Laryngeal Bioreactor

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UW BMF Design

Abstract

Tissue engineered organs have the potential to overcome several obstacles associated with organ donation, such as limited supply and immune rejection. We have created a **novel bioreactor** for the **decellularization** and **recellularization of laryngeal tissue** composed of polycarbonate and other sterilizable materials. It is fitted with **electronics** that have been seen to consistently **control pumps** that supply the bioreactor with media, and **turn the larynx** to vary its exposure to media and air. We have **verified the efficacy** of the use of the bioreactor through **modeling**, and have seen its **competence in *in vitro* decellularization protocols**. Future work with this device involves investigating recellularization procedures and improving modeling techniques.

Background & Motivation

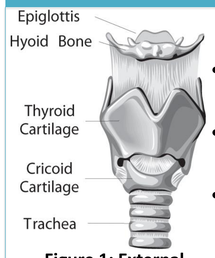

Figure 1: External larynx anatomy (3)

Larynx and Therapies

- Larynx: complex organ in an airway that houses vocal cords
- Laryngeal cancer affects 136,000 individuals worldwide each year (1)
- Very low success of complete larynx transplant due to immune rejection of the allograph (2)

Laryngeal Tissue Engineering Background

- No known occurrence of tissue engineered larynx
- Tissue engineered tracheal cartilages and vocal folds grown in vitro and implanted (4,5)

Figure 2: Tissue-engineered trachea (5)

Design Criteria

Client Requirements for Design

- Single bench top unit for decellularization and recellularization
- Allow separate environments for inner lumen and organ exterior
- Provide researcher access to larynx
- Sterilizable and operable in a refrigerator and an incubator
- Allow variable access of the tissue to air and media

Prototype Redesign

Changed Prototype Features

Accessibility Changes

- Bioreactor dimensions enlarged from 25x12x15 to 25x12x20 (cm)
- Cage lengthened from 13cm to 17cm
- Drainage site moved from bottom to the side
- Notches made in bioreactor sides

Stability and Durability Changes

- Legs removed for stability
- Superior sealant used
- Removable cage attachment added to bioreactor wall
- Floor of motor housing removed

Accuracy of Fabrication Changes

- Pieces cut with mill instead of band saw
- Custom O-ring created

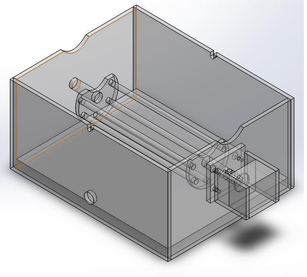

Figure 3: SolidWorks model of redesigned prototype

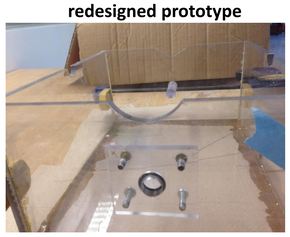

Figure 4: Custom O-ring and removable cage attachment

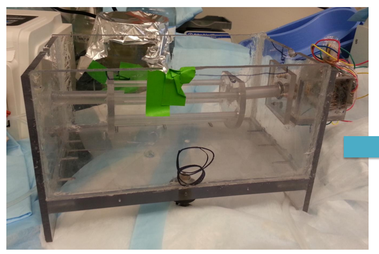

Figure 5: Original prototype

Figure 6: Redesigned prototype

Discussion

- Changes made to sealant and bioreactor structure to increase longevity of bioreactor and electronics
- Changes made to bioreactor geometry to put less stress on tissue and tubing
- Redesigned prototype allows greater customization of experimentation and future bioreactor use

Device Modeling

Background

- Use of ANSYS-Fluent CFD software
- Created mesh of laryngeal lumen in SolidWorks
  - Based on MRI data
  - 37x 0.5mm slices joined together
- K-ε model: turbulent kinetic energy and kinetic dissipation
- Set for viscosity, density of 1% SDS flowing through inner lumen
- Tested at 1, 15 and 50 mL/min volumetric flow rates

Results

- At 1 mL flow cases, no turbulent flow regimes seen within larynx; most portions also see laminar flow at 15 and 50 mL/min cases
  - Velocities reach 4.7 m/s near vocal folds in 50mL/min case; equates to an Re=10,000
- At 50 mL/min flow, shear stresses reach 5.6 kPa near vocal folds; 6 times *in vivo* stresses seen (6)

