UNIVERSITY OF WISCONSIN-MADISON
DEPARTMENT OF BIOMEDICAL ENGINEERING
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PERFUSION DECELLULARIZATION-RECELLULARIZATION BIOREACTOR FOR LARYNGEAL TISSUE ENGINEERING

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TEAM

Arman Grabowski – BWIG

Taylor Milne – Communicator

Brett Napiwocki – Co-Leader

Sara Schmitz – Co-Leader

Ben Smith – BSAC

CLIENT

Dr. Nathan Welham

ADVISOR

Professor Tom Yen

Professor, Biomedical Engineering

ABSTRACT

Tissues and organs of the human body can be partially damaged or rendered completely dysfunctional when subjected to trauma or disease. In the case of tissues with minimal regeneration capabilities, the only option for regaining function is transplantation. This procedure is very expensive and there is a shortage of qualified donors. Even if a donor is found and the transplant performed, the immunosuppressants that must be taken by the recipient can lead to complications and the organ or tissue may be rejected. Recent tissue engineering research has shown promising evidence that tissues and even whole organs can be generated using the recipient's cells. This is done by utilizing a decellularization-recellularization process. The decellularization step lyses the cells of the donor organ, turning it into an acellular scaffold. This scaffold is then recellularized by exposing it to organ-specific cells. This process has been performed on by whole organs and tissues. Of specific interest to this design project is whole organ regeneration of the larynx. Previously only partial laryngeal engineering has been successful. A key component in successfully engineering the larynx is proper bioreactor design. The bioreactor is the unit that will house the larynx throughout the decellularization and recellularization processes. Design of a quality bioreactor includes determining optimal orientation of the larynx and techniques for recellularization of the scaffold.

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PROBLEM STATEMENT

The purpose of this project is to design a sterile bioreactor for whole organ tissue engineering of the human larynx, as well as comparable large animal models such as the pig or dog larynx. The bioreactor must be capable of performing two different processes: perfusion-decellularization of the larynx to create an acellular scaffold, and perfusion-recellularization of the acellular scaffold using vocal fold fibroblasts and other cell sources.

BACKGROUND

Larynx Anatomy

The larynx is the organ that is located at the superior aspect of the airway channel, connecting the trachea and pharynx, ultimately leading to the oral cavity. Although the larynx is a relatively small organ, it has many important functions. The primary functions of the larynx include the production of voice (phonation), regulation of the flow of air into the lungs, and preventing the passage of food or other foreign matter into the airway during swallowing [1]. Other functions include coughing, the Valsalva maneuver, and providing sensory information [2]. Figure 1 shows three different views of the larynx and highlights its gross anatomical structure.

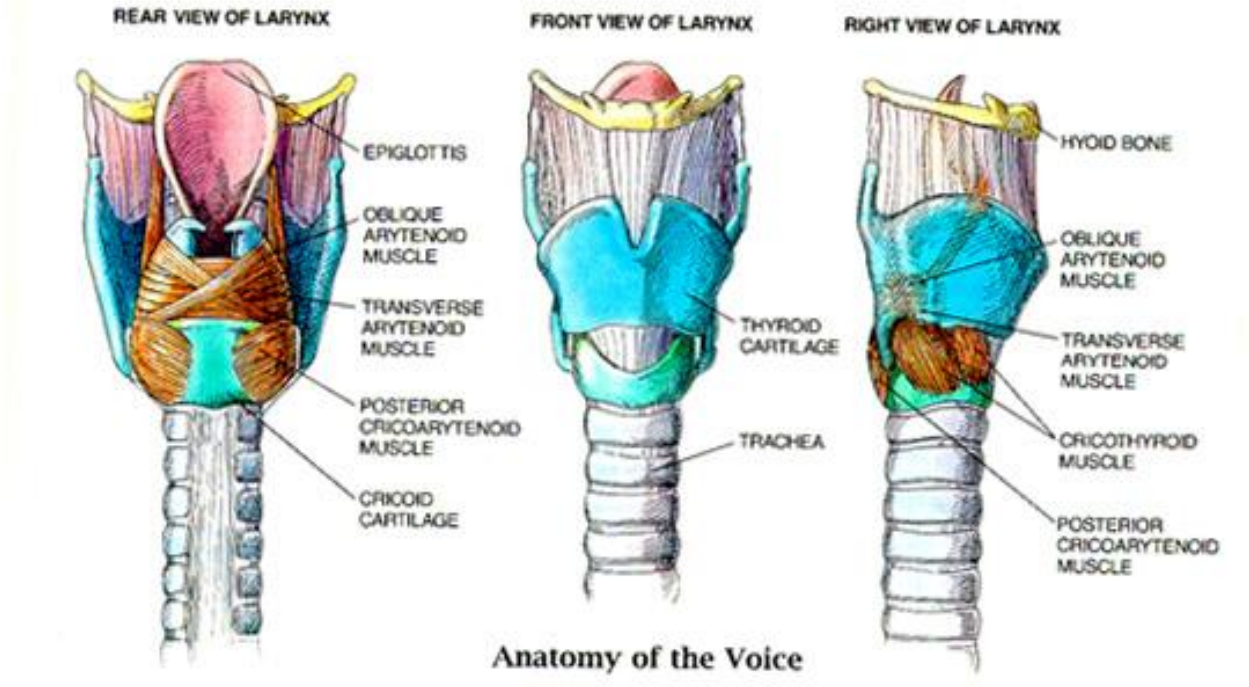

Figure 1: Major anatomical structures of the larynx. [http://www.edoctoronline.com/media/19/photos_040EAD64-F02E-4068-A04D-1B1C94AFDB10.jpg]

