# Bioreactor Cassette for Autologous Induced Pluripotent Stem Cell Production

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## ABSTRACT

Autologous induced pluripotent stem (iPS) cells show great potential for use in patient-specific medical therapies. In order for this therapy to be effective, a bioreactor cassette system is required that is capable of providing conditions for growing multiple iPS cell samples from individual patients without exchanging media between samples. Last semester we focused on basic design concepts, material selection, and flow analysis. This semester we collected data regarding cassette function and cell behavior within a simulated bioreactor system, and found that the formation of bubbles ($3.6 \pm 1.1$ mL of air after four days of operation) within the system prevented optimal performance of the bioreactor cassette. To address bubble accumulation, we designed a bubble trap better suited to our needs than similar commercially available products, characterized its performance, and confirmed its usability in the bioreactor system with ergonomic testing. Initial studies with HEK-293 cells in the bioreactor cassette system and bubble trap show improved cassette performance, but revealed areas for ergonomic improvements in the design. Future work includes replicating the initial cell study with the bubble trap in the bioreactor system and improving the ergonomics of the cassette's design.

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# Background

## iPS Cells

Stem cells, through which any of the body's approximately 200 cell types can be derived, are a source of great therapeutic potential. Their power comes from their pluripotency, or the ability to be maintained indefinitely in culture until signaled to differentiate into a specific, specialized cell type (Ling Yi & Lin 2009). Most public controversy surrounds the use of human embryonic stem cells (hESCs), or lines derived from the inner cell mass of a 3–5-day old blastocyst. Induced pluripotent stem (iPS) cells are adult somatic cells that have been reprogrammed back into a pluripotent state. These cells avoid this ethical obstacle and by their nature make stem-cell therapy a more individualized practice (MedicalNet 2010).

Stem cell-based therapeutics, or treatments in which induced differentiation of stem cells is used to repair damaged or destroyed cells or tissues, are showing great progress in their potential for use in a clinical setting (Ling Yi & Lin 2009). Until recently, stem cells have been used primarily to model cell growth and function or to perform drug screening. As the potential for implantation and use in a patient approaches, however, researchers need to be able to grow stem cells under individually controlled conditions. Specialized culture chambers called bioreactor cassettes allow this to occur.

Bioreactor cassettes are small, specialized culture dishes used to maintain undifferentiated stem cells. They can be attached in a network to a bioreactor (Appendix A), which incubates and provides them with a continual, though variable, supply of media appropriate for growth. Once a sufficient number of cells have grown, they can be subjected to specific chemical or mechanical signals that direct their differentiation when a certain cell type is needed. In order to make individualized stem cell therapy accessible on a large scale, it will be necessary to have an economically and ergonomically viable cell culture cassette to connect with a bioreactor system. Because it will be more cost effective to use one bioreactor to culture multiple iPS cell samples, the cassettes must be capable of growing iPS cells from individual patients in parallel by keeping the samples independent. This ensures that cultures in each cassette are composed purely of cells derived from a specific patient and are not cross-contaminated with other patients' cells through

transferred media. The bioreactor cassette method of individualized stem cell culture would make such an endeavor feasible and cost-effective for large-scale therapeutic operations.

## Problem Statement

Autologous iPS cells show great potential for use in patient-specific medical therapeutics. The most common approach to iPS cell growth is to take a skin biopsy and grow the cells in conditions that induce pluripotency; they can later be controllably differentiated into the desired cell type. However, for this therapy to be cost-effective, it is necessary to have a bioreactor cassette capable of providing conditions to grow and monitor multiple iPS cell samples from individual patients without exchanging media between samples. After developing and testing our cassette with the perfusion bioreactor system, we discovered an unacceptable accumulation of gas in the body of the cassette. This accumulation of gas must be eliminated to facilitate healthy, uniform cell growth. We will continue to modify our cassette and perfusion interface to address these emerging demands.

## Current Solutions and Competition

Due to the novelty of the field of stem cell therapeutics, iPS cells have not been cultured on a large scale in undifferentiated form. However, since cells have been used in the biotechnology industry for a long time, there exist methods, bioreactors, and cassettes that offer competition.

Currently, culturing cells under static conditions is the simplest solution to the problem. In static cell culture, the cells adhere to the bottom of a polystyrene flask and are constantly bathed in culture media. Media replacement is manually performed in bulk. Static cell culture flasks can be

**Figure 1: Static culture flasks sold by Corning (Product #3814) are frequently used to grow cells. The cells adhere to the bottom and are bathed in liquid media as shown**

*Image Courtesy of Corning*
*http://www.corning.com*

purchased from Corning (Figure 1) or other manufacturers. While static cell culture is a common way to grow cells, there are many limitations that render it inadequate for large-scale iPS cell culture. First, for the cells to remain undifferentiated, they require constant supply of autocrine factors produced by the cells, which would be removed when the media is exchanged in bulk (Zandstra and Nagy 2001). Second, the buildup of waste products prior to media exchange could cause differentiation or prevent self-renewal (Zandstra and Nagy 2001). For culture to be useful in large-scale therapeutic applications, an automated process is desired.

A common way to grow large quantities of cells in culture is in a batch mode stirred flask bioreactor (Zhang *et al.* 2010). In this system, the cells are grown in suspension until they reach a high density. As cells start to die from lack of nutrients and too much waste, they are harvested for the desired product. In order for most cells to grow in suspension, they must be adapted to lose anchorage-dependence. This is undesirable for therapeutics, because loss of anchorage-dependent growth is associated with malignant cancers. Additional problems associated with this method are the harsh conditions of nutrient-starvation, waste accumulation, and shear stress from stirring that would likely cause stem cell differentiation (Zhang *et al.* 2010).

One solution to these problems is to use a perfusion bioreactor. In a perfusion bioreactor, fresh liquid media is continuously pushed through the cassette, allowing efficient waste removal and nutrient addition. Many variations of perfusion bioreactors have been employed to culture cells, and some of them involve growing the cells in suspension with a method for separation of cells from media at the output. One patent on such a perfusion bioreactor solves the separation problem by enclosing the cells in a semipermeable bag (impermeable to cells) (Singh 2003). However, because the cells must still be adapted to grow in suspension first, this method would not be ideal for growing iPS cells for therapeutic purposes. Another perfusion bioreactor that has been patented solves the problem of suspension growth by placing microcarrier beads in the medium, which allow the cells to adhere, similarly to a normal tissue culture substrate, but to still get the nutrients and oxygen that they require for healthy growth (Schwarz and Wolf 1992). While this system alleviates the problem of anchorage-independence, these harsh conditions are

likely to cause differentiation of stem cells. Additionally, the cells may not be exposed to pluripotency factors secreted by other iPS cells, also promoting differentiation.

