Supplementary Materials

Design Alternatives – Fall 2013 Semester

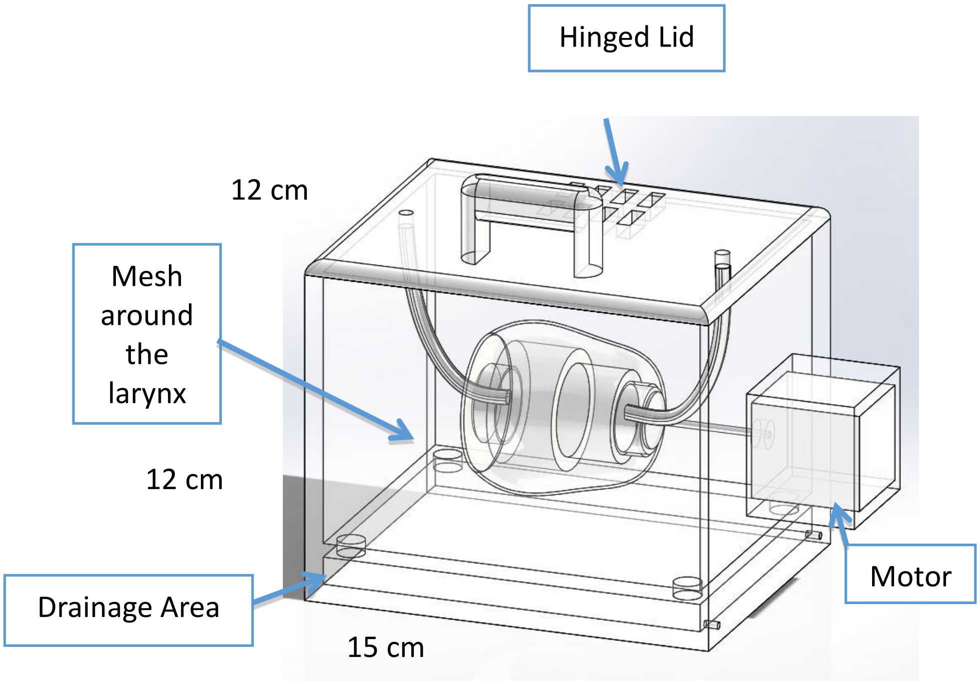

Figure 8: Design Alternative 1 – A plastic mesh covers the larynx and is attached to a stepper motor. Endotracheal and catheter tubes enter the device through holes in the lid, and separate slits allow air to penetrate into chamber.

Design Alternative One: Mesh Design

The first bioreactor design consists of a 12 cm wide by 12 cm high by 15 cm long box with a hinged lid. The box is made out of polycarbonate that is 0.5 cm thick. These dimensions were chosen to decrease the cost of the use of the bioreactor versus the previous design. As shown below, these dimensions reduce the volume of media used by up to 71% versus the previous design. This decrease will also result in a corresponding decrease in cost per experiment.

Table 1: Size comparison of the previous team's design to the new design

Design	Height (cm)	Width (cm)	Depth (cm)	Volume of Media needed to fill Device (cm^3)
Previous (without inserts)	25	15	20	7500

Current	12	12	15	2160

The hinged lid is designed with eight air holes to allow movement of air during recellularization and decellularization. The lid is on hinges that ensure that the lid is never misplaced and is always positioned correctly. Also, because the lid is removable, it allows easy access to the inside of the bioreactor. Easy access is important for placement, removal, and adjustment of the larynx and other internal features. The lid has a handle so that it can be pulled up by the researcher.

In the bottom three cm of the bioreactor there is a drainage area that is connected to the main area via drainage holes. The drainage area has a hole in the side of the bioreactor that connects to one of the pumps, which allows for the change of inner media. This pump will connect back to the top of the bioreactor to allow flow and recycling of the inner media.

The lumen of the larynx needs separate media that is constantly flowing. This design addresses this issue by including a tube that runs through the center of the larynx. This tube connects back up on both ends to the top of the lid and then to the second pump, enabling the constant flow of media. The section of the tube inside of the larynx itself will have slits or holes (depending on flow testing) that allow the media to perfuse through the inner lumen. This tube also has the additional function of providing structural support for the larynx during decellularization.

The main feature of this design that differentiates it from the other alternatives is a polymer mesh. This mesh surrounds the larynx, provides support, and enables the perfusion of media in and out. The mesh would be made out of high-density polyethylene or another suitable polymer. The mesh would be stiff enough to provide structural support, while also form-fitting the larynx. The mesh would scrunch up to the right of the larynx around a metal washer. To put the larynx in the mesh you would hold the larynx in place and then slide the mesh around the larynx. The metal washer on the right side of the mesh interfaces with the motor.

On the right side of the bioreactor there will be a 4x4x4 cm cube that houses the motor to control rotation of the mesh and larynx. The motor will be a servo or stepper motor. These motors allow fine angular control of rotation and can be controlled via basic programs and electrical work that could potentially be housed next to the motor. The motor could connect to a LCD screen, so the researcher has full control of the motor at the touch of a button. The full design can be referenced in figure 8 above.

