Final Design Report - Bioreactor for Vocal Fold Tissue Engineering

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BME Design 200/300
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December 12, 2007

Table of Contents

Abstract

In order to effectively study vocal fold tissue outside of the human body, a bioreactor needs to be constructed that appropriately stimulates vocal fold tissue to behave as naturally as possible. Such stimuli include, but are not limited to, vibration, tensile stress, changing angles between the vocal tissue, and pressure variation of the tissue. Previous bioreactors have been made, both by researchers and a previous biomedical engineering student team, but did not sufficiently mimic the human vocal fold environment. Our objective for this semester is to improve upon the pre-existing bioreactor designs by completing the design and constructing a new bioreactor which has one or more improved cellular substrates, vibratory stimuli, tensile stress, and changing angles between each pair vocal tissue strips.

Problem Summary

The aim of this project is to re-design and improve upon a previous version of a bioreactor that will be used for the culturing of human vocal fold fibroblasts. The bioreactor needs to provide appropriate stimulation to fibroblasts in order to elicit behavior typical of *in vivo* human vocal fold tissue. The bioreactor will be used for studying healthy and diseased states of vocal fold fibroblasts, as well as researching possible therapies that may be applied to injured vocal folds.

The previous bioreactor design was able to vibrate two pairs of cell-seeded strips under tensile stress, but had design flaws that needed improvement, including keeping the bioreactor leak-proof, subjecting the cells to additional stimuli (including tensile stress and angular motion), and allowing the equipment providing the stimuli to be controlled by a computer. Our goals include finishing the design and fabrication of this new model, to obtain a substitute for the cellular substrate, Tecoflex, and to test the bioreactor and cellular substrate for optimal design and operating conditions.

Background Information

Bioreactor

A bioreactor is a system or device that supports a biological environment (Wikipedia). In this project, a bioreactor will be used for growing and maintaining fibroblasts in conditions that closely resemble the *in vivo* environment. To grow as fibroblasts, the cultured cells require a sterile environment with a constant temperature of 37° Celsius, a high level of humidity, and a 5% CO_2 level. To sustain or obtain properties of vocal fold fibroblasts, the cells require similar stimuli occuring in the larynx, such as tensile stress and vibration (Titze).

Vocal Folds

The vocal folds are a pair of elastic tissue found horizontally inside the larynx (Figure 1). When air is exhaled through the lungs and reaches the closed vocal folds, the folds open and close many times per second, causing a “mucosal wave”, with vibrations that can be manipulated by the throat, mouth, and lips into speech (Altman)(Figure 2). The vibration of human vocals folds could naturally occur at the frequency ranging from 100-1000Hz, at an amplitude of 1mm (Titze). The length of male vocal folds ranges from 17 and 25 mm, whereas the length of female vocal folds is between 12.5 and 17.5 mm (Wikipedia).

The vocal folds mainly consist of mucous membranes, however there is also a layer of extra-cellular matrix (ECM), which is attached to the cell surface and provides traction and positional recognition to the cell (Titze). Fibroblasts are critical for creating and maintaining the ECM in the vocal folds (Wikipedia). When the ECM is not in the proper condition, pathologies often result because of changes in viscoelasticity.

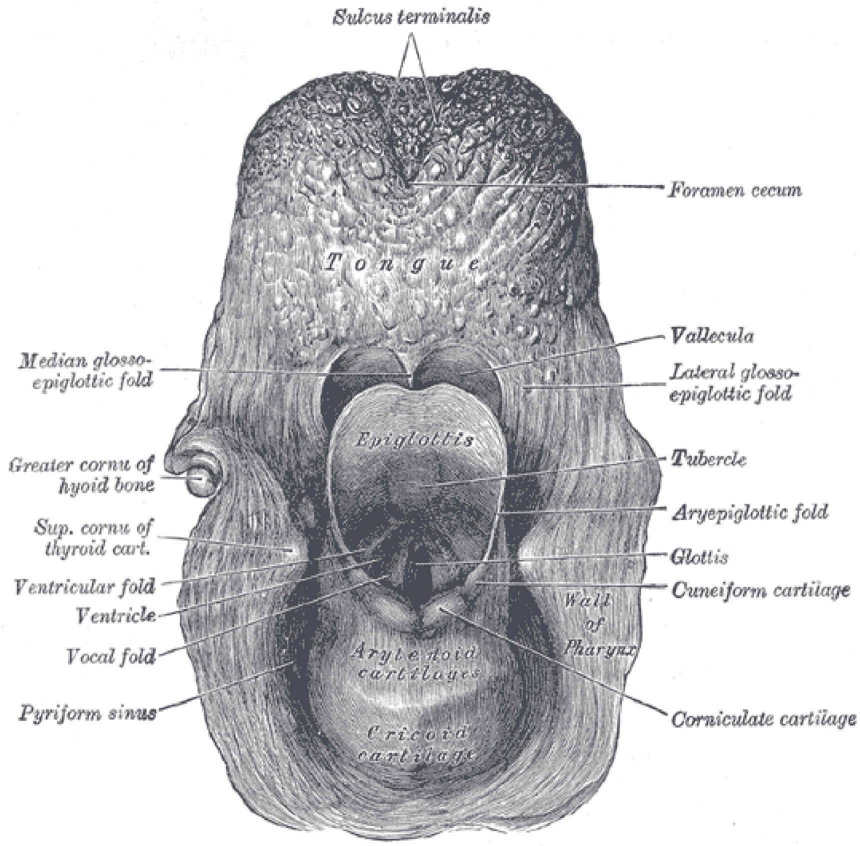

Figure 1: View of vocal folds inside larynx as seen from the back of the throat.

