BME489 PROJECT PROPOSAL
ORGAN GROWTH CHAMBER

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Client: Dr. Ian Rogers

Date: September 28, 2016

Executive Summary

Bioengineered organs are being researched as a potential solution to the costly problem of donor organ shortage. Currently, the majority of the 123,000 patients waiting for an organ in the US are waiting for a kidney at significant cost to the health care system. Accordingly, this proposal outlines a system for research use at Mount Sinai Hospital to develop a platform for porcine kidney recellularization and culture that could one day address this shortage. A prototype system for kidney recellularization and culture is currently in use at the Lunenfeld-Tanenbaum Research Institute (Mount Sinai Hospital) due to lack of a commercial one. Given the cost of $35,000 to recellularize one porcine kidney, it is crucial to have a more robust and reliable system than the prototype to improve control over organ culture conditions. A system is needed to allow for the seeding of cells into a decellularized kidney under controlled vacuum in a chamber, and subsequent organ culture in the same chamber under controlled oxygenated media perfusion. It is crucial that such a system maintains sterility throughout the organ culture process.

The proposed design includes an improved organ culture chamber able to withstand vacuum, as well as five ports in the chamber for media recirculation, an oxygenator to oxygenate media to reduce organ damage due to necrosis, a bubble trap to prevent emboli, a peristaltic pump to maintain media, a pressure gauge to monitor pressure in the chamber, and medical grade tubing to connect all the system modules. The modular nature of the system permits for future modifications and improvements. The estimated cost of the proposed design is $750, with $500 allocated to producing a suitable organ culture chamber as it is a core component of the system. Available pumps and oxygenator will be used in the design initially but can be easily replaced if the client chooses to purchase new equipment.

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Project Description

Background and Motivation

In the United States, more than 123,000 patients are on the waiting list for an organ transplant [1], the majority of whom are waiting for a kidney [2]. Patients on the waiting list typically receive dialysis at a significant cost to the health care system, up to $89000 per patient per year, and as a result treatment of patients with end-stage renal disease accounts for 1.2% of total health care expenditure in Canada [3] [4]. With a worldwide organ shortage, many patients - nearly 6000 in the US in 2014 - die on the waiting list while the rest pose significant economic burden [1] [2]. Bioengineered organs could address this shortage, with the potential added benefit of saving patients from a lifetime of anti-rejection medication as organs could be generated from a patient's own cells [1].

In 2008, Ott et al created a system that was able to successfully decellularize a rat heart, opening the doors for generating a biocompatible organ in the lab [5]. More recently, in 2015, Uzarski et al produced a bioreactor capable of maximizing cell seeding and maintaining cell survival in extended culture for rat liver and kidney tissue [6]. However, this study did not examine kidney function, and the ability to scale the organ to a human sized model is a key unaddressed consideration. Groups have previously created organ growth chambers by modifying existing laboratory equipment such as Falcon™ tubes or Nalgene™ bottles. However, these systems are too small for porcine kidney culture (a human-sized model system) and thus a new chamber must be developed. Dr. Ian Rogers' group at Mount Sinai Hospital has been developing a system for this purpose and has modified a Tupperware® container to include a port for creating a vacuum during cell seeding, and several input and output lines which connect to a pump which recirculates media. Challenges faced by this system include lack of media oxygenation (which can lead to necrosis in the recellularized organ), bowing of the lid upon vacuum application, and minimal ability to sample key parameters such as oxygen level and pH [6]. These challenges limit the ability of researchers to achieve consistent, successful recellularization, and

lack of monitoring precludes the determination of the cause of failed recellularization. As such, a system that addresses these challenges is necessary to create recellularized organs for in vivo experimentation and ultimately transplantable organs which could save human lives.

Project Goal

The goal of this project is to develop a device to facilitate porcine kidney recellularization. A system that permits close monitoring and control of conditions, such as pressure, media oxygen content, and pH during recellularization could lead to a more viable recellularized kidney construct.