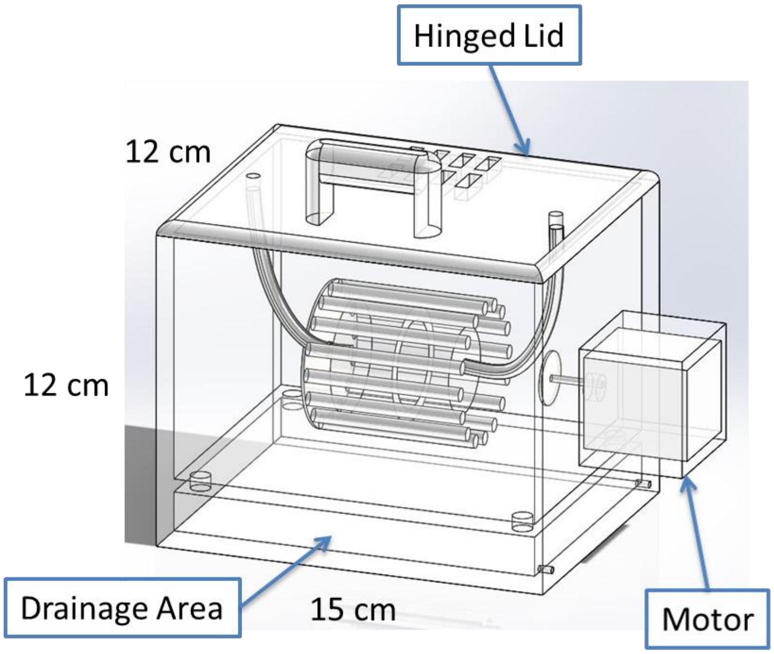

Figure 9: Design Alternative 2 – A cage attached to the motor surround the larynx. Tubes enter the device through the lid, drainage occurs through outlets at the bottom.

Design Alternative Two: Cage Design

The second design alternative features the same lid, 12x12x15 cm box size, motor, and drainage area as the first design alternative. This design can be seen in Figure 9 above.

The differentiating aspect of this design is the cage component. The cage is made out of firm plastic rods that are attached to each other via a circular plate on both ends (only the left plate is pictured in the image). This plate connects to the motor and enables the rotation of the cage and larynx. Because, the cage only rotates 90 degrees in each direction (clockwise and counter clockwise), the cage doesn’t rotate a full 360 degrees, thus avoiding tangling of the tubes. The device will be set up so that there is enough slack in the tubes delivering media so that these can be rotated with the larynx. Additionally, there is a gap on the top of the cage that allows the inner lumen tubes to go through to the top of the bioreactor. The cage design provides support to the larynx and the gaps between the cage bars allow free movement of the media in and out of the cage.

The cage is not as flexible as the polymer mesh and therefore is not as form fitting, so the larynx will be sutured to the cage with loops in two locations. This will keep the larynx in line with the cage as the motor rotates it and prevent extra movement of the larynx in the cage. Additionally, cage bars on the top of the larynx (from the

10 o’clock position to the 2 o’clock position) will be made to be removable so that the user can easily reach inside the cage from the top of the device to attach or adjust the organ.

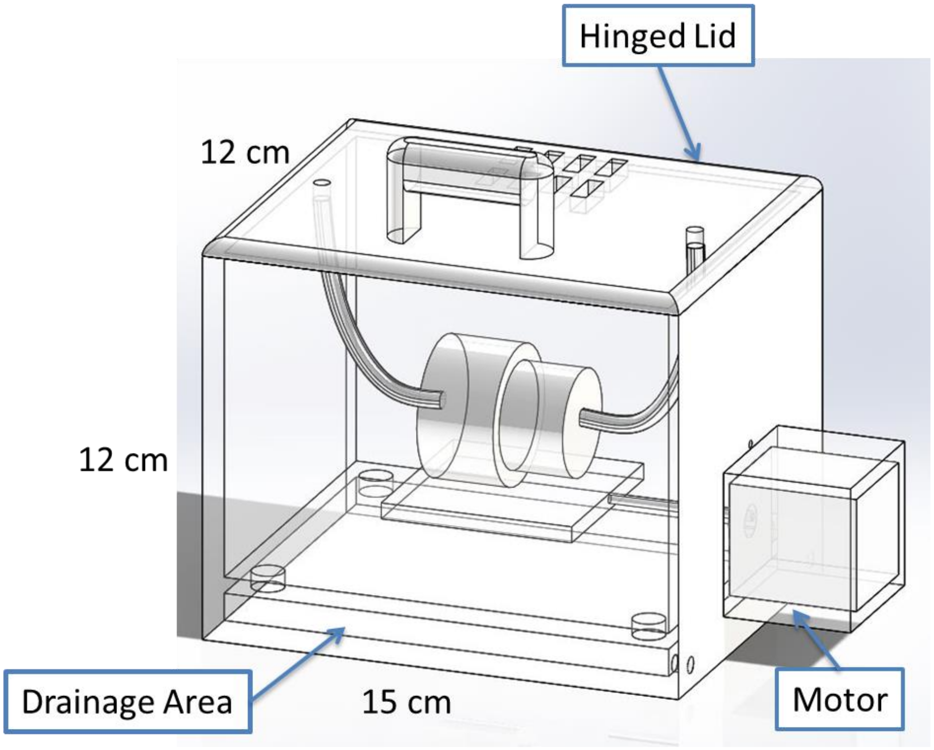

Figure 10: Design Alternative 3 – The larynx is pinned to a plastic table attached to the stepper motor. An additional rod is used to support the weight (not shown).

Design Alternative Three: Operating Table

The third design alternative features the same lid, 12x12x15 cm box size, motor, drainage area, and inner lumen tubes as the first design alternative. This design alternative, described below, can be seen in Figure 10 above.

