connection between the syringes that hold the perfusion solutions and the tubing line. Other materials incorporated into the system include a water bath, bubble trap, cannula, lab stand, clamps, and syringes. The cannula that the client has provided is essentially a shortened Eppendorff pipette tip. The lab stand and clamps are incorporated into the system for purposes of support and upholding the water condenser.

Materials used in the thermistor/LED display circuit to measure temperature include components used from previous projects (referring to 22-gauge solid core wires, 5 LED lights, various resistors and a battery pack) as well as a thermistor, UA741CN operational amplifier, and Arduino Mega board. See Appendix B for a breakdown of all materials and associated cost.

The client proposed that perfusate solutions be manually recycled after having been run through the excised heart. With this request in mind, the design team evaluated all materials that come in direct contact with the perfusate solutions on a number of criteria that pertain to ensuring the purity and sterilization of the perfusate solutions upon reaching the heart. These criteria included biocompatibility, protein absorption, chemical inertness, and thermal stability. See *Appendix C* for a comprehensive discussion explaining why each of the chosen materials are suitable for this application.

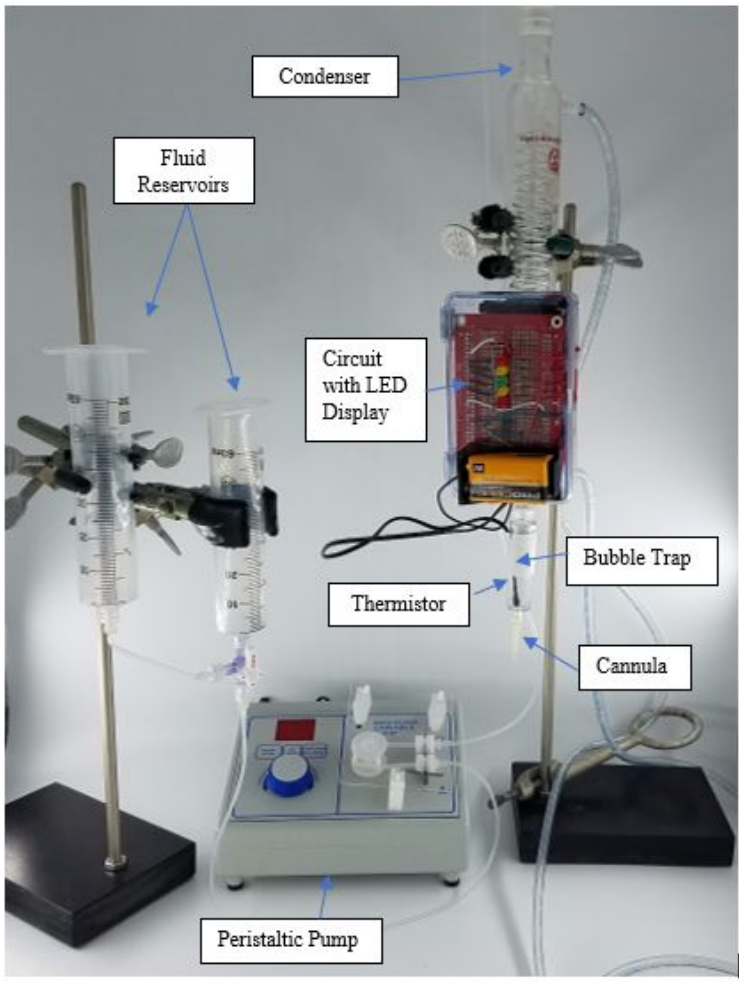

Figure 5 - Image of our final design. Solutions will be held in the two large syringes fixed to the rings stand. Silicon tubing leading from each fluid reservoir is joined to a three-way valve. The tubing exiting the outlet of the three way valve is connected to the peristaltic pump tubing. The flow rate achieved by the peristaltic pump can be chosen by adjusting the dial on the pump. See the discussion of flow rate testing on page 15 for an exact relationship between the arbitrary pump setting and flow rate. The perfusate solution is passed through a water-jacketed condenser in order to achieve a temperature of 37℃ upon entering the cannulated heart. (The water bath is not pictured, but will be positioned above the device). To ensure that the perfusature solution is at the desired temperature, the thermistor measures the temperature of the perfusate in the bubble trap. The LED display will depict whether or not the perfusate temperature is within the desired range. LED display signals: bottom red (Temperature < 36.5), bottom yellow (36.5 ≤ Temperature ≤ 36.75), green (36.75≤ Temperature ≤ 37.25), upper yellow (37.25 ≤ Temperature ≤ 37.5), upper red (Temperature > 37.5).

Methods

In terms of the device setup, the team confirmed the material performance through literature research ensuring the setup follows general lab safety requirements including but not limited to biologically inert materials. The final project setup requires confirmation of functionality for two major systems. As was mentioned previously, our design focuses on the consistency of the temperature near 37℃ upon reaching the cannulated heart and flow rate of the perfusate upon entering the cannulated heart. For a more detailed description as to how this device is intended to be used see the user protocol located in *Appendix D.*

Temperature of the perfusate solution is altered as it travels through a warmed water condenser. Thus, it was necessary to confirm the correlation of the desired perfusate temperature and the necessary temperature of the warming fluid in the water condenser. To determine this correlation the team tested various water bath temperature settings and the corresponding flowing fluid temperature. Setting the water bath to increments of 1℃ beginning at 39℃, the team determined that the likely most suitable water bath temperature was 39℃ for more details on these results see *Appendix F.* Results of the testing are discussed in the proceeding section. After this temperature bath setting was determined, three timed tests were run, collecting temperature values as a function of time.

The flow rate testing began with setting the pump setting to 35 and measuring how long the system took to output 5mL. From this time value, the flow rate in mL/min was determined using MATLAB. For additional details regarding the MATLAB code see *Appendix D* - Protocols. Three tests were run for each setting up to a setting of 65. From this a linear fit equation was concluded. Results of this test are also shown below.