Perhaps the most significant competition for us is a cell culture cassette that is designed to interface with a perfusion bioreactor. This CLINIcell cassette (Figure 2) can be used to grow culture adherent cells. It provides a better solution than those noted previously, but it still fails in several ways. Lack of flow guiding features at the front and back of the rectangular cassette does not optimize media flow; this results in dead space as seen in our spring 2010 rectangle design results (Johnson *et al.* 2010). This dead space might cause stem cell differentiation in some areas due to lack of media exchange. Stem cells are sensitive to nutrient exchange and require careful control of culture conditions. Second, the plates of this cassette are thin and permeable to oxygen. This is problematic for iPS cells because they require specific oxygen concentration in the media. At the low perfusion rate needed for stem cell culture, the media would spend a significant amount of time in the gas permeable CLINIcell cassette; thus, the oxygen concentration of the media would equilibrate with the surrounding air. Third, although the CLINIcell cassette provides an acceptable growth area of 250 $cm^2$, it accepts 160 mL of media, which is much greater than the client's requirement of a 60 mL total cassette volume. This smaller volume minimizes waste of expensive media and makes priming possible using only one syringe maneuver. The smaller volume also lowers the risk of contamination during priming,improves the ease of cassette operation, and minimizes media use. Finally, this cassette does not yet incorporate a means to monitor the metabolism of the cells. This feature would be necessary if this cassette were incorporated

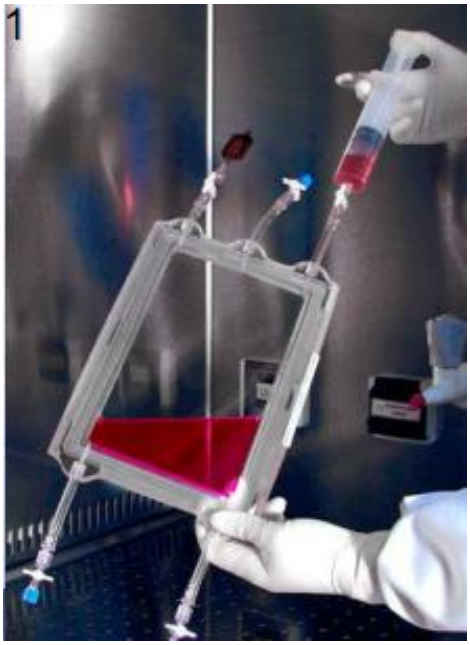



**Figure 2: The CLINIcell cassette provides the most competition to our design proposal in that it allows perfusion culture with adherent cells. However, the geometry of the cassette is not optimized for flow distribution, the plates are too thin, allowing oxygen permeability, and the cassette requires a high volume of media per square centimeter of growth area.**

*Image Courtesy of INNOMEDITECH*
*http://www.innomt.com/*

into a large-scale bioreactor cell growth system, most of which have hardware for measuring pH.

## Ethical Considerations

The knowledge that cells grown in these bioreactor cassettes may be injected into the human body is of primary ethical concern. Cell growth and activity, especially as induced from a pluripotent state, is extremely sensitive to external stimuli. During the entire design process, our team has paid special attention to the presence of material extractables, or chemicals that could leach out of the materials forming the cassette. We have reduced the conditions under which they are more likely to form and optimized flow dynamics, which could otherwise encourage prolonged cellular exposure to them. In this way, we can ensure that cells harvested and used for research or clinical therapeutics are of the highest and safest quality possible, as standardized by the FDA. This will ultimately be determined through various immunostaining and functional assays that match with results from control, natural cells of that particular type.

Along with safety, it is also important that the bioreactor cassette affords security to the person receiving or administering the therapy. Any commercial product should be disposable to limit chances of patient-to-patient cross-contamination or sample alteration due to poor sterilization.

We will also consider the individual nature and patient specificity inherent to iPS cells. Cells from different patients do not necessarily grow at identical rates, so the design must allow for observation, as well as lag time in the feeding schedule of each sample, so that individual cultures are given the best chance to grow successfully. This avoids multiple visits or biopsies of the patient, and limits unnecessary suffering and expense. Finally, it will be important to develop a cost-effective device so that promising clinical therapeutic research and implementation can move forward efficiently and productively.

## Ergonomic Considerations

The bioreactor system is designed to be largely self-sufficient. Therefore, after culture, user interaction with the cassette will be primarily to monitor iPS cell growth.

Accordingly, the bioreactor cassette will have a transparent cell adhering surface so microscopic viewing can be done while maintaining sterility in the cassette. Similarly, valve line connections from the cassette to the media and cassette assembly should be easily and securely engaged and disengaged and a frame must be established in the incubator so that individual cassettes can be removed and replaced without disturbing nearby cassettes. Each cassette must be marked clearly with patient identification so that cultures at various stages can be attended to appropriately. In order to monitor metabolism of the cells easily, a metabolism indicator associated with the device must be mounted so that problems can be detected visually and regulation of flow input can be easily accomplished.

## Design Criteria

The bioreactor cassette will provide appropriate conditions to culture multiple samples of iPS cells (from different patients) without exchanging media between samples. The cassette will be translucent or transparent with a transparent growth plate and have a cell growth area around 225 $cm^2$, in order to maximize the use of a polystyrene growth plate cut from a pre-existing culture flask. It will facilitate confluent healthy growth and adherence by encouraging appropriate fluid flow coverage of the cell growth area. The perfusion interface will allow variable control of flow rate and volume of nutrient media supplied to each cassette, while maintaining physiological growth conditions within them. A trap mechanism will be included before or incorporated within the cassette to remove bubbles that may occlude flow.

The cassette will be designed such that priming and loading of cells can be done in a sterile, straightforward, and user-friendly manner without significant disturbance of the process by bubbles. Components will be sterilizable with gamma irradiation or steam. Materials will be disposable and composed of polymers known to not affect stem cell fate, having USP Class VI certification. The cassette and interface will be designed such that sterility can be maintained if iPS cells need to be removed from the bioreactor for microscopic analysis.