As shown in Figures 1 and 2, the larynx is composed of many different structures and tissue types. This allows for the many precise and intricate movements that are necessary to achieve its aforementioned functions. A brief overview of the gross anatomy of the larynx follows, highlighting pertinent information for the understanding of this report and design project.

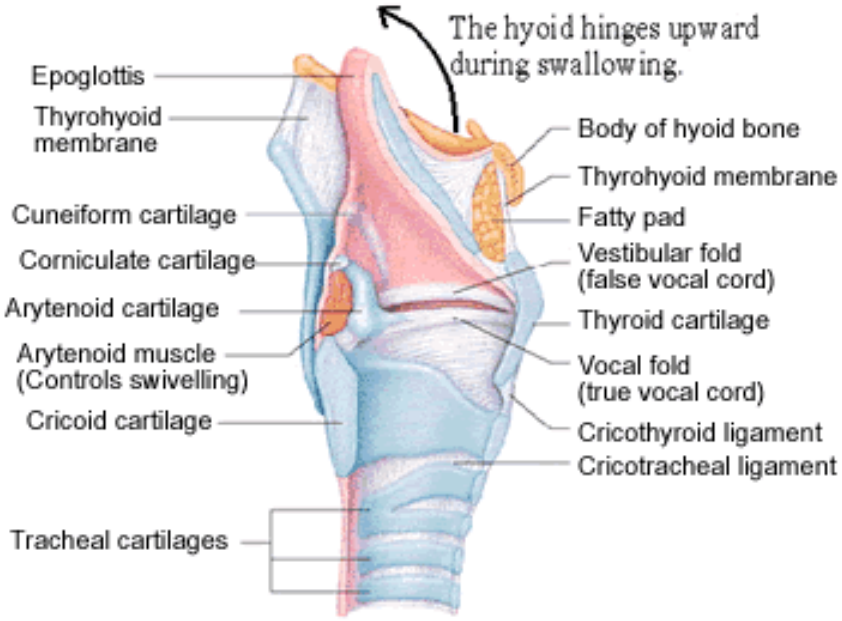

Figure 2: Additional laryngeal anatomy [http://www.edoctoronline.com/media/19/photos_167BFD91-F36A-473D-A56E-ACBAB131E39C.gif]

Structural support for the larynx is provided by six cartilages: three large unpaired cartilages and three smaller, paired cartilages. Briefly, the thyroid, cricoid, and arytenoid (paired) cartilages serve as attachment points for many muscles in the larynx. The epiglottis is a leaf-shaped cartilage that protects the vocal folds and airway during swallowing, as it folds over the opening to the vocal folds. The corniculate (paired) cartilages are smaller in size and extend the arytenoid posteriorly and medially. Finally, the cuneiform (paired) cartilages are also much smaller and are responsible for the small whitish projections above the arytenoid cartilages (see Figure 2) [2].

The vocal folds, which are concerned with phonation, are composed of two strong vocal ligaments and the vocalis muscle [2]. This structure is covered by four layers of tissue with distinguishable structures: the epithelium and superficial, intermediate, and deep lamina propria [3]. Two classes of muscles attach to the larynx and are defined by their points of attachment. The extrinsic muscles pass between the larynx and its surrounding structures; the intrinsic muscles are confined entirely within the larynx. Both the extrinsic and intrinsic muscles allow for the finely tuned pitch and frequency control of the voice as well as respiratory functions. Muscles are innervated by the superior laryngeal nerve and the recurrent branch of the vagus nerve [2].

To accelerate the decellularization and recellularization process, the vasculature is used to perfuse media and detergent into tissues [4]. The left and right common carotid arteries feed blood into the smaller thyroid artery, as shown in Figure 3.