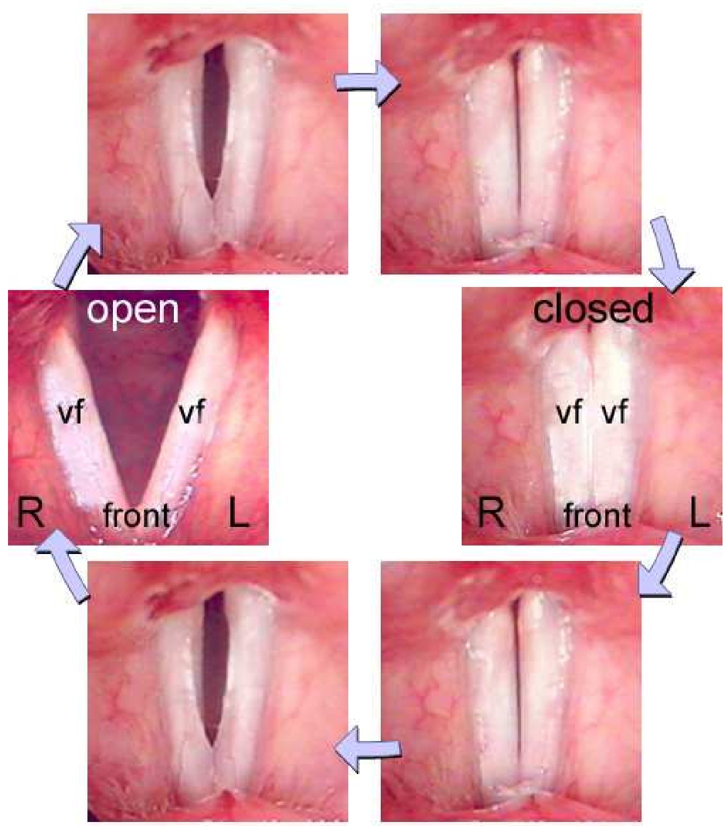

Figure 2: Photographs illustrating the movement of healthy vocal folds when speech is occurring.

Current Device

A literature search was performed to see if other similar bioreactors have been built, and it seems that the only previous bioreactor for vocal fold tissue that has been tested was designed by Ingo Titze *et al* in 2004. It was this bioreactor design that was modified by a design team spring semester of 2007, and is being modified and improved by this team. Recently, it has been learned that Xinqiao Jia and her colleagues have made a bioreactor that combines vibration, tensile stress, and pressure, but has not been tested quantitatively or qualitatively (New Tissue).

The previously built bioreactor (Figure 3) incorporated the use of an electromagnetic voice coil actuator and a stepper motor to provide stimulus to the cell-seeded Tecoflex strips immersed in hyaluronic acid medium and housed completely within a T-75 culture flask. This device, despite its long lifespan, was composed of a life-limiting actuator and motor components, while the T-flasks containing the cell cultures were properly sealed and disposed of after several weeks of use. Periodic maintenance of the actuators and cleaning (sterilization) of the components were essential to both accurate data collection and performance ability. The approximate size of this device was roughly that of the T-75 culture flask, the stepper motor and the electromagnetic voice coil actuator.

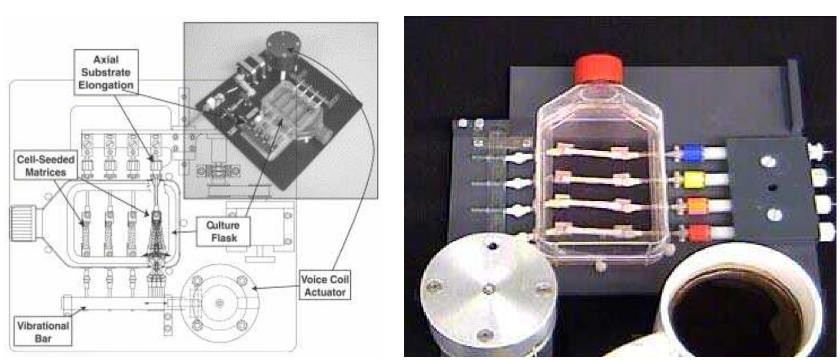

Figure 3: From left to right: Original bioreactor schematic diagram and fully operational bioreactor without a stepper motor.

Problem Motivation

Motivation for the bioreactor stems from the increasing need for knowledge of healthy and dysfunctional vocal fold tissue, in addition to increasing therapeutic options available for those with injured vocal folds. Because of the small size of the vocal folds, and the difficulty in obtaining healthy tissue for research from individuals, a bioreactor that can induce and sustain properties of *in vivo* vocal fold tissue is needed. Such a device would allow research of healthy and diseased vocal fold states, in addition to providing a source of healthy vocal fold fibroblasts that may be injected into injured vocal fold tissue to improve its condition.

Team Goals

The goal of the team for the semester is to finish the design and complete a version of a bioreactor that is an improvement upon a pre-existing bioreactor design. The previous bioreactor did not implement efficient vibration, and had absolutely no other stimuli, which does not accurately simulate the human vocal fold environment. The cellular substrate, Tecoflex, was too porous, and had an uneven porosity. To allow for more flexibility in testing and research, the design has two separate T-flasks with 2 pairs of strip in each flask. To more closely resemble the *in vivo* environment, this design has vibration, tensile stress, and changing angles between each pair of strips. It also has programmable motors that will be controlled by a computer, allowing the fibroblasts to be subjected to varying levels of stimuli. In addition, the cellular substrate will be replaced by one or more substrates that more closely mimic the environment experienced by the vocal fold fibroblasts.