VI.Results

Flow Rate

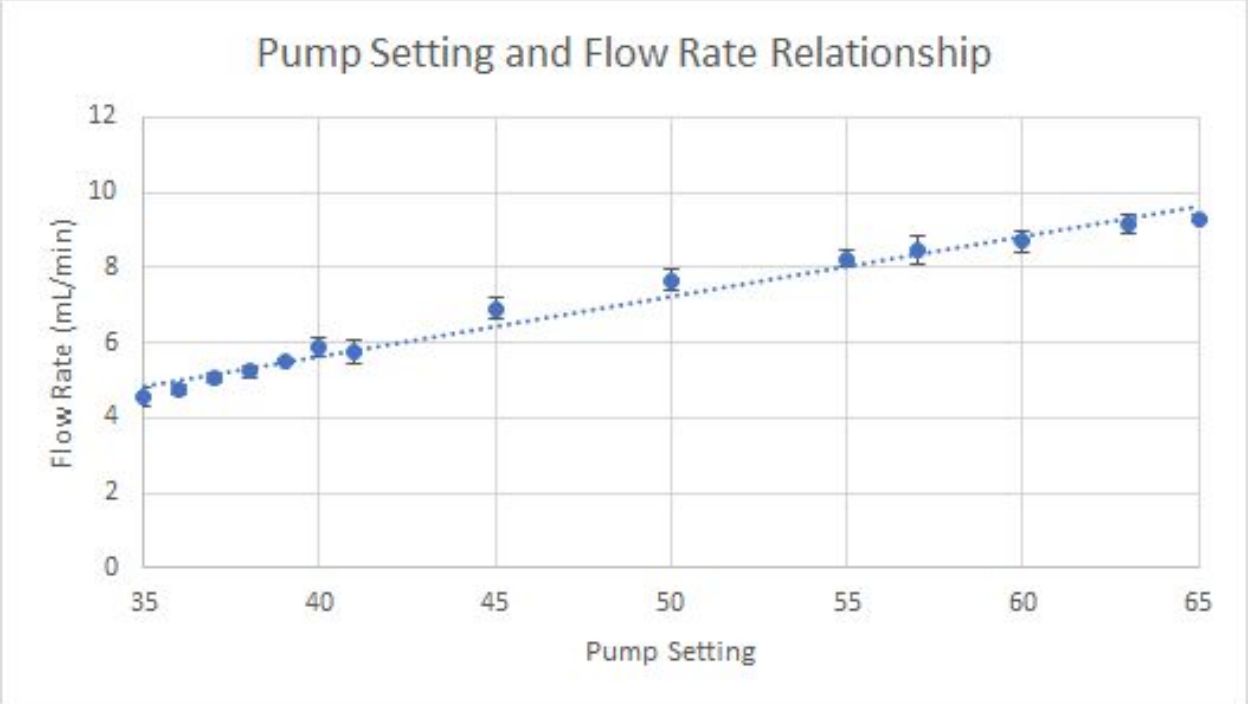

Figure 6. Arbitrary pump setting number and its corresponding relationship with the flow rate produced with our system setup

Through testing it was determined that the pump setting numbers had a linear relationship with flow rate. This data was interpreted and the following equation was produced:

$$N_{pump} = FlowRate * 6.2814 + 4.53077$$

The accuracy of our linear model is assumed by an R^2 value of 0.9785 which indicates that nearly all of the variability of our data is accounted for by our model. With this value in mind, and the relationship between pump setting and the respective flow rate is well-represented by a linear equation. Considerations for experimental error must also be taken into account in reference to the R^2 value. The consistency of flow rate was addressed by measuring the time each our system to fill each 1mL of a beaker for 10mL the standard deviation of this time was found to be 0.5 seconds. For additional details, see raw data and calculation methods in *Appendix E.*

Perfusate Temperature

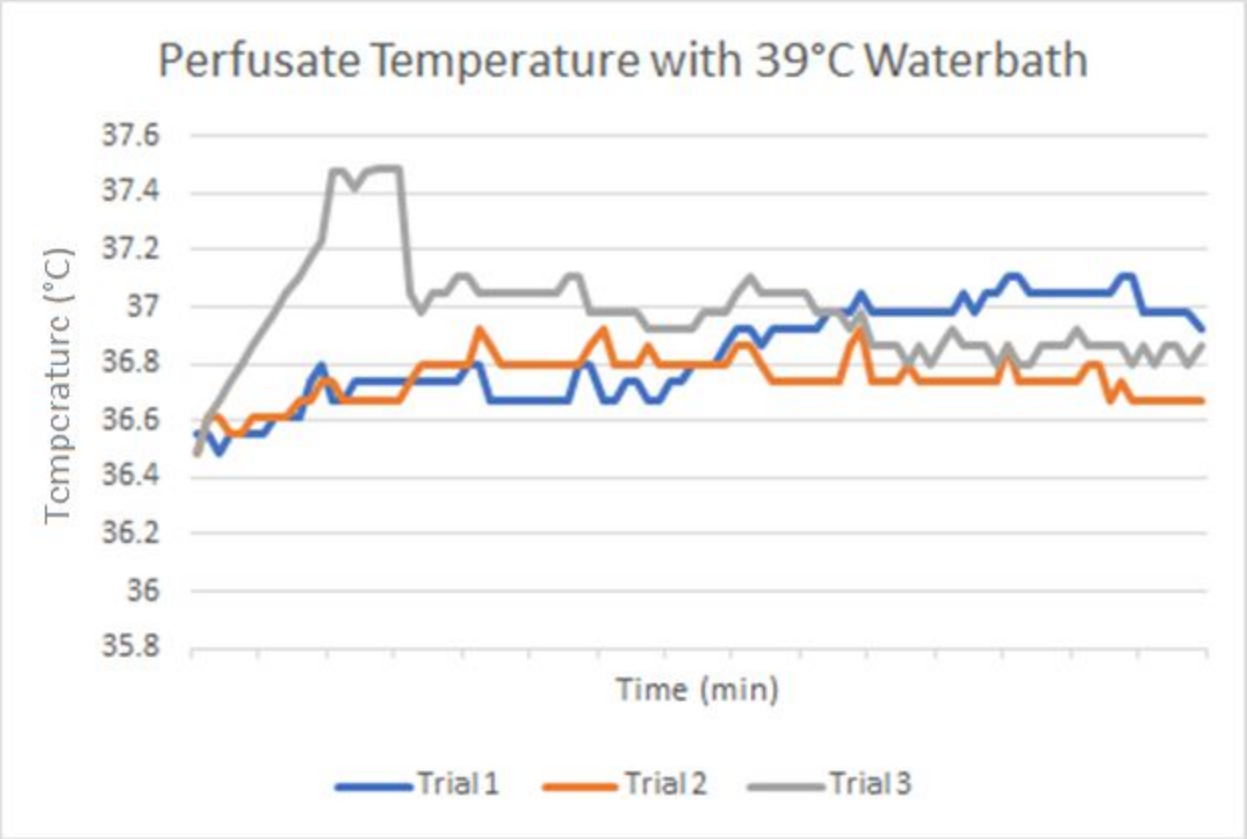

Figure 7. Perfusate temperature measured by the thermistor in the bubble trap of the the Langendorff apparatus to closely represent the temperature exiting the cannula.

The water bath temperature of 39°C was determined as the optimum temperature of the water bath using the raw data seen in *Appendix F.* Temperature data was collected every 10 seconds over the course of a 15 minute time period. The large spike in temperature for Trial 3 can most likely be attributed to the fact that the water bath was still heating to 39°C at the beginning of the test. Note that despite random fluctuations, all of the temperature data falls

within the desired temperature range of 37±0.5°C. The accuracy of the thermistor can be see through the thermistor calibration protocol seen in *Appendix G*.

