Tissue Engineering Bioreactor

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Abstract

In the interest of further researching into vocal fold cells, our client would like to have a device that best simulates the environment and the motion of vocal folds. An improved bioreactor that best simulates the vocal fold environment needs to be developed. The simulation includes an even distribution of the vibration along the vocal fold cell-seeded strips, while the strips are coming into contact. A design of one single-sided vibration bar with two pairs of cell-seeded strips was selected, which largely improves the extent of vibration.

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Background

Bioreactor

A bioreactor is a system or device that supports a biological system. The purpose of this device is to simulate an active environment that is similar to a biological system. In this system, organisms or tissues of organisms are applied with known variables, such as pressure, temperature or stress ("Biochemical Engineering"). The results due to the variables are observed. A bioreactor can be in various sizes. Its size could range from cubic meters to cubic centimeters, which is as small as a Petri dish. The material of the bioreactor could be stainless steel or polyethylene and many other materials.

As the purpose of this project, an environment that is very similar to the larynx was simulated. A T-75 (Figure 1) culture flask made of polyethylene was used as the bioreactor body, with an opening on the upper surface of the flask in order for the materials to be placed inside the flask. A voice coil actuator was be attached to the T-75 flask to apply appropriate vibration (variable) to the system, which was then connected to the interior of the flask through drilled holes on the sides of the flask (Titze, 2004). The fibroblast (tissue for culturing) of the extra-cellular matrix (ECM) of the vocal folds was placed on the simulated ECM structure, Tecoflex, inside the flask, and submerged in fluid that is similar to the fluid at the larynx. Another function of the fluid is to provide necessary nutrition to the cell for cell culturing. The variable that will be applied to this system would be the vibration caused by the voice coil actuator. As the vibration is continuously applied to the fibroblasts, the alterations of the fibroblast will be observed (Webb, 2004). This will be a closed system, with flapped on lids to keep dust or molecules from contacting the culturing fibroblast. However, the larynx is just one type of the biological system simulated by the bioreactor. The bioreactor could be further adjusted to simulate any other parts of the living organisms.

Figure 1: Two T-75 flask showing, stacking one on top of the other. This is the typical culturing flask for tissue engineering.

Vocal folds

Vocal folds (Figure 2), also known as vocal cords, are two pieces of stretchable structure located inside the throat. This structure mainly consists of mucous membranes. There is also a layer of ECM, which is a "filamentous structure attached to the cell surface and provides traction and positional recognition to the cell" (Titze, 2004). The type of cell that synthesizes and maintains the ECM is called the fibroblast. Fibroblast provides a structural framework for tissues and is also critical in wound healing. Moreover, vocal pathologies are often determined by the conditions of the ECM. These conditions are often due to the alterations, which affect the mechanical properties of the tissue between the epithelium and the muscles of the vocal folds (*Ib.*). Furthermore, there is a cartilaginous tissue around the vocal folds that controls the vibration and stretching of the vocal folds. The vibration of human vocals folds could naturally occur at the frequency ranging from 100-1000Hz, at and amplitude of 1mm (*Ib.*). However, the frequency that they mostly vibrate at is a range from 0-400Hz.

The vocal folds are horizontally stretched across the larynx. When vibration is applied to the vocal folds, the larynx is exposed to high inertial stress (*Ib.*). The stretching and relaxing of these two membranes determine the pitch of one's phonation. The two vocal folds come in contact and produce a "mucosal wave" and produce the sounds, and also allow the exiting of the air from the lungs (Altman, 2002).

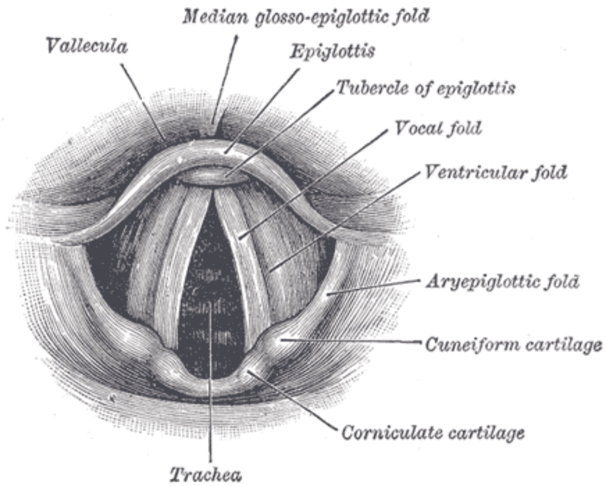


Figure 2. The left figure indicates the diagram of the vocal folds, with parts of the vocal folds labeled. The right figure is an actual screen shot of the vocal folds. Notice the two mucous membranes with the opening in the middle.