This design is set apart by the platform rotation mechanism, upon which rests the larynx. The larynx is pinned in four places to the platform, which has little screws on the corners to anchor the pins in place. The platform itself would be made out of polycarbonate. On the right side of the platform a rod connects the motor to the platform. This rod, when rotated by the motor, would tip the platform from side to side and along with it the larynx. The motor will only allow 90-degree rotation in each direction of the platform. This ensures that each side of the larynx is covered by media half the time and prevents the tubes from tangling. The platform has another rod extending from the left side that connects to the side of the bioreactor and provides additional support.

Design Matrix Analysis

Table 2: Design matrix for the three design alternatives for the laryngeal bioreactor. The highest scores per category are highlighted in a darker color. The cage design garnered the most points.

	Weight	Mesh		Cage		Operating Table	
Precision of turning	5	2	2	3	3	4	4
Safety	5	4	4	4	4	2	2
Ease of use	10	3	6	4	8	3	6
Manufacturability	10	4	8	3	6	4	8
Cost	10	3	6	3	6	2	4
Compatibility with various environments	10	3	6	3	6	3	6
Durability	15	2	6	4	12	3	9
Support	15	2	6	4	12	5	15
Access to media	20	3	12	4	16	1	4
Sum	100	56		**73**		58	

The three design alternatives were evaluated on nine different criteria. Precision of turning and safety were given low weights since fluids will be perfused through the entire vasculature of the larynx and because there are no dangerous components in the designs. The operating table design scored highest in precision because the larynx will be rigidly pinned to the surface, whereas in the mesh design the larynx would have the ability to slide slightly. The operating table makes use of pins to attach the larynx to the device, which increases the chance for accidental injury.

Ease of use for the client, ability for the team to manufacture the device, cost, and compatibility with different lab environments were all weighted the next lowest following safety and turning precision. The cage mechanism was deemed easiest for the client to use, but most difficult for the team to manufacture. The cage allows the user easy access to the larynx and tubes due to the removable cage components and the fewer attachment points; however, the mesh and table could potentially be bought, and the cage will most likely need to be custom fabricated. The cost of the table design was predicted to be the highest, as it would require more support and possibly a stronger motor for the entire table to be turned. In regards to operating environment, all three designs must function in a standard lab environment, a refrigerator, and an incubator as per the product design specifications. For this reason, all three designs scored equally in this category.

The highest weighted criteria were durability of the device, support of the larynx during the process, and the ability for the larynx to access media (which was weighted slightly higher). The device is used for up to three weeks at a time, and therefore must be durable enough to not break during that period. Furthermore, the geometry of the larynx changes throughout the three weeks, especially as it decellularizes. It is important that the design supports the larynx during this time. Most importantly, the larynx must have access to media and surrounding fluids in order for the decellularization and recellularization to be successful.

The mesh design received the lowest score in both durability and support categories. The mesh will not be as durable as the other designs since it would be made of a flexible polymer and have the potential to tear. In addition, it would not provide any rigid support and could possibly allow the larynx to sag. The cage was determined to be the most durable, as pins would not be inserted into it like in the table design and because the motor would have less strain on it. The table was considered to be the most supportive because it reinforces the entire length of the larynx. The design that allowed for the greatest access to media was the cage design. The operating table design would pin the entire dorsal side of the larynx to the table and would not allow media to effectively reach this area. In a similar fashion, the mesh would have to be relatively tight and would thus leave small areas where media could not diffuse into the larynx. For this reason, the cage was given the highest score for access to media.

Final Design – Fall 2013 Semester

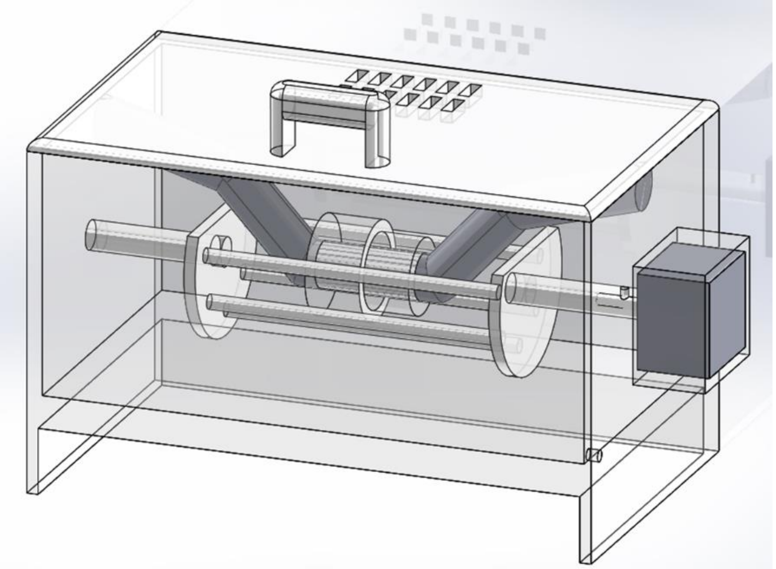

Figure 11: Solidworks of final design: Final design showing rotatable cage with larynx and tubes displayed.

Compiling the results of the design matrix analysis reveals that the cage design is the best choice to adequately meet the needs of the client. This design received the most points overall, and high scores in the three highest weighted categories. The lowest score it received was in manufacturability, as it will most likely need to be fabricated as opposed to purchased and assembled. However, this had a minimal effect on its score and the cage design is ultimately the design the team will move forward with.