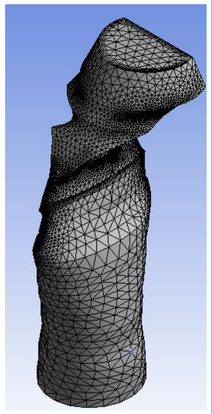

Figure 7: Completed mesh of laryngeal lumen anatomy

Discussion

- Greatest stress occurs near vocal folds: experimental fluid velocities used must account for stresses in these areas
- Based on normal *in vivo* conditions, a flow rate of at or below 15 mL/min or less is recommended (6)

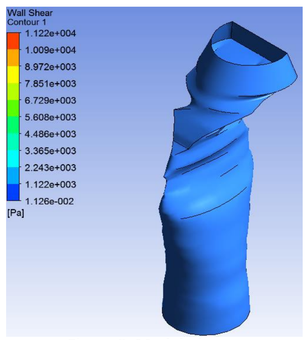

Figure 8: Modeled stress profiles within laryngeal lumen

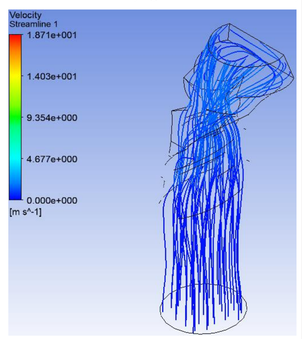

Figure 9: Modeled velocity profiles within laryngeal lumen

Initial Decellularization

Experimental Setup

- Initial trials: perfuse SDS through inner lumen, arteries
- Perform biopsy after decellularization trial
  - •Gross visual
  - •Histological analysis
  - •Proteomic analysis

Results

- Gross examination shows lack of color, especially at vocal folds
- Histological examinations show lack of nuclei in tissue
- Proteomic analysis reveals decellularization:
  - Reduces protein content by 97.5% (wt %)
  - Reduces protein types present by 31%

Discussion

- 1% SDS + 96 hour trial yields successful apoptosis and cellular tissue degradation
- Current detergent concentration very harsh on tissue

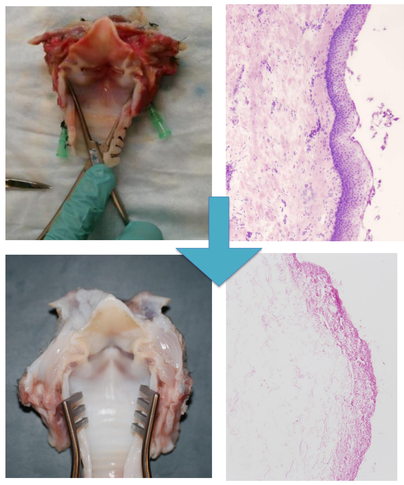

Figure 10: Gross anatomy (left) and H&E stains (right) of normal (above) and decellularized tissue (below)

Protein Remaining After Decellularization

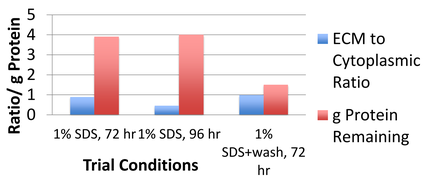

Figure 11: Ratio of ECM to cytoplasmic proteins remaining and g protein remaining after each decellularization trial

Budget

Details	Cost
Fall 2013 Expenditures	$1538
Bioreactor Raw Materials: Polycarbonate	$65
Electronics: Arduino Uno, Stepper Motor, etc.	$75
Miscellaneous: Hardware, seals, glue, etc.	$27
Total Cost	**$1705**

Future Work

- Refine decellularization protocol for optimal cellular apoptosis while maintaining integrity of ECM
  - Vary SDS concentration and duration of perfusion
- Recellularization testing: injection of fibroblasts
  - Histological staining, RNA assays
- Develop method to mimic *in vivo* stress conditions
- Further develop model to reflect conditions on exterior of tissue and in vasculature

References

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