Laryngeal Bioreactor

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Abstract

Organ transplantation is used when organs are rendered dysfunctional due to trauma or disease; however, this procedure is quite expensive and donor organs are limited in supply. Tissue engineering is an emerging field that has the potential to solve the current problem at hand. For our project, we created a bioreactor that was capable of performing two distinct processes necessary for the regeneration of a human larynx: decellularization and recellularization. The decellularization process aims at creating an acellular larynx scaffold which will support new cell growth during the recellularization phase. Upon completion of the recellularization phase, we hope to create a fully functional larynx that has the potential to be used in organ transplantation.

Problem Motivation

- Each year almost 136,000 patients are diagnosed with laryngocarcinoma and require partial or complete laryngectomy (1)
- The final treatment strategy for many organs is transplant. In 2009 alone, 29,346 organ transplants were conducted in the United States (2).

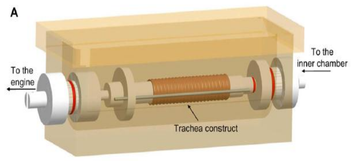

Figure 1: Example of tracheal bioreactor (3)

- Organ transplants are often limited due to lack of available donor organs.
- In 2009, a double-chamber rotating bioreactor was used for the decellularization and recellularization of a human trachea which was successfully transplanted into a patient (3).

Background

Functions
- Phonation
- Regulation of airflow into lungs
- Prevention of food entering airway during swallow

Anatomy
- Structural support provided by six main cartilages
- Vocal folds responsible for phonation
- Carotid arteries provide blood

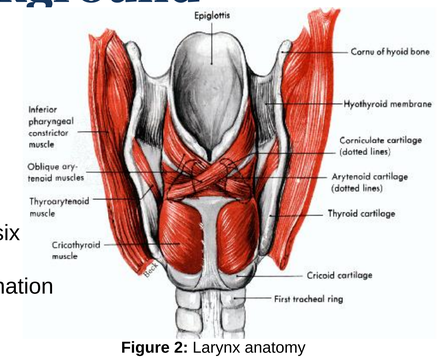

Figure 2: Larynx anatomy

Requirements

- Sterilizable or replaceable components
- Continuous function in incubator environment
- Single unit for decellularization and recellularization
- Separate environment for larynx lumen and exterior

Final Design

Figure 3: Final bioreactor

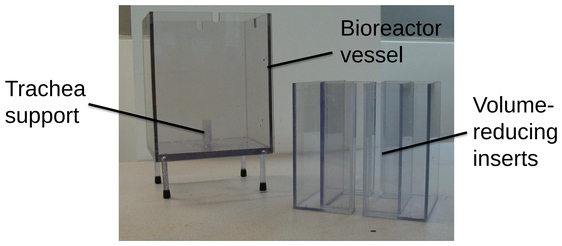

Figure 4: Bioreactor without inserts in place

- Vertically oriented larynx
- Larger outer box with smaller inserts
  - ➢Larger outer box allows hands to fit in bioreactor
  - ➢Inserts reduce inside volume
- Three environments: inner lumen, external chamber, vasculature
- Pump to control media in inner lumen
- Pump to perfuse media through vasculature

Materials

Component	Material (Manufacturer)	Cost
Bioreactor	Polycarbonate (Grainger, Midland Plastics	$126.55
Pumps (perfusion, vasculature)	Peristaltic pumps (Langer Instruments)	$1,329.00
Miscellaneous & accessories	Stainless steel (McMaster), Trach Tubes	$47.61

Total = $1503.16

Fabrication

Highlights
- Machined edges for water-tight seal
- Drill & tap threaded pipe insert
- Personalized trachea support spun on lathe
- Solvent polycarbonate bonding cement-fast seal!
- Clamping, time, & teamwork!

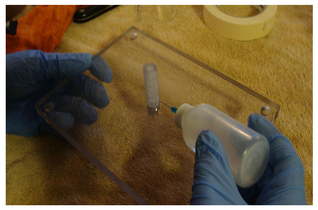

Figure 5: Gluing of fixed cylinder

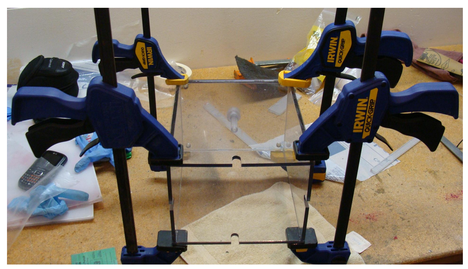

Figure 6: Cementing of outer walls

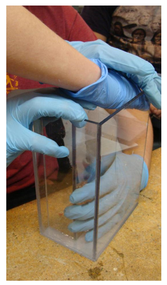

Figure 7: Teamwork!

Testing

- Overall functionality of bioreactor
- Water tight seal
- Recorded fill time for pump tubing and 25mL tube
- Pump tubing was emptied prior to each test

Figure 8: Testing set up with inner lumen pump

Results

Results for vasculature pump

Speed (rpm)	10	20	30	40	50	60	70	80	90	100
Fill Time (seconds)	773.1	387.0	257.6	193.2	153.9	128.5	108.8	96.30	84.50	77.47

Results for inner lumen pump

Speed (mL/min)	63.63	53.63	43.63	33.63	23.63	13.63
Fill Time (seconds)	30.49	36.54	44.98	58.32	82.99	143.5

Future Work

- Determine effectiveness of device in decell-recell process
- Decell: Detergent (SDS) perfused through vasculature, lysing cells (estimated time: 24-48 hours)
- Recell: Seed decellularized larynx with various types of cells (cartilage, smooth muscle, epithelial, endothelial)
- Estimated time for recell: 3 weeks
- Wash period in between decell and recell (estimated time: <1 day)
- For decell, test to see whether or not perfusion process is successful in lysing cells
- For recell, test to see whether different cell types are surviving/proliferating

Acknowledgements

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References

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