

Preliminary Design Report - Bioreactor for Vocal Fold Tissue Engineering  
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October 10, 2007

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## **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 cell-seeded strips, and pressure variation. Previous bioreactors have been made, both by researchers and a previous biomedical engineering student team, but did not sufficiently mimic the stimuli provided by the human body. Our objective for this semester is to improve upon the pre-existing bioreactor designs by completing the design and construction of a new bioreactor which has one or more improved cellular substrates, vibratory stimuli, tensile stress, and changing angles between each pair of cell-seeded 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 humans with 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 more stimuli, and allowing the equipment providing the stimuli to be controlled by a computer. Our goals for this semester 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.

The bioreactor will be made from two T-flasks, two moving magnet linear voice coil actuators, two rotary stepper motors, and two linear stepper motors. A total of four pairs of cell strips will be immersed in a buffer in two T-flasks, 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 sterilizable parts, fit inside a standard incubator, and have a capacity to generate vibrations within the frequency range of 50-400 Hz.

## **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<sub>2</sub> level. To sustain or obtain properties of vocal fold fibroblasts, the cells require stimuli that occur 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). However, the frequency that they mostly vibrate at is a range from 50-400Hz. 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 (Engineering).

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 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 to grow vocal fold tissue (New Tissue).

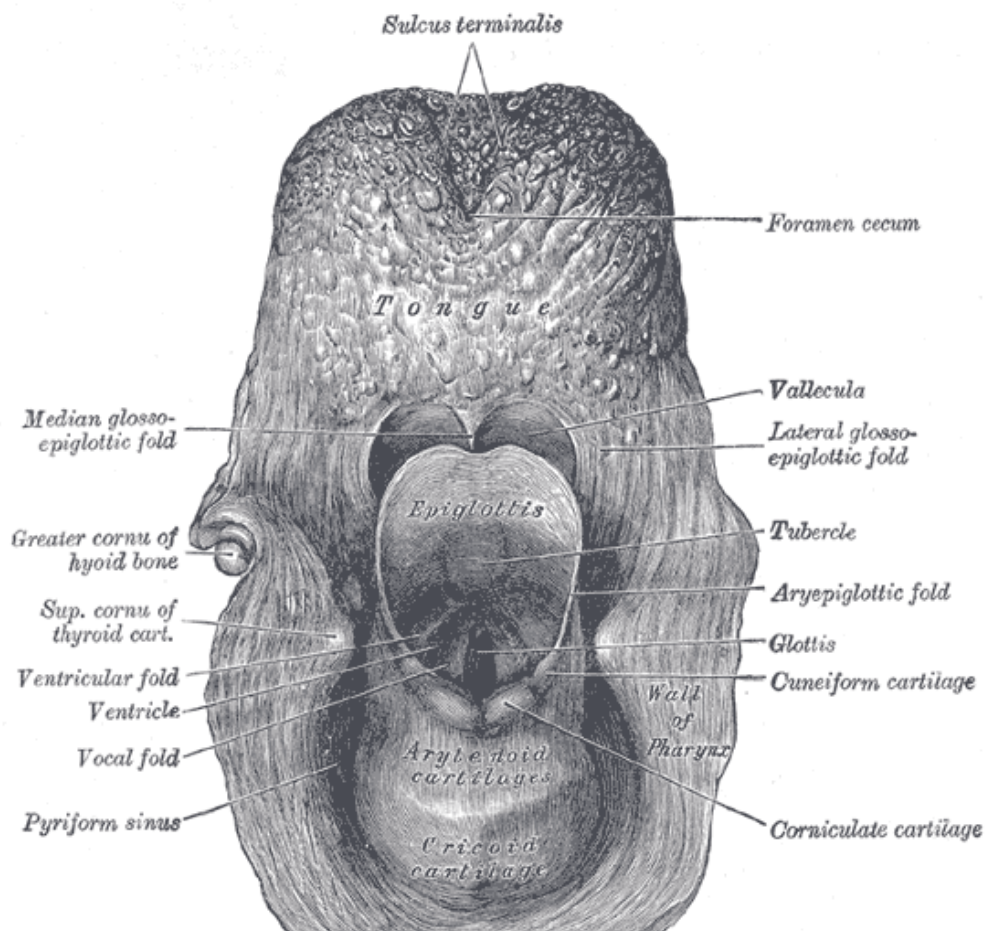


Figure 1: View of vocal folds inside larynx as seen from the back of the throat.