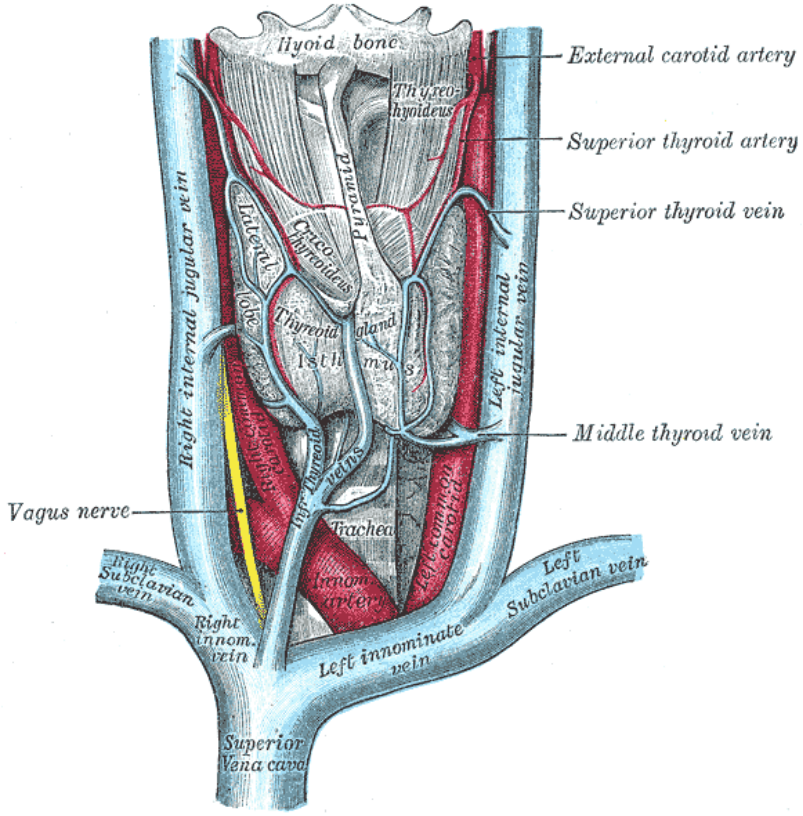

Figure 3: Blood supply to the larynx
[http://www.fpnotebook.com/_media/entLarynxAnteriorVesselsGrayBB1174.gif]

Tissue Engineering

Stem cells are remarkable for their ability to differentiate into one of many other cell types in the body. The major hallmarks of stem cells are their lack of specialization and their ability to differentiate into more specialized cell types, such as neurons, muscle cells, red blood cells, and others. This process is shown in Figure 4. For this reason, stem cells tend to function as an internal repair system, dividing and differentiating to replace damaged tissue. This is especially useful in organs such as the stomach, in which cells are regularly damaged and destroyed, and stem cells can easily replace the damaged cells to maintain function [5]. Stem cells are of great interest in tissue engineering for their unique ability and versatility. With their ability to differentiate into any other cell type, both embryonic and adult stem cells could be used to replace damaged cells in a body part and to help cure diseases.

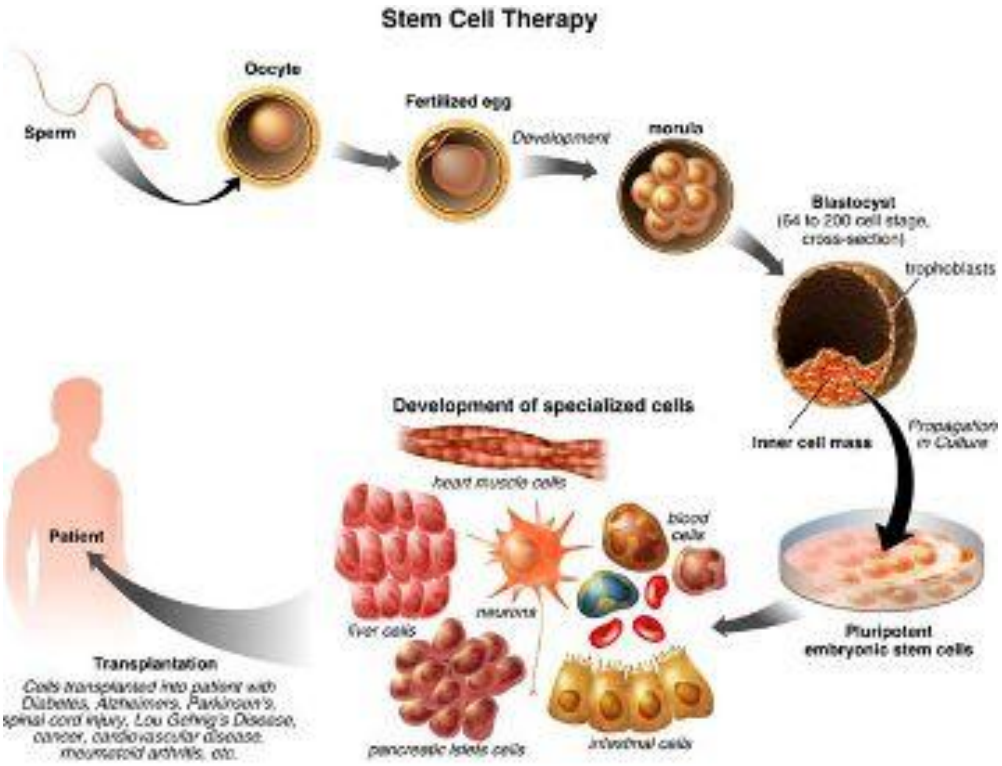

Figure 4: Differentiation of stem cells into specialized cells such as neurons and blood cells [http://nanobiotechnews.com/wp-content/uploads/2011/07/stem_cells.jpg]

The use of a decellularized matrix to repair damaged tissues is quickly becoming very popular in tissue engineering. By taking a donated tissue, using perfusion techniques to lyse cells within the tissue, then repopulating it with healthy cells (either stem cells or specialized cells of the body part in question), it is possible to restore lost function. An example of this is shown in Figure 5, which shows a rat kidney both before and after the decellularization process. This process is especially popular in whole organ tissue engineering, being used on donor organs such as the heart, lungs, and liver in order to provide an environment in which healthy cells can thrive [6].