Problem statement

The larynx simulating bioreactor is currently operating under conditions that do not fully simulate the motion of the vocal folds. The existing device models the vibration of one vocal fold under tensile stress and provokes the elongation of fibroblasts and the subsequent formation of an extra-cellular matrix, characteristic of vocal fold fibroblasts. The goal is to improve upon this model by simulating contact between two vibrating vocal folds and distribute even vibration across the two vibrating vocal folds. The bioreactor will be made from a T-flask with elastic strips immersed in a buffer, which will function as vocal folds. A vibration motion from an actuator will be directed to the strips to provide vibration. This device needs to be easily replaced with disposable parts, along with 2 pairs of artificial vocal folds and a capacity to generate vibrations at frequencies ranging from 0-400Hz.

Problem motivation

The focus of this project is to construct a working prototype of a bioreactor that would better simulate the vocal fold motions. The vocal fibroblasts could be cultured for research purposes by subjecting the cell-seeded matrices (attached to Tecoflex strips) to certain stimuli. Such stimuli include both ‘violent’ vibrations and tensile stress that would effectively mimic the human vocal fold environment when vibrations are produced in the larynx. Research will be conducted while the environment of the bioreactor is maintained and monitored by a function generator and a signal amplifier. For best results, the distribution of uniform vibrations to the cell-seeded strips would be imperative to the success of a working bioreactor.

Design constraints

For this project, our client would like the bioreactor to distribute the vibration motion generated by an actuator across the cell-seeded Tecoflex strips while the strips contact each other. This encompasses a list of important criteria: performance requirements, safety, accuracy and repeatability.

The bioreactor must satisfy the performance requirements. The device must be able have a function generator that creates a frequency ranging from 0 to 400Hz. This vibration needs to be continuously provided to the Tecoflex strips for at least one full testing period, typically 2 to 3 weeks. Moreover, the vibration needs to spread along the whole strip to simulate the vocal fold motion. The strips also need to come in contact with one another.

Safety is also a concern when operating any devices. For a bioreactor, the actuator must not over heat during vibration. A lid covering the T-flask should be provided in order to prevent spillage of the culturing solution during operation. Furthermore, the T-flask will be placed in an incubator during usage, thus it needs to sustain at least 37°C without cracking or disassembling.

Accuracy and repeatability is always an important aspect for researches. The strips and the culturing solution need to be replaceable, and the T-flask should be easily sterilized for successive experiments. By accurately repeating the experiments, the client would be able to collect comparable data for analysis.

The cost of all materials, including poster printing needs to be under $5,000, which is how much the client funds this project.

Current devices

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 a nutrient solution 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.

Competition

There has only been one official bioreactor of this kind constructed. This device has since

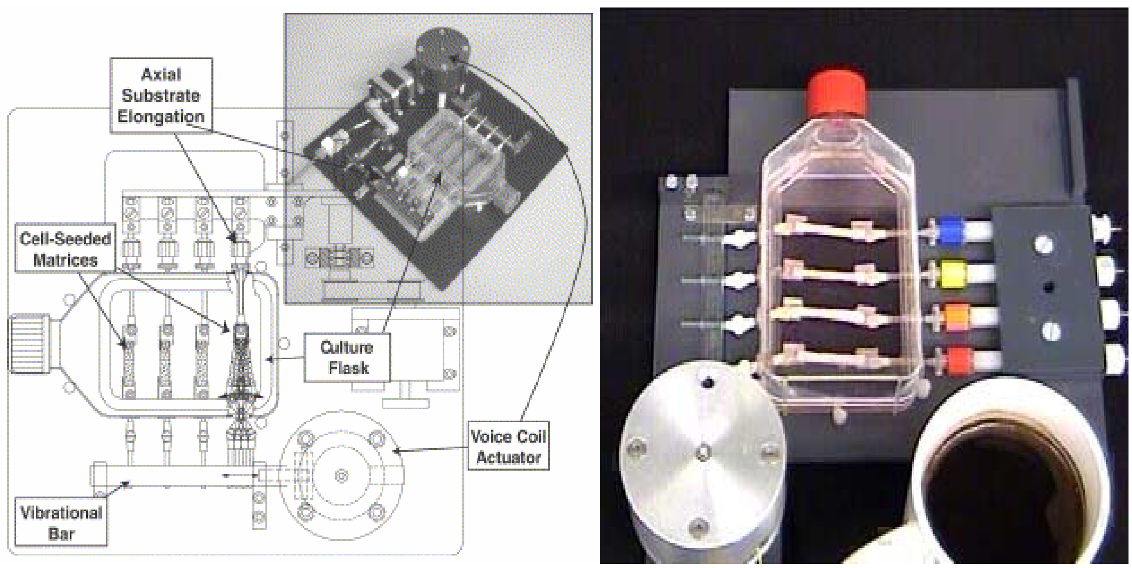


Figure 3: From left to right: Original bioreactor schematic diagram and fully operational bioreactor without a stepper motor.

been disassembled, however there is probably more than one institution attempting to build a new and improved version of the bioreactor for research purposes. 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 Mar 2007). 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 Feb 2007). 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 7 Mar 2007).