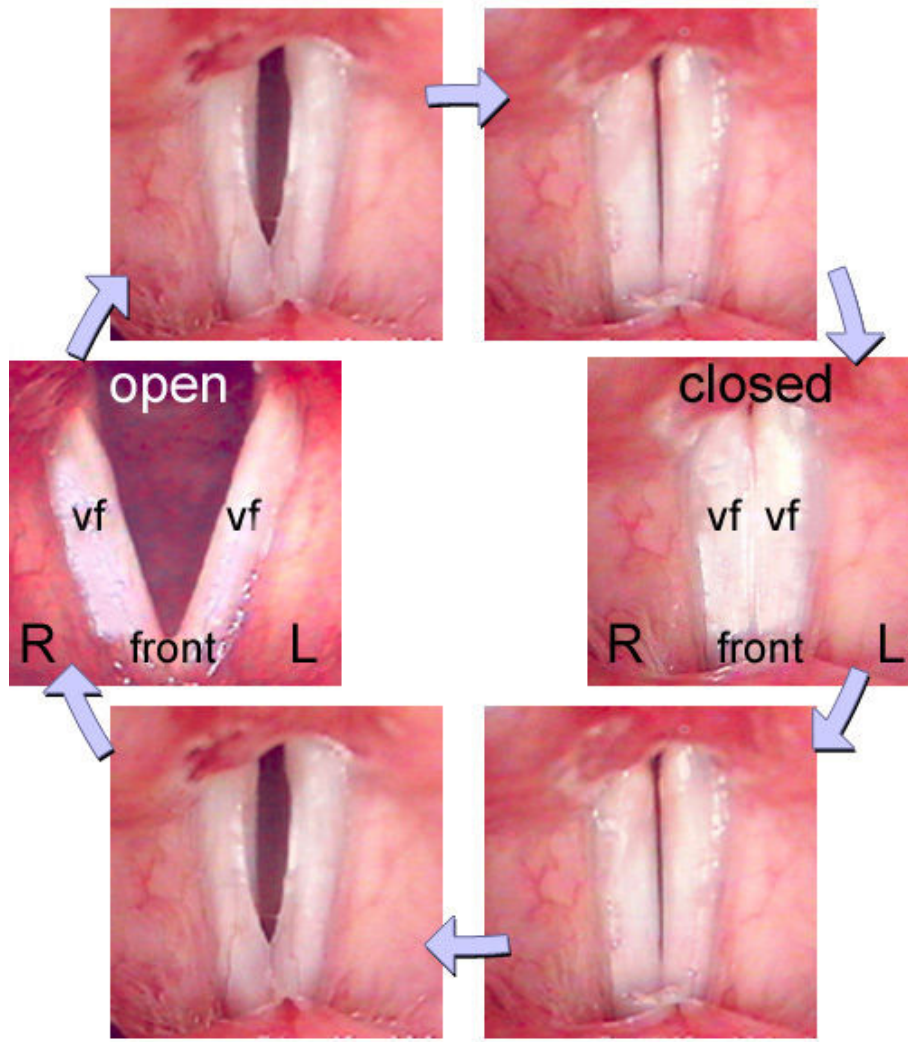


Figure 2: Photographs illustrating the movement of healthy vocal folds when speech is occurring.

Additional information needed will be dependent on the testing results of the current bioreactor design. If the present uses of motors do not properly stimulate the fibroblasts, more research pertaining how to more closely resemble the *in vivo* vocal fold environment will be needed. In addition, other environmental factors that determine vocal fold properties besides the stimuli addressed in this bioreactor design may be researched in preparation for the next version of the bioreactor.

### *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 completion of a version of a bioreactor that is an improvement upon a pre-existing bioreactor design. To allow for more flexibility in testing and research, the design also 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, formerly Tecoflex, will be replaced by one or more substrates that more closely mimic the environment experienced by the vocal fold fibroblasts.

### *Design Constraints*

The bioreactor needs to 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, and each pair of strips needs to vibrate and stretch as well. The motors need 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.

### *Current Device*

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, although having had a 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. Weight, however, had no effect on the function of the bioreactor.