To reiterate, the cage design has a semi-circular shape (when the top bars are removed) that allows the user easy access to the tubes and larynx. The tubes have a small chance of decannulating during rotation, so it is important that the client can remedy the situation easily. Rotation of the cage 90 degrees in either direction exposes all surfaces to the exterior media, while continuing to support the larynx as its geometry changes over time. The plastic sides of the cage will be durable enough to withstand the weight of the larynx, while light enough to refrain from stressing the motor and cage attachment. The Solidworks model for the final design can be found in figure 11 above.

Testing

This semester, several tests were conducted on different portions of the prototype in order to determine their individual efficacy and reliability as well as to optimize their use within the final prototype. These tests helped to ensure that each component in the prototype works effectively and predictably alone. In the future, the team will focus on testing that incorporates all aspects of the design and will evaluate how these different components function together to form a complete, functional prototype.

Pump Correlation and Initial Flow Rates Testing

The first test, which examined pump flow rates, was conducted for two reasons. First, the flow rates of the low flow rate peristaltic pump are displayed in rotations per minutes instead of mL/minute. This is not a very useful unit of measurement for characterizing flow through the tissue; thus, it was desired to develop a mL/min flow profile of the pump. Second, though pump speeds will be constant over long periods of time, initial pump speeds will seem slower because the pumps need to fill the tubing before they can perfuse media through the tissue. This could potentially be problematic if not accounted for because if the pumps are not turned on early enough, the tissue could be lacking in media for longer than expected, which could lead to cell death. Therefore, this test was used to develop “initial flow rates” for the pumps, or more literally, the time taken for the pumps to fill up the tubing that will be used in the device plus the maximum foreseeable volume of the area that they will be filling up.

Procedure

Each pump was tested separately. For each pump, a large water reservoir was placed at one end of the empty tubing, and the other end of the tubing was secured to a 15 mL beaker for the small pump, and a 50 mL graduated cylinder for the large pump. Then, beginning at the maximum speed for each pump (100 RPM for the small pump and 64.53 mL/min for the large pump), the time taken for the pump to fill up the tubing plus a specified volume (10 mL for the small pump and 25 mL for the large pump) was measured. The speed of the pump was then decreased by 10, (10 RPM for the small pump, and 10 mL/min for the large pump) the tubes were emptied of fluid, and the test was repeated. The measurement was conducted at each speed 3 times. A table shows the differences in procedure for the large and small pumps below.

Table 3: Pump Differences: Several key differences between the two pumps used in the prototype, including volume of fluid measured in testing and maximum speeds, were accounted for in the pump testing.

Pumps	**Small (Vasculature)**	**Large (Inner lumen)**
Speed display units	RPM	mL/min
Maximum speed	100 RPM	64.53 mL/min
Volume measured for testing	10 mL	25 mL

Results and Discussion

As expected, the small pump displayed a linear relationship between RPM and flow rate. Additionally, as expected, the “initial flow rates” of the pumps are slower than the displayed flow rates, but the difference between the initial flow rate and the displayed flow rate decreases with increasing pump speed. Somewhat interestingly but not unpredictably, this decrease in time correlates to a logarithmic trendline. This is most likely due to the fact that at higher speeds, the pumps fill the tubing more quickly, so there is a shorter lag time between when the pump is turned on and when fluid initially reaches the tissue. Graphical correlation between RPM and flow rate for the small pump can be seen in Figure 17 below. Additionally, correlations between the displayed flow rates and the time to fill the containers can be seen in Appendix D in supplementary Figures 1 and 2.

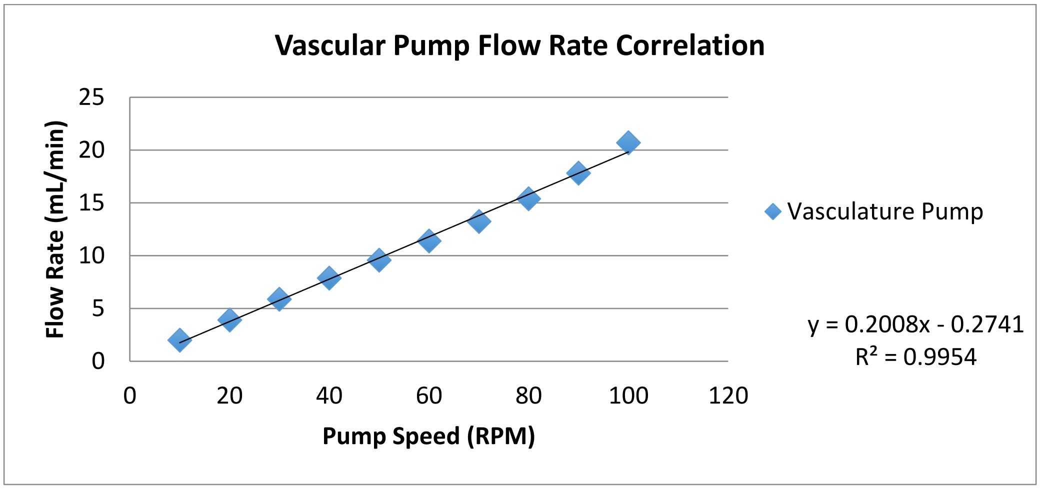

Figure 17: Vasculature Pump flow rate correlation: The pump that will be used to perfuse media through the vasculature in this prototype displays a linear correlation between displayed pump speed (in RPM) and flow rate (in mL/min)

Note: Error bars not shown because, since this test measures the output of digital devices, standard deviations are very small.