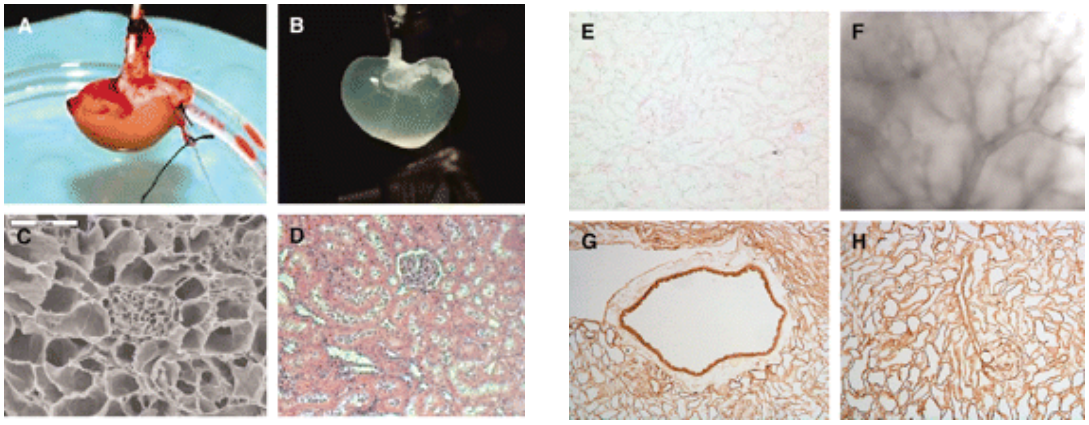

Figure 5: Rat kidney both before and after decellularization. [http://jasn.asnjournals.org/content/20/11/2338/F1.expansion.html]

Naturally, such a process is difficult to perform *in vivo*; thus, an *ex vivo* environment must be created to simulate the conditions of the physiological environment. This is the purpose of a bioreactor, or a device in which biological processes develop in a controlled environment. Bioreactors were traditionally used in industrial processing and waste management until recently, when the popularity of perfusion-decellularization tissue engineering led to a demand for devices such as bioreactors to create an optimal environment in which cells could grow and develop outside the body [7]. There are many different types of bioreactors, as shown in Figure 6, which support different physiological conditions, cell types, organs, and other factors. Regardless of the type, bioreactors are a very useful tool for controlling the environment in which cells grow and proliferate. And when used in conjunction with decellularized matrices and stem cells, there is a lot of potential to engineer healthy, functioning tissues and organs.

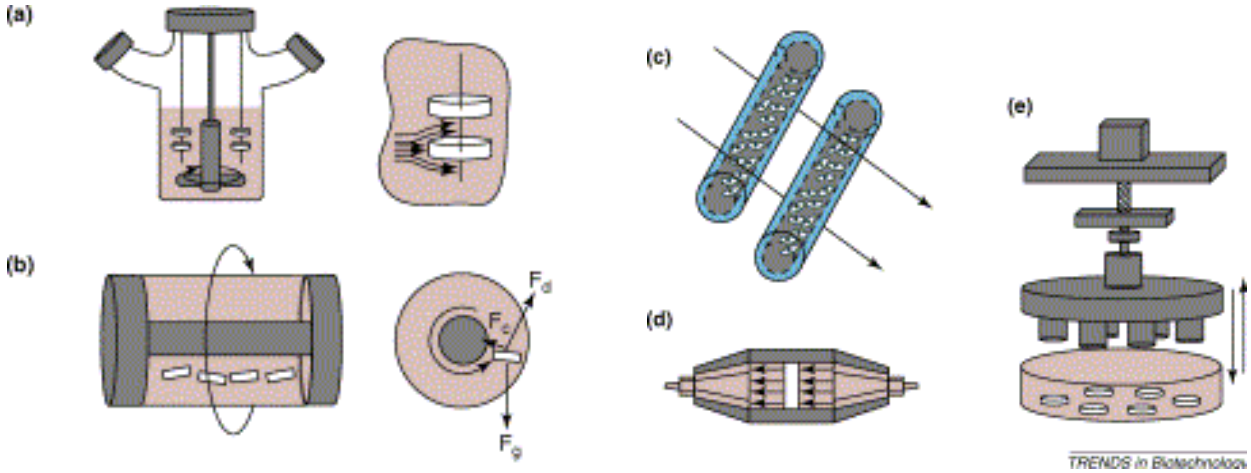

Figure 6: An example of different bioreactor types, including a rotating bioreactor (b) and a direct perfusion bioreactor (d) [http://ars.els-cdn.com/content/image/1-s2.0-S0167779908000346-gr2.jpg]

Significance

As with other organs, the larynx may be rendered dysfunctional from disease or trauma and current therapeutic techniques are ineffective. Each year almost 136,000 patients are diagnosed with laryngocarcinoma (laryngeal cancer) and require partial or complete laryngectomy, or removal of the laryngeal tissue [4]. Retaining function of the larynx following these procedures may not be possible, leaving patients mute and/or with respiratory problems. The only plausible treatment at this point is allographic transplantation.