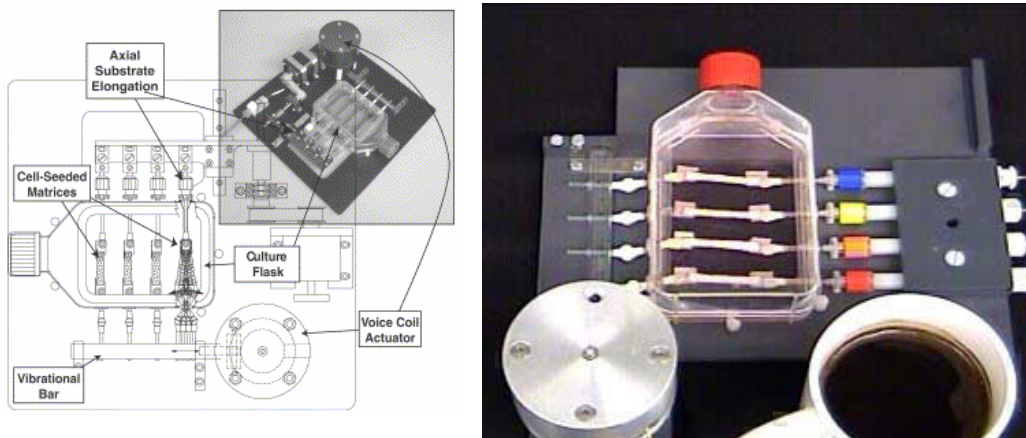


Figure 3: From left to right: Original bioreactor schematic diagram and fully operational bioreactor without a stepper motor.

### *Competition*

There has only been one bioreactor of this kind constructed. This device has since been disassembled, however there is probably more than one institution attempting to build a similar device. The goal for this semester is to build a single working prototype, however the future plan would be to have this device assembled in series with others like it for simultaneous research work. There is no plan to patent this design, and there are no other patents related to this specific bioreactor, nor are there any other designs besides ideas created by the original design team (Thibeault 2 Feb). 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). Furthermore, the ideal objective would be to create a more cost-effective design, as the previous bioreactor had a production cost of \$15,000 (Hitchcock).

### **Alternative Design Descriptions**

Though the overall design of the bioreactor was in place, several design elements needed to be addressed. One of the greatest flaws 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 other problems. A brainstorming session developed several ideas for resolution of this moment. These ideas include a pneumatic system, using 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, which could 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

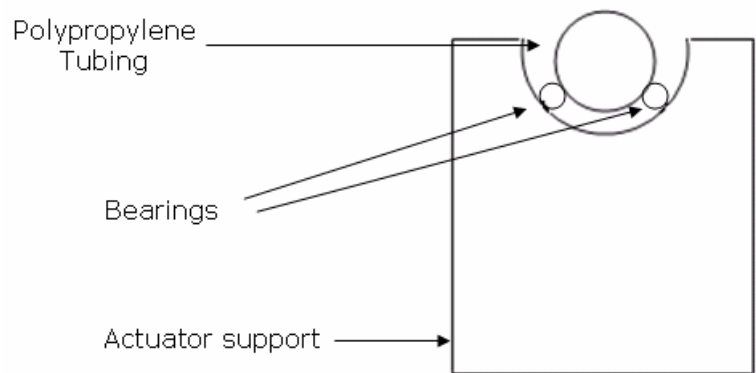


Figure 4: A bioreactor design with bearings allowing the moment to be expressed

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.

### *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. Being that the client wants a functioning bioreactor, this design is simply unacceptable.

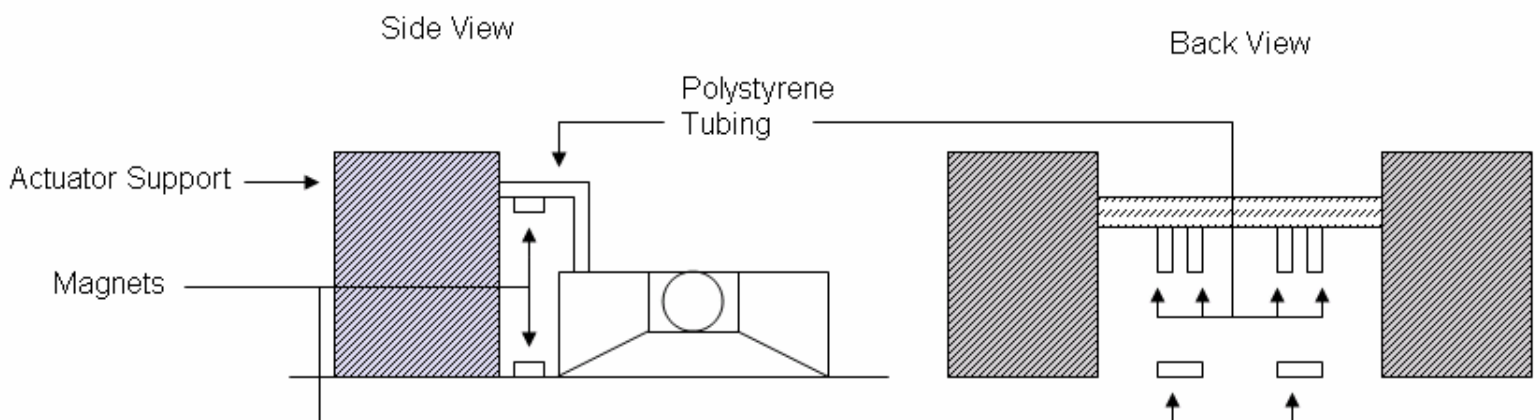


Figure 5: A bioreactor design using magnetic repulsion to oppose the moment. Displays both the side and back view