PWM and Flow Rate Correlation Testing

The second test conducted correlated the pulse width modulation (PWM) that was coded into the Arduino IDE program and the flow speed of the pumps as a result of this PWM. PWM, as explained above in the pump automation section, is a method that the Arduino uses to vary its analog output voltage between 0 and 5 V, and involves the continuous switching between these two values. PWM can be coded to be any whole number between 0 and 255, and each of these integers results in a different flow speed. Therefore, it is necessary to develop a correlation between PWM and flow speed so that the client can predictably program specific and predetermined flow speeds into the computer. Using this information, the client can more easily optimize flow rates through the tissue. It is important to note that this prototype is only intended to automate the large pump for perfusion through the inner lumen, but it is still useful to gather this information for the small pump in case changes need to be made in the design.

Additionally, the use of a low pass filter is necessary to smooth the analog voltage from the Arduino to the pump. However, the use of the low pass filter results in a reduction in the maximum voltage output of the device. This reduction will also result in a reduction of the maximum flow speeds attainable using the analog control. The extent of this reduction must be verified in order to determine whether a redesign of the low pass filter is necessary, or, conversely, if satisfactory pump speeds can be attained with the current low pass filter.

Procedure

Each pump was tested separately in the same manner. Beginning with zero and increasing by 25 each time, a PWM was coded into the Arduino IDE. This program, which turned on the pump, ran it clockwise at the specified speed for 1 minute, and then turned off the pump, was then uploaded to the pump. Finally, after the code was uploaded, the resulting flow rate displayed by the device was recorded. This test was conducted 3 times at each PWM.

Results and Discussion

For each pump, there was a very visible linear correlation between PWM and pump speed, which was observed to be consistent across multiple trials. This result illustrates that pumps respond in the same manner each time a voltage is applied to them, and demonstrates that pump function can be programmed in a predictable manner. Specifically, using this low pass filter design, pump speed can be predicted from the following equations:

$$y = 0.1941x + 0.0909$$

Equation 1: Small Pump Flow Rate to PWM correlation: Where y is the pump speed (in RPM) and x is the PWM input

$$y = 0.1247x + 0.2308$$

Equation 2: Large Pump Flow Rate to PWM correlation: Where y is the pump flow rate (in mL/min) and x is the PWM input

These equations are based off of trendlines fitted to a scatterplot of the data of this test. Both equations have an R^2 value of >0.999, which further demonstrates the linearity of the data. The scatterplots of this data with the associated trendlines can be seen in Appendix D, supplementary figures 3 and 4.

It is also important to note that the maximum flow rates achieved by these pumps using this low pass filter are approximately half of the actual maximum flow rates of the machines themselves. The small pump shows a speed of 48.5 RPM associated with a PWM of 250, while the large pump shows a speed of 31.5 mL/min associated with a PWM of 250. While it is not thought that these pump speeds will be problematic, if the clients desire a faster pump speed than this, the low pass filter will need to be redesigned, possibly with the use of an operational amplifier.

Additionally, after this test was conducted, the efficacy of the low pass filter was verified. When the pump was connected directly to the Arduino speed output, a transient speed was observed. This means that the speed did not stay constant over time, but varied rapidly over a range of approximately 4 mL. This contrasts sharply with the results seen above, and supports the hypothesis that the low pass filter is necessary for the proper operation of the prototype.

Motor Turning Testing

To verify the efficacy and consistency of the turning of the motor, the motor's ability to accurately and reproducibly rotate to multiple angles and back was tested. During the testing, the motor was programmed to turn 90, 120, and 180 degrees at varying speeds, and with varying pauses between each turn. The motor was checked at several intervals over each test to determine if it maintained continued accuracy in its turning. Thus, if differences became apparent, they would be compounded from the first observation to the last, demonstrating the inefficacy of the motor.

Procedure

In order to verify the consistency and precision of the motor, the following procedure was used. First, a drinking straw was affixed to the motor with painters tape. Next, the motor was secured to a standard sheet of white paper using painter's tape, and this paper was taped to a table, also using painter's tape. After that, a 180 (or 120 degree or 90 degree) degree line was drawn on the paper using a protractor, denoting the current position of the edge of the propeller. This experimental setup can be seen in Figure 18 below. Finally, the motor was programmed to turn 800 (or 533 or 400) steps every five seconds for fifteen minutes. Every five minutes, a group member paused the program and measured the distance from the propeller to the line on the page, thus, measuring the discrepancy between the motor turning distance and 180 degrees (or 120 or 90 degrees) of rotation. Additionally, at the 180 degrees (400 steps) direction, the stepping speed was varied, and trials at 400, 600 and 1000 us between steps were completed. Also at 180 degrees (400 steps) frequency between turning was varied, and trials with pauses for 1, 3, and 5 seconds were completed. It should be noted that the default trial was 180 degrees (400 steps) with a stepping speed of 1000 us between steps and a pause between turns of 3 seconds. From this default, only one variable was changed at a time.

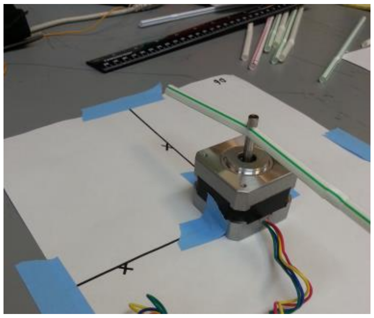

Figure 18: Experimental Setup for the motor turning test: This test demonstrates that the motor used in the experimental prototype consistently and predictably rotates at a rate of 0.225 degrees per step taken.