Alternative designs

Since the goal of the project was to reproduce and improve an existing bioreactor, the ability to create completely novel designs was limited. However, several ideas for improving the existing bioreactor were brainstormed. These ideas focused on the improvements the client desired in the new bioreactor, including providing impact between two cell-seeded matrices, and improving the distribution of vibrations across the length of the strips.

V-shaped strips

The first solution to the bioreactor involved putting the strips at an angle to each other, having them joined at one end and separated at the other. The bioreactor would be able to accommodate two pairs of "nested" strips this way (Figure 4). This design would be similar to the appearance of vocal folds in the human body when they are at rest. To simulate the movement of the vocal folds when they are vibrating, the design was enhanced by allowing the angle to be changed to various smaller angles. This design would allow the client to study vocal folds that are not functioning properly, and would allow her to customize the angle and the needs of the specific research objective quite easily.

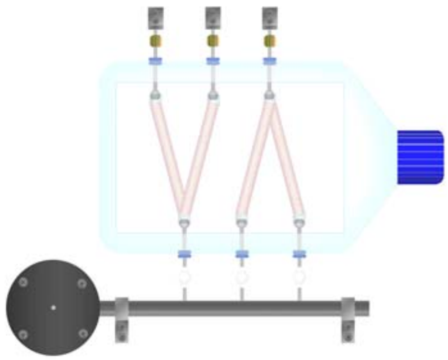


Figure 4: T-75 flask containing 2 pairs of angled strips, with a single-sided voice coil actuator.

The drawbacks to using angled strips included the complexity of the equipment needed to make it possible. Because the T-flask would be filled with fluid, the components would have to be waterproof and disposable after several uses. The T-flask would also have to maintain its leak proof properties while allowing the angle between the strips to change. In addition, it was important to the client for the strips to make contact while vibrating, to optimize fibroblast production of the cells. The amount of contact between the strips would be greatly reduced if they were at an angle to each other, thereby limiting fibroblast production.

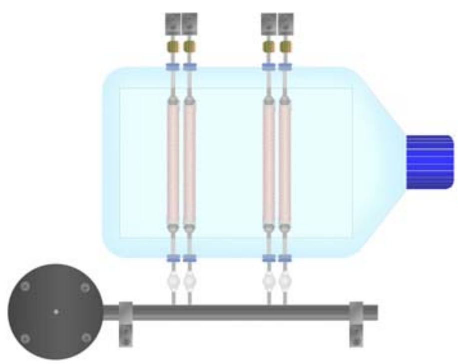


Figure 5: A design of the bioreactor, with 2 pairs of parallel strips, and a single-sided voice coil actuator.

Parallel strips

To eliminate the complexity of the design, the next idea involved implementing parallel strips instead. The client expressed the importance of the strips making contact, and placing the strips parallel to each other (at a 0-1mm distance) optimized fibroblast production of the cells (Figure 5). Although this design does not have as many research possibilities of the design involving angled strips, the design is much simpler and still satisfies the objectives of this project.

Rotating strips

Another idea pertaining to the orientation of the cell-seeded matrices would allow them to rotate completely. This would more closely simulate the rolling, wave-like action of the human vocal folds as they make contact and produce sound. However, the components required to create this design and the difficulty of ensuring the strips make sufficient contact would be very challenging.

Double-sided vibration

The client expressed dissatisfaction with the old bioreactor pertaining to the uneven distribution of vibrations along the length of the strips. It was noted that vibration was strongest on the end of the strip connected to the vibration bar, but dwindled along the strip's length as the distance from the vibration bar increased. The first idea to solve this problem involved having vibration on both sides of the T-flask (Figure 6), instead of on just one side, as previously was implemented. This idea would provide more even vibration along the length of the strips, but it had several complications.

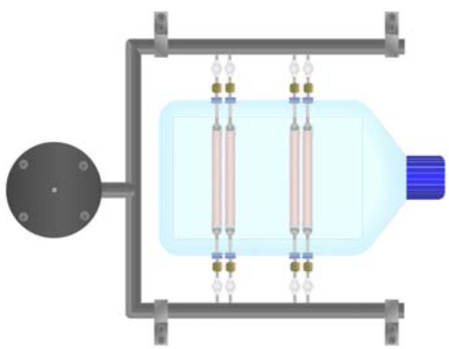


Figure 6: Another bioreactor design, with 2 pairs of parallel strips and double-sided voice coil actuator.

The first consideration with this idea involved how to connect vibration on the other side of the T-flask. It was determined that two vibration bars would be needed, but it was debated whether the amounts other pieces of equipment would need to be doubled or not. Since the previous bioreactor had a stepper motor providing tension to the strips on the side opposing the vibration bar, it was also unsure whether this new design would allow tension to be provided in the strips at all. Another uncertainty expressed in using double-sided vibration involved the reaction of the strips to vibration from both sides. It was not known whether the oscillations of the strips would cancel each other out or reinforce each other.