Project Requirements

Functional Requirements	
1	System must be able to withstand and sustain vacuum of at least -40mmHg gauge pressure for a minimum of 30min. The ability to sustain vacuum is essential for even cell seeding.
2	System must be able to fit one full porcine kidney submerged in media. The system is intended for research on scale up of the recellularization protocol, and a porcine kidney is the model.
3	System must allow for media recirculation at rates up to at least 150 mL/min. Media must be circulated to ensure cell growth and potentially to allow for recellularization of the vasculature
4	System must be sterilizable and maintain a sterile environment once recellularization is in progress. In order to successfully culture an organ, the device must remain free from bacteria.
5	System must have a minimum of five in/out ports that can be sealed when not in use. Ports will be used for seeding cells, recirculating media and creating a vacuum environment.
6	System components that are to be placed in an incubator must be able to withstand 37°C in 5% CO_2, 95% relative humidity for at least 15 days.
7	System must allow both cell seeding and subsequent organ culture in the same vessel. Moving the kidney would potentially expose it to bacteria and/or disrupt cell attachment and growth.
8	System must allow air bubbles to escape from the media before it is perfused into the kidney. Air bubbles must be removed to ensure perfusion is not hindered, as this could disrupt cell growth.
Constraints	
1	System must not require regular manipulation of the organ undergoing recellularization. This will help ensure sterility of the culture system.
2	System modules that require aseptic handling, must not exceed the total size of 18” in height and 12” in the other dimensions to ensure they can fit into a biosafety cabinet.
3	Total cost of producing the system must not exceed $750.
	Objectives

1	System should allow for media sampling with low contamination risk. Sterility is crucial for successful organ culture.
2	System should allow for media oxygenation (to prevent necrotic cell death in the tissue).
3	System should be modular. Modularity would allow for easy modification as needed.
4	System parts should be either off-the-shelf components or readily available for custom order. This will allow for easy modification as needed.
5	System should allow for closed loop circulation of cell suspension. This will allow for experiments on seeding renal vasculature.

Validation and Acceptance Tests

Functional Requirement Validation and Testing	
1	Seal system, connect to vacuum pump, and apply vacuum until pressure gauge reads -40mmHg. Assess ability to sustain vacuum. Assess system components for any structural failure.
2	Place porcine kidney in culture vessel and fill with media. If no overflow, the system passes.
3	Fill the culture vessel with media and connect system to a perfusion pump. Circulate media at rates up to 150mL/min. Inspect system for leakage or other signs of failure.
4	To check for sterility, sample media from the system over a minimum 3 days and place media samples in agarose gel. Assess bacterial colony number after 24h and 48h incubation.
5	If system has 5 media/air flow ports, the system passes. Place closed valves or stoppers in the ports when vacuum is applied.
6	Place required system components in a cell culture incubator for 15 days. Check for corrosion or mechanical failure. Alternatively, obtain confirmation from manufacturer stating the component is rated for performance under incubator conditions.
7	If the kidney must be transferred between vessels after seeding, the system fails
8	Visually inspect the media being perfused into the kidney for air bubbles for 5 min to ensure no air bubbles are observed. Perform the test over the entire range of flow rates.
Constraint Validation	
1	If the kidney undergoing recellularization is physically disturbed between being placed in the organ culture vessel and the end of the organ growth period, the system fails.
2	If the system cannot fit into a biosafety cabinet, the system fails.
3	If the total project cost exceeds $750, the system fails.
Objective Validation and Testing	
1	After media sampling, obtain another media sample, place a few drops of the sample on agarose plates, and count the number of bacterial colonies after 24h and 48h of incubation.

2	If the system permits an oxygenator to be connected, the system passes.
3	If modules can be replaced without affecting the system, the system is modular and passes.
4	If all parts can be readily ordered from supplier stock or custom-made, the system passes.
5	If media does not leak out of closed loop, the system passes.