## Bioreactor Cassette

We have developed a basic cassette design that consists of two main parts – a frame and cell growth plate (Figure 3). The frame has luer lock connections for input and output of media. The cell growth plate locks into the bottom of the frame, using a thin silicone gasket and screws to create a tight seal, allowing a surface on which the iPS cells grow and proliferate. Both the cell plate must be translucent or transparent so the researcher can visually monitor the growth of the cells.

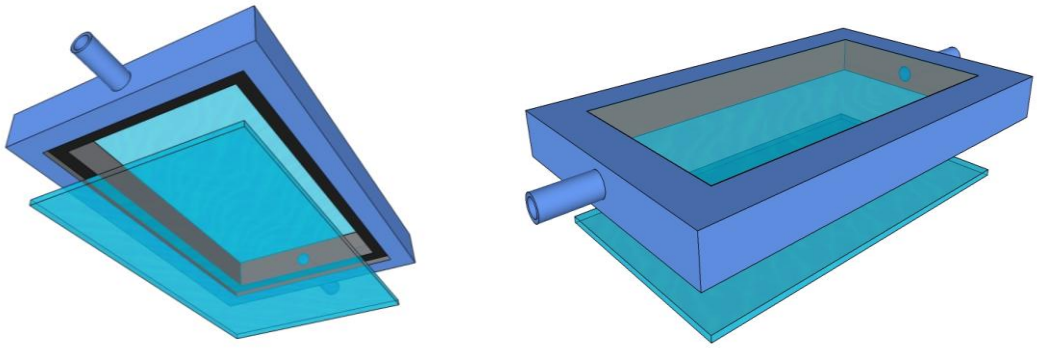


**Figure 3: Top (left) and bottom (right) view of cassette design concept. The cassette consists of a frame and cell growth plate. The cell growth plate is translucent.**

Materials for the cassette frame, cell plate and gasket were researched and tested in order to create a system that is sterilizable, optically translucent, and free of potentially harmful extractables that could cause cell death. PC-ISO (polycarbonate) was selected for the cassette frame because it is autoclavable, USP Class VI certified, and compatible with rapid prototyping (Stratasys Inc 2009; 3D Systems Inc 2007). We also completed a successful study with H9 embryonic stem cells to ensure that PC-ISO did not negatively influence cell viability and growth. For the cell growth plate, tissue culture polystyrene was selected because it is USP Class VI certified and is already used to grow iPS cells successfully. A maximum 238 $cm^2$ rectangle was cut from a Corning 500 $cm^2$ Cell Culture Dish (Cat. #431110) to fit into the cassette's frame. In the future, these polystyrene pieces will be resterilized, apart from the cassette frame, by gamma irradiation. Finally, we selected a thin silicone gasket to create the seal between the cassette frame and cell plate.

Silicone is commonly used in medicine and many formulas are USP Class VI certified (Columbia Engineered Rubber Inc 2010). The silicone selected was provided by our client and has previously been used with stem cells successfully.

Before creating any physical prototypes, we simulated and tested several different designs using computational fluid dynamics, but focused on the basic rectangle, the straw design and the balanced runner design (Johnson *et al.* 2010). These three were manufactured with rapid prototyping techniques and tested with dye to assess the fluid flow profile over time within the cassette. Our results uncovered several complications, including rapid prototyping error, bubbles, and recirculation zones.

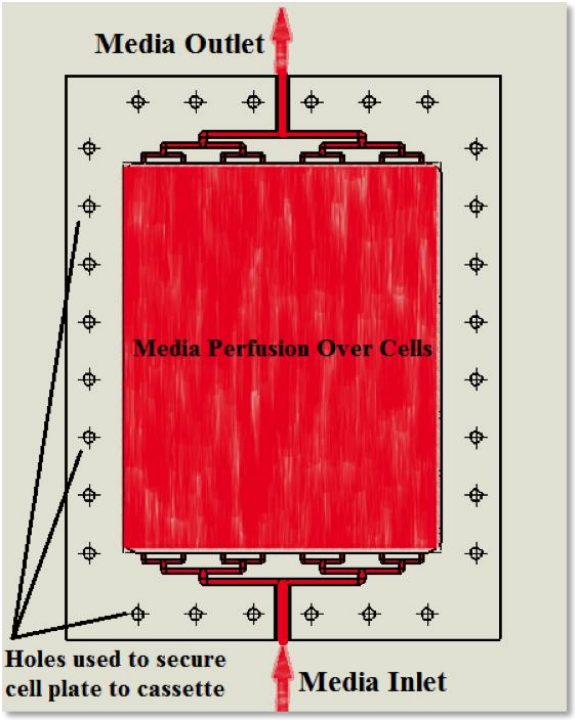



**Figure 4: Current bioreactor cassette design with mirrored balanced runners. See Appendix C for dimensions.**

To resolve some of the undesirable flow patterns observed during dye testing, further modifications of the balanced runner

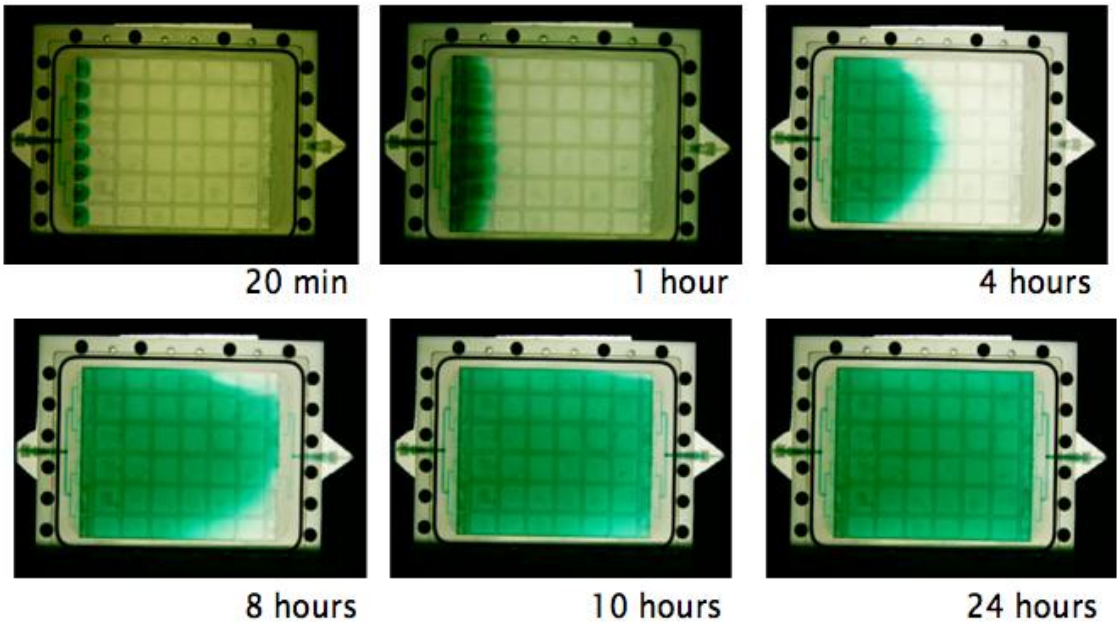



**Figure 5: Flow patterns of mirrored balanced runner cassette. This design avoids backflow and recirculation zones (images courtesy of Bill Kreamer).**

design were completed to mirror the inlet to the outlet (Figure 4). This design appears to avoid backflow and recirculation zones, providing a more even flow than previous tests (Figure 5). It is this cassette design we continued to worked with test this semester.