Design matrix

The design matrix (Table 1) included the four permutations of strip configuration and type of vibration as follows: 1) double-sided vibration with angled strips, 2) double-sided vibration with parallel strips, 3) single-sided vibration with angled strips, and 4) single-sided vibration with angled strips. Considerations included in the design matrix included cost effectiveness (weighted 10%), frequency distribution (weighted 30%), realistic imitation of environment (weighted 20%), feasibility/practicality of design (weighted 30%), and contentedness of client (weighted 10%). One-sided vibration with parallel strips was rated high for cost effectiveness and feasibility, and somewhat lower for frequency distribution, realistic imitation, and client contentedness. However, the implementation of cartilaginous support for the strips should increase the frequency of distribution. In addition, the feasibility of the design is essential for the ability to create a working bioreactor in the specified amount of time.

	Cost Effectiveness (1-10)	Frequency Distribution (1-30)	Realistic Imitation of Environment (1-20)	Feasibility / Practicality of Design (1-30)	Client Contentedness (1-10)	Total (100)
Two-sided Vibration V-shape Strips	5	21	18	12	10	66
Two-sided Vibration Parallel Strips	7	23	16	16	7	69
One-sided Vibration V-shape Strips	7	18	14	18	7	64
One-sided Vibration Parallel Strips	**10**	**18**	**14**	**24**	**7**	**73**

Table 1. Design matrix that indicate the scoring of various design possibilities. The designs in black are alternatives, whereas the design in blue achieved the highest score, which will be the design in the future.

Final design

The final design of the bioreactor consisted of a function generator used to create sine waves amplified by a linear amplifier (Figure 7). Before the alternating current reached the moving magnet voice coil actuator, the frequency was variable between 20 and 400 Hz. The actuator was held in place by an enclosing wooden base (lined with some traction cloth to prevent it from sliding), attached to the actuator base with two screws. Because the magnet of the voice coil actuator did not stay centered on its own, losing energy as it reached its maximum displacement; one spring was placed on each side of the actuator to center it as the current alternated. The magnet running through the central axis of the actuator was attached on one side to a stainless steel barbell and a plastic actuator rod. The back-and-forth motion of the magnet was translated to side-to-side motion by way of four polystyrene pipettes placed through the side of the actuator rod. The pipettes (Figure 8) had an axis of rotation along the outside edge of the T-flask by way of one metal pin placed through each of the pipettes. The pins were held in place by the actuator base and a bent metal flange, and were easily removed in order to take out the T-flask. Two pairs of Tecoflex strips (each pair placed 1 mm apart) were held in place and vibrated by the polystyrene pipettes. Tension in each of the strips was adjusted by one plastic screw, one plastic nut (on the outside of the T-flask), and a spring (on the inside of the T-flask). When each nut was loosened, the tension in the Tecoflex strips was lowered because of the increasing length of the spring. Tension was increased by tightening each nut. The T-flask was held in place horizontally by 3 bolts attached to the actuator base, and was easily lifted vertically for removal. Fluid was retained inside the T-flask by one plastic tent-like apparatus placed on both sides of the T-flask to cover the holes that were drilled. The tent was taped to the inside edge of the T-flask and around each of the pipettes. A large square opening was cut into the top of the T-flask and covered with a slightly larger piece of transparency. The transparency cover was easily removed and re-attached to the T-flask by using a glue stick as an adhesive on the

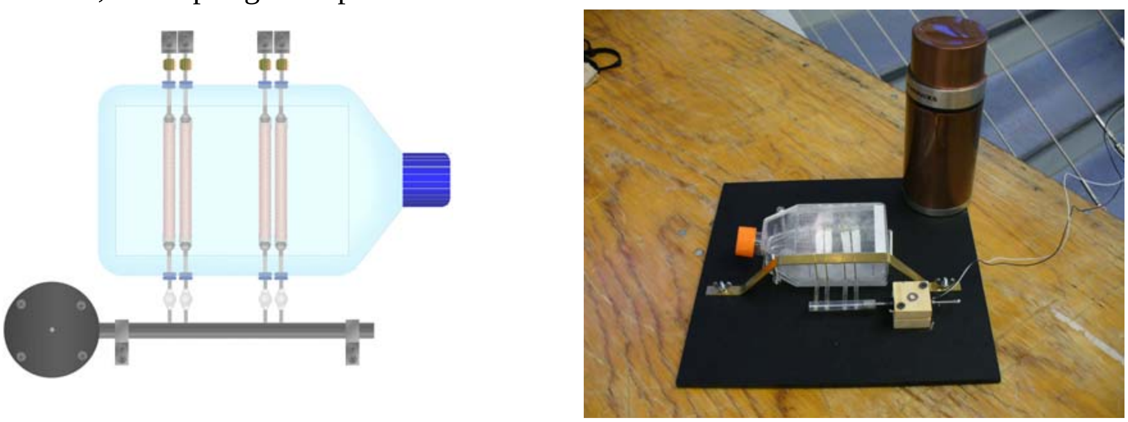


Figure 7: The diagram on the left represents a schematic drawing of the proposed bioreactor design. The diagram on the right is the final prototype.

sides and was kept in position by superglue at the bottom edge.