VII. Discussion

Biomedical design carries inherent ethical considerations. In the application of the designed Langendorff apparatus, considerations heavily focus on the ethics of research as their implications reach farther than the isolated scope of the work. As was mentioned previously, this lab has conducted research that laid the groundwork for various FDA regulations on drug use through tests performed on cardiomyocytes. In the scope of the design team's work, it is essential that the guaranteed metrics of the studies to be conducted on the perfused hearts are confirmed by our design team as the downstream effects could include something as impactful as FDA regulations.

Research done with Langendorff apparatuses are much farther reaching than the few studies researched for fabrication technique. This method has been proven to work effectively in the isolation of the cardiomyocytes, so the proof of the method itself isn't necessary, rather the design team worked to prove the effectiveness of the metrics desired from the client as these metrics define the functionality paralleled with the Langendorff technique.

There is yet to be a confirmed research protocol to include this Langendorff apparatus. For this reason, general desired characteristics and models were discussed before design. The design team gathered the general information and continued with fabrication and testing to not only accurate deliver the metrics desired for the Guinea pig model, but also cater to expanded research techniques. This aids in confirming consistently accurate perfusion techniques despite altered research protocol. This expandability is promising for maintaining the relevance of the studies conducted as a result of the cardiomyocyte perfusion.

VIII. Conclusion

In summary, the goal of this project was to design a Langendorff apparatus in which solutions could be retrogradely perfused through an excised Guinea pig heart, with the purpose of preparing the cardiomyocytes for isolation. Motivation for the project stems from the client's need, as a postdoctoral researcher, to isolate cardiomyocytes in such a way that retains the cells' physiology and contractile functionality. The final design achieves constant flow of the perfusate solutions using a peristaltic pump. The design also provides the user with a measurable temperature of the perfusate solution within the desired range.

Beginning at one of the two perfusate chambers, the perfusate flows through a 3-way valve and then travels to meet the peristaltic pump, which allows the fluid to be propulsed to meet the water condenser. Water of approximately 39ºC flows through the water condenser,

warming the perfusate solution as it flows through the innermost coil of the condenser. After flowing through the condenser, the perfusate reaches the bubble trap, where the temperature of the solution is measured before flowing through the attached cannula into the excised heart.

Testing was conducted to develop an equation that the client can use to determine which arbitrary pump number corresponds to the desired flow rate. This equation allows the user to determine the appropriate pump number by simply inputting the desired flow rate into the linear function. Testing was also conducted to identify what temperature the water bath must be to achieve the desired perfusate temperature of 37±0.5°C, and it was determined that the ideal water bath temperature is 39°C.

After completing the design, the level of simplicity achieved can be appreciated; all design criteria have been met, while maintaining ease of use and reliable functionality. The beauty of such a design can been seen best in the setting that motivated this project. Research projects, like our client's, tend to change quickly for a variety of reasons; the simplicity of this design allows for ease in advancement of the design as our client's research project specifications, and consequently, the client's needs, may change. While all components of the current design are acceptably functional and ready for use, particular design adjustments the design team feels would be beneficial, assuming time and budget allowed, are discussed in the following section.

Future Work

Although all design and client requirements have been addressed and delivered upon in the final design, there is certainly prospect for future work in regard to improving overall ease of use and specificity of the design. First, the implementation of a digital temperature display, replacing the LED light signals, would offer precise readouts of temperature values. By having a digital output with the exact numerical temperature, the user could more easily identify precisely how much the perfusate temperature varies from the desired temperature, ultimately allowing he or she to make fine-tuning adjustments until the digital output reads the desired temperature.

Another item to be considered in future work is the implementation of a mechanism for oxygenation of the solutions prior to being a perfused throughout the system. The client indicated that oxygenation of perfusate solutions had already been incorporated into the protocol for preparing for use of the Langendorff system; however, it could prove useful to design a component within the Langendorff system that accomplishes this task. Another component that could be introduced into the existing Langendorff system is a mechanism for recycling perfusate solutions back into their respective reservoirs. The design team anticipates this could be done by simply incorporating a second peristaltic pump into the system. Budget guidelines restricted the ability to incorporate a secondary pump into the final design as presented; however, adding this component would likely be a straightforward and easy addition. One consideration that must be had regarding the implementation of this component would be the potential sterilization or filtration of the perfusate solutions before returning to their original reservoirs. Both of these

mechanisms discussed above, for oxygenation and recycling of the perfusate solutions, not only enhance the function of our Langendorff design, but also make the preparation protocol simpler for the user.

Another element of future work could be the implementation of a pressure transducer, or some other type of pressure sensor, to allow for monitoring of the pressure of the perfusate solutions as flows through the heart. The monitoring of pressure would not replace the constant flow parameter on which our design is based, but rather serve as an additional metric to ensure that the chosen flow rate of perfusion solution also corresponds to a desirable pressure.

Finally, future work with this design could focus on comparing the viability of the isolated cardiomyocytes against various flow rates of the perfusate solutions. One metric that could be used to quantify the viability of the isolated cardiomyocytes is the cell's retained contractile function, which could be analyzed using cellular impedance assays. Additionally, the viability of the cells could be quantified by their metabolic activity, using MTT assays [8]. The purpose of conducting such testing models and interpreting those results would allow the client to determine precisely which flow rate through this particular Langendorff apparatus will yield optimized viability of the cardiomyocytes. While literature reviews suggest a wide range of acceptable flow rates, comparing flow rate against viability for this specific design would undoubtedly provide constructive data for the client and allow for the use of this apparatus in the most efficient way.

VIII. Acknowledgements

The Langendorff apparatus design team thanks Dr. Erick Ríos Pérez, along with Dr. Gail Robertson and colleagues, for providing the opportunity to work on this project and providing lab space. The team also thanks Dr. Wan-Ju Li for providing excellent guidance and advice, paired with dedication and enthusiasm.

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X. Appendices

Appendix A - Preliminary Design Specifications

Function:

Use retrograde perfusion techniques to prepare cardiomyocytes for isolation with an increased calcium tolerance to prevent loss of contractility function upon re exposure to physiological conditions.

Client Requirements:

Create a Langendorff apparatus that perfuses a Guinea pig heart with buffer and enzyme solutions to prepare primary cardiomyocytes for functional isolation. The solution must be maintained at a constant temperature and meet the perfusion rate criteria. Overall, the design must meet our budget of $200.