Technical Design

Possible Solutions and Design Alternatives

Design 1: The Ideal All-in-One System

A self-heating organ culture chamber with built-in pH meter, oxygenator, oxygen sensor, media reservoir, perfusion pump, thermostat, oxygen and CO2 control would be the ideal design (see Figure 1A). This system would feature the suspended organ undergoing recellularization in media to prevent flattening of the organ under its own weight. Integrated sensors would display physiologically and experimentally relevant measurements in real time without need for media sampling. A user interface would allow the user to check and adjust any relevant parameters. This type of system would be self-contained, not requiring the use of external pumps or an incubator, and thus could permanently reside in a biosafety cabinet. All components of the system that come in contact with media would be autoclavable. Unfortunately, such a system is beyond the scope and budget of this project.

Design 2: Current "Standard"

This design is a direct derivative of the current Tupperware prototype used by Dr. Rogers. In this system, the organ undergoing recellularization is laying on the bottom of the culture vessel which has several ports drilled in the side to allow for connection to the pumps (vacuum and perfusion) (see Figure 1B). This system fails to address the issue of the organ flattening under its own weight during recellularization, which is not physiologically representative and thus should be avoided. Also, this design does not include an oxygenator, which may lead to necrotic cell death in the tissue.

The improvement over the current design would be the growth chamber, which would be designed to be autoclavable, more sturdy and to ensure ease of tubing installation. While the current

"standard" design requires tubing to be pulled through holes in the walls of the glass culture vessel, the improved design would include ports allowing quick connection of the tubing to the vessel. In addition, the improved design would have a sturdy glass lid which would allow the vessel to better withstand the vacuum applied during cell seeding.

Exclusive focus on improving the growth chamber makes this design easily achievable. Furthermore, this design would improve the ease of use and reliability of the system by allowing the user to quickly set up the organ for culture while being better able to withstand the vacuum applied during seeding. However, lack of active oxygen delivery limits this design's potential for success.

Design 3: Breathing Floating Kidney

This design is an intermediate between the previous two. While not an all-in-one system, it is a modular system that permits easy future modification. This system depends on an incubator to remain at 37°C, and on an external perfusion pump to recirculate media (see Figure 1C). A built-in pressure gauge would permit pressure monitoring during vacuum application. In this system, the culture vessel is re-designed as in Design 2 to permit easy tubing connection and improved performance under vacuum. Inlet and outlet ports will be designed with valves and Luer locks to allow redirection of the flow without having to remove the kidney from the growth chamber, which would allow various seeding routes, and both closed and open loop media circulation. This versatility provides the researchers freedom to optimize media flow patterns over the course of recellularization. The vessel is also designed to allow the kidney to be submerged in media so that it is experiencing at least some buoyancy, preventing flattening. In addition, an oxygenator is connected to the system to oxygenate media before it is pumped into the kidney along with a bubble trap to prevent emboli, which can prevent complete perfusion of some kidney sections. To summarize, this design should improve ease of use, be more durable and provide crucial oxygenation to improve kidney recellularization.

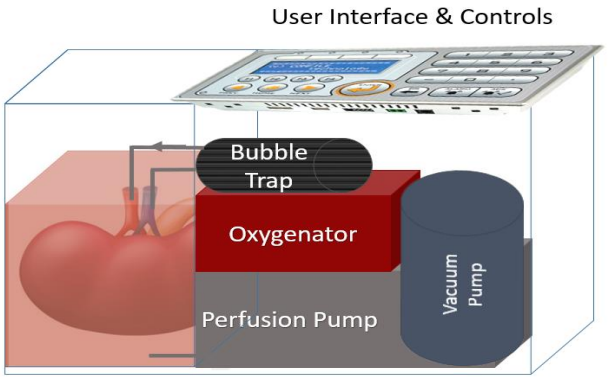


Figure 1A. The ideal system would be self-contained, requiring no external vacuum, oxygenator, heating, perfusion pump, or sensors (pH, oxygen). Such a device would be convenient as it would provide easy control and monitoring of all organ culture conditions.

