Tissue Engineering Bioreactor

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Abstract:

In the interest of pursuing further research into vocal fold cells, a bioreactor was constructed to effectively simulate the environment of the vocal fold tissue within the human body. The purpose of the design was to subject cell-seeded strips (vocal fold substitutes) to a variety of vibration and tensile stimulation so that vocal fold cells may be studied more in-depth. Only one such bioreactor is known to exist, however the objective for this design project is to reconstruct a similar bioreactor and make a few modifications to the device that would enhance its performance for research. For this project, the single-side vibration bar with parallel strips will be constructed due to its high score in the design matrix.

Background:

Bioreactor:

A bioreactor is a system or device that supports a biological system. The purpose of a bioreactor is to simulate an active environment that is close to a biological system. In this system, organisms or tissues of organisms are applied with known variables, and the results due to the variables would be observed. This system has various sizes, could be as large as cubic meters and as small as a Petri dish. The material of the bioreactor could be stainless steel or polyvinylchloride (PVC).

In this case, an environment that is very close to the larynx will be simulated. A T-75 culture flask made of PVC will be 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 will be attached to the T-75 flask to apply appropriate vibration (changes) to the system, which will be connected to the interior of the flask through drilled holes on the sides of the flask (Titze, 2004). The fibroblast of the ECM will be placed on the simulated ECM structure 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, where no air or other molecules come in contact with the culturing fibroblast.

Vocal Fold Fibroblasts:

Vocal folds (Figure 1), 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 extra-cellular matrix (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 a fibroblast. The 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 (Titze, 2004). Furthermore, there is exists 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 (Titze, 2004). However, the frequency that they mostly vibrate at is a range from 0-400Hz.

The vocal folds are horizontally stretched across the larynx. Due to the vibration of the vocal folds, the larynx is exposed to high inertial stress (Titze, 2004). The stretching and relaxing of these two membranes determine the pitch of one's phonation. The two vocal folds come in contact and create a "mucosal wave", which produces sound and also allows the exiting of air from the lungs (Altman, 2002).

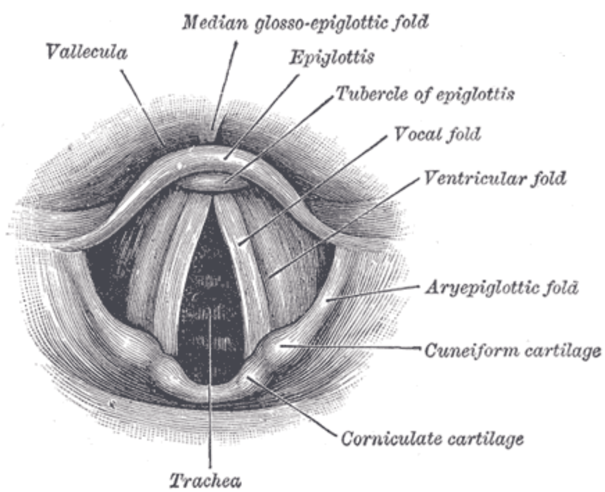

Figure 1. The left figure indicates the diagram of the vocal folds, with parts of the vocal folds labeled. The right figure is an actual screen shot of the vocal folds. Notice the two mucous membranes with the opening in the middle.

Problem Motivation:

The focus of this project is to construct a working prototype of a bioreactor that would be able to culture vocal fibroblasts for research purposes by subjecting the cell-seeded matrices (attached to Tecoflex strips) to a specific range of stimuli. Such stimuli include both 'violent' vibrations and tensile stress that would effectively mimic the human vocal fold environment when vibrations (sounds) are produced in the larynx. Research will be conducted while the environment of the bioreactor will be maintained and monitored by software interface, the Labview software in particular. For best results, the distribution of uniform vibrations to the cell-seeded strips would be imperative to the success of a working bioreactor.

Problem Statement:

The main goal of this design project is to improve and reproduce the current design for the vocal fold bioreactor. The current design does a decent job of culturing vocal fold fibroblasts while promoting characteristic elongation and creation of an extracellular matrix through tensile and vibrational stimuli (Titze et al. 1527). However, there are a few things that could use some improvement. First, there is no contact between the vibrating strips. To more accurately recreate the vocal fold environment, a third 'contact' stimulus will be included in addition to the tension and vibration produced by the bioreactor. Second, there is an uneven distribution of vibration along the cell-seeded strips. The single-sided vibration causes more vibrational strain to be exerted nearest the source, and different ideas will be experimented with to more evenly distribute the vibration along these strips. Finally, the strips themselves are made of Tecoflex, which is a porous substrate. Unlike real vocal folds which are lined with smooth, elongated cells, Tecoflex looks like a sponge under a microscope and makes cell culturing more difficult (Thibeault 2 Feb 2007). In other words, the goal is to recreate the vocal fold fibroblast bioreactor in a manner that better simulates the actual environment of human vocal fold tissue. This project is directly for research purposes, so mimicking the actual environment will help researchers further study the growth of human vocal folds in a controllable manner.