### *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.

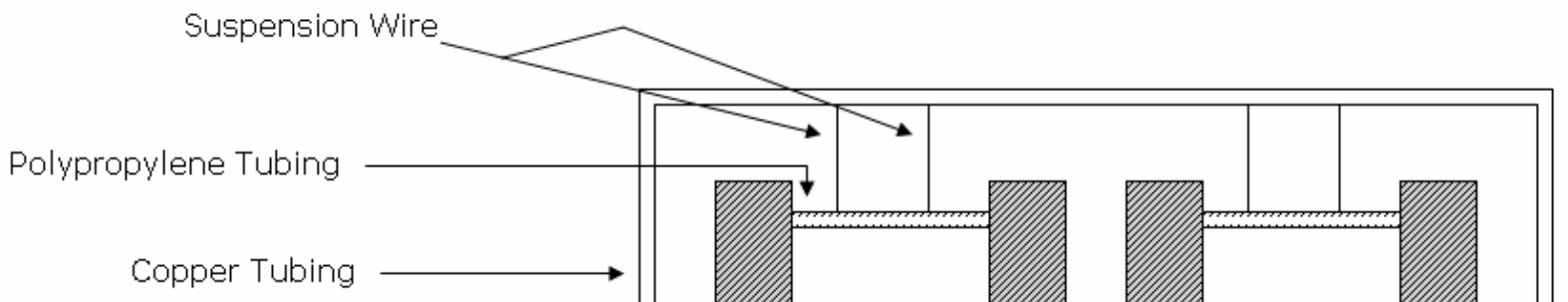


Figure 6: A bioreactor design that employs a suspension system to counteract the unresolved moment.

## 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” applies to the simplicity of the design for implementation, 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 in cost effectiveness, simplicity, effect on the rest of the system, and ease of construction. 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.

## **Proposed Solution**

After completing the design matrix, the best option was determined to be the suspended string design. This design involves a copper tube that will extend horizontally from one end of the bioreactor to the other. The tube will be approximately two inches above the base of the bioreactor and will be anchored down at the two ends. There are four groupings of aluminum tubes that need to be suspended, necessitating that four holes be drilled through the copper tubing. Fishing line will then be threaded through the holes and wrapped around the aluminum tubing. The fishing line loop will be adjusted to the necessary size to hold the aluminum tubing in the proper position.

This fishing line suspension will allow the vibrations to occur while preventing the aluminum tubes from rotating downward. While some friction will be created between the string and the aluminum tubing, it should not 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 is 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 downside, 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 made need to be found. There are other solutions to this problem as well, such as

increasing the frequency of the vibrations and realizing that some energy is lost to the fishing line. 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.

### **Future Work**

For the rest of the semester, the plans are to finish constructing the bioreactor so that testing using cells can begin. To assemble the bioreactor, the suspension system will need to be put into place, which includes attaching the copper tubing and fishing line to the system. Also, the side of the bioreactor that isn't finished yet will have to be held stationary for now in order for testing to begin. On this side of the bioreactor, polystyrene tubes will be made that go down into the T-flask, so the cellular substrate has something to attach to. The polystyrene tubes on the other side of the bioreactor will also have to be attached to the system.

The Tecoflex substrate needs to be ordered and the gel that goes on top of it is being shipped from Utah. Once the Tecoflex and gels are received, the cellular substrate will be made. When all of this work is complete, testing with cells will begin. The testing will involve first growing the cells on the substrate and then placing them into the bioreactor to see how the vibratory force affects them. Once testing starts, trouble shooting and redesigning will occur as needed. Also, while preliminary testing is going



on, the changing angle apparatus, including the brass forceps and linear and rotary stepper motors, can be finished and attached to the 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 the cell-seeded strips. In order to accomplish this goal, a suspension system to prevent undesirable rotation was decided upon. This system will be constructed and implemented and the rest of the parts of the bioreactor will be put together. In addition, new materials are being ordered to use as the cellular substrate which will allow for testing of the prototype with cells to begin.

## **Appendix A: Product Design Specifications**

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

Group members: Kara Barnhart, Joel Gaston, Rachel Mosher

*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 experiments 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.

## **Appendix B: References**

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<[www.wikipedia.org](http://www.wikipedia.org)>.

### 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		