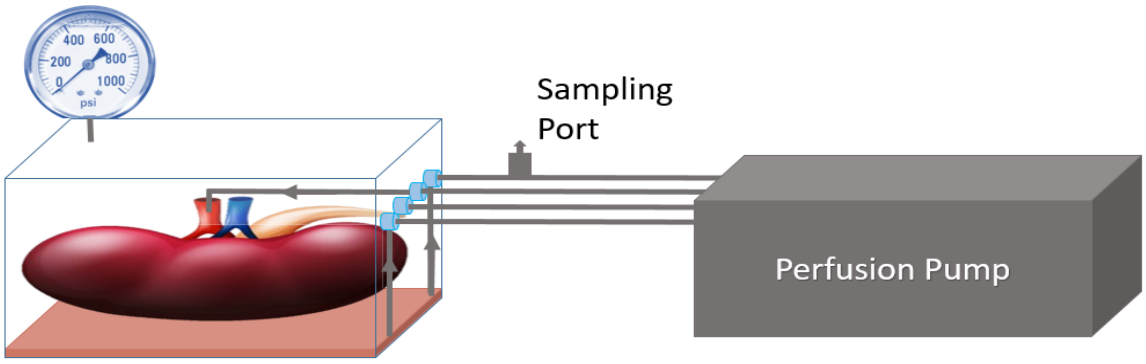


Figure 1B. The current "standard" involves a modified Tupperware container used to house the kidney, connected to a perfusion pump and pressure gauge. Such a system permits the application of vacuum but does not withstand vacuum well due to weak structural components. A lack of media oxygenation also leads to tissue death due to necrosis.

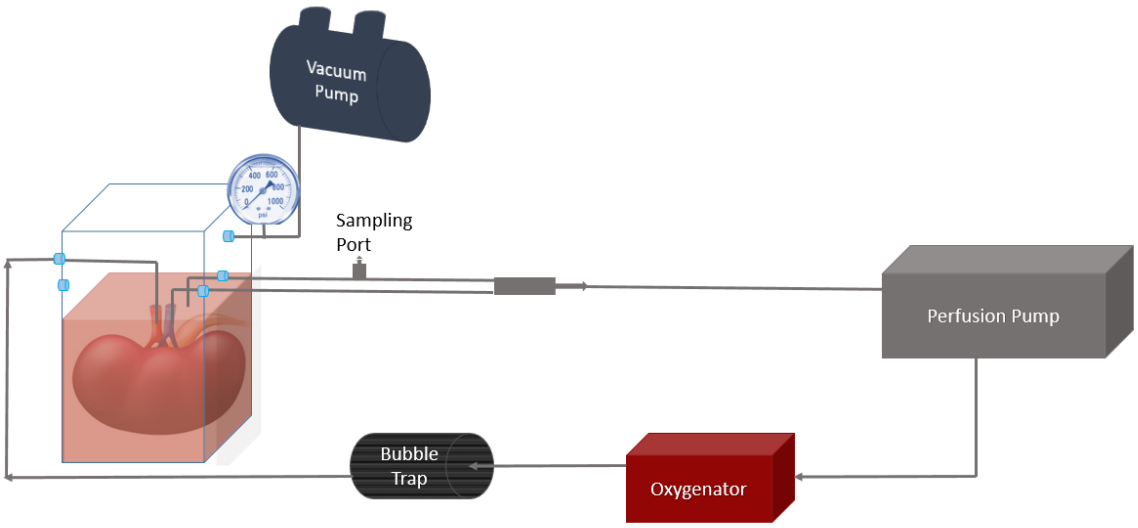


Figure 1C. While the ideal design incorporates all desirable functions into one self-contained unit, the current standard provides very few of the desired features. Therefore, an intermediate design is proposed that improves the organ culture chamber to withstand vacuum and adds oxygenation to the media while providing a modular, easily upgraded system for future modifications.