Design Constraints

The bioreactor must fit into a standard-sized incubator and have sterile disposable parts and/or permanent parts that can be sterilized. The angle between each pair of strips needs to have the ability to change, allowing strips to make contact along the entire length, and can be adjusted to make contact at only one end. Each pair of strips must be vibrated within the frequency range of 50-400 Hz, as well as undergo tensile stress. The motors are required to be programmable and controlled by a computer. In addition, an improvement upon the previously used cellular substrate, Tecoflex, needs to be obtained and tested for its ability to help re-create the *in vivo* vocal fold environment. Since primary fibroblasts from mice will be used in testing, there are some ethical issues to consider, however, these cells were provided by the client and the choice of cells to be used is not under our discretion.

Competition

There are two different labs, Susan Thibeault's and Xinqiao Jia's, which are working on bioreactors for vocal fold tissue engineering; however there is collaboration rather than competition. Therefore, there is no plan to patent this design, and there are no other patents related to this specific bioreactor. The production value of this design is not significant, and only an estimated few dozen research groups around the world would be interested in having such a bioreactor (Thibeault 2 Mar).

Alternative Design Descriptions

Though the overall design of the bioreactor was in place, several design elements needed to be addressed. The greatest flaw of the current design is a moment due to the motion of the actuator. This moment will cause the polystyrene tubing to bend down into the T-flask, inhibiting the motion of the actuator, as well as causing the cell-seeded strips to come in contact with the bottom of the T-flask. A brainstorming session developed several ideas for resolution of this moment. These ideas include a pneumatic system, roller bearings, magnetic repulsion, and suspension by wire.

Pneumatic System

The first solution presented is a pneumatic system that would blow air onto the tubing, keeping the tube up with air pressure. This design offers one major advantage in the fact that it is frictionless. The fact that air is keeping the tubing up means that no energy is being lost to the environment in the form of friction. However, this solution has several major flaws. First and foremost, it would bring outside material into the bioreactor. This would contaminate the cells, giving the client neither accurate nor correct data. This design is also very difficult to construct and implement. A design would need to be constructed that included a high pressure air pump enclosed in an area that already has little free space. This would most certainly cause problems due to time and material constraints. Finally, a pneumatic system would be expensive and not very cost effective compared to other solutions.

Bearing design

The second resolution is a bearing located on the actuator support, allowing the moment to be expressed (Figure 4). This design has several benefits that the previous idea did not. It is a very simplistic solution, and as such would be easy to construct and implement. This idea is also an improvement in the fact that it is very cost effective. This design has one major drawback caused by the friction of the bearing with the polypropylene tubing. This friction not only causes energy to be lost, but also induces a great deal of wear and tear on the system. The actuator must run 50-400Hz frequency, which would result in the breakdown of material. This would significantly shorten the shelf life of the bioreactor. Although the actuator support has already been made, significant machining would be needed in order for this design to work.

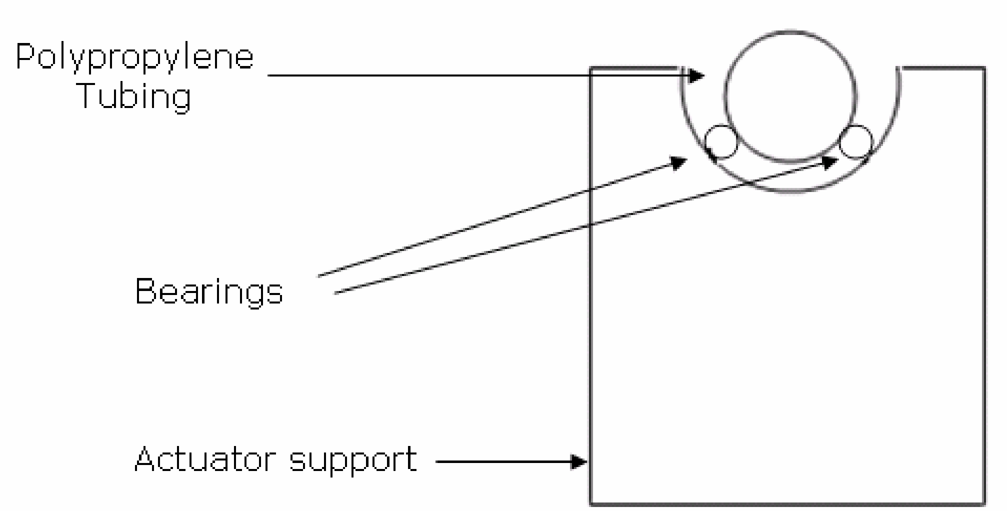

Figure 4: A bioreactor design with bearings allowing the moment to be expressed

Magnetic Repulsion

The next idea involved using magnets to keep the polystyrene tubing from rotating. By attaching two magnets with the same poles (one to the polystyrene, and one to the aluminum base), magnetic repulsion could be used to keep the tubing in place, and therefore opposing the moment (Figure 5). This design has several advantages. First of all, there would be no friction, allowing no other forces to come into play. Secondly, a magnetic repulsion system would be fairly simple to design, construct, and implement. This idea is also cost effective due to its relative simplicity and lack of materials. However, magnets induce magnetic fields, which interact with circuits and electronics. The bioreactor uses many electronic components to produce the effects of the stimuli on the cells, which would be adversely affected by the magnetic fields created by a magnetic repulsion system. Due to the fact that the client wants a functioning bioreactor, this design is simply unacceptable.