Physical and Operational Characteristics:.

a. *Performance Requirements:* The Langendorff apparatus must provide a means of retrogradely perfusing a various solutions through a cannulated Guinea pig heart. The apparatus is to utilize either a constant flow or constant pressure system to achieve perfusion. The desired flow to be maintained is approximately 8.6 ± 3.6 mL/min, or the desired pressure must be in the range of 60-80 mmHg for pressure[1]. Additionally, the apparatus must allow for the perfusion solutions to be maintained at a temperature of 37 degrees Celsius [2]. The system must also be able to function without mechanical or operating error for a minimum of ten minutes, which the typical length of time needed for completing the Langendorff protocol. Furthermore, care should be taken to ensure that components of the perfusion apparatus can be easily sterilized with ethanol to minimize the introduction of bacteria and other contaminants that may reduce the number of viable isolated cells.

b. *Safety:* There are minimal risks to a user operating the Langendorff system. The operator must take care to adhere to all chemical and biosafety protocols while working with the perfusion solutions, Guinea pig blood, and Guinea pig heart.

c. *Accuracy and Reliability:* As described in the performance requirements, the Langendorff system must function without mechanical or operational error for a minimum of ten minutes. In order for the solutions to be reliably perfused throughout the system, it is critical that all tubing connections are securely fastened, as to prevent any leakage from the system. Also, the system must maintain an accurate temperature of the perfusion solution, in order to prevent temperature shock to the cardiomyocytes and the adverse effects temperature shock could cause.

d. *Life in Service:* Ideally, the Langendorff apparatus will be utilized by researchers until a different isolation technique is desired.

e. *Operating Environment:* The Langendorff apparatus will be used within a laboratory setting and will be operated by researchers.

f. *Size:* The apparatus must be small enough to fit on a lab table but large enough to hold an adult Guinea pig heart. As long as it fills these size requirements and can perform its desired function, any size in this range is acceptable.

g. *Power Source:* The device will be stationary and will be powered through a direct connection to the wall. This will ensure that the power input for the Langendorff apparatus stays constant.

h. *Weight:* The Langendorff apparatus will be designed to be light enough where it is possible for one person to move without struggling or be able to be taken apart and transported. The apparatus must be structurally sound enough to hold all of its components and not collapse under its own weight.

i. *Materials:* The materials used in the Langendorff apparatus must sturdy enough to support itself and the Guinea pig heart. The tubing used will be made of silicon rubber and the other materials must be resistant to reacting and degrading from exposure to the buffer and enzyme solution..

j. *Aesthetics, Appearance, and Finish:* The appearance and aesthetics of the Langendorff apparatus will not play a large role in development. The main goal is functionality with aesthetics being addressed as a concern at the end of the project.

k. *Product Characteristics:*

1) Target Product Cost: The budget for this product is $200. The client will provide the water bath and any necessary reagents and solutions. The only projected team expenses will be tubing and a pump if the constant-flow perfusion method is chosen.
2) Quantity: The client requires one functioning Langendorff apparatus.

Miscellaneous

a. *Competition:* There are Langendorff systems available on the market from several different distributors. These apparatuses have capabilities to convert between constant pressure or constant flow systems, whichever may be preferred by the customer. The constant pressure perfusion model allows operators to visually detect changes in coronary resistance by looking at volumetric levels of fluid . The constant flow model may be present some advantages because it could enable recirculation of perfusate, and thus less operator involvement [3]. Companies that sell Langendorff systems include Harvard Apparatus®, World Precision Instruments®, Adinstruments®, Radnoti®, Experimentria Ltd. All of these systems can perform the requested task given by the client and can precisely monitor all aspects of the perfusion, however they cost well over $20,000. Transonic® also provides pumps and flow sensors with tips for assembling a Langendorff system.

b. *Client:* Dr. Ríos Pérez, a post-doctoral researcher in the Gail A Roberson lab.

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Appendix B - Cost Chart

Items To Buy	Cost Per Unit	Quantity	Total
Bubble Trap	N/A	1	$0
Glass Water Condenser	$36.00	1	$36.00
Polypropylene Tubing Fittings	N/A	7	$0
Lab Stand	N/A	1	$0
Clamp	N/A	2	$0
Cole-Parmer HDPE stopcocks	N/A	2	$0
Misc Circuit components (Op amp, Wire, resistors, switch, LED lights)	N/A	N/A	$0
Arduino Mega	$38.49	1	$38.49
~~Segment display**~~	$9.94	1	$9.94
Thermistor	$3.01	1	$3.01
Breadboard	$4.95	1	$4.95
Total			92.32

Figure 8. All materials where cost is listed as non-applicable are materials that have been provided by the client. Therefore, the cost of these materials was not considered as a cost of the design. **The segment display was not included in our final design due to overloading the display during fabrication and testing of the circuit. This ultimately rendered the display unusable.**

Appendix C - Materials Selection Justification

Introduction

When choosing materials that will be in used in preparing a cell culture, it is important to ensure that the material properties will not negatively impact the integrity and function of the cells. There are numerous plastics and polymers that are commonly used in biomedical devices; Each of them have their strengths and weaknesses depending on the intended application. During our literature search we evaluated a variety of plastics commonly used in biomedical applications focusing on protein adsorption, biocompatibility, thermal stability, and chemical reactivity. Our client has expressed interest in recycling perfusate solutions, however the design team does not anticipate that considerable amounts of cells will be passed through the tubing of the Langendorff-like apparatus. Despite this, protein adsorption to the material is still an important criteria to consider. Protein adsorption can result in the formation of biofilms, ultimately presenting contamination concerns [1]. For our analysis, we decided that hydrophobicity given by the water contact angle would be our primary criterion for assessment of protein adsorption. Proteins prefer attachment to hydrophobic surfaces. A water contact angle greater than 90° indicates that a material is hydrophobic, while a water contact angle less than 35° implies hydrophilicity [2]. Biocompatibility refers to material properties that do not elicit a biological response in a living system. For this criterion, we decided to focus on the common biomedical applications of the material. Additionally, thermal stability of the candidate materials was analyzed to ensure that the materials would not degrade upon exposure to perfusate solutions at 37°C. While the client plans to sterilize the apparatus with 70% ethanol, we decided that materials that capable of autoclave sterilization would be given preference in case the client decided to change his sterilization protocol. Our final criteria for analysis was chemical reactivity, with a main focus on reactivity upon exposure to alcohols since all components of the design will be sterilized with ethanol following each isolation experiment. The materials that were selected for use in our design are listed below with their respective justifications.

Silicone Rubber

Use in Design: peristaltic pump tubing and mainline tubing

Justification:

Silicone rubber is widely used in biomedical devices such as balloon catheters, shunts, valves, and tubing for feeding and drainage [3]. Silicone rubber is used in a variety of biomedical applications for several reasons. First, silicone rubber has an inorganic backbone, a key feature to its biocompatibility. Since it is inorganic, the material cannot be metabolized by cells [4]. This contributes to the overall chemical inertness of the material, as it minimizes the likelihood of chemical interactions with bodily fluids and cells. These characteristics are important for our purposes because it is essential that the perfusate solutions running through the tubing do not react with the tubing material.

Silicone rubber can also withstand high temperatures (up to 250°C) and pressures, which is important when considering sterilization methods and its ability to withstand the autoclaving process [4]. Having high temperature resistivity also alleviates any concern regarding perfusate solutions of high temperatures running through the system. Furthermore, the material has no reactivity with alcohols, specifically ethanol. This was a critical factor to our decision because the client has indicated that the primary sterilization method for the tubing will be by running through 70% ethanol through the tubing [5].

Due to the properties discussed above, silicone rubber is widely accepted for use in biomedical applications, and the design team feels it is a suitable material option for the tubing in the apparatus.