## Sources of Variability in the System

The system in which this cassette will ultimately function is complex; it was important to maintain this complex configuration in our testing setup so that recorded results would be relevant to the final bioreactor system, and so that any problems with the cassette–bioreactor interface could be detected and resolved early. Preliminary tests, which perfused media through the entire bioreactor system, revealed significant bubble formation ($3.6 \pm 1.12$ mL after 4 days of perfusion) between the cell growth plate and frame in the bioreactor cassette. This bubble formation is unacceptable when growing cells on the plate, so several pilot tests were run to analyze sources of variability and to determine potential sources of bubble formation in the cassette system.

### Tubing and Valve Connections

Tubing and 3-way valves function to connect all major components of the simulated bioreactor testing system. Bubbles lodged in the tubing during setup, as well as leakage drawn from loose tubing to component connections, are possible sources of bubble formation. These bubbles contribute to inconsistencies in observed results. We determined that identical tubing and valve configuration between test replicates, coupled with a complete media flush of tubing before perfusion began, would minimize this variability. However, significant bubble formation still existed during pilot testing.

### Hollow Fiber Gas-Exchanger

The hollow fiber gas-exchanger facilitates oxygenation of the fresh media before it is delivered to the cassette. Testing suggested that incomplete rinsing of the hollow fiber unit after sterilization with Minncare may have contributed to bubble formation in the cassette. Testing the hydrogen peroxide content of flow-through from the hollow fiber unit (using MinnTech strips) after several washes revealed that extensive rinsing including air

pressure was required to remove residual Minncare. All future tests will include a washing procedure with PBS and air pressure until the Minntech strips confirm ≤1 ppm hydrogen peroxide in the hollow fiber unit.

### Oxygenator Pump

In the simulated bioreactor system, a Tetratec AP150 aquarium pump provides gas to the hollow fiber gas-exchanger for media gassing. Pilot tests indicate that the level of bubble formation in the cassette is proportional to the rate at which this pump operates; since our client has not standardized this rate for the eventual bioreactor system, we configured this pump to run such that it creates the highest risk for bubbles in the cassette during all testing runs. This pump setting may increase the accumulation of gas in the cassette as well. If the pump is forcing gas into the media at a high pressure and oversaturating it, it is possible that the media is off-gassing in the cassette as a result.

### Variability Conclusions

After analysis of the sources of variability in the bioreactor cassette system, it was determined that integration of a bubble trap before the cassette was necessary to alleviate part of the problem of bubbles affecting the environment of the cells in the cassette. Thus, we focused on researching and designing a bubble trap to be used in both testing procedures and in the final bioreactor configuration.

## Bubble Trap

### Commercial Bubble Traps

There are numerous commercial bubble traps available, though none suit the requirements for containment of bubbles in the bioreactor system. The Braun Three Tower Trap (Figure 6a) accepts bubbles into the tower and displaces media of an equivalent volume until this initially primed reservoir has been depleted (approximately 3 mL collected gas). At that point, accumulated bubbles overwhelm the trap, and it must be disconnected from the system to remove the accumulated gas. The maximum gas collection

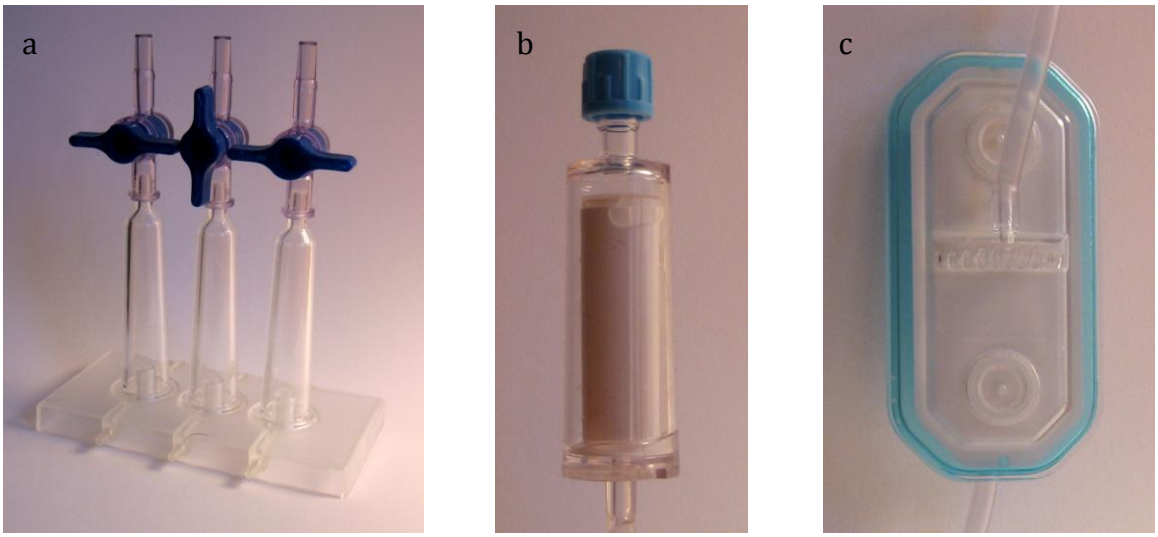



**Figure 6: Commercial bubble traps; (a) Braun three tower trap, (b) 0.22 micron downstream high pressure extended life filter by Baxter and (c) Clearlink System non-DEHP extension set**

of 3 mL is insufficient to collect the amount of air generated in the bioreactor system without daily manual maintenance, and the peaked shape of this trap is unstable and would waste space in a stacked cassette system.