A final treatment strategy for the kidney, pancreas, heart, lung, liver and intestine is transplant. In 2009 alone, 29,346 organ transplants were conducted in the United States [8]; however, due to anatomical complexities leading to difficulties in surgical technique, only two full-larynx transplants have ever been recorded [9,10]. Researchers have suggested that eliminating the need for immunosuppressants would increase the rate of laryngeal transplantation [4]. A recellularized laryngeal scaffold would not require the same level of immunosuppression as normal transplantation because they are grown from patient-specific cells. Thus, this project would be a big step in providing implantable larynges that would require minimal immunosuppression to patients.

PREVIOUS WORK AND CURRENT DEVICES

The concept of decellularizing and recellularizing an organ is not novel. Several research teams have successfully completed the task in other organs, including the lung [11], heart [12], liver [13], and kidney [14]. Researchers and clinicians have taken this a step further with the trachea. In 2009, the first successful full organ transplantation was completed using this decellularization-recellularization technique. Clinicians matched the geometry of the patient with a donor using MRI and CT scans to obtain a perfect match. After decellularization of the donor scaffold, the tissue was slowly rotated in a double-chamber bioreactor (Figure 7) that separated the inner lumen and outside of the trachea. This separation allowed for the different conditions of cellular development, promoting successful growth for the luminal epithelial cells and the cells on the outer surface [15]. The simple, tubular geometry of the trachea simplified many aspects of bioreactor design and definitely contributed to its published success. While bioreactors are commercially available, none exist that support whole organ laryngeal generation.

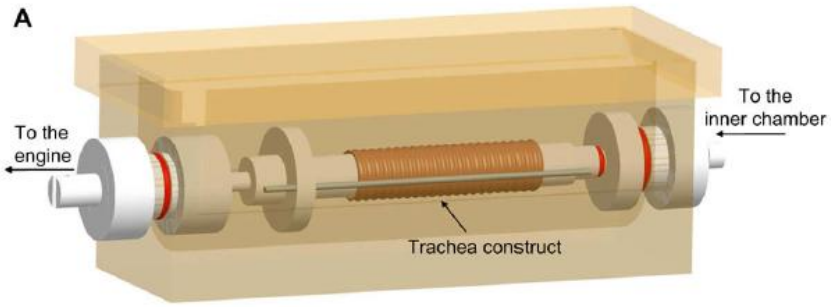

Figure 7: Example of currently available tracheal bioreactor [Asnaghi et al, 2009]

CLIENT DESCRIPTION

Dr. Nathan V. Welham (PhD, CCC-SLP) is an Assistant Professor in the Division of Otolaryngology-Head and Neck Surgery at the UW School of Medicine and Public Health. Clinically, Dr. Welham is certified in speech-language pathology and the evaluation and treatment of patients with disorders of the voice, resonance, swallowing and airway disorders. He practices as the Voice and Swallowing Clinic and the Pediatric Voice and Swallowing Clinics at UW Health. In addition an established clinical career, Dr. Welham has had extensive research experience [16]. Dr. Welham has 31 citations listed on PubMed and most recently published on proteome analyses, vocal fold scarring and treatment, and surgical treatment of sulcus vocalis. He has also worked with and developed animal models to study vocal fold scarring [17].

CLIENT REQUIREMENTS

In accordance to the wishes and specifications of our client Dr. Welham the laryngeal bioreactor will be made to meet the following criteria. It is essential that any portion of the bioreactor that

comes in contact with the larynx or cell culture media be sterilizable. Autoclavable components are ideal, though where this is not possible, replaceable components, to be changed after each use, are acceptable as well.

The bioreactor will be required to function continuously for days at a time within an incubator environment, which maintains temperatures of approximately 37ºC with high humidity levels. This continuous function must be upheld throughout the entire decellularization and recellularization processes, which combined are estimated to take no more than 4 weeks.

In order to streamline this process as much a possible, a single unit will be used to house the larynx for both decellularization and recellularization, with an intermediate wash cycle. It is necessary that this unit provide easy access to the larynx to allow for cell seeding, cell media replacement and any additional adjustments that need to be made. This will be achieved while maintaining dimensions that can easily fit within a Forma Series II Water-Jacketed CO_2 incubator from Thermo Scientific.

It is also required that the bioreactor maintain separate environments for the lumen of the larynx and the exterior. This separation needs to be maintained so that different cell media can be delivered to the larynx interior and exterior. In addition, the interior must be exposed to air in order to aid in appropriate cell development.