Polypropylene

Use: Tubing fittings, valves, syringe

Justification:

The design team has determined that polypropylene is a suitable material for the following reasons. Polypropylene is a widely used material in a variety of biomedical applications including: heart valve sutures, meshes, sutures, disposable syringes, and oxygenators[6]. Polypropylene also has an "excellent" chemical resistivity rating in regard to reactivity with ethanol. As described above, this is a critical characteristic for chosen materials to have, since the clients' preferred sterilization process is the use of 70% ethanol [5]. Additionally, polypropylene has a high temperature resistivity (minimum thermal decomposition temperature: 513 K), meaning it can withstand autoclaving processes if necessary [7]. Having high temperature resistivity also alleviates any concern regarding perfusate solutions of high temperatures running through the system. Furthermore, polypropylene is a very inert material and does not present issues for degradation *in vivo*. Also, the water contact angle of polypropylene is approximately 102°, indicating that the material is hydrophobic [7]. Despite concerns related to the hydrophobicity of the material, the team is confident that our perfusate solutions will not react with the polypropylene components.

Borosilicate Glass

Use: Water Condenser

Justification:

Borosilicate glass is a very inert material as a result of the strong Si-O bonds that are the main component of its structure. It is chemically resistant to nearly all substances, with the exception of phosphoric acid, hydrofluoric acid, and hot strong caustic solutions [8]. Due to these chemical properties, borosilicate glass does not present any reactivity issues with ethanol. Furthermore, another consequence of the inertness of glass is that triggering a response in the cells is very unlikely. In addition to its excellent chemical properties, borosilicate glass can withstand temperatures upwards of 520°C prior to softening [9]. This high thermal stability

allows glass to be autoclaved without concern of degradation. While information regarding the water contact angle is difficult to obtain, glass is widely used in tissue cultureware, ultimately implying that it is a relatively hydrophobic surface that permits strong cell adherence. The hydrophobic nature of borosilicate glass is not a major concern due to the fact that the recycled perfusate solution should contain very few cells during the isolation process. Aside from the hydrophobicity of the material, the team feels that borosilicate glass is a suitable material choice for use in our Langendorff-like apparatus.

Final Thoughts:

All three selected materials present virtually no concerns with respect to chemical reactivity upon exposure to ethanol during sterilization. Additionally, all proposed materials have excellent thermal stability, which would allow the client to autoclave all components of the design if he chooses to alter the sterilization protocol. Despite these favorable material properties, the design team recognizes that protein adsorption may be a minor concern for the polypropylene valves and borosilicate glass condenser. In order to mitigate the potential of the cells adsorbing to the materials, the design team advises that the client to filter the recycled solutions with a cell strainer before placing them in the fluid reservoirs. The team has also explored the possibility of using surfactants to reduce the hydrophobicity of the materials. During our literature search, we found a paper that explored abilities of a variety of surfactants to reduce protein adsorption to glass beads and polypropylene tubing. The paper concluded that cationic surfactants such as SDS and DTACl did not markedly reduce protein adsorption to the materials; however, proposed that non-ionic surfactants like Tween-20 effectively increase the hydrophilic content of the materials [10]. While some papers have highlighted the use of Tween-20 and its low potential for toxicity, a paper by Eskandani *et al.* proposed that Tween-20 may inhibit cell growth through the induction of apoptosis and DNA fragmentation [11]. Furthermore, the team is unsure how often the materials would need to be coated or if fluid flow could lead to degradation of the coating and ultimately unwanted introduction of Tween-20 into the perfusate solutions. Due to these concerns, the team has decided to forgo the use of surfactants in our design; however, we will mention the possibility of using them to our client in case he is greatly concerned with protein adsorption.

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Appendix D - Protocols

Arduino Thermistor Calibration

To calibrate our thermistor to give an accurate temperature readout. We must set up a circuit for our microcontroller such that a change in resistance in the thermistor can be measured as a change in voltage (see circuit design). The Arduino will first be setup to read in a value across a thermistor through an Analog Input port (A0). Using the official values from Arduino website we determined the raw Analog read data must be multiplied by a scalar of $\frac{5}{1023}$ to achieve output voltage measured. The code set up is to allow the Arduino IDE to continually output voltage. This data can be recorded and manipulated in MATLAB to create an equation which we can then use as a function on the arduino to display the temperature.

Calibration Procedure:

1. Set up multiple water baths with different known temperatures in the range of 20-40℃ (The more the better) we will use approx. 8
2. A multimeter with a thermistor attachment will be placed in each water bath to achieve true temperature
3. The tip of our thermistor attached to Arduino will be lowered into each water bath and the voltage seen through Arduino will be recorded after it reaches a constant value
4. Record a data point for each water bath set up. Using the true temperature of the water bath and the output voltage.
5. After all data collection use MATLAB to create a fit line for the data and develop an equation that corresponds voltage and temperature.

The equation that corresponds temperature and voltage will later be used later in developing an output algorithm to show temperature on the display board.

Matlab Code

```
Thermistor = //Imported Data

mdl = fitlm(Thermistor(:,1)',Thermistor(:,2));
X = min(Thermistor(:,1)'):max(Thermistor(:,1))';
Y= 6.8327-0.079025*X;
plot(X,Y)
figure;
hold on;
scatter(Thermistor(:,1)',Thermistor(:,2));
plot(X,Y);
```

```
xlabel('Temperature');
ylabel('Voltage');
```

Circuit Schematic

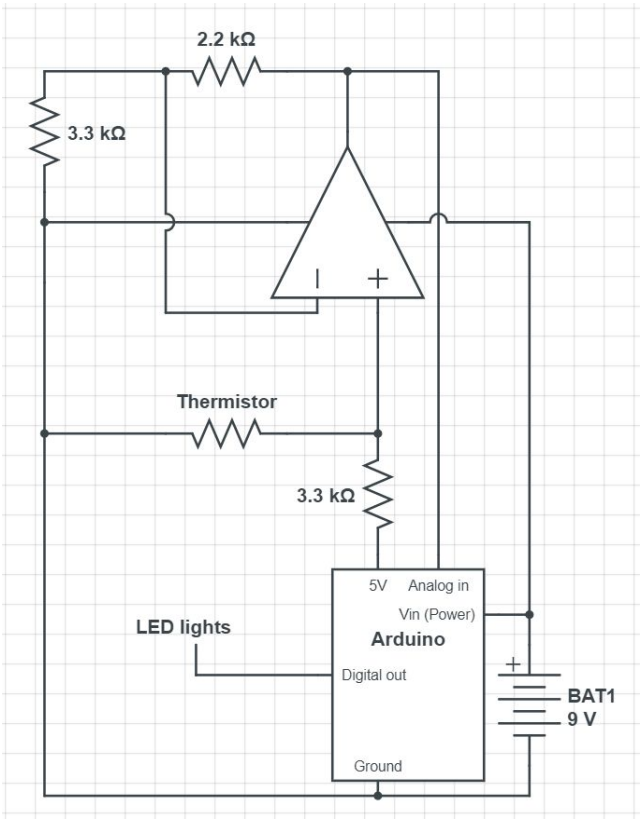

Figure 9. Complete circuit schematic outlining the details of the thermistor, power provision to the microcontroller as well as the operational amplifier, and the LED output.

