**Cardiomyocyte Isolation Langendorff Apparatus**
**Preliminary Product Design Specifications**
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**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 calcium-free Tyrode, Tyrode, and low calcium 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 7 mL per minute [1]. If designing a constant pressure Langendorff system, the pressure must be approximately 70-80 mm Hg [2]. Additionally, the apparatus must allow for the perfusion solutions to be maintained at a temperature of 37 degrees Celsius [1]. The system must also be able to function without mechanical or operating error for a minimum of ten minutes. Furthermore, care should be taken to ensure that components of the perfusion apparatus can be easily sterilized 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 polyethylene and the other materials must be resistant to reacting and degrading from the Tyrode solution, calcium free Tyrode and low calcium solutions.

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 doc in the Gail A Roberson lab.

[1] J. Tytgat, "How to isolate cardiac myocytes", Cardiovascular Research, vol. 28, pp. 280-283, 1994.
[2] R. M. Bell, M. M. Mocanu, and D. M. Yellon, “Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion,” Journal of Molecular and Cellular Cardiology, vol. 50, no. 6, pp. 940–950, Feb. 2011.
[3] "Scisense Pressure Technical Note", Transonic.com, 2016. [Online]. Available: https://www.transonic.com/resources/research/ex-vivo-isolated-langendorff-heart-model-for-cardiac-assessment-using-pressure-measurements/. [Accessed: 21- Sep- 2017].