DESIGN ALTERNATIVE: SPRAY

The first bioreactor design consists of a 10x10x20cm box with a removable lid. The removable lid will allow easy access to the inside of the bioreactor which will help during initial placement of the larynx onto the support tube and during exterior media changes. As previously mentioned, there will be a support tube inside the bioreactor that is also removable. The support tube will be taken out of the bioreactor so that it can be attached to the excess trachea below the larynx. The two will be sealed together using a cable tie and then placed back into the bottom of the bioreactor. To stabilize the larynx, sutures will be run through the superior cornu of the thyroid cartilage and fixed to the bioreactor walls. This will aid in supporting the larynx during the decellularization-recellularization process.

Once the larynx is attached inside the bioreactor, a balloon will be inserted through the epiglottis side of the larynx and positioned below the vocal cords. Once below the vocal cords, the balloon will be inflated to create two separate culture environments: one below the balloon on the luminal side of larynx and another environment above the balloon which is exposed to the exterior media that surrounds the larynx.

The media applied to the inner environment will be sprayed through a nozzle tip that is located on top of the support tube. The nozzle is attached to a tube that runs the inner length of the support tube and is connected to a pump. When the pump is turned on, media will be sprayed through the nozzle tip at various time intervals. By atomizing the media, the cells will be exposed to both air and media which is a design requirement. After the inner media is sprayed onto the cells, it will trickle down the wall of the lumen and collect in a void below the support

tube. By collecting the media at the bottom, the other end of the pump can be attached to the void and the media can then be recycled.

The exterior media will be added by removing the lid of the bioreactor and filling it until the larynx is completely submersed in media. Likewise, to remove the exterior media the lid will be removed and the media will be aspirated out of the bioreactor. In the wall of the bioreactor near its base will be a tube that draws exterior media into to it (Figure 8). This exterior media will then be perfused through the left and right carotid arteries by pumps to simulate blood flow through the larynx and provide oxygen and nutrients to the cells located within the extracellular matrix. Once the media passes through the internal vasculature, it will spill out the bottom of the larynx and into the exterior media. The exterior media can then be pulled back in by the pumps and the cycle repeats.

Figure 8: External media will be pumped in through the bottom and perfused back out through the left and right internal jugular veins (not shown) on the top of the larynx.

DESIGN ALTERNATIVE: FILL-REFILL

Like the first bioreactor design, the second bioreactor will be a 10x10x20cm box with a removable lid. A balloon will be inserted below the vocal cords and inflated to create two separate environments like in the first bioreactor design. The exterior media will be added in a similar way as it was in the first design; however, the inner media will not be delivered by spraying the cells on the inside of the lumen.

Instead, media will be pumped in and out of the lumen so at any given time, the cells are either exposed to media or to air. To perform this action, a reversible pump will be used. The pump will line the support tube and push media out until the lumen is full of media. After a given amount of media has been expelled from the tube, the tube will be sealed so that media cannot flow back down. This seal will trap the media on the inside of the lumen and expose all the cells that line the lumen to this interior media. To expose the cells to air, the tube will be opened and media will be allowed to flow down. To expose the cells to media, the pump will push more media in and the cycle will continue. This design is advantageous over the first design because

all cells are more equally exposed to media, and it is more controllable than the spraying technique of the first design.

DESIGN ALTERNATIVE: ROTATION

The third design, shown in Figure 9, more closely emulates the tracheal bioreactor currently available. Thus, this design is similar to that of the tracheal bioreactor, but modified to fit the size and shape of the larynx. The larynx is mounted on the inside of the chamber and media is perfused through the vasculature via pumps, just like with the previous two designs. The main difference between this design and the previous two, other than the fact that it is horizontally oriented, is that this is a rotational bioreactor. The larynx exterior is completely surrounded by media, while the inner lumen is half full with media and half full with air. The bioreactor will be designed to rotate slowly, guaranteeing full exposure of the inner lumen to media and to air. Unlike a traditional continuous unidirectional rotation, this bioreactor will rotate a set distance in one direction before changing directions and rotating in the opposite direction. This will ensure that the larynx is fully exposed to media, without the additional complications that a full rotation would bring, such as tangling the perfusion tubing and vasculature.

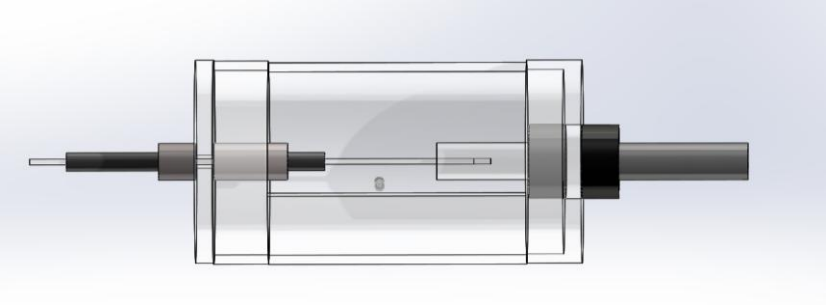

Figure 9: The third design alternative, which utilizes rotational movement and is horizontally oriented.