Flow Rate Testing Protocol

For the purpose of perfusing the guinea pig heart. We must keep the keep the perfusate flow in the range of 8.6 ± 3.6 ml/min. The goal of this protocol is to establish a method to determine the Cole-Parmer© Medium Flow Peristaltic pump speed settings and verify the correct perfusion rate. The pump flow is correlated to a number displayed on the pump and is adjustable by the knob located on the top of the pump. Our client, Dr. Erick Rios Perez has told us to assume that the solutions to be perfused have similar fluid properties of water. To improve the accuracy of our system this protocol could be replicated with the actual perfusate.

Materials:

- Langendorff system with Cole-Parmer© Medium Flow Peristaltic pump
- 20mL syringe with luer lock cap (measuring syringe)
- Water

- Stopwatch with lap time
- Computing software such as MATLAB

Procedure:

1. Fill the Langendorff system perfusate containers with water.
2. Place empty 20mL syringe with Luer lock cap under the bubble trap where the cannula will be attached
3. Begin running the pump at a lower setting (a numerical value of about 30 displayed on the pump).
4. Once the measuring syringe reaches 1 mL begin timer
5. For the first Trial only, Record the time by using the lap function on stopwatch every 1 mL
6. Stop pump and timer once the measuring syringe reaches 11mL
7. Using computing software such as MATLAB or EXCEL calculate the flow rate and record the data in a table and pair it with the pump numerical value setting
8. Adjust the pump speed setting up by a numerical value of 3
9. repeat steps 4-7 until at least 10 data points are taken and the desired flow rate is contained within the data
10. Plot the numerical value of pump versus the calculated flow rates in a scatterplot using the same computing software
11. We will assume a linear relationship for this data such that

$$Num_{Pump} = FlowRate*C$$

Where C is an unknown constant, use the computing software's fit function evaluate this value of C. This Equation will serve as a reference to determine flowrate for the numerical value shown.

12. Calculate Num_{Pump} for the desired 8.6 flow rate and test to confirm using methods described 4-7 to confirm this is the actual flow rate within a smaller tolerance range than listed above

Water Bath Testing Protocol

In order to prepare a Guinea pig heart for functional isolation of primary cardiomyocytes, we must keep the perfusate temperature at at 37℃. It is essential to maintain this physiological condition in order to reduce the likelihood of cell stress over the course of the isolation procedure. Furthermore, this temperature is needed for optimal enzyme performance. For the purpose of temperature control, our device utilizes a water jacketed condenser . The goal of this protocol is to establish a method to determine the optimal water bath temperature in order to ensure that the temperature of the perfusate is 37 ± 0.5℃ upon reaching the cannulated heart. For the purpose of testing, our client, Dr. Erick Ríos Pérez, has told us to assume that the experimental perfusate solutions will have similar properties to water. This protocol can be adopted to test the optimum water bath temperature for the actual perfusate solutions.

Materials

- Langendorff system equipped with Cole-Parmer peristaltic pump
- Water (can be substituted for actual perfusate solution)
- Thermistor circuit
- Laptop with Arduino IDE and Excel
- 12 L water bath

Protocol

1. Fill 12L water bath equipped with a thermometer to capacity. Heat the water bath to desired temperature.
2. While water bath is heating to the desired temperature, heat the perfusate solutions in a separate water bath set to 37℃. Allow the perfusate solutions to heat for a minimum of 15 minutes. Do not remove the perfusate solutions from the water bath until completing the experimental set-up.
3. Make sure pump is plugged in and pump is set to desired setting.
    a. Pump setting 56 is recommended for just over 8 mL/min
    b. Pump setting equation:
        i. $N_{pump} = FlowRate * 6.6007 + 2.7314$
4. Run the bottom hose from the condenser to the water bath and let the top hose drain into the sink.
5. Turn on the Pump. Pour the warmed perfusate solution into the fluid reservoirs and allow the system prime itself to avoid the creation of air bubbles.
6. Ensure the temperature of the water bath has stabilized. Use the large 40 mL syringe to create a siphon in the condenser. Negative pressure will cause the condenser to fill from the bottom, allowing water to drain from the top tube into the sink.
7. While the system is running, turn on the circuit and run the thermistor code in the Arduino IDE. Allow data collection for a minimum of 15 minutes.
8. Following data collection, make a plot of time vs temperature in Excel to better visualize the change in temperature over the course of the experiment.
9. Make adjustments to the temperature of the water bath and repeat the testing protocol until the desired temperature has been reached. It is recommended that you start running the protocol at a lower temperature and then make adjustments by increasing the water bath temperature.

When running this testing protocol with water as our perfusate temperature, we determined that 39℃ was the optimum temperature; however, this may be different if the properties of the actual perfusate solutions differ from those of water.

User Protocol

Sterilization notes

It is highly recommended that the system be sterilized before and after each use. Pipette tips used as the cannula and in the bubble trap can be replaced and cut to the desired size. Recommended sterilization for the current system consists of perfusion of 70% Ethanol through the system followed by water.

Preparation

1. Heat perfusate solutions in a separate 37◦C water bath for 15 minutes. Do not remove perfusate solution from water bath until the experiment is ready to run.
2. While the perfusate solution is heating, set the water bath to be used in conjunction with the water condenser to 39◦C. Ensure the temperature of the water bath has stabilized at 39◦C before beginning the experiment.
3. Make sure pump is plugged in and pump is set to desired setting.
    a. Pump setting 56 is recommended for just over 8 mL/min
    b. Pump setting equation:
        i. $N_{pump} = FlowRate * 6.6007 + 2.7314$
4. Ensure the hose attached to the bottom of the condenser is placed at the bottom of the 39◦C water bath
5. Run the hose from the top of the condenser into the sink.
6. Using the large 60 mL syringe, create a siphon in the condenser. Do this by placing the tubing over the end of the syringe. Pulling the handle of the syringe back to 60mL and then removing the tube. Negative pressure will cause the condenser to fill from the bottom, allowing water to drain from the top tube into the sink. If the water bath is full initially, this syphon will last about 60 minutes.
7. Turn on the Pump, let the system prime itself to avoid the creation of air bubbles, and make sure the perfusate solution is at the desired temperature of 37◦C. Ths should take around 5min. Ensure that the perfusate holders do not completely empty to avoid air bubbles.
8. Once the system is primed and it has been ensured there are no air bubbles the excised guinea pig heart can be secured to the cannula using a string.
9. The three way valve allows for the user to easily switch between types of perfusate by turning the handle titled “Closed” towards the tubing they wish to be closed.