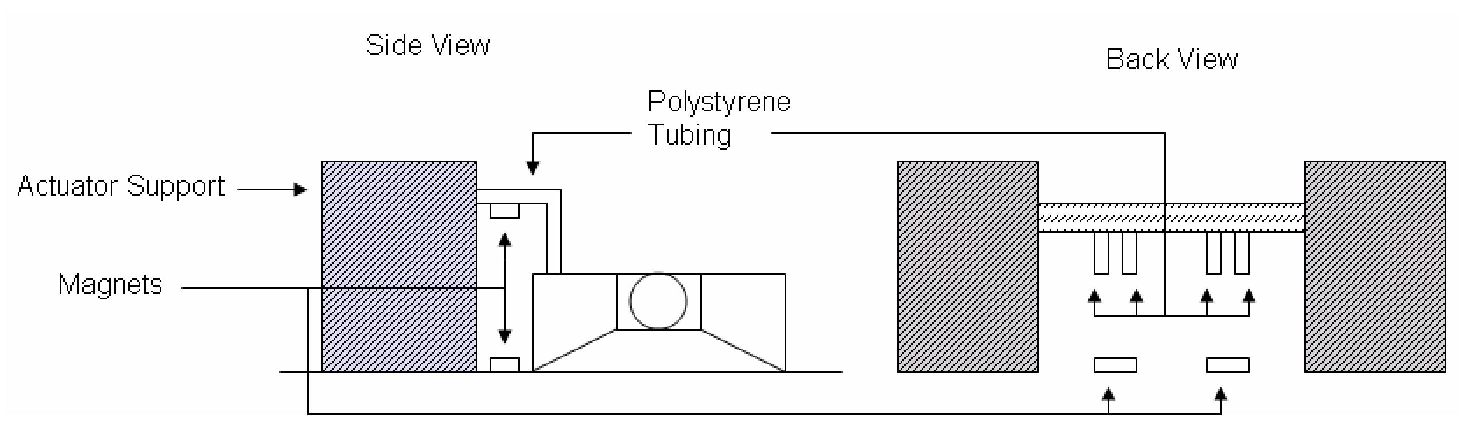

Figure 5: A bioreactor design using magnetic repulsion to oppose the moment. Displays both the side and back view

Suspension System

To eliminate negative effects on the bioreactor, a design involving a suspension system was developed (Figure 6). By stretching a bar across the length of the bioreactor, wires can be extended down from the bar to hold the polystyrene tubing up. This design is simple, cost effective, and easy to construct. The materials needed include copper tubing and fishing line. Most importantly, it has a minimal effect on the rest of the bioreactor. By only touching the polystyrene tubing, the suspension system cannot come in contact with the rest of the system. The fact that it does touch the system means that friction is produced, causing energy to be lost. While this is a disadvantage, the main problem encountered with this particular design is that it is unknown what effect the vibrations will have on the wire. Resonance may occur, as well as a number of other effects, due to the many stimuli produced by the bioreactor. However, this design allows testing to be done with a minimal effect on the bioreactor. If problems do arise that cannot be resolved, the entire system can be removed or replaced with no effect on the bioreactor.

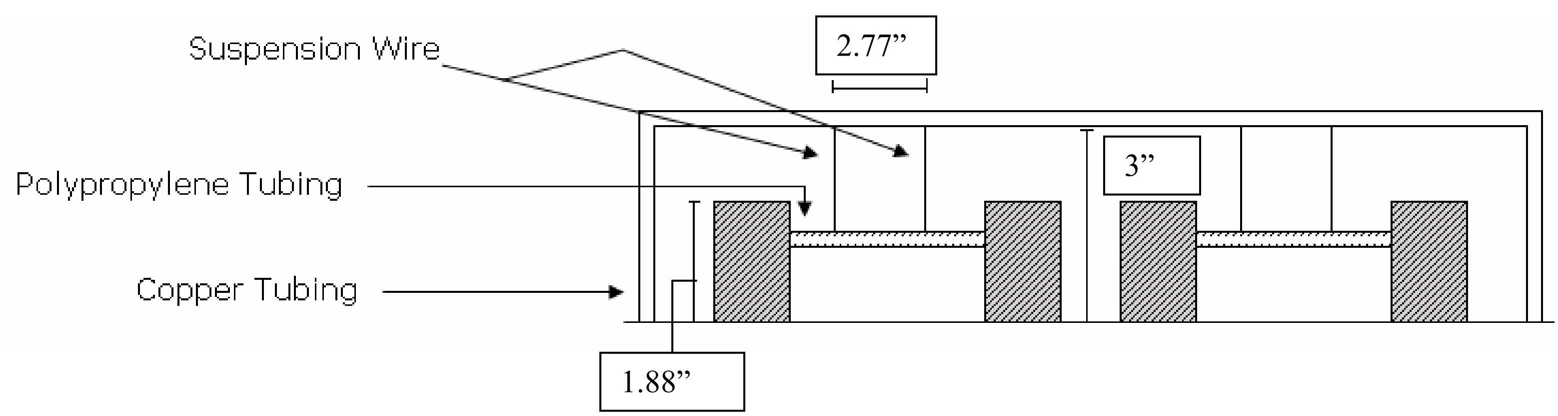

Figure 6: A bioreactor design that employs a suspension system to counteract the unresolved moment.