DESIGN MATRIX AND EVALUATION

	Weight	Design 1 Spray	Design 2 Fill-Refill	Design 3 Rotation
Cost	0.1	9	9	4
Decellularization-recellularization	0.3	7	7	8
Physiological accuracy	0.2	6	8	7
Adaptability	0.15	9	9	9
Maintenance	0.25	8	8	5
Total	**1**	**0.75**	**0.80**	**0.68**

Table 1: Summary of Design Matrix

The three design alternatives were evaluated and compared in the categories of cost; how conducive the bioreactor is to the decellularization-recellularization process; physiological

accuracy of cellular development; adaptability of the infrastructure; and media maintenance of the bioreactor. Cost was given a low weight since the most expensive component of the designs will be the perfusion pumps, a feature in all three designs. Since perfusion through the vasculature is a client requirement, pumps are included in all designs. The rotation design features additional mechanical motors and therefore will be more costly than the other designs.

Since the main objective of the bioreactor is to decellularize a larynx into an acellular scaffold followed by recellularization into a functional organ, this criterion was given the highest weight. The decellularization process will be the same in all designs and therefore the major difference between the alternatives is in the recellularization process. While all designs provide a favorable environment for recellularization, the rotation design received a slightly higher rating because this technique has proven successful in similar recellularization processes.

Physiological accuracy was defined as the similarity between the bioreactor environment and the *in vivo* laryngeal environment. This includes orientation of the larynx as well as the air and media exposure techniques utilized in each design. The fill-refill design will best achieve the *in vivo* environment since the larynx will be secured in the anatomical position. The technique of having the inner lumen entirely exposed to either air or media is a more realistic representation of the *in vivo* conditions.

Adaptability encompasses two areas: the ability to secure larynges of varying sizes and alterations to be made to the bioreactor. All designs feature securing methods that allow for different sized larynges. Since the recellularization process is largely unknown at this point, the bioreactor must be able to handle experimental variations on media composition and levels in addition to minor mechanical alterations to optimize the recellularization process. All three alternatives were designed with this capability and therefore all received the same score.

The final important criterion, receiving the second highest weight, is maintenance of the bioreactor. This was characterized by the ease of maintaining the different medial levels and compositions in the external, internal, and perfusing environments. While the maintenance of the external and perfusing environments will be similar in all three designs, the internal environment will be the most difficult to access and the media composition will be most frequently altered. The similarities in the spray and fill-refill technique allow for the easiest access to the internal environment, thus enabling the media to be easily replaced.

FINAL DESIGN

Compiling the results of the alternatives in the design matrix, the fill-refill design is the clear choice for the final design. This design received high rankings in all matrix categories. The lowest score it received was in the decellularization-recellularization category; however this is only due to unknown nature of what defines a successful recellularization process for the larynx. What separates the fill-refill design from the other two alternatives is the physiological accuracy. The air exposure technique allows for the inner lumen to be entirely exposed to media, as it would be in the embryonic development, or to air, as it would be in the postnatal environment.

ETHICAL CONSIDERATIONS

Ethical considerations for this project lend themselves to future use of the bioreactor. The tissue engineered larynges could cause harm if not properly attended to in the decellularization-recellularization process. If components are not properly sterilized throughout the entire process, contaminants could introduce unwanted pathogens to the donor recipient. This could stimulate a negative immune reaction to the engineered larynx.

FUTURE WORK

Testing will be performed on the effectiveness of sealing techniques used to separate the larynx lumen from the exterior. As this differentiated environment is essential to the overall function of the bioreactor, the results of these tests will be vital in determining if any modifications need to be made to the final design before prototyping.

Once it has been confirmed that the larynx lumen can be sufficiently isolated from the exterior culture environment, focus will be redirected towards acquiring materials and assembling the prototype. Perfusion pumps will need to be purchased, as well as material for the construction of the bioreactor itself. Translucent or transparent autoclavable plastics will most likely be utilized for this purpose, as they provide visualization of the larynx and are capable of withstanding the high temperatures and pressures of autoclaving.

The assembled prototype will then be tested under the conditions the final model will need to function under. Tests will be performed within an incubator environment and for the duration of decellularization (estimated at 24-48 hours with perfusion) and recellularization (estimated at approximately 3 weeks). Iterative design will be used to address any palpable limitations found in the testing process.

REFERENCES

[1] Gray's Anatomy Online. *Splanchnology*- The Larynx. http://education.yahoo.com/reference/gray/subjects/subject/236. Online. Accessed 10/22/12.

[2] *Larynx Anatomy*. Emedicine.medscape.com/article/1949369-overview. Online. Accessed 10/22/12

[3] Titze, IR. *Principles of Voice Production*. National Center for Voice and Speech. Iowa City, IA. 2006

[4] Hou N, Cui P, Luo J, Ma R, Zhu L. Tissue-engineeed larynx using perfusion-decellularized technique and mesenchymal stem cells in a rabbit model. Acta Oto-Laryngologica, 2011; 131:645-652.