Assessment of Proposed Design

Consideration of the three design alternatives outlined above within the context of the project requirements and time frame led to the conclusion that the third concept, in which the growth chamber is incubator-dependent and the kidney is suspended within the chamber, was the most appropriate. Building incubator capacity into the design, as described in the first alternative, would considerably increase the convenience of using the device by eliminating the need for a dedicated incubator for the duration of the experiment. However, since enabling inbuilt incubation requires both heating the media and maintenance of an elevated humidity and 5% carbon dioxide environment within the chamber, it is unlikely that this functionality would be implementable within the time frame of the project. Moreover, it is unlikely that this functionality would substantially increase the ability of the device to produce viable organs, whereas controlling other aspects of the chamber environment (oxygen content, pH) might produce better outcomes in this regard. The second and third designs are principally differentiated by the suspension of the kidney and the addition of oxygenation. The client reports that previous attempts at kidney recellularization using a non-suspended kidney resulted in the flattening of its chamber-contacting face, which is undesirable as nutrient and oxygen delivery may be affected, and functional deficiencies may occur. Suspending the kidney in media should remedy this concern. Furthermore, the implementation of media oxygenation should decrease necrotic or abnormal tissue compared to the current standard and the second proposed design, allowing for improved potential organ function overall.

System-Level Overview

The system consists of a culture chamber made of laboratory glass that acts as a suspension vessel and includes five ports in the sides of the container. The chamber will be connected to the vacuum pump with a pressure gauge attached to the vessel in order to monitor the pressure inside the chamber, which will be controlled by an adjustable valve. The lid and ports will be sealed to ensure a

closed system. An external perfusion pump will be connected to the ports, which connect to the kidney vasculature, to circulate media. An oxygenator will oxygenate perfusion media to prevent tissue death due to necrosis. A bubble trap will be attached to ensure removal of bubbles that could affect tissue viability. A three-way port will be connected to the tubing to allow media sampling. Figure 1C provides an overview of the proposed system.

Module-Level Descriptions

- External perfusion pump allows for delivery of media and oxygen into the kidney at a controlled rate using tubes that connect to the inlet and outlet of the bioreactor chamber.
- Glass chamber houses the kidney and protects it from the nonsterile external environment. The chamber contains ports that connect the perfusion system to the kidney vasculature. The media in the chamber will provide buoyancy to the kidney and prevent it from flattening.
- Oxygenator adds oxygen to the perfusion media to prevent necrosis of the kidney.
- Bubble trap allows for removal of air bubbles which may compromise media flow in the kidney.
- Sampling port allows for sampling the media to monitor media conditions non-invasively.