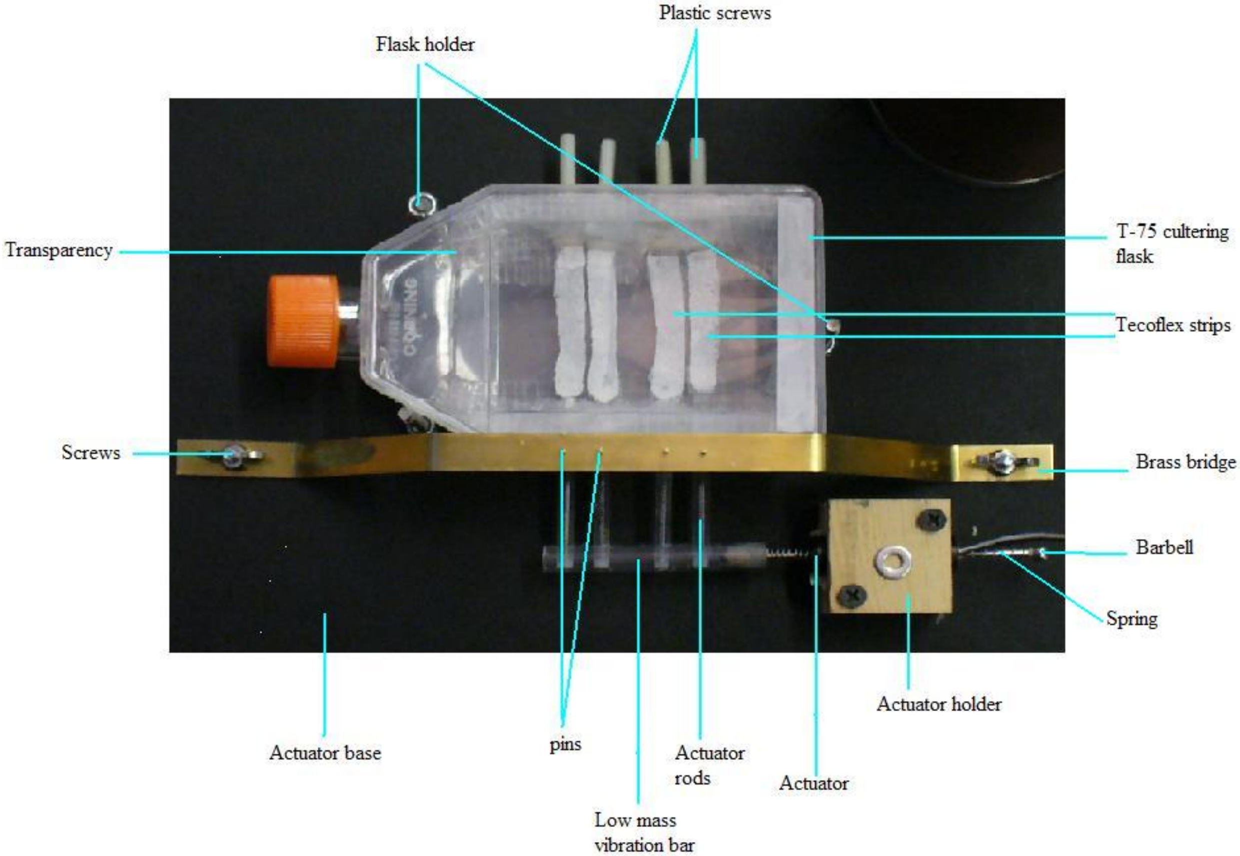


Figure 8: A diagram explaining the various components of the final bioreactor prototype.

Testing and results

As of the current bioreactor, there is no specific way of testing the distribution of vibration across the vibrating strips. However, it is possible to measure the amplitude of the vibrating strips. The maximum amplitude is measured by using a ruler to approximate the change of vibrating strip movement. The two different stages of the tension were measured: the strips at 100% tension and the strips at 50% tension. When the strips are at 100% tension, the screws on the side of the T-flask are screwed to the tightest, whereas the 50% tension refers to the default tension of the strips, not too lose or too tight. We also tested the amplitude of vibration in air and in solution. Although the testing method is neither precise nor accurate, some data were still collected (Appendix B). From the testing results, it is deduced that strips at 100% tension could produce larger amplitude than the strips at 50% tension. This result applies to both vibrations in air and in solution. Also, at lower frequencies, the strips at both tensions generate larger amplitudes relative to the amplitude at higher frequencies.