Design Constraints:

For this project, the client would like the bioreactor to exert concerted bar vibrations (ranging from 0-400Hz frequency) along the lengths of the cell-seeded Tecoflex strips. This device should also be designed such that contact between the strips will exist exclusively during vibration in order to best imitate the vibration of the human vocal folds. A cartilaginous structure on one side of each cell-seeded strip may be constructed in order to evenly distribute the vibrations across the strips. Apart from function, the bioreactor should also incorporate disposable parts, and components that are easy to sterilize (with alcohol). Should time permit, the use of a polyurethane substrate alternative in lieu of Tecoflex would be used as the cell-seeded strip, to perhaps better distribute vibrations and produce better research data. These constraints should be adhered to in conjunction with a target cost of $5,000, which does not include the construction of an incubator to maintain constant temperature at 37˚C and the Labview software to control vibrations and tensile stress.

Current Device:

The previously built bioreactor (Figure 2) 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.

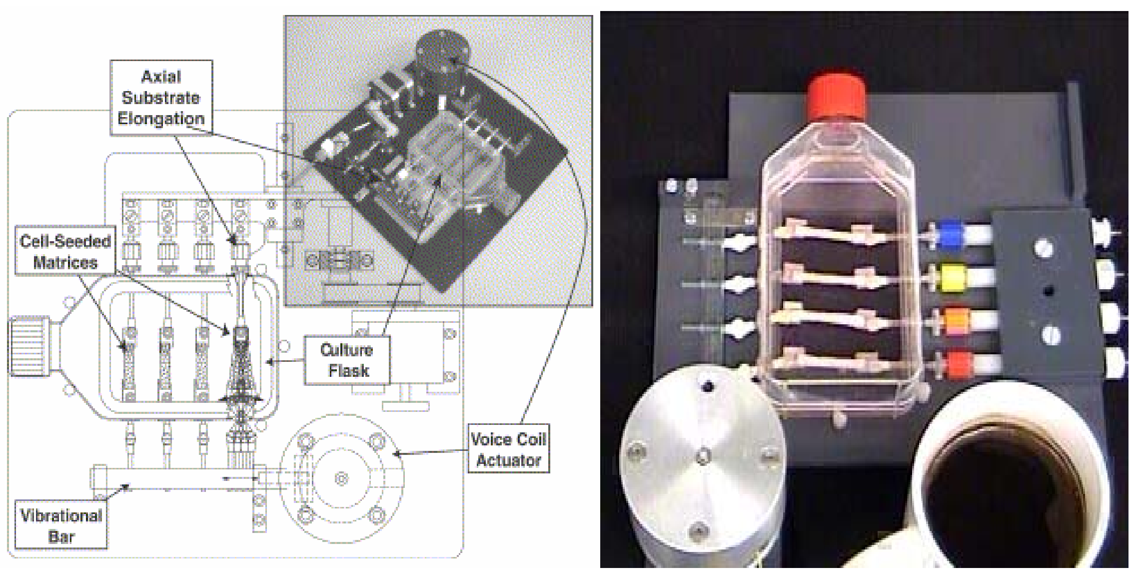

Figure 2. From left to right: Original bioreactor schematic diagram and fully operational bioreactor without a stepper motor.

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 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 Design Descriptions:

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.

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. 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. See Figure 3 below:

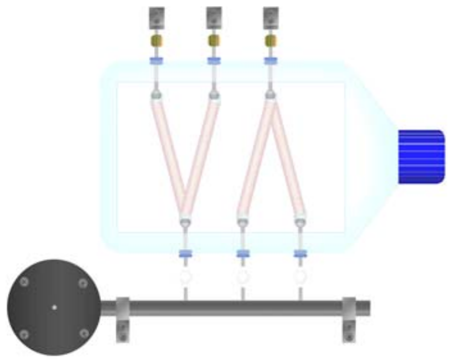

Figure 3. T-75 flask containing 2 pairs of angled strips, with a single-sided voice coil actuator.

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.