Work Plan

Work Breakdown Structure

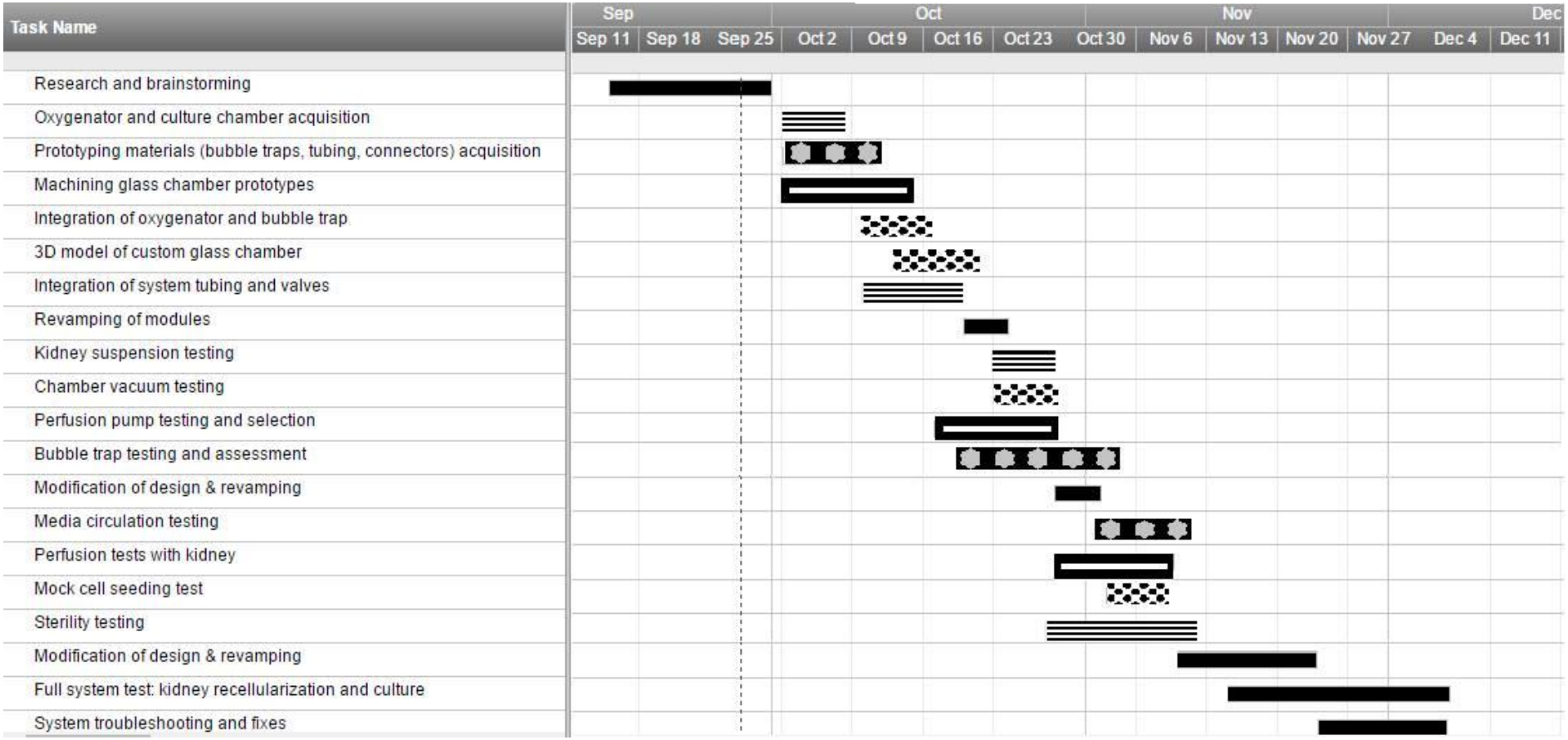
Task	Task	Alexander	Anastasiya	Natalie	Niema
#	**Materials and Equipment Acquisition**				
1	Acquisition of media oxygenator		R	A	
2	Purchase of tubing and accessories			R	A
3	Purchase bubble trap or components	R			A
4	Purchase and/or modify organ culture chamber		R	A	
	Fabrication				
5	Machining of glass chamber prototypes	A		R	A
6	Attachment of ports and tubing to chamber		R	A	
7	Connection and integration of oxygenator	R	A		

8	Design bubble trap, if not purchased	A			R
9	Bubble trap construction and integration	R			A
10	3D model for custom chamber, if necessary	A			R
	Component Testing/Evaluation				
11	Kidney suspension/flotation in media test		R		A
12	Vacuum tolerance test on chamber			A	R
13	Test two available pumps, make selection	A	A	R	A
14	Flow-through test without kidney	R			A
	System Testing/Evaluation				
15	Connection of kidney tubing	R	A	A	A
16	Re-assess flow rates with kidney resistance			R	A
17	Mock seeding under vacuum		A		R
18	Sterility testing	A	R		
19	Assessment of bubble trap efficacy	R			A

Feasibility Assessment

Our design is intended to provide an organ growth chamber which will allow scientists to further their work on developing a reliable kidney recellularization protocol and platform. This particular application creates unique feasibility concerns for the project which are addressed in subsequent sections.

Gantt Chart



Legend

- All group members
- Anastasiya Martyts
- Natalie Landon-Brace
- Alex Gulka
- Niema Binth Mohammad