The 0.22 micron Downstream High Pressure Extended Life Filter by Baxter (Figure 6b) also accepts approximately 3 mL of air before failure, as indicated by backpressure into the inlet valve from the trap. Like the tower trap, the cylindrical trap cannot hold a sufficient volume of air to be effective without daily maintenance. This system also cannot stand without support, inhibiting potential integration into a stackable system. One limitation of this system is that if primed incorrectly, it completely loses its function of trapping bubbles.

Baxter's Clearlink System Non-DEHP Extension Set (Figure 6c) is typically used to eliminate bubbles in IV intravenous lines with a chamber system using air filters. This bubble trap also has the limitation of losing its function when primed incorrectly.

## Bubble Trap Design Process

Since preliminary testing revealed that bubble formation was a significant problem, we designed an external bubble trap. The purpose of this bubble trap is to capture bubbles in the tubing prior to entering the cassette. All of the designs that were considered had a similar framework that involved a rectangular chamber with an inlet, outlet and bubble filter mechanism.

The inlet and outlet location was one of the first design aspects to consider. It was decided to place the inlet higher than the outlet. Since the bubbles forming in the perfusion system are less dense than the media, the bubbles will move upward and gather in the top of the trap. By having the inlet higher than the outlet, a bubble entering the system will be less likely to immediately exit the trap because it would have to move downwards through a more dense liquid.

Several different bubble removal mechanisms were also considered while designing our bubble trap. One of our first designs involved several channels that the media would have to flow through (Figure 7). As the media moves through the channels, it is expected that bubbles will move upward and sequester at the top of the chamber. A potential flaw with this design is that the inner channels will gather more bubbles than the outer channels. This could cause the inner channels to be filled with air quicker, and cause bubbles to stick to the sides of the chambers, preventing air from moving to the edges of the cassette. Potentially, with all of the bubbles sequestering in the center of the cassette, the air could then easily reach the outlet before the entire trap is filled with air.

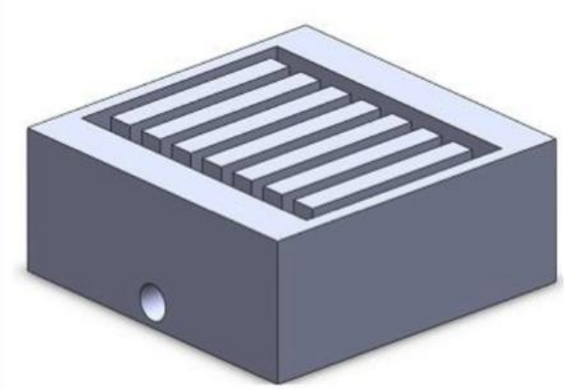


**Figure 7: External bubble trap design that involves tall, thin channels to filter bubbles from the media.**

**Our second design mechanism involved splitting the bubble trap into two chambers with a diagonal screen (**

Figure 9). The screen has holes to allow media to perfuse through the trap while limiting bubble passage, by bubble size selection. Similar to the channel design, this concept is based on the expectation for the bubbles to move upward. When a bubble enters the trap, the screen will limit bubble flow to the outlet and redirect the bubbles upward. A potential problem in this design is that bubbles could enter the lower chamber through the pores on the upper portion of the screen. This could allow bubbles to sequester right above the

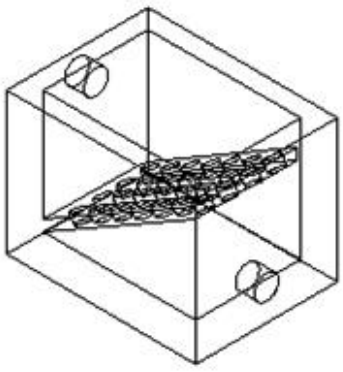


**Figure 8: External bubble trap design that involves two chambers and diagonal screen.**

outlet, therefore increasing the chance that one may move past the trap and into the cassette.

**The diagonal screen design was modified for our third and final design so that only the lower portion of the screen has holes (**

Figure 10). The holes, with a 2.85 mm diameter, have collective larger cross-sectional area than the outlet so as not to create pressure gradients. As bubbles enter the trap, they are expected to sequester in the upper chamber until it is nearly full and reaches the lower holes. A potential problem with this design is that the bubble volume in the bioreactor system could be larger than the volume of the upper chamber. To address this issue, the upper chamber can be modified to fit the expected bubble volume of the bioreactor system.

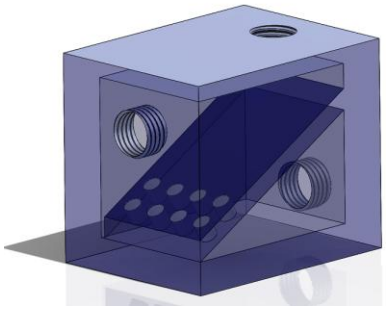


**Figure 9: The final bubble trap design, which incorporates two rows of holes and a diagonal screen. See Appendix D for dimensions.**

# Methods

## Bubble Trap Efficiency Testing

A 50-bubble efficiency test was completed for our final external bubble trap design. The trap was primed with media using clear tubing at both the inlet and outlet. A syringe filled with media and air was then connected to the inlet tubing. To form bubbles (with a volume of approximately 0.07 mL, based on observed diameter of about 5 mm), the syringe was tipped, allowing air to enter the inlet tubing. The number of bubbles entering and exiting the trap was counted in order to calculate the bubble trap's efficiency. Efficiency (E) was defined as the following:

$$E = \frac{(bubbles_{enter}) - (bubbles_{exit})}{(bubbles_{enter}) + (bubbles_{exit})} \times 100\%.$$

## Cell Testing

This semester, all cell testing was done using a terminally differentiated cell line HEK-293 instead of an iPS cell line to provide proof of concept and identification of problems before more difficult culturing methods were attempted. A HEK-293 cell bank

was prepared for testing purposes, comprised of 15 vials of 1 x $10^6$ cells/vial. The cassette was seeded by pushing the cells into the inlet using a 60 mL syringe. The shear stress along the walls of the runners while loading the cells into the cassette is approximated to be around 1.24 dyn/$cm^2$ (Appendix E). Since a shear stress of 9.84 dyn/$cm^2$ has been shown to not cause differentiation of human ES cells (Fok and Zandstra 2005), the decision to load the cassette in this manner is reasonable with regard to maintaining viability and undifferentiated status of our cells.