Conclusion

Overall, it is clear that the bioreactor has many complicated components. The main purpose of this device is to simulate a biological system, and in this case it will be the vocal folds. The vibration applied to the system is an essential factor for this bioreactor, since the changes of the fibroblast are dependent on the vibration. The final working prototype of the bioreactor could better simulate the vibration of the vocal folds, and even further model the contacting motions.

In the future, we wish to improve the leakage problem on the sides of the T-75 flask. The plastic tent method does not effectively prevent the solution from leaking out of the flask. Moreover, a better testing method needs to be developed in order to examine the accuracy and reliability of the vibration generated by the bioreactor. Even better to improve this device, a computer monitored system could be implemented for automated control instead of the current manual adjustments. A stepper-motor could also be used to provide varying tensile stress to the system over an extended period of time without need for manual adjustment. Another future consideration would be to make the actuator housing out of copper or aluminum with perforations to effectively ventilate the actuator and keep it from overheating. Even with foreseen improvements, there are no ethical considerations involved with the setup and implementation of the bioreactor device.

Appendix A: Product design specifications

Product Design Specifications for BME 201 group 35: Bioreactor

(May 9, 2007)

Group members: Karen Chen, Rachel Mosher, Richard Bamberg, and Dustin Gardner

Problem Statement: The larynx simulating bioreactor is currently operating under conditions that do not fully simulate the motion of the vocal folds. The existing device models the vibration of one vocal fold under tensile stress and provokes the elongation of fibroblasts and the subsequent formation of an extra-cellular matrix, characteristic of vocal fold fibroblasts. The goal is to improve upon this model by simulating contact between two vibrating vocal folds and distribute even vibration across the two vibrating vocal folds. The bioreactor will be made from a T-flask with elastic strips immersed in a buffer, which will function as vocal folds. A vibration motion from an actuator will be directed to the strips to provide vibration. This device needs to be easily replaced with disposable parts, along with 2 pairs of artificial vocal folds and a capacity to generate vibrations at frequencies ranging from 0-400Hz.

Client requirements: (improvement of design)

- Vibrator Bars on both sides of T-flask
- Vibration Frequency 0-400Hz
- Contact between each pair of cell-seeded strips during vibration (2 pairs in T-flask)
- Simultaneous concerted vibration of Vibration Bars
- Cartilaginous structures around cell-seeded strips
- Easily sterilized with disposable parts
- Find alternative to Tecoflex substrate
- Cost limit $5,000

Design requirements: Since the device has already been designed, our focus for this project is to improve the current design and construct a better working model. The first improvement will be the construction of vibrator bars on both sides of the T-flask, which would better distribute the vibration evenly across the strips. Also, each pair of cell-seeded strips and the attached cartilaginous structures must come into contact with each other during vibration, to further mimic the action of the vocal folds. The Tecoflex substrate needs to be replaced by a better material to eliminate the porosity of the strips. Furthermore, the parts of the device should be disposable and easily sterilized. The vibration frequency should range from 0-400Hz. Overall, the expenses should not exceed $5,000.

a. *Performance requirements:* The device is meant to culture and research the reactions of vocal fold fibroblasts to various stimuli, such as duration of vibration, tensile stress, angle of vibration and other experimentally-controlled environments.

b. *Safety:* This device does not need to be sterilized often because the T-flask is disposable and other components require periodic cleaning. The device must be sealed properly to prevent leakage of cell-culturing medium.

c. *Accuracy and Reliability:* The device will be operating in a medium that is denser than air therefore a different frequency of vibrations will be applied to the system. The current substrate Tecoflex poorly distributes vibrations because of its porosity. The angle between the vibrating cell-seeded strips (matrices) should be adjustable prior to vibration but fixed during vibration.

d. *Life in Service:* The T-flask and its interior are disposable components of the Bioreactor that are disposed of after each use (several weeks per use). The life-limiting factor of this Bioreactor would be the vibration-generating motor.

e. *Shelf Life:* The Bioreactor itself has a long shelf life. The T-flask and its internal components are disposed after each use. Periodic cleaning and maintenance of the device is required to avoid contamination of the system and therefore prolong shelf life.

f. *Operating Environment:*
Temperature range: Incubator (37°C)
Pressure: Negligible differences in pressure (only ½ psi difference between atmosphere and inside the body).
Corrosion from fluids: Any corrosive fluids would be retained in the T-flask and therefore would not come into contact with the rest of the Bioreactor.
The device must effectively simulate the environment of human vocal folds by uniformly providing ample vibrations to the cell-seeded strips in the T-flask.

g. *Ergonomics:* In the endeavor to imitate the inner body environment around the vocal folds, gas flowrates, temperature, and pH should all be constant inside the system. These internal environmental factors would be monitored and controlled by a programmable logic controller (PLC). Most of the Bioreactor will be computer software controlled, however the periodic replacement and sterilization of the device will be conducted manually.