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 0-2mm distance) optimized fibroblast production of the cells. 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. See Figure 4 below:

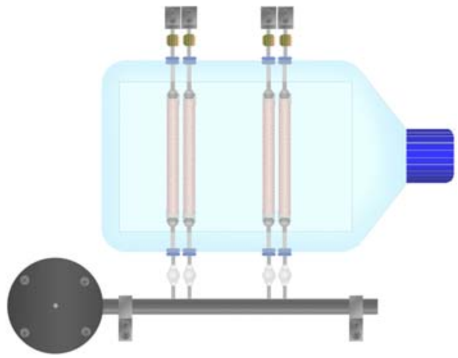

Figure 4. A design of the bioreactor, with 2 pairs of parallel strips, and a single-sided voice coil actuator.

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.

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, 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. See Figure 5:

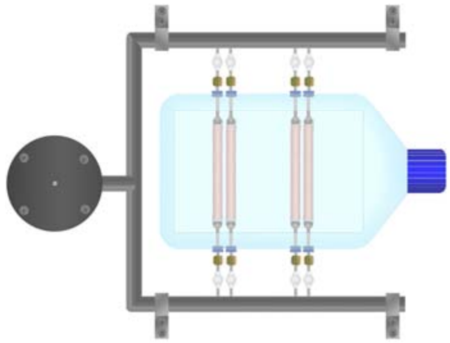

Figure 5. Another bioreactor design, with 2 pairs of parallel strips and double-sided voice coil actuator.

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.

The complexity of using double-sided vibration was solved with an alternative solution of creating vibration on only one side of the T-flask. This eliminated the complexity and uncertainty involved with double-sided vibration, and ensured the ability to implement tension in the strips with a stepper motor. It was determined that the concern the client had regarding uneven distribution of vibrations would need to be solved with a different method. See Figure 6 below:

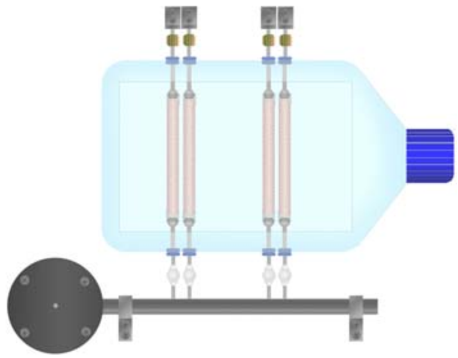

Figure 6. A design with single sided vibration bars with 2 pairs of parallel strips.

Solutions to providing even vibratory distribution focused on changing the design of the cell-seeded matrices. It was imperative that the alternative design transferred vibrations effectively along the outer side of the strip, but it was also important for the structure not hinder vibration on the rest of the strip, in particular, the inner side. It was also favorable for the structure to allow a wave-like motion from the outer edge of the strip to the inner edge to more accurately simulate the movement of human vocal folds. Ideas include attaching a thin metal rod to the outer edge of the strip, imbedding a spring into the strip, molding indentations into the strip, and creating a wedge-shaped strip. The design chosen, if any, will be determined based on experimental evidence involving a working bioreactor.

Design Ideas:

The alternative solutions that were used in the design matrix involved using parallel or angled strips and one- or two-sided vibration. Parallel strips were ultimately chosen because they would be able to make contact along their entire length, contributing to the highest amount of fibroblast production. If angled strips were used they would only make contact near the joined end, not allowing for much contact between the strips. Although the most accurate model of human vocal folds would involve angled strips that could have an adjustable angle, the ability to adjust this angle and maintain a leak-proof T-flask would be quite a challenge. Thus, feasibility and optimizing the contact between the strips were the most important reasons for determining to use parallel strips.

Single-sided vibration was chosen over double-sided vibration for various reasons. Implementing one-sided vibration in the bioreactor is less complex, easier to construct, and is less expensive. Unfortunately, it may not provide as even of vibratory distribution as double-sided vibration. Although this improvement to the existing bioreactor was very important to the client, utilizing double-sided vibration on this bioreactor is not practical at this time. As an alternative solution to creating even vibratory distribution, the idea of having a cartilaginous structure attached to the cell-seeded matrices is being considered.

Design Matrix:

The design matrix 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

Materials:

PART	# NEEDED	DETAILS
T-75 Culture Flask	~10	
Actuator Base	1	
Clear Polyester Lid	~5	Fralock, San Carlos, CA
Elastomeric Boots	~20	Tecoflex SR-80A, Thermedics, Inc. Fit holes in flask.
Actuator Rods	1-2 sets of 4	Also called linear actuators? Transfer mechanical stimuli into flask; go through holes in flask
Threaded Connectors	~20-30?	Connect flask and substrates (cell-seeded strips)
Threaded Rods		Contained in actuator base, manually adjusted to apply various amounts of tension to strips
Electromagnetic Voice Coil Actuator (Can be linear or rotary, cylindrical or rectangular)	1-2	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.
Lever	1-2	Attached to moving coil of actuator and a low-mass

		vibrational bar. Same as manual valve actuator?
Low-Mass Vibrational Bar	1-2	Attached to lever, moves vibrator arms of flask that drive cell-seeded matrices.
Labview Software	1	National Instruments. Controls vibration actuator base.
Analog Output Board	1-2	Controls vibration actuator
Two-Stage Amplifier	1	Amplifies output from computer
Position Feedback Sensor	1-2	Provides vibrational information to voice coil actuator

Table 2. The list of components that will be ordered. Note that this is just a tentative list, things could subject to change.