Design matrix

The design matrix included all four ideas on the resolution of the moment due to the actuator (Table 1). These ideas include the pneumatic system, bearings on the actuator support, magnetic repulsion, and a suspension system. The designs were evaluated using five criteria: "least amount of friction", "cost effectiveness", "simplicity", "effect on the rest of the system", and "ease of construction". These were weighted 25%, 6.25%, 18.75%, 25%, and 25%, respectively. It should be noted that "simplicity" refers to the simplicity of the design idea (while "ease of construction" refers to actual machining), and that a higher score given for "effect on the rest of the system" correlates to a small effect on the bioreactor. The design decided upon, the suspension system, was rated highest overall. The suspension system was rated much lower for least amount of friction, due to the fact that it will be subjected to strong vibrations, causing considerable amounts of friction.

	Least Amount of Friction (1-20)	Cost Effectiveness (1-5)	Simplicity (1-15)	Effect on Rest of System (1-20)	Ease of Construction (1-20)	**Total (80)**
Pneumatic System	20	2	3	1	3	**29**
Bearing/Wheel	15	4	10	15	10	**54**
Magnetic Repulsion	20	5	10	1	15	**51**
Suspension by String/Wire	12	5	13	17	16	**63**

Table 1: Design matrix that indicates the scoring of the design possibilities. The highlighted design achieved the highest score, and will be the design used in future work.

Final Design

After completing the design matrix, the best option was determined to be the suspended string design. This design involves a copper tube that extends horizontally from one end of the bioreactor to the other. The tube is three inches above the base of the bioreactor and anchored down at the two ends. There are four groupings of aluminum tubes, with only two (one for each actuator) suspended. Fishing line was tied in a custom knot, and actually tied to the copper tubing itself, not through the holes drilled into the copper. The fishing line loop was adjusted to the necessary size to hold the aluminum tubing in the proper position.

This fishing line suspension allows the vibrations to occur while simultaneously preventing the aluminum tubes from rotating downward. While some friction is created between the string and the aluminum tubing, it does not seem to have a large effect on the system. Most importantly, this design does not interfere with the cell environment inside of the T-flasks, allowing the bioreactor to be used for extensive research. Another advantage to this design is that it was relatively easy to construct, requiring simple materials and minimal machining needed to put the system in place. Lastly, this approach is very cost effective since there are only a few easily obtainable materials involved. On the other hand, this design has several possible problems.

One potential problem is that the fishing line may interfere with the vibratory stimuli. Since there will be some friction created between the fishing line and the aluminum tubing, the fishing line may dampen the effect of the vibrations. In the occurrence of this event, a material other than fishing line that doesn't interfere as much may need to be found. This also could be solved by allowing the fishing line to move horizontally along the copper tubing so that it was moving with the vibrating rods. Another problem that may occur is that the fishing line might not be strong enough to withstand the vibrations for a long period of time. If this turns out to be the case, a new, stronger material may have to be found or the fishing line could be wrapped around the aluminum tubing several times to add strength.

Another important aspect of the final design is the material used to mimic the vocal folds themselves. As shown in figure 7, the vocal fold is made up of the looser layer known as the superficial lamina propria, and the more rigid vocal ligament.

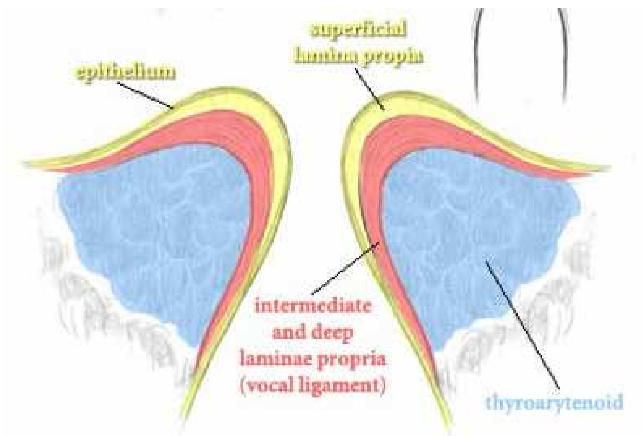

Figure 7: Vocal Fold Structure (Voice Problem)

In order to effectively simulate this structure, two materials with varying properties had to be found. In order to mimic the vocal ligament, Tecoflex SG-80A was used. Shown in figure 8, this material is a thermoplastic polyurethane and when it is dissolved in DMAC, combined with Pluronic 10R5, and lyophilized, it becomes an elastic material very similar to the vocal ligament. This specific material was chosen since it has been used to mimic ligaments in the past and has been specifically used to mimic the vocal ligament in previous research (Titze). The other material needed was something looser then the Tecoflex that would simulate the superficial lamina propria. After consultation with Dr. Glenn Prestwich of the University of Utah, it was decided that the Extracel hydrogel would be a suitable material to mimic the superficial lamina propria. It is not as rigid as the Tecoflex and allows for the seeding of cells either on top or within the hydrogel. In order to make the layered structure found in human vocal folds, Tecoflex strips were cut (10 mm x 100 mm x 2 mm) and the hydrogel at a thickness of approximately 2 mm was poured on top. Cells were then seeded on top of the hydrogel and the strips were able to be placed in the bioreactor.