[5] Battey, James F., and Laura K. Cole. "A Stem Cell Primer." Pediatric Research 59.Supplement 1 (2006): 1R-3R.

[6] Badylak, Stephen F., Doris Taylor, and Korkut Uygun. "Whole Organ Tissue Engineering: Decellularization and Recellularization of Three-Dimensional Matrix Scaffolds." Annual Review of Biomedical Engineering 13.1 (2010): 110301095218061.

[7] Martin, I. "The Role of Bioreactors in Tissue Engineering." Trends in Biotechnology 22.2 (2004): 80-86.

[8] Organ Procurment and Transplantation Network. http://optn.transplant.hrsa.gov/organDatasource/. Online. Access 10/23/12

[9] Birchall MA, Lorenz RR, Berke GS, Genden EM, Haughey BH, Siemionow M, Strome M. Laryngeal Transplation in 2005: A Review. Am J or Transplantation, 2006;6:20-26.

[10] "Extraordinary larynx transplant restores voice to California Woman". http://www.ucdmc.ucdavis.edu/welcome/features/2010-2011/01/20110126_larynx_transplant.html. Online. Access 10/23/12

[11] Petersen, T.H., et al., Tissue-engineered lungs for in vivo implantation. Science. 329(5991): p. 538-41.

[12] Ott HC et al., Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med. 14,213(2008)

[13] Gilbert TW, Sellaro TL Badylak SF, Decellularization of tissues and organs. Biomaterials. 2006;27:3675.

[14] Baptista PM et al. Whole organ decellularization - a tool for bioscaffold fabrication and organ bioengineering. IEEE Eng Med Biol Soc. 2009;31:6526.

[15] 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.

[16] UW School of Medicine and Public Health Faculty Profile. http://www.surgery.wisc.edu/profile/nathan-welham. Online. Accessed 10/22/12.

[17] PubMed Author search: Nathan V Welham. http://www.ncbi.nlm.nih.gov/sites/entrez. Online. Accessed 10/22/12.

APPENDIX: PRODUCT DESIGN SPECIFICATIONS

Perfusion decellularization-recellularization bioreactor for laryngeal tissue engineering

September 26th, 2012

Armand Grabowski, Taylor Milne, Brett Napiwocki, Sara Schmitz, Ben Smith

Problem Statement

The purpose of this project is to design a sterile bioreactor for whole organ tissue engineering of the human larynx, as well as comparable large animal models such as the pig or dog larynx. The bioreactor must be capable of preforming two different processes: perfusion-decellularization of the larynx to create an acellular scaffold, and perfusion-recelluarization of the acellular scaffold using vocal fold fibroblasts and other cell sources.

Client Requirements:

- The bioreactor must be sterile and have parts that can be replaced or autoclavable
- The bioreactor must easily be able to fit inside of the incubator
- The bioreactor should be capable of decellularizing and recellularizing the larynx
- The bioreactor must be able to function for days at a time

Design Requirements:

1. Physical and Operational Characteristics

a. *Performance Requirements:* The bioreactor needs to be capable of decellularizing a larynx to produce an acellular scaffold and subsequently provide an environment to recellularize the scaffold. This will be accomplished through perfusion pumps through the vasculature and trachea.

b. *Safety:* The device needs to be sterile if the larynx will be used in future transplantation. Additionally, the device should contain sufficient safeguards against user chemical exposure.

c. *Accuracy and Reliability:* The flow rate through the vasculature should be similar to physiological values and be able to maintain function throughout the entire decellularization/recellularization process.

d. *Life in Service:* For the decellularization process, the bioreactor should perform for a minimum of two days. For the recellularization process, the bioreactor needs to be able to perform for a minimum of 3 weeks. The bioreactor must also be reusable.

e. *Shelf Life:* The bioreactor should be capable of performing for 5 years.

f. *Operating Environment:* The bioreactor should be fit to operate in an incubator environment. Typical conditions include 37degrees C, 5% CO_2 and humidity. The device will also be exposed to various chemicals and liquids which are commonly found in a bioreactor environment.

g. *Ergonomics:* The bioreactor needs to movable and reasonable for one person to carry. It also must open to provide easy access to the tissue specimen.

h. *Size:* The reactor itself must be able to house a human or a large animal model larynx. The dimensions of the bioreactor must not exceed 50.8 x 54.1 x 68.1 cm.

i. *Weight:* The product must be handled easily by one person without excessive strain.

j. *Materials:* All materials used in the bioreactor must be sterile or autoclavable. None of the materials should degrade after exposure to detergents and other chemicals used during the decellularization and recellularization process.

k. *Aesthetics, Appearance, and Finish:* The bioreactor must look like it fits in a laboratory setting.

2. Production Characteristics

a. *Quantity*: 1 deliverable

b. *Target Product Cost*: $1-3000

3. Miscellaneous

a. *Standards and Specifications*: N/A

b. *Customer/Patient related concerns*: N/A

c. *Competition*: There are companies that already make bioreactors but none that are specifically made for the larynx.