A "set it and forget it" approach was used for the bioreactor test, as that is how the device will be used in the future. After sterilizing the entire bioreactor system with a 1% Minncare solution, both our cassette and static culture control T225 $cm^2$ flask were seeded with passage 7 HEK-293 cells at a density of 2 x $10^4$ cells/$cm^2$. After leaving the cell suspension undisturbed for at least 60 minutes at 37˚ C to facilitate cell adhesion to the polystyrene, we commenced perfusion, supplying DMEM/Glutamax plus 10% dialyzed FBS media. The media perfused at approximately 1.5 volume changes per day. Then, perfusion was stopped after four days, as pilot static culture studies have indicated that nearly confluent growth is achieved in a 225 $cm^2$ flask after this time period.

End point analysis of the perfusion cassette was performed on the fourth day after seeding, when the cells were at 90-95% confluent. Analysis included a system assessment and cellular assays. We first observed and evaluated any flaws in design that have inhibited appropriate function of the cassette when interfaced with the bioreactor system over the four-day trial, including ergonomic issues, sterility, and cell compatibility with the setup.

A Trypan blue stain was executed on the flat culture surface in both the cassette and control growth areas to relate viability to location on the cassette. After removing the media from the control flask/cassette, the cell surfaces were washed with 10 mL of PBS. 3 mL of Trypan blue was then added and allowed to sit for one minute. After removing the excess Trypan blue, the flask/cassette was washed two more times with 6 mL PBS. Finally, 8 mL of PBS was added to the cell surface to keep the cells moist during imaging. To get a representation of the viability of different areas of the cassette and control flask, the growth area was divided into 6 sections. Within each of these 6 sections, 3 samples were

imaged randomly within the area. These images can then be used to observe cell density and live/dead percentages.

In order to visually determine the success of our seeding procedure, we completed a crystal violet stain after the Trypan study was complete. We fixed the same cells with 10% formalin and stain with 0.05% crystal violet in order to compare the confluency and growth patterns macroscopically. Since crystal violet stains extracellular matrix and cytoskeletal proteins, this test allowed us to qualitatively observe any areas of insufficient seeding. The general testing procedure will follow the flow chart (Figure 10).

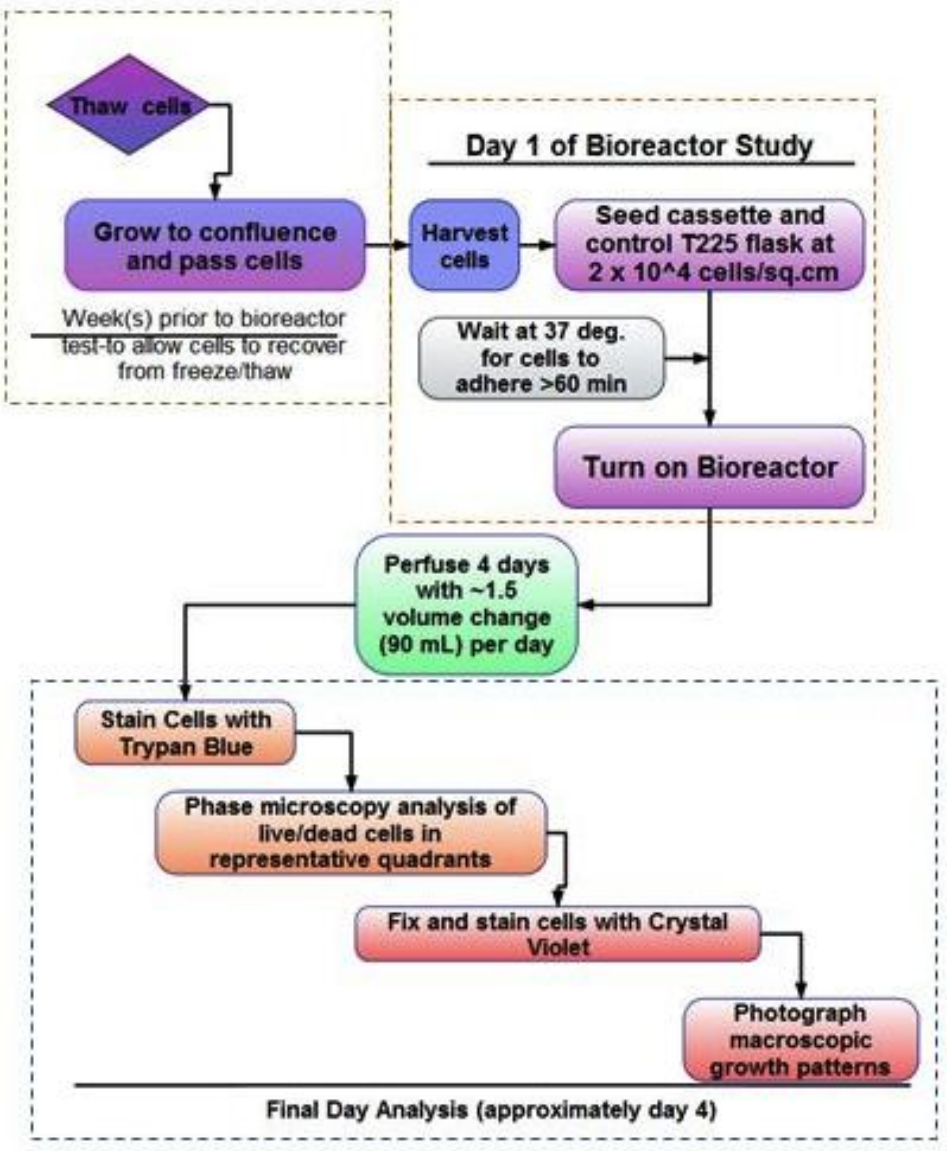



**Figure 10: This flowchart summarizes the cell studies to test the cassette.**

## Bubble Trap Ergonomics Testing

In order to evaluate the ergonomics of our bubble trap compared to those commercially available, we designed a study paired with a survey (Appendix F) to test our bubble trap design. In the study, subjects were asked to prime our bubble trap (Referred to as "Cube"), as well as the commercially made Clearlink System non-DEHP extension set (Referred to as "Flat") and Baxter high-pressure filter (Referred to as "Cylinder.") We authored instructions for priming our prototype; instructions for both commercial bubble traps were copied directly from their packaging. All directional sheets were typed and provided to participants (Appendix F.)