** All testing was performed using a perfusate solution of water. Flow rate and water bath testing of desired experimental perfusate solutions can be reproduced following the testing protocol **

Fabrication Protocol

For the purposes of assembling the Langendorff-like apparatus the following materials must be acquired. The following procedure should be followed to complete the assembly process.

Materials:

- 0.8mm diameter tubing
- Tubing Adaptors
- Metal Clamps
- Ring Stand
- 3-way Valve
- Peristaltic Pump
- Water Jacket

- Water Bath
- ¾ inch Tubing
- Bubble Trap
- Cannula

Procedure:

1. Connect 3-way Valve into syringe
2. Place both syringes into their respective clamps
3. Attach 2 adaptors to each of a 2-inch long piece of 0.8mm diameter tubing
4. Attach one end to the syringe without the 3-way valve and the opposite end to the side opening of the 3-way valve
5. Create a piece of 0.8mm diameter tubing that is of appropriate length to connect the 3-way valve to the peristaltic pump
6. Attach an adaptor into one end of this tube and attach it to the bottom end of the 3-way valve
7. Find the appropriately sized pump adaptor for the 0.8 diameter and attach it to the free end of the tube attached to the 3-way valve
8. Place the pump adaptor into its position on the peristaltic pump
9. Create another piece of 0.8mm diameter tubing of appropriate length to connect the pump to the top of the water jacket
10. Attach this tube to the open end of the pump adaptor and attach the other end of the tube to a regular tubing adaptor
11. Place this tubing adaptor with tube into the top of the water jacket
12. Attach bubble trap to the bottom of the water jacket
13. Attach the cannula to the bottom of the bubble trap
14. Cut two pieces of ¾ tubing to appropriate lengths to connect the water bath to the bottom, perimeter opening of the water jacket and from the top, perimeter opening of the water jacket to the sink

Appendix E - Flow Rate Testing

Raw Data

Pump Number	Flow Rate (mL/min)				
	Trial 1	Trial 2	Trial 3	Average Flow Rate	Standard Deviation
35	4.526935265	4.8	4.32152118	4.54948548	0.24003517
36	4.721435316	4.623921085	4.87725573	4.740870711	0.12778072
37	4.948453608	5.16973979	5.09294627	5.070379889	0.1123558
38	5.204718945	5.416629051	5.08001016	5.233786052	0.1701815
39	5.521303028	5.504082194	5.40491848	5.476767899	0.06281635
40	5.970149254	6.065507481	5.58555204	5.873736258	0.25408838
41	5.902606985	5.424464334	5.95829196	5.761787758	0.29345447
45	6.914035492	7.192519779	6.64010624	6.915553838	0.2762099
50	7.832898172	7.870916962	7.32421875	7.676011295	0.30525375
55	8.084074373	8.474576271	8.13228517	8.230311939	0.21290812
57	8.056935679	8.866558298	8.47577341	8.466422464	0.4048923
60	8.602150538	8.987417615	8.472183	8.687250384	0.2679518
63	8.87442686	9.139375476	9.38526513	9.13302249	0.25547839
65	9.213759214	9.372071228	9.36621917	9.317349871	0.08975984

Figure 10. Raw data of the pump number, time to fill the measuring beaker to 5ml and the calculated flow rate. This data is seen above in a graph, refer to *Figure 6.*

Time to per 1 mL

Time (s)
11
9.32
10.81
10.23
10.63
10.57
9.56
10.3
10.18
9.6

Figure 11. To ensure the consistency of the flow rate time measurements were taken every 1 mL to ensure the intervals were relatively the same. Any variation is likely due to human error due to the difficulty to read the volume as water was dripping in.

Matlab Code

```
%%Initialize
clear all
Flow1 =
[132.54,35;100.5,40;86.78,45;76.6,50;127.08,36;121.25,37;115.28,38;108.67,39;101.65,41;74.22,55;69.75,60;65.12,65;67.61,63;74.47,57]
Flow2 = [125,35;98.92,40;83.42,45;76.23,50;70.8,55;66.76,60;64.02,65;129.76,36; 116.06,37; 110.77,38; 109.01,39; 110.61, 41;
65.65,63;67.67,57];
Flow3 = [138.84,35; 107.42,40;90.36,45;81.92,50;73.78,55;70.82,60;64.06,65; 123.02,36;117.81,37;118.11,38;111.01,39;
100.70,41;63.93,63;70.79,57];
Flow = [Flow1(:,1)', Flow2(:,1)', Flow3(:,1)'; Flow1(:,2)', Flow2(:,2)', Flow3(:,2)']';
%%Calculate FlowRate
for i=1:size(Flow,1)
   Flow(i,3) = (Flow(i,1)/600)^-1
end
%%Find Fit Line and Graph
g = fittype( @(k1,k2, x) k1*x+k2,'independent', {'x'},...
    'dependent', 'y','coefficients', {'k1','k2'});
options = fitoptions(g);
options.StartPoint=[1,-1];
options.MaxIter = 1e-100;
results = fit(Flow(:,2),Flow(:,3), g, options);
k = coeffvalues(results);
k1 = k(1);
k2 = k(2);
X = min(Flow(:,2)):max(Flow(:,2));
Y = X*k1+k2
figure
hold on;
scatter(Flow(:,2),Flow(:,3));
plot(X,Y);
xlabel('Pump Number');
ylabel('Flow Rate');
K = [1/k1, -k2/k1];
disp(sd(Trial2));
```

Appendix F - Water Bath Temperature Data

Time (s)	41°C	40°C	39°C
60	37.42	36.8	36.55
120	37.73	37.05	36.8
180	38.04	37.23	36.74
240	38.04	37.48	36.74
300	38.34	37.42	36.67
360	38.28	37.42	36.8
420	38.28	37.54	36.67
480	38.1	37.73	36.86
540	38.16	37.73	36.92
600	38.1	37.66	37.05
660	38.1	37.66	36.98
720	38.1	37.79	37.05
780	38.1	37.73	37.05
840	38.1	37.79	37.11
900	38.1	37.85	36.92
Average	38.02167	37.47789	36.83644

Figure 12. Summarized data of the water bath temperatures tested and their corresponding mean temperature data of the perfusate solution. This was the data used in determining the rest of the testing should be done with a water bath set at 39 °C