Financial Plan

Item	Vendor	Cost	Buyer	Justification	Item Priority and Contingency Plan*
Glassware and Plasticware	Ikea, Home Hardware	$20	NLB	Glass vessels are needed to develop prototype of the culture chamber.	Priority: 3. Back-up: team members contribute any suitable glassware they may have at home
Diamond drill bit	Canadian Tire	$20	AM	To cut ports in glassware.	Priority: 3. Back-up: other drilling techniques may be attempted to machine glass
Various sizes of tubing	Cole-Plamer, Med Store,VWR,	$15	AG	To connect system modules, both in prototypes and final product.	Priority: 2. Back-up: attempt to repurpose tubing found in available labs
Tubing connectors, adapters, ports, valves, etc.	Med Store, VWR, Fisher Scientific	$95	AM	To connect system modules; ports for media sampling; valves for directing flow	Priority: 1. Back-up: use components from hardware stores- cheaper but not autoclavable, which may increase operational cost.
Misc. supplies	Dollarama	$10	NM	Supplies for prototyping	Priority: 5. Back-up: members contribute their own
Organ growth chamber	Corning	$500	All	If custom-made, the chamber will be autoclavable and reusable - an important objective.	Priority: 1. Back-up: single-use laboratory plastic (custom may be difficult to obtain) or modified autoclavable, off-the-shelf products.
Oxygenator (single unit or membrane & oxygen tank)	Radmoti, Medtronic, Surgimedics	$0	Client	While an oxygenator will likely be borrowed for the duration of this project, the client may choose to purchase one for permanent use.	Priority: 1. Back-up: if no oxygenator is available, the system can be constructed with ports to connect an oxygenator at a later time. This may require modification to the testing plan as only short-term system testing will be achievable.
Bubble Trap		$80	NM	To allow air bubbles to escape from perfusion media.	Priority: 2. Back-up: a handmade bubble trap can be used, but such a device would be less reliable and durable.
Perfusion Pump		$0	Client	To circulate media	Priority: 1. Back-up: one of client's pumps will be used or borrow from another lab.
Pressure Gauge		$10	AG	To measure vacuum during cell seeding.	Priority: 2. Back-up: testing can be conducted to determine threshold vacuum levels, which can be used for the duration of experimentation.
Testing Facilities and Supplies	N/A	$0	N/A	Materials and facilities will be required to test the design.	Priority: 1. Back-up: System can be tested at a U of T lab.
DesignSoftware	N/A	$0	N/A	To design custom components	Priority: 4. Back-up: use U of T licensed software
TOTAL:		**$750**			*Priority rankings on a scale of 1-5, 1 = highest priority, 5 = low priority

Each group member is expected to contribute $187.50 to the project (to be reimbursed by IBBME). The client may purchase other high cost items that the client will retain.

Risk Assessment & Mitigation

The design and development of the organ growth chamber carries considerable risk. Firstly, the system is being developed for use in a recellularization protocol where the ideal operating conditions are still being determined [KARCZEWSKI]. Accordingly, even in a system which meets all of the requirements, it may be discovered that other conditions are needed for creating a viable kidney . Addressing these challenges is beyond the scope of the project. It is, however, important to consider that even an organ chamber which improves on the current system and meets all requirements may not result in successful recellularization. In this case, the goal of the project should be shifted to providing researchers ability to improve their protocols. Furthermore, a custom organ growth chamber may be beyond the budget, or require time to manufacture beyond the span of the project. This risk can be mitigated by purchasing an off-the-shelf laboratory glassware product and modifying it to meet project needs. Should this also be unattainable, laboratory plasticware can be modified to prototype the design. Another large risk to the project is the limited opportunity for functional testing due to the timescale and expense of organ culture. During testing, it may be determined that the system fails to meet key functional requirements, but time and budget constraints might limit ability to modify and re-test the system. Should the system fail to perform in key functional tests (without ability to conduct further testing), we will focus on creating a modified prototype which specifically addresses these deficiencies and create a detailed experimental plan for future testing with accompanying modifications to address potential shortcomings.

References

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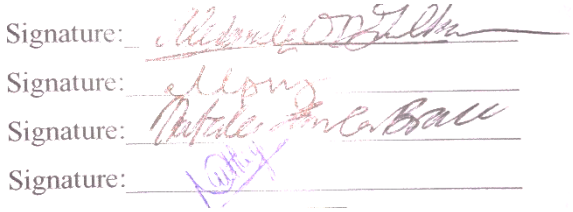
Appendix A - Report Attribution Table

AG - Alexander Gulka

AM - Anastasiya Martyts

NLB - Natalie Landon-Brace

NM - Niema Mohammad



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Figures and Diagrams	NM, AM