NB: No Stepper Motor will be used for this semester because of time constraints for design process.

<u>Future Research:</u>

As previously mentioned, the primary objective of this design project is to improve the previous bioreactor design for research purposes. The latter part of this semester will be dedicated to constructing one working prototype. If continued for another semester, plans to redesign and reconstruct this bioreactor will be set into motion with hopes of having multiple working products. With multiple bioreactors, simultaneous experiments could be conducted for different stimuli scenarios. This would tremendously help the research aspect since one single experiment could take several days to weeks in order to effectively culture fibroblasts with vocal fold characteristics.

<u>Future Work:</u>

After considering many different ideas, the future work for the design team is very clear. Plans are underway to immediately begin ordering some advanced parts and fabricating other parts in order to construct a working prototype. The main part of concern is the electromagnetic actuator voice coil. Considerations exist for using an old speaker core, but ultimately a more reliable actuator such as the one used in the previous design from BEI Kimco would yield better results (Titze et al. 1523). Also, there are plans to order some peel-back T-75 flasks in order to concentrate design efforts on more challenging aspects, rather than worrying about creating custom lids and airtight seals for regular T-75 flasks. The parts that will need to be fabricated and/or assembled include all of the necessary rods, boots, joints, and lure connectors that will be mounted on the bioreactor plate, which may have to be ordered from a company like US Plastics or simply cut from a ¼ inch plate of PVC.(Hitchcock 7 Mar 2007). After all of the necessary components are gathered, this semester's goal is to assemble the bioreactor and test it, using an amplified source for power and a function generator to produce the required frequency for vibration. There is hope for some insightful data by the end of the semester, which can then be further applied to another semester's work granted this project continues.

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. According to the design matrix, the single-sided vibration of parallel strips achieved the highest scores. This will become the design that the team will go about for this semester. In the near future, the parts of the bioreactor will be ordered and a working prototype should be fabricated.

References

Altman, K. W. (2002). Vocal fold histology. Retrieved March 3, 2007 from http://www.voice.northwestern.edu/vfhisto.html

Hitchcock, Robert.	Personal Interview	7 Mar. 2007.
	Personal Interview	2 Mar. 2007.
Thibeault, Susan	Personal Interview	2 Feb. 2007.

Titze, Ingo R., et al. "Design and validation of a bioreactor for engineering vocal fold tissues under combined tensile and vibrational stresses." *Journal of Biomechanics* 37 (2004): 1521-9.

Webb, K., Hitchcock, R. W., Smeal, R. M., Li, W., Gray, S. D., & Tresco, P. A. (2006). Cyclic strain increases fibroblast proliferation, matrix accumulation, and elastic modulus of fibroblast-seeded polyurethane constructs. Journal of Biomechanics, (39), 1136-1144. Retrieved January 29, 2007

www.globalspec.com

www.wikipedia.org

Appendix A

Product Design Specifications for BME 201 group 35: Bioreactor

(March 13, 2007)

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

Problem Statement: The aim of this project is to redesign and reproduce a pre-existing Bioreactor that is specific to the culturing of human vocal fold fibroblasts. The existing design models the vibration of a single 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. Our goal is to improve upon this model by simulating contact between two vibrating vocal folds and potentially finding a better substitute for Tecoflex, because its porosity is non-uniform and the pore size is too large. The bioreactor will be made from a T-flask with elastic vibrators immersed in a buffer, which will function as vocal folds. This device needs to be easily replaced with disposable parts, along with 2 pairs of artificial vocal folds and a capacity to generate (computer controlled) vibrations at a frequency (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, the 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, Elastomeric Boots, Actuator Base and Rods, Threaded Connectors and Rods, Lever, Low Mass Vibrational Bar, Labview Software, Analog Output Board, Two Stage Amplifier, Position Feedback Sensor, Servo Drive.
Actuators: Electromagnetic Voice Coil Actuator and Vibrational Actuator

k. *Aesthetics, Appearance, and Finish:*
Texture: 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.