The results of this experimentation are insipid and are therefore not shown in quantitative format in this analysis. To summarize the experimentation, 0 deviation was seen in any trial during the experiments. The motor consistently and accurately turned to the direction desired under all conditions. This verifies that the motor is accurate for the purposes of the experimentation desired by the client. The clients also corroborated in this assertion of accuracy, claiming that the motor was functional for and accurate for the 4-5 days required for an experimental decellularization trial.

Reynolds Number Calculations

Many models model the area directly anterior to the closed vocal cords as a small cylinder with a 5.1 mm radius. Using the following equation to determine the characteristic length or hydraulic diameter (D_h):

$$D_h = \frac{4\pi r^2}{2\pi r}$$

Equation 3: Hydraulic Radius of a cylinder; where r is normal radius of the cylinder

The characteristic length can be determined to be 10mm. Using this data, the Reynolds number can be derived from the following equation:

$$Re = \frac{QD_h}{\nu A}$$

Equation 4: Reynolds number. Note that equations 1 and 5 are equivalent, but that, often, one is more convenient to use based on given parameters.

where Q is the volumetric flow rate, υ is the kinematic viscosity, and A is the cross sectional area of the section of interest. Using volumetric flow rates of 1mL/min, 15mL/min, and 50mL/min yields Reynolds numbers of 126; 1,903; and 6,344 respectively.

Ethics

As with any bioengineered tissue, there are several facets of ethical concerns associated with this project. First, xenographic tissue will be used in experimentation, and animal rights must be respected (according to the national guides for laboratory animal welfare) for bioreactor use. This means that any tissues used should not be wasted. Additionally, the bioreactor is intended to be used with allograft tissue that will be decellularized and reseeded with the patient's own adult stem cells. The donor of tissue that will be used must have their wishes respected and their privacy maintained if desired. Additionally, the use of adult stem cells, while less ethically objectionable than the use of embryonic stem cells, still leads to ethical concerns associated with the profit of a company from someone else's tissues, as well as the patients ownership of their own tissues (including patents) (21). In any case, the wishes and privacy of the patient should be respected in future applications of this product in order to avoid these issues.

Specifically for the case of the bioreactor itself, there is another set of ethical concerns. Any larynges could cause a great deal of harm to a patient if they are not properly decellularized and recellularized prior to implantation. If components of the bioreactor are not properly sterilized before use, as well as if the larynx is not grown in a sterile environment, unwanted pathogens could be introduced into the tissue, leading to a heightened immune reaction in the recipient of the tissue. Additionally, researchers must ensure that there is no longer any foreign media or non-laryngeal tissue in the regrown organ prior to implantation, as this could lead to complications for the recipient.

Budget

The client had no specified budget for this project. The team collectively decided, after looking at raw material costs, that a goal budget of $300 for an initial prototype would be appropriate. The materials for the structure of the bioreactor included sheets and rods of polycarbonate plastic, which ranged from $10 to $30 dollars, depending upon thickness and sizes. An Arduino Uno microcontroller was used to control the turning of the stepper motor and cage, and cost approximately $30. The price of the stepper motor and motor driver for the project also cost approximately $30. Finally, sealant and miscellaneous hardware components cost around $20. Thus, the materials needed to fabricate the bioreactor cost $230; a figure below the team's goal budget. The client, however, requested that the team purchase a set of peristaltic pumps used to circulate fluid through the inner lumen of the laryngeal tissue and to pump media into the laryngeal vasculature. These two pumps collectively cost nearly $1,300, which resulted in a total amount spent in the fall semester of $1,525.

In the spring semester, the team created a new prototype. Thus, many materials needed to be re-ordered. The raw materials for the device cost approximately $63, which is considerably less than their cost last semester due to the team's use of the excess materials from last semester. The second prototype has an equivalent electronics system to the first, but these products also cost less than in the first prototype due to the malfunction and subsequent need to re-order products that was problematic in the fall and can now be avoided in the spring. The electronics for this device cost $70. Finally, the miscellaneous materials for the spring semester, including sealant, hardware, and a drainstop, cost approximately $40, which is greater than the cost of these items in the fall. This increased cost is due to purchasing of better quality materials as well as the incorporation of new products to prevent leaking. A detailed list of components purchased in the fall as well as a budget for the spring can be found below.

Fall 2013 Budget

Component	**Price**	**Supplier**
Peristaltic Pump, Model BT100-2	$350.00	Langer Instr.
Ultra low flow rate heads, Model DG-2-10	$140.00	Langer Instr.
3-stop pump tubing	$39.00	Langer Instr.
Peristaltic Pump, Model BT100-1F	$598.00	Langer Instr.
Medium flow rate heads, Model YZ1515X	$168.00	Langer Instr.
.5" thick 12x12" PC sheets	$29.30	Grainger
.236" thick 12x12" PC sheets	$60.00	Grainger
.5" diameter, 1 ft long PC rods	$3.72	Grainger
.25" diameter, 3 foot long PC rods	$12.96	Grainger
0-5V analog pump input	$45.00	Langer Instr.
Arduino Uno Microcontroller	$29.95	Sparkfun
Stepper motor Easydriver	$14.95	Sparkfun
Stepper motor with cable	$14.95	Sparkfun
Hardware (screws, sealant, etc.)	$20.00	TruValue
Total	$1,525.83	

Spring 2014 costs

Component	**Cost**	**Supplier**
.236" thick 12x12" PC sheets	$60.00	Grainger
0.5” diameter, 1ft long PC rods	$3.72	Grainger
Arduino Uno Microcontroller	$29.95	Sparkfun