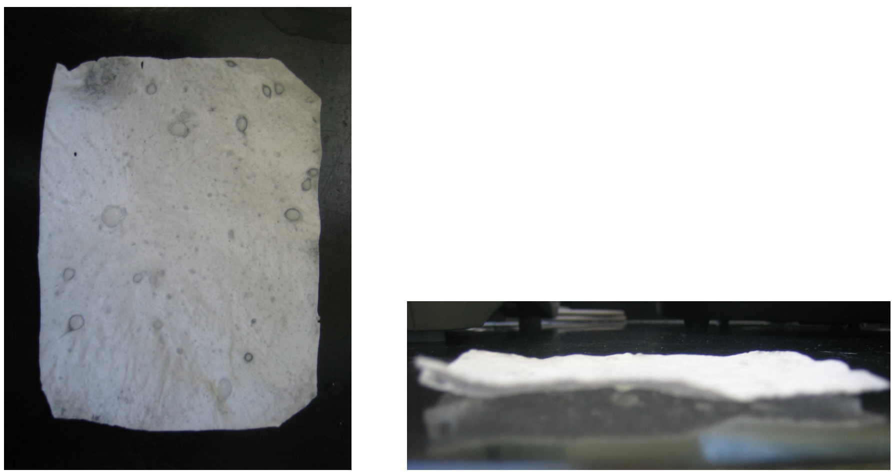

Figure 8: Tecoflex top and side views

Figure 9 shows the completed working prototype. The linear voice coil actuators, encased by the aluminum blocks, produce vibrations (with the aid of a function generator and amplifier) in the cell-seeded strips via the vibration bar, aluminum rods, and polystyrene pipettes. The linear stepper motors, attached to the long rails (platens) in back, stretch the cellular substrate (Tecoflex and hydrogel), located in the T-flask. This provides varying tension in the strips which is found in normal human speech. The rotary stepper motors, located on top of the linear motors, cause the angle between each pair of cell seeded strips to change. The rods are alternately left- and right-hand threaded so that when the rods are rotated, the aluminum scissors will open and close depending on the direction the motor shafts rotate. During normal human speech, these angles change, so this function of the bioreactor is integral for properly simulating the vocal fold environment. The linear stepper motors and rotary stepper motors still need to be programmed with a computer, so they are not fully functional yet.

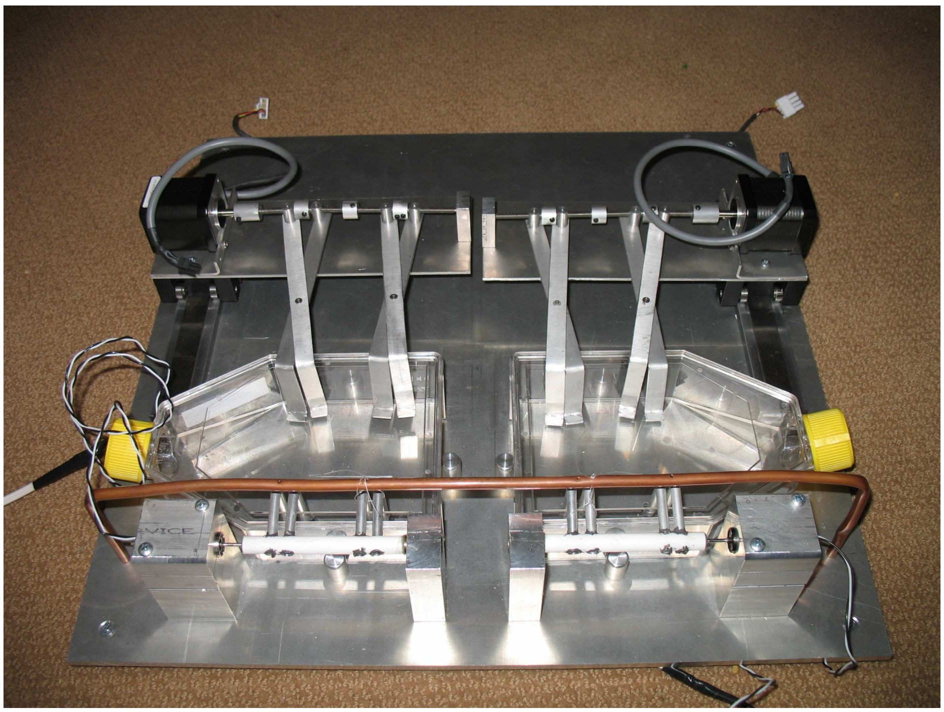

Figure 9: Working Prototype

In order to complete the final design, the tasks were divided in the following manner: Kara worked on the cellular substrates and cell culture; Joel worked on the cell culture and testing; Rachel machined the bioreactor. The estimated cost of materials for this semester was about $300 and the total hours spent (as a team and as individuals) has been estimated to be several hundred.

Testing

In order to see if cells would grow on the material, the hydrogel was poured on top of a Tecoflex strip and cells were seeded on top of this layered structure. The material was then placed in an incubator overnight, and after 15 hours the cells were checked to see if they were still alive. By looking under the microscope, living cells could be identified, which showed that cells could survive on the material. Figure 10 shows living cells on the Tecoflex and hydrogel material after incubation.

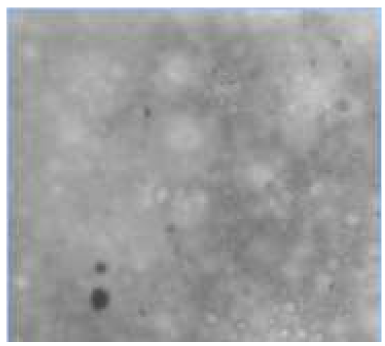

Figure 10: Cells on Tecoflex/hydrogel

Testing was also conducted on the ability of the bioreactor to vibrate the Tecoflex strips. To do this, Tecoflex strips were attached to the bioreactor using o-rings and the system was turned on. A high speed camera which photographed at 2000 frames/sec, filmed the Tecoflex strips vibrating at a frequency of 180 Hz. A frequency of 180 Hz was chosen because it is a typical and common frequency of the human voice. By looking at the videos, it was apparent that the Tecoflex strips were touching and creating a wave pattern characteristic of human speech. In addition, a stroboscope was used to record the Tecoflex vibrating. The film from the stroboscope produced similar results, and it was even easier to see the wave motion. Also, the test was conducted with the strips under water since eventually the strips will be immersed in media to keep the cells alive. In this test, the strips still touched and a wave motion was produced once again. However, there was a visible decrease in vibration due to drag. Overall, the testing was very successful and showed the bioreactor had the capability to produce motion similar to what is naturally found in humans.