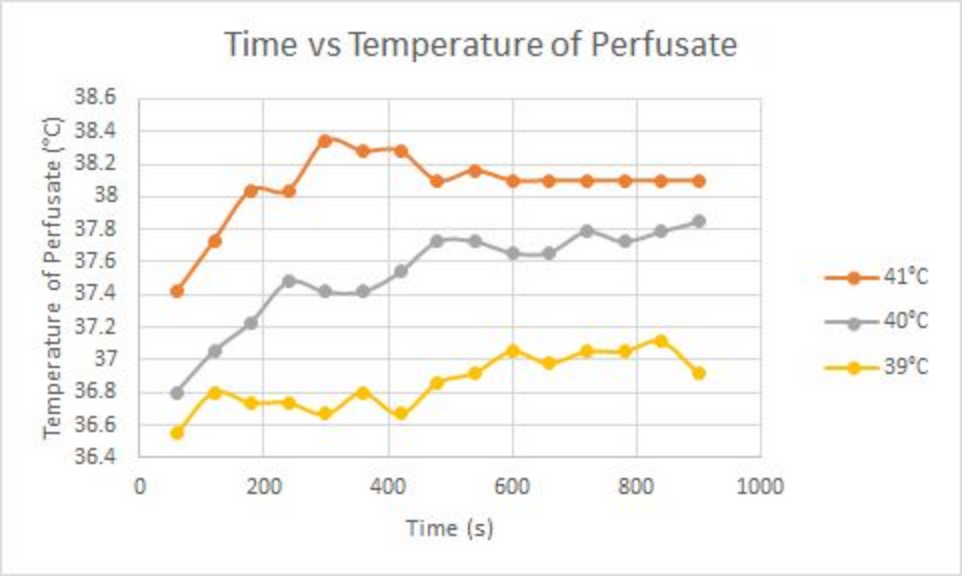

Figure 13. Graph of preliminary water bath testing data from Figure 12. Data was collected every minute over the course of 15 minutes. Ultimately this helped the team conclude that 39 °C would be the optimum temperature to achieve a 37°C output temperature of the perfusate solution entering the cannulated heart.

Appendix G - Thermistor Calibration Data

Raw Data/Graph

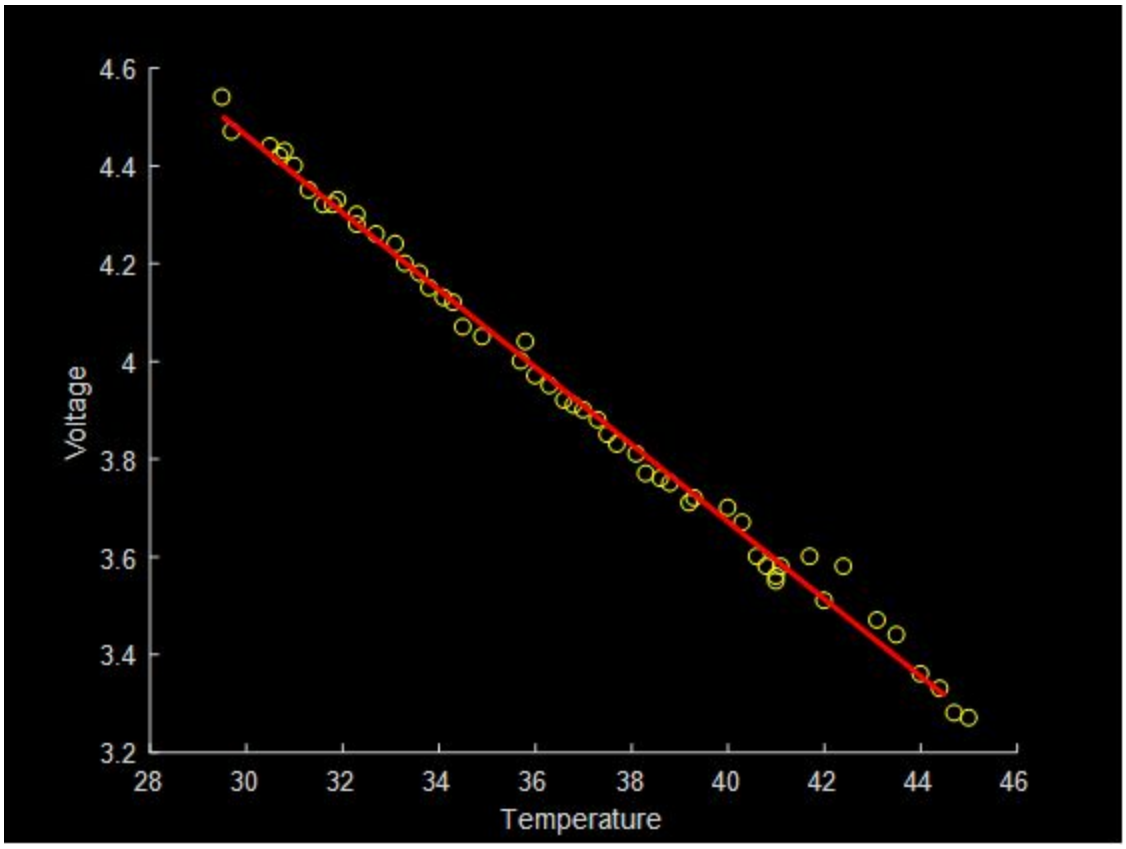

Figure 14. The relationship between temperature and voltage determined during the thermistor calibration procedure. The data yields an R-squared of 0.994 and the resulting equation used in the arduino code T = -12.6542*V+86.4625 where T represents the Temperature in degrees Celsius and V represents voltage read by the arduino through the analog input.

Final Arduino code

```
float raw = 0;
void setup()
{ Serial.begin(9600);
  pinMode(8, OUTPUT);
  pinMode(9, OUTPUT);
  pinMode(10, OUTPUT);
  pinMode(11, OUTPUT);
  pinMode(12, OUTPUT);
for(float i=1;i==3; i++){
  digitalWrite(8, HIGH);
  digitalWrite(9, HIGH);
  digitalWrite(10, HIGH);
  digitalWrite(11, HIGH);
  digitalWrite(12, HIGH);
  delay(100);
```

```
 digitalWrite(8, LOW);
  digitalWrite(9, LOW);
  digitalWrite(10, LOW);
  digitalWrite(11, LOW);
  digitalWrite(12, LOW);
  } }
void loop()
{
  raw = analogRead(A0);
  raw = (5*raw)/1023;
  float T = -12.6542*raw+86.4625;
  Serial.println(T);

if(T > 36.75 && T < 37.25){
  digitalWrite(8, LOW);
  digitalWrite(9, LOW);
  digitalWrite(10, HIGH);
  digitalWrite(11, LOW);
  digitalWrite(12, LOW);
}
if(T > 36.5 && T < 36.75){
  digitalWrite(8, LOW);
  digitalWrite(9, LOW);
  digitalWrite(10, LOW);
  digitalWrite(11, HIGH);
  digitalWrite(12, LOW);
}
if(T < 36.5){
  digitalWrite(8, LOW);
  digitalWrite(9, LOW);
  digitalWrite(10, LOW);
  digitalWrite(11, LOW);
  digitalWrite(12, HIGH);
}
if(T > 37.25 && T < 37.5){
  digitalWrite(8, LOW);
  digitalWrite(9, HIGH);
  digitalWrite(10, LOW);
  digitalWrite(11, LOW);
  digitalWrite(12, LOW);
}
if(T > 37.5){
  digitalWrite(8, HIGH);
  digitalWrite(9, LOW);
  digitalWrite(10, LOW);
  digitalWrite(11, LOW);
  digitalWrite(12, LOW);
}
}
```