Participants in the study were given a brief overview of the purpose of a bubble trap in a system, as well as the importance of correctly priming a bubble trap. They were randomly assigned to begin at one of the 3 bubble trap stations, each of which contained an instructional sheet, syringe, 150 mL of water, and the appropriate bubble trap. They were asked to prime the trap using the provided materials. We moderated each station and noted whether or not each trap was primed correctly.. After completing all 3 stations, the participant filled out the survey, evaluating and comparing their experience with each trap, either on a scale of 1-5 (easy to difficult) or through direct commentary. If the participant asked how successful they had been, they were told to assume that all attempts had been successful, though this may not have been the case.

A second instructional set was prepared which explained how to remove a bubble from just our bubble trap prototype, an activity that was separately rated by participants in the evaluation survey. This activity was carried out under observation at our bubble trap station. Participants were also asked to indicate on the survey their level of experience with both cell culture and bubble trap use.

Results of the survey were analyzed first for the 11-member sample group as a whole, and second as a comparison of two separate groups divided based on experience with cell culture or bubble traps. Ratings were analyzed and compared quantitatively, while qualitative commentary was evaluated to explain observed trends. Statistical significance was analyzed using a 2-way ANOVA with no replication.

# Results

## Bubble Trap Efficiency Testing

Our efficiency test revealed that of 50 bubbles injected, no bubbles were detected leaving the outlet of the trap, giving it an efficiency of 100%. In this test, 50 bubbles were not enough to overwhelm the trap; however, if the total bubble or air volume exceeded 15 mL, bubbles entered the outlet tubing. From the observed runs with the cassette, the accumulated air volume was about $3.6 \pm 1.1$ mL in four days. This would suggest that our trap could accommodate our cassette's needs for about two weeks before failing. However, to increase this allotted time, modifications to the trap could include increasing the volume of the upper chamber of the bubble trap.

## Cell Testing

During our first cell test this semester, we unveiled a problem with bubbles or air accumulating in the cassette. After addressing this problem with the design of a new bubble trap, we performed a new cell study. However, due to setup error, we were unable to get the desired imaging data for the cassette. Although we did not get the desired results, we discovered a variety of new information about our design, setup, and analysis methods.

The most important piece of information that we obtained from this test is also the reason we did not obtain successful results from this study: the bottom side of the cassette is not obvious. Since the tissue culture polystyrene plate is screwed onto the bottom of the cassette, as designed currently, the cassette sits on the heads of the screws when set up

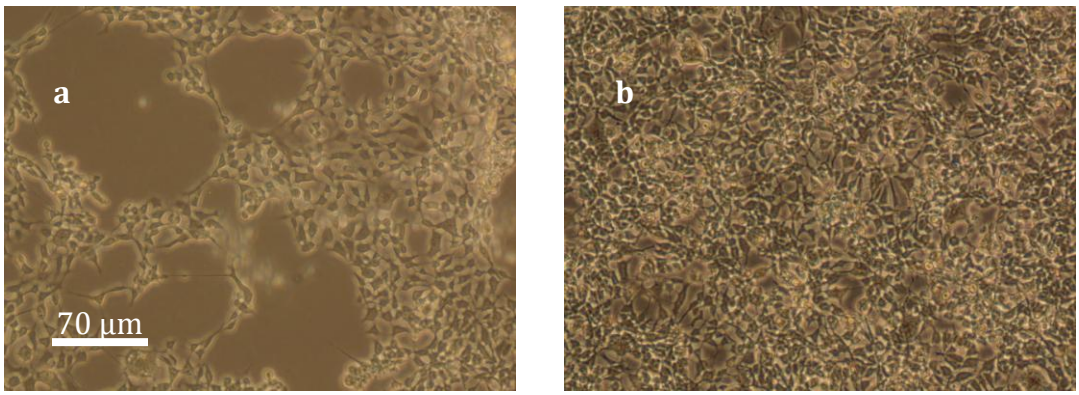



**Figure 11: Trypan blue microscopy (a) Representative sample of one side of the flask; approximately 60% confluent. (b) Representative sample of other side of the flask; approximately 100% confluent.**

correctly. However, this is counter-intuitive and not user friendly. The user is not able to see through the plate from the top, making quick analysis more difficult. Thus, for our bubble studies with the cassette, we had to run the system with the cassette upside-down in order to observe bubble formation clearly. After moving to the cell study, we neglected to change this procedure, leading to cells being seeded to the cassette frame rather than the intended polystyrene plate. The cassette resting on the screw heads creates an uneven surface for the cell plate, which was suggested by our Trypan and crystal violet stain studies of our control flask (which we were able to carry out). Our control flask was placed on top of the cassette in order to serve as a control for the positioning of the polystyrene plate. In this flask, we observed cell density differences on opposite sides of the cassette: one side was completely confluent (Figure 13a) after the four-day study, while the other side was about 60% confluent (Figure 13b). This was also evident in our crystal violet study (Figure 13). This result is not completely conclusive, however, because the method of staining caused many cells to detach. Thus, we will need to address this crystal violet protocol in the future, perhaps using a different fixation method such as methanol.

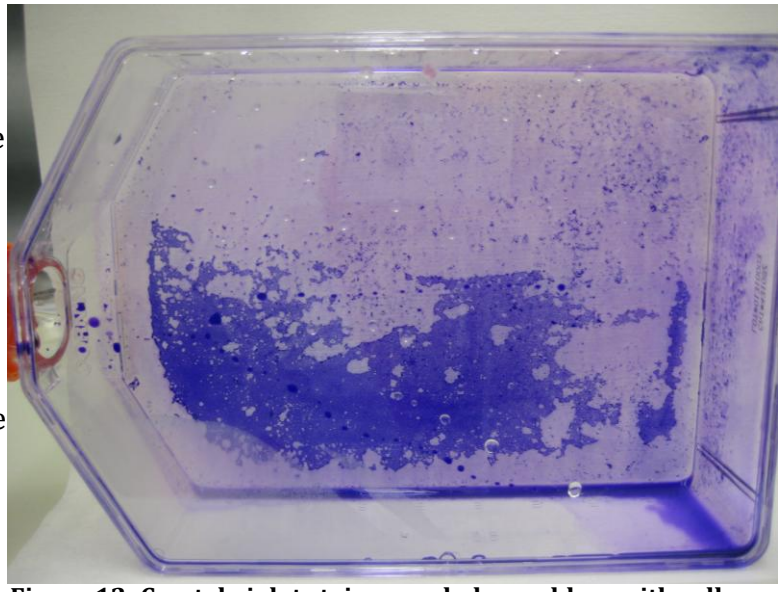


**Figure 12: Crystal violet stain revealed a problem with cells delaminating. The top is representative of Figure 11a while the bottom is Figure 11b.**

Before disassembling the cassette from the cell test, we noticed two more important issues. First, there were still many small bubbles (about 2 mm in diameter) throughout the cassette, though the total volume (about 1.5 mL) was much smaller than previously seen in tests without our bubble trap. Evidence suggests that the media was degassing in the system because we observed few bubbles in both the upper and lower chambers of the bubble trap without it being overwhelmed with air. Since the oxygen and carbon dioxide concentrations resulting from different gassing settings are unknown, it is possible that we

could simply turn down the gas supply to alleviate this problem. Additionally, we observed a color gradient in the cassette, with the media being more red toward in the inlet and in the center of the cassette and more yellow on the edges and toward the outlet (Figure 13). This indicates a pH difference, with yellow being more acidic. There are many possible causes for this, including inconsistent seeding, uneven flow patterns, bubbles blocking some of the runners., or complications due to the outlet runners.