Stepper motor Easydriver	$14.95	Sparkfun
Stepper motor with cable	$24.95	Sparkfun
Miscellaneous Hardware (screws, sealant, etc)	$40.00	Dorn TruValue/DAP
Total Cost	**$173.57**	

Spring 2014 Timeline

Task	January		February				March					April			
	20	27	3	10	17	24	3	10	17	24	31	7	14	21	28
Testing															
Decellularization		🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	X	X	X	X	X	x
Model Creation							🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	🟩 X	X	X
Prototype and Electronics testing									🟩 X	🟩 X	🟩 X	🟩 X			
Second Prototype															
Design reiteration	🟪 X	🟪 X	🟪 X	🟪 X											
Ordering Materials			🟪	🟪 X	🟪 X										
Fabrication				🟪 X	🟪 X	🟪 X	🟪 X	🟪 X	🟪 X	🟪 X	X	X	X	X	
Client Feedback								🟪	🟪	🟪	🟪	🟪	🟪	🟪	X
Deliverables															
Progress Report	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X	🟧 X
Meetings															
Client	X	X				X			x		X			x	x
Team	🟨 x	🟨 X	🟨 X	🟨	🟨	🟨 X	🟨 X	🟨	🟨 X	🟨 X	🟨	🟨 X	🟨 X	🟨 X	🟨 x

User Guide for Laryngeal Bioreactor

Physical Descriptions and Problems with Bioreactor

Removable items within the bioreactor:

- Rod attaching cage to wall (non-motor side) can be removed.
- Rod attaching cage to motor can be removed using screw and Allen wrench. Should ensure this screw is tight before starting device.
  - Ensure that the motor is **All the way pushed in** before tightening the screw with the Allen wrench
- The plate on the wall (motor side) can be removed with a screw driver

If the cage will not turn, and you suspect a physical problem (ie you hear the motor running), ensure the following:

- Is the motor pushed all the way into the rod connecting it to the cage?
  - If not, unscrew the screw and push the motor in further
- Is the rod connecting the cage to the bioreactor wall slipping on the cage?
  - If so, push this rod further into the cage construct

Electronics Descriptions and Problems with Bioreactor

If the cage is not turning, and you suspect a problem with the electronics hardware (ie you **DO NOT** hear the motor attempting to turn), you should take the following steps:

- Ensure that the circuitry of the bioreactor is set up correctly:
  - If wires become removed from the stepper motor, on easy driver (red board) the following connections should be made:
    - A+ pin connects to red wire
    - A- pin connects to green wire
    - B+ pin connects to yellow wire
    - B- pin connects to blue wire
  - If wires are disconnected from the arduino (blue board), the following connections should be made:
    - Pin 11 should be connected to the Step input on the red board

        - Pin 13 should be connected to the Direction input on the red board
        - The ground pin should be connected to the ground pin collection on the breadboard (3 pins in series)
    - If wires are disconnected from the pump, the following connections should be made:
        - Pump input 1 should be connected to arduino pin 5 output. This should be connected to the breadboard between the resistor and the capacitor
        - Pump input 2 should be connected to arduino pin 9 output
        - Pump input 3 should be connected to arduino pin 10 output
        - Pump input 4 should be connected to ground pin collection on breadboard
        - Pump input 5 should be connected to arduino pin 6 output
- If this does not fix the problem, check the following:
    - Is the power light on the arduino on? (It should be orange and be solid)
        - If not, the arduino needs to be plugged in OR a new arduino may need to be ordered
    - Is the power light on the stepper motor driver on? (It should be orange and solid)
        - If not, the stepper driver needs to be plugged in OR a new stepper driver needs to be ordered.
    - Is the program light on the arduino functioning? (It should be green and BLINKING)
        - If not, the program is not correctly transferring to the arduino. See software troubleshooting below.