Future Work

While a lot of progress has been made, there is still a considerable amount of work that needs to be done before the bioreactor is complete. The Tecoflex and hydrogel material fabrication method needs to be perfected to create a uniform substance that stays together when subjected to stimuli in the bioreactor. In addition, a mold for the Tecoflex that does not dissolve from DMAC needs to be fabricated so that plastic from the mold does not dissolve in the Tecoflex. Also, a better method of attaching the Tecoflex strips to the bioreactor needs to be found. Currently o-rings are used, however it is very difficult and cumbersome to get the strips within the o-rings. Another problem that needs to be addressed is sometimes the actuator rods stick while vibrating, which produces a non-uniform vibration. Hopefully, the use of springs on the side of the actuators will correct this problem. Also, programming of the motors needs to be completed so the bioreactor can be easily controlled by the user. Lastly, a pressure stimulus needs to be incorporated into the bioreactor. In human speech air passes through the vocal folds, and this is not taken into account in the current bioreactor.

Conclusion

The goal is to create a bioreactor that accurately mimics the vocal fold environment so that meaningful research can be done. The stimuli used to do this include vibratory forces, tensile stress, and changing angles between each pair of cell-seeded strips. In order to accomplish this goal, a suspension system to prevent undesirable rotation was created and installed. In addition, new materials to mimic human focal folds were found. The ability of the material to sustain cells and the ability of the bioreactor to vibrate the strips appropriately was tested with successful results. Work will continue on the bioreactor so it can be completed and used in vocal fold research.

Appendix A: Product Design Specifications

Product Design Specifications for BME 200/300 group 18: Bioreactor

Group members: Kara Barnhart (communicator & BSAC), Joel Gaston (BWIG), Rachel Mosher (team leader)

Problem Statement: The aim of this project is to re-design and improve upon a previous version of a bioreactor that will be used for the culturing of human vocal fold fibroblasts. The previous design was able to vibrate two pairs of cell-seeded strips under tensile stress, but had design flaws that needed improvement, including keeping the bioreactor leak-proof, subjecting the cells to more stimuli, and allowing the equipment providing the stimuli to be controlled by a computer. Our goals are to finish the design and fabrication of this new model, to obtain at least one substitute for the cellular substrate, Tecoflex, and to test the bioreactor and cellular substrate for optimal design and operating conditions. The bioreactor will be made from 2 T-flasks, 2 moving magnet linear voice coil actuators, 2 rotary stepper motors, and 2 linear stepper motors. A total of 2 pairs of strips will be immersed in a buffer in each T-flask, and subjected to vibration, tensile stress, and angular changes between each pair of strips. This device needs to be easily replaced with disposable and/or sterilized parts, fit inside a standard incubator, and have a capacity to generate vibrations within the frequency range of 50-400 Hz.

Client requirements: (improvement of design)

- Even vibration across length of cell-seeded strip
- Vibration frequency of 50-400 Hz
- Contact between each pair of cell-seeded strips during vibration.
- Easily sterilized and/or disposable parts
- Obtain and test alternative(s) to Tecoflex substrate
- Cost limit $5,000

1. Design requirements

Since this device is an improvement upon a previous bioreactor (which most of the new design and some of the machining has already been accomplished), the focus for this project is to improve and finish building the current design, and to extensively test it along with one or more new cellular substrates. In addition to testing the bioreactor for design flaws, two support systems for the device must be designed and machined. A Tecoflex substrate needs to be replaced by a more optimal material, such as a crosslinked hydrogel. Furthermore, the parts of the device need to be disposable and/or easily sterilized. The vibration frequency should range from 50-400 Hz. Overall, the expenses should not exceed $5,000.

a. *Performance requirements:* This device is meant to culture and research the reactions of vocal fold fibroblasts (and potentially other cells) to various stimuli, such as frequency and duration of vibration, varying tensile stress, and changing angle between each pair of strips.

b. *Safety:* Cell culture procedures will need to be followed. The reusable portions of this device will need to be disinfected periodically. The disposable parts of the bioreactor will need to be replaced frequently. Fluid from inside the T-flask cannot leak out onto the base of the bioreactor, which could potentially make contact with the electrical motors and cause a safety (and financial) risk.

c. *Accuracy and Reliability:* The system may need calibration when operating under computer-controlled conditions.

d. *Life in Service:* The T-flask and most of its interior parts are disposable components of the bioreactor that are disposed of after each use (at least several days at a time). The life-limiting factors of the bioreactor will probably be the motors.

e. *Shelf Life:* The T-flask, polystyrene pipettes, cellular substrate, and o-rings are disposed of after each use. The other components, including the aluminum parts and motors, should have a shelf life of at least several years.

f. *Operating Environment:*

- Temperature range: Incubator (37° C)
- Pressure: Negligible differences in pressure.
- Corrosion from fluids: The humidity in the incubator may compromise the capabilities of the motors and the quality of the metal components over time.

g. *Ergonomics:* The T-flask should be easy to remove and replace.

h. *Size:* The bioreactor must be able to fit inside a standard incubator, therefore it is limited to 18” x 18” in width and length.

i. *Weight:* The weight of the bioreactor will probably be several pounds, as well as the amplifier and function generator(s) required to operate the voice coil actuators.

j. *Materials:* Two T-150 cell culture flasks, 2 moving magnet linear voice coil actuators, 2 rotary stepper motors, 2 linear stepper motors, vibration bars and connectors, spring holders, cellular-seeded strips, moving forceps, left- and right-handed threaded rods, and base plates. If the system will be computer controlled, there will also be two power sources, 2 data cards, and 4 MicroLynx controllers. If not, 1-2 function generators and one amplifier will be used.

k. *Aesthetics, Appearance, and Finish:* Although the aluminum parts are easy to machine, lightweight, and are great conductors of heat, they are also aesthetically pleasing.