**Figure 13: We observed a color gradient in the cassette with the middle being red (neutral pH) and the outside being yellow (more acidic pH).**

## Bubble Trap Ergonomic Testing

Ease of priming, straightforward nature of directions, and manageability in a sterile hood were rated by study participants on a scale of 1-5 (1=Very Easy, 5= Very Difficult) for each type of bubble trap. When evaluated for all participants, ratings show no distinguishable trend between bubble trap type (Figure 15).

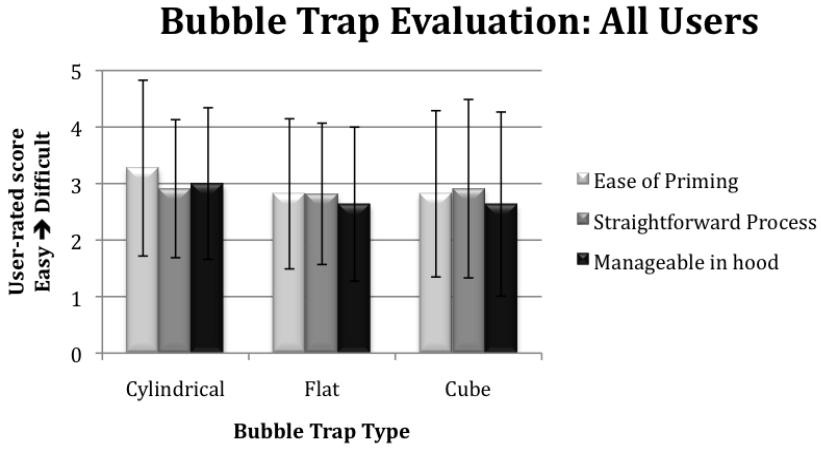



**Figure 14: There was no significant difference (F(2,2)=2.11, p=0.24) in ratings for ease of priming, straightforward nature of directions, and manageability in the hood of each trap when all participants were evaluated. n=11**

When responses from only those participants without cell culture experience were evaluated, our bubble trap was consistently rated the most difficult to manipulate; however when responses from only those participants with cell culture or bubble trap experience were evaluated, our bubble trap was consistently rated easiest to manipulate, though these trends are not statistically significant (Figure 16.)

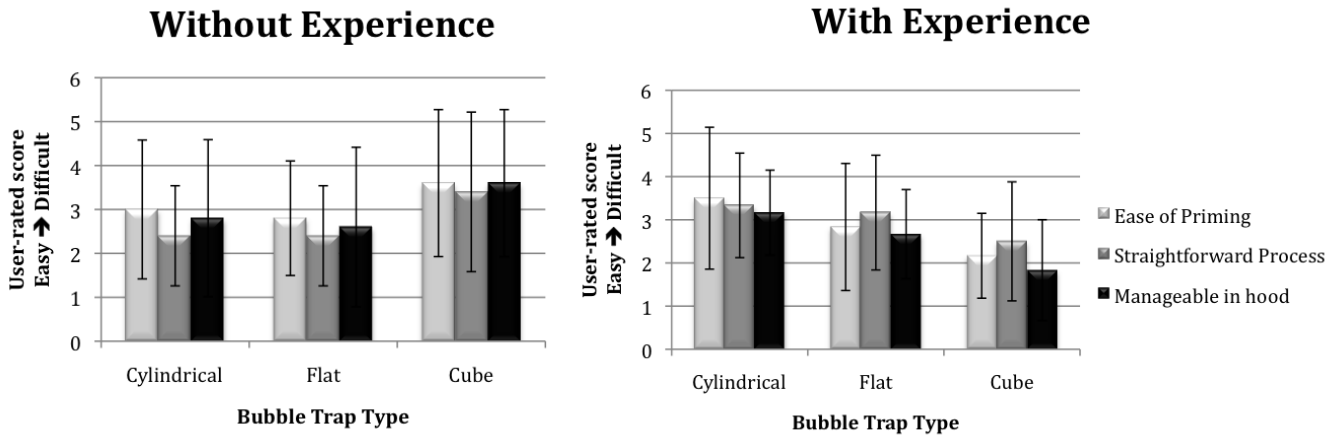



**Figure 15: Users without cell culture experience (left) rated our bubble trap (cube) as most difficult to prime, understand as a process, and manage in the hood, and instead preferred the Clearlink System non-DEHP extension set (flat), though differences between trap type ratings are not statistically significant ($F(2,2)=11.2$, $p>.02$). Users with cell culture experience (right) rated our bubble trap as easiest to prime, understand as a process, and manage in the hood, though trends are not statistically significant ($F(2,2)=5.76$, $p>.06$). n=11**

In addition to the user-rated score, participants were asked to choose specifically which bubble trap would be easiest to prime, most difficult to prime, and easiest to operate under sterile conditions (Table 1, *Appendix F for questions*.) This provided additional insight, since a user-rated scoring system allowed the participant to give multiple devices the same score.

**Table 1: Percent of participants who chose given trap type when asked specifically which bubble trap was easiest to prime, most difficult to prime, and easiest to operate in a sterile hood. When forced to choose, 45% of participants thought that the cubic trap was easiest to prime, 9% thought that it was the most difficult to prime, and 64% thought that it would be easiest to operate in a sterile hood. n=11**

| Percent of Participants | | | |
|---|---|---|---|
| | *Ergonomic Criteria* | | |
| *Trap Type* | Easiest to Prime | Most Difficult to Prime | Easiest to Operate in Sterile Hood |
| Cylindrical | 36 | 45 | 27 |
| Flat | 18 | 45