Laryngeal Bioreactor

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Abstract

Organ transplantation and tissue grafts are used when function is lost due to trauma or disease; however, this procedure is quite expensive and donor tissues 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. This bioreactor has been shown to successfully decellularize most laryngeal tissues. Following successful decellularization, the acellular scaffold is primed for recellularization of patient-specific cells.

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).
- 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).

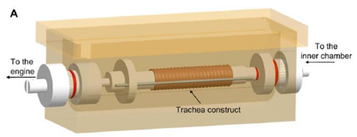


Figure 1: A tracheal bioreactor that has produced first tissue implanted in patients (3).

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

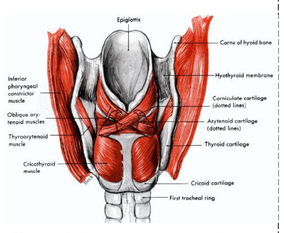
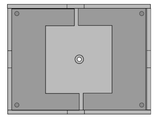


Figure 2: Larynx anatomy (4)

Client Requirements

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



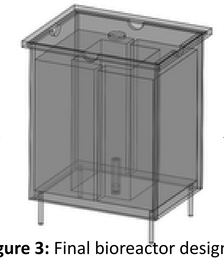


Figure 3: Final bioreactor designs

Bioreactor Design

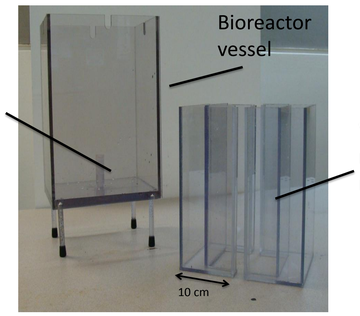


Figure 5: Bioreactor without inserts in place

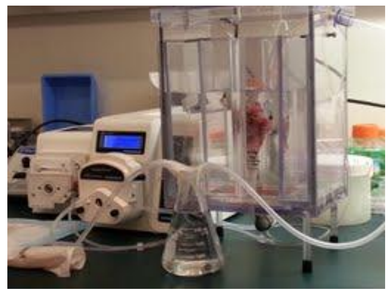


Figure 6: Bioreactor setup with pumps

- 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 to the inner lumen
- Pump to perfuse media through vasculature

Automation

- Improve the user interface
- Inner lumen pump during recellularization:
  - Vary air exposure time
  - Vary flow rates
- Arduino Uno
- External Control Interface
- Low pass filter

Figure 7: The Arduino Uno used for pump automation (5)

Materials

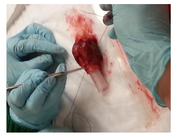
Component	Material (Manufacturer)	Cost
Bioreactor	Polycarbonate (Grainger, Midland Plastics)	$126.55
Pumps (perfusion, vasculature)	Peristaltic pumps (Langer Instruments)	$1,329.00
Miscellaneous & accessories	Microcontroller, electronics, endotracheal tubes, etc.	$137.92

Total = $1,593.47

Procedure & Testing

Procedure

- Primary excision
- Add tracheal insert, cannulas



1) Tissue Prep

- Perfuse detergent
- Check scaffold

2) Decell

- Flush detergent
- Seed cells
- Perfuse media

3) Recell

Figure 8: Images from trial 3

Testing

Trials

- **Trial 1:** Lumen perfusion only
- **Trial 2:** Vasculature perfusion only
- **Trial 3:** Lumen & vasculature perfusion
  - Included control tissue

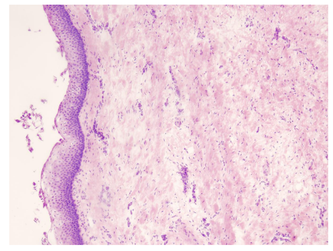
Decellularization Agents

- 1% sodium dodecyl sulfate (SDS) detergent
- 1% antibiotics

Washing Agents

- 3M NaCl
- DNase1 (50U/ml)
- dH2O

H&E Analysis



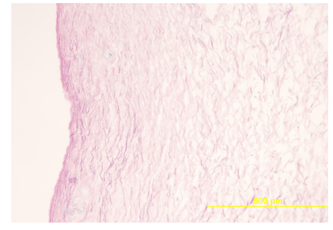


Figure 9: Images (L) and histology (R) from decellularization trial 3

Future Work

- Recellularization
  - Fibroblasts/media perfused throughout scaffold
  - Perform H+E staining
  - Observe where fibroblasts are seeded
  - Incorporate different cell types
  - Aim to direct cell seeding + perfusion

References

1) Hou N, Cui P, Luo J, Ma R, Zhu L. Tissue-engineered larynx using perfusion-decellularized technique and mesenchymal stem cells in a rabbit model. Acta Oto-Laryngologica, 2011; 131:645-652.
(2) Organ Procurment and Transplantation Network. http://optn.transplant.hrsa.gov/organDatasource/. Online. Access 10/23/12
(3)Asnaghi et al. A double-chamber rotating bioreactor for the development of tissue-engineered hollow organs: From concept to clinical trial. Biomaterials, 2009; 30:5260-5269
(4) www.quora.com/Human-Anatomy/What-is-the-purpose-of-an-Adams-apple-and-why-do-only-men-appear-to-have-it
(5) Image used from www.sparkfun.com