Software Description and Problems with the Bioreactor

To operate

- Specify variables in the Arduino IDE program refer to the code below. The highlighted regions are code lines that can be changed to manipulate the program, and are explained below the program.

```
////////////////////////////////////////////
//13 controls direction
//11 controls stepping motion
// 3 controls speed of pump - blue to board
// 11 starts 0 or stops 255 - purple
// 10 controls direction CCW 225 CW 0 - yellow
// 5 COM of speed - orange
// GND - COM of start stop and direction - green to board
```

```
/////////////////////////////////////////////////////////////////
//Authors: Rebecca Stoebe, Peter Guerin, Dan Thompson, Kyle Anderson
//Program turns pump on then turns the larynx 180 degress.
////////////////////////////////////////////////////////////////

//Note: Larynx will turn ~120 degrees each time void loop is run.

int dirpin = 13; //pin 13 controls stepper directio
int steppin = 11; //pin 11 controls stepping motion
int directionInput = 10; // pin 10 controls CW/CCW direction
int startStop = 9; // pin 9 controls pump start/stop
int speedcom = 6; // pin 6 com of speed
int speedrpm = 5; // pin 5 controls pump speed
int distance = 0; //for start of program motor is at position 0
int timeinput = 5; //CHANGE FOR TIME PAUSE
int timedelay = 1000; //CHANGE FOR SPEED OF ROTATION
int distancegoal = 400; //CHANGE FOR ANGLE OF ROTATION


void setup() {
  pinMode(dirpin, OUTPUT); //sets pin 13 as output
  pinMode(steppin, OUTPUT); //sets pin 11 as output
  pinMode(speedcom, OUTPUT); // sets 6 as output
  pinMode(startStop, OUTPUT); // sets 9 as output
  pinMode(directionInput, OUTPUT); // sets 10 as output
  pinMode(speedrpm, OUTPUT); // sets 5 as output

  digitalWrite(dirpin, LOW);  //Sets the direction pin to clockwise
  digitalWrite(steppin, LOW); //Sets the stepper pin to off


  int pumpspeed = 500; //sets the pump speed. Refer to chart

   analogWrite(speedcom, 0); // sets com of speed
   delay(1000); // waits 1 second
   analogWrite(directionInput, 225); // sets direction CW
   delay(1000); // waits 1 second
   analogWrite(speedrpm, pumpspeed); //Sets the speed of the pump
   delay(1000); // waits 1 second
   analogWrite(startStop, 0); // turns pump on

}
```

```
void loop() {
  int time = timeinput*1000; //changes the input time to seconds

  //The next four lines take 1 step and record the position
  //Movement is slowed so larynx will not be harmed.
  for (int x = 0; x < distancegoal; x++) {
    digitalWrite(steppin, HIGH);
    //this delay was added to slow movement and also because the
    //motor freaks out when switching from high to low very rapidly
    delayMicroseconds(timedelay);  //THIS CHANGES SPEED OF ROTATION
    digitalWrite(steppin, LOW);
    delayMicroseconds(1000);
  }
   delay(time); //waits desired time with pump on

   digitalWrite(dirpin, HIGH);

    // Now pause for a second
   delay(1000);
    //The next four lines take 1 step and record the position
  //Movement is slowed so larynx will not be harmed.
  for (int x = 0; x < distancegoal; x++) {
    digitalWrite(steppin, HIGH);
    //this delay was added to slow movement and also because the
    //motor freaks out when switching from high to low very rapidly
    delayMicroseconds(timedelay);  //THIS CHANGES SPEED OF ROTATION
    digitalWrite(steppin, LOW);
    delayMicroseconds(1000);
  }
   delay(time); //waits desired time with pump on

   digitalWrite(dirpin, LOW);

    // Now pause for a second
   delay(1000);

}
```

- Explanation of highlighted regions:

```
int timeinput = 5; //CHANGE FOR TIME PAUSE
```

  - This changes the time paused between each turn. The number is the time spent between each rotation (in seconds)

```
int timedelay = 1000; //CHANGE FOR SPEED OF ROTATION
```

  - This changes the time taken between each step taken by the stepper motor, that is, it changes the speed of rotation. As this number is increased, the speed of turning increases. DO NOT DECREASE THIS NUMBER TO LESS THAN 400

```
int distancegoal = 400; //CHANGE FOR ANGLE OF ROTATION
```

  - This changes the number of steps taken in each turn. 800 steps is equivalent to a 360 degree rotation

- Do not change anything else in the program unless you are experienced in Arduino programming. Information and tutorials on programming in Arduino can be found here (http://arduino.cc/en/Tutorial/HomePage)

- If it is desired to also program the pumps using the arduino, the SIMPLE PROGRAM code should be used. For another copy of this code, as well as for help with operation of this code, contact Rebecca Stoebe (becca.stoebe@gmail.com)

If you suspect a problem with the bioreactor software, try the following steps:

- Verify the code (press the checkmark in Arduino IDE)
  - If Arduino sees an error in the code, it will alert you.
  - If there is an error in the code, it has likely resulted from the deletion of a key element of the code or in a syntax error.
    - A common example of this is “Expected ‘;’ before ‘command‘ “, however, these errors can take on hundreds of forms.
    - The simple solution to this problem is to copy and paste the above code into the Arduino IDE and re-format your variables.
    - If you desire to fix the code in a more complex/personalized manner, information on Arduino IDE error messages can be found here (http://diyroboticslab.wordpress.com/2009/06/05/correcting-arduino-compiler-errors/) and here (http://arduino.cc/en/guide/troubleshooting)
- Upload the code (press the arrow pointing to the right in Arduino IDE)
  - If there is an error in uploading, it will alert you.
  - Check to make sure the USB is connected to the computer and the arduino
    - The arduino will try to sync through your PRIMARY usb port. If it cannot, the error message will read something like “Serial port /dev/cu.usbmodem cannot be found.” This indicates that the USB is most likely plugged into the wrong place.
    - If this does not work, ensure the Bluetooth on your computer is turned off.

  - If the code uploads correctly, the green light should be solid and then blink three times. If this does not occur, there may be a problem with the arduino board itself.

Parts List and Replacements

The parts for the bioreactor can be replaced from the following manufacturers:

Electronics

- Arduino (green board): https://www.sparkfun.com/products/11021
- Stepper Driver (red board): https://www.sparkfun.com/products/10267
- Stepper Motor: https://www.sparkfun.com/products/9238 (Here is one that is slightly stronger and more durable: https://www.sparkfun.com/products/10847)
- Power cord: https://www.sparkfun.com/products/10273
- Power connector: https://www.sparkfun.com/products/119

Hardware:

- Polycarbonate rods: http://www.grainger.com/product/Rod-Stock-WP143581/_/N-c1nZ1z0o3vs?_=1399471379217&s_pp=false (we used 0.5” diameter)
- Polycarbonate sheets: http://www.grainger.com/product/LEXAN-Polycarbonate-Sheet-Stock-WP143617/_/N-c1p?s_pp=false (We used 0.236” thick)
- Set screw: dimensions are 8-32, 0.5", available at any hardware store
- Removable screws: 8-32, 0.5"-0.75", available at any hardware store
- O-ring: Inner diameter of 0.5”, available at any hardware store
- Standard acrylic sealant, available at any hardware store