h. *Size:* For this project, the design will be focused mainly around the T75-culture flask, its contents contained within and the required electrical components to provide vibration to the system. Thus the actual size of the device will be somewhat slightly larger than the T-flask itself.

i. *Weight:* Actual weight of the bioreactor will depend on the amount of cell-seeded strips that could fit within the T-flask. A weight cannot be specified at the moment; however, the weight of the electrical and mechanical components of the device will be of little consequence to the function of the Bioreactor itself.

j. *Materials:* T-75 Culture Flask, Clear Polyester Lid, Plastic tent, Actuator Base and Rods, Plastic screws, Actuator rods, Lever, Low Mass Vibration Bar, Analog Output Board, Power Amplifier, Position Feedback Sensor, Servo Drive.
<u>Actuators</u>: Electromagnetic Voice Coil Actuator and Vibration Actuator

k. *Aesthetics, Appearance, and Finish:*
<u>Texture</u>: The T-flask should be smooth on the inside and outside. No extra furnishings would be necessary. The focus of this design is function over aesthetics.

2. Production Characteristics

a. *Quantity*: Although we are making only one prototype, the client has shown interest in using several devices simultaneously in experiments. 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 several dozen at most.

b. *Target Product Cost*: The cost of a similar existing bioreactor was around $15,000, thus it is expected our bioreactor would have a comparable cost. Because our bioreactor will probably not be complete with all the elements the previous bioreactor had (such as software), our device is expected to cost less than $5,000.

3. Miscellaneous

a. *Standards and Specifications*: The product of this design 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.

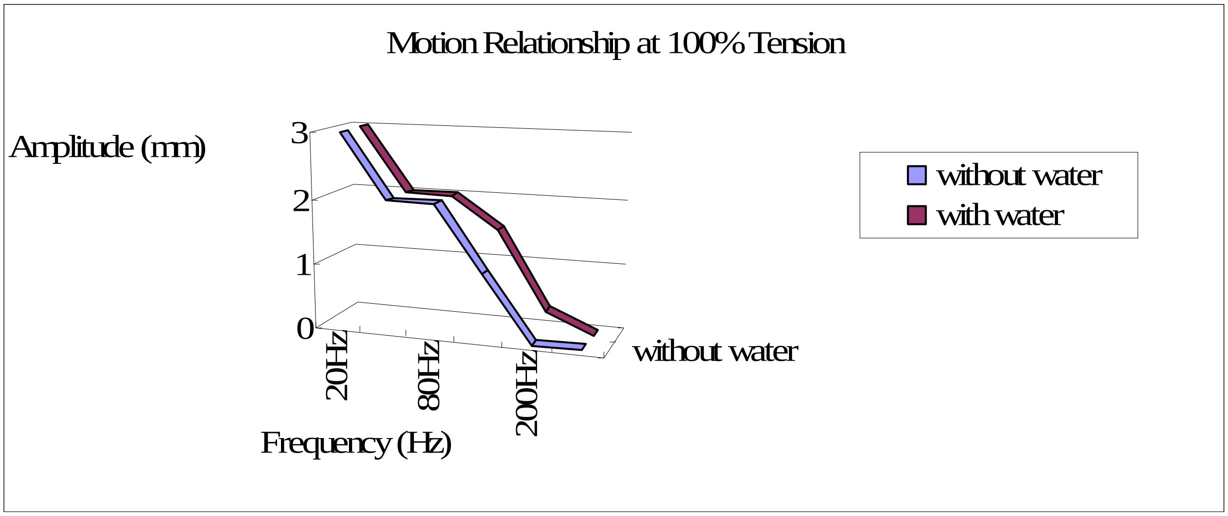
c. *Patient-related concerns:* Since this device is not used for human contact, patient-related concerns do not apply.