2. Production Characteristics

a. Quantity: Although we are only making one prototype, the client has shown interest in having several replicas in order to perform experiences with many variables. Because of the specific characteristics of the device and the small number of institutions involved in this particular type of research, production of the bioreactor would be limited to a dozen at most.

b. *Target Production Cost:* The cost of a similar (yet simpler) existing bioreactor was around $15,000, thus it is expected our bioreactor will have a comparable cost. Because the majority of the parts of the bioreactor were purchased over the summer, our costs for the semester are expected to be much less than $5,000.

3. Miscellaneous

a. Standards and Specifications: The bioreactor will not be used for human contact and therefore doesn't need to adhere to any international or national standards.

b. *Customer:* Anyone operating this device will be using it for research purposes, particularly with vocal fold fibroblasts. Because of its nature, strong computer and cell-culturing knowledge is required to obtain meaningful results.

c. *Patient-related concerns:* Since this device is not used for human contact, patient-related concerns do not apply.

d. *Competition:* Because of the very limited demand for this product, there is no intent by any party to patent this design.

<u>Appendix B: References</u>

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Appendix C: Parts Inventory

Part #	Part	Price	Purchased at	Manufacturer	Description
1	BNC to RCA cable	$19.95	Svideo.com		1 ft long
2	AL base plate	~$90	MSCDirect.com	MSC Industrial Supply Co.	.250" x 18" x 18" 6061 AL
3	Servo amplifier	~$120	Radioshack	Samson Audio	
4	Function generator	$259	Bkprecision.com	BK precision?	1 output .2 Hz – 2MHz
5	T-150 flasks	~$154	Midsci.com	TK	Box of 54
6	Rotary Stepper Motors	$19 each (2)	Automationdirect.com		NEMA-17 bipolar
7	Vibration Bar	~$3	Hobby Docktor (Odana Rd, Madison)	StripStyrene Evergreen scale models Woodville, WA	3/8" Polypropylene Tube
8	8/32"screws	$.16 ea (4)	Ace Hardware (Milton)		2.5" long
9	8/32" screws	$.08 ea (4)	Ace Hardware (Milton)		1.25" long
10	8/32" screws	$.08 ea (8)	Ace Hardware (Milton)		1" long
11	0-80 washer	$.19 ea (8)	Ace Hardware (Milton)		
12	0-80 hex nut	$.25 ea (4)	Ace Hardware (Milton)		
13	0-8 x 3/8" flat head	$.19 ea (4)	Ace Hardware (Milton)		
14	AL square for actuator holders, supports	$17.86	The Metals Depot	Ledford Steel Company (Winchester, NY)	1-1/2" x 1-1/2" 6061-T6 AL, 12" long
15	7/32" AL tube	$1.10 ea (4)	Uptownsales.com	KW	12" long Connects pipettes and vibration bar
16	13/32" brass tube	$2.30	Uptownsales.com	KW	12" long telescopic
17	7/16" brass	$2.60	Uptownsales.com	KW	12" long

	tube				telescopic
18	15/32" brass tube	$2.70	Uptownsales.com	KW	12" long telescopic
19	Brass strip	$1.40 ea (4)	Uptownsales.com	KW	.064" x .25" 12" long
20	Moving Magnet Linear voice coil actuator	$711 ea (2)	H2wtech.com	H2W Technologies	Model # NCM02-05-005-4JB .15" stroke, housing length 1.67"
21	Linear Stepper Motor	$490 ea (2)	H2wtech.com	H2W Technologies	Model # STS-0213-R 2-lb force
22	Roller Bearing Stepper Platen	$500 ea (2)	H2wtech.com	H2W Technologies	Model # STP-13-016-R
23	MicroLYNX - 4 Integrated Motor Drive and Controller	$453.30 ea (4)	All Control		# MX-CS101-401
24	24V Power Supply	$149.80 ea (2)	All Control		#ISP200-4 Up to 4 Amps
25	PCI Analog Output Board	$695 ea (2)	CyberResearch.com		#CYDDA 02HRP, 2 channels of 80 kHz, 16-bit D/A
26	Polystyrene pipettes	~$15	Fischer scientific		
27	RCA Cable	$9.99	Radio Shack		3 ft.
28	Phono Plug Coupler	$3.99	Radio Shack		
29	RCA Y Cable	$13.99	Radio Shack		Female to 2 Males
30	#8-32 Right Hand Rod	$7.95	Smallparts.com		TRX-0832 24"
31	#8-32 Left Hand Rod	$9.45	Smallparts.com		TRLX-0832 24"
32	BNC to RCA Cable	$20.45	Svideo.com		3 ft.
33	Copper Tubing	$15.00	Ace Hardware		
34	Fishing Line	$2.00	Ace Hardware		
35	Tecoflex SG-80A	Free Sample	Thermedics		

36	DMAC – 500mL	$36.10	Sigma-Aldrich		
37	Pluronic 10R5	Free Sample	BASF		