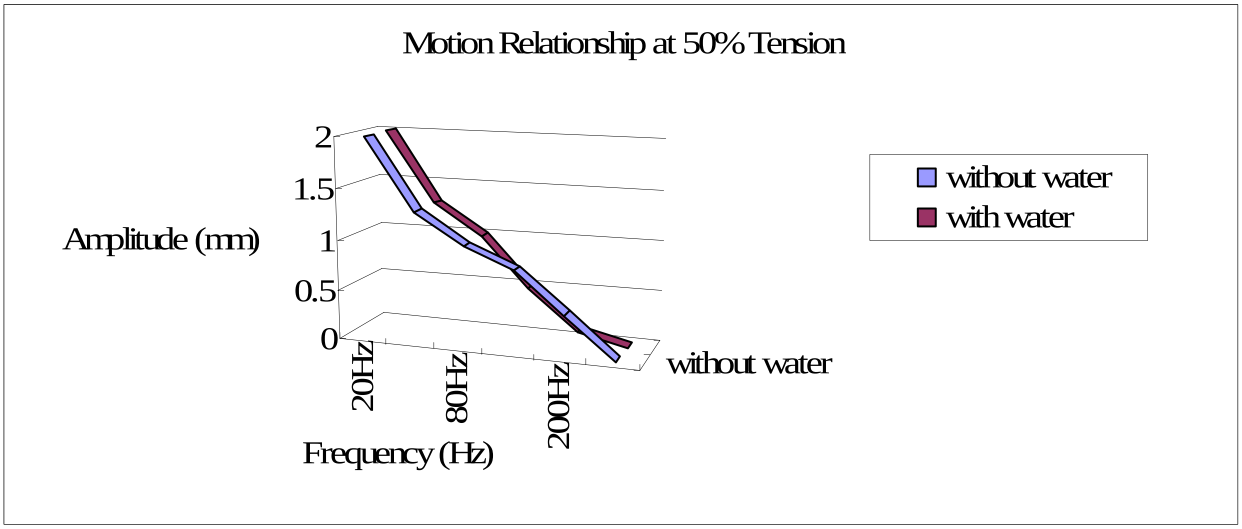
d. *Competition:* This device is to be improved from a previous design, which was also for research purposes only. There is no intent by any party to patent this design.

Appendix B: Testing results

	20Hz	40Hz	80Hz	100Hz	200Hz	400Hz
max tension with solution	3	2	2	1	0	0
max tension without solution	3	2	2	1.5	0.3	0
50% tension with solution	2	1.3	1	0.5	0.1	0
50% tension without solution	2	1.3	1	0.8	0.4	0

units: mm





Appendix C: List of materials

PART	AMOUNT	DETAILS
T-75 Culture Flask	~10	Contains media and Tecoflex strips
Actuator Base	1	Frame to hold everything in place
Clear Polyester Lid	1	A piece of transparency
Plastic sheets	~20	Acts as a "tent" to prevent leakage of the buffer solution inside the flask.
Actuator Rods	1-2 sets of 4	Also called linear actuators Transfer mechanical stimuli into flask; go through holes in flask
Screws and nuts	4 each	To act as the fixed end of the Tecoflex strips. Attach to the other end of the strips, could also adjust the tension of the strips
Electromagnetic Voice Coil Actuator	1	Aerflex, GEEPLUS, H2W Technologies, *BEI*, Equipment Solutions, Motran Industries, Schneider Electric SA, Tinitron, Western Components, Inc. BEIKimco.com. Utilizes cyclic strain, provides vibrations via a flat, photoetched spring fitted into a custom machined housing.
Low-Mass Vibrational Bar	1	Attached to lever, moves vibrator arms of flask that drives cell-seeded matrices.
Wave function generator	1-2	Controls vibration actuator
Power Amplifier	1	Amplifies output from wave function generator
Barbells	2	Connects actuator to vibration bar to restrain position
Traction cloth	1	Keeps actuator in place
Wooden block	2	Custom actuator housing
Springs	6	Stabilize actuator motion

Appendix D: Budget and expenses

Statement of Accounts (BME-Spring 2007)			
Date	**Type**	**Amount**	**Balance**
	Starting Amount	0	5,000.00
16-Mar-2007	Function Generator	-256.00	4,744.00
16-Mar-2007	Power Amplifier	-200.00	4,544.00
17-Mar-2007	Barbell	-31.65	4,512.35
17-Mar-2007	Electromagnetic Voice Coil Actuator	-711.00	3,801.35
17-Mar-2007	Actuator Rods (Pipettes)	-16.14	3,785.21
18-Mar-2007	Low Mass Vibrational Bar	0.00	3,785.21
10-Apr-2007	Metal Screws with Plastic Caps	-4.11	3,781.10
12-Apr-2007	Plastic Screws with springs	-4.22	3,776.88
21-Apr-2007	Flat Spring Rollers	-6.32	3,770.56
21-Apr-2007	Hex Bolts	-1.46	3,769.10
22-Apr-2007	Dropcloth 9x(12.5mil)	-1.36	3,767.74
22-Apr-2007	Solid Brass Bar 1x(3/64")	-1.36	3,766.38
27-Apr-2007	Paint Brushes	-2.01	3,764.37
27-Apr-2007	Duct Tape	-6.32	3,758.05
27-Apr-2007	Paint	-3.47	3,754.58
29-Apr-2007	Lock Nuts	-4.14	3,750.44
30-Apr-2007	Projector Slides	-0.80	3,749.64
30-Apr-2007	Super (Krazy) Glue	-2.30	3,747.34
30-Apr-2007	Wing Nuts	-0.44	3,746.90
30-Apr-2007	Washers	-3.10	3,743.80
30-Apr-2007	Metal Short Screws	-4.98	3,738.82
2-May-2007	Barbell	-21.10	3,717.72
2-May-2007	Grip Cloth	-1.01	3,716.71
3-May-2007	Final Project Poster	-179.35	3,537.36

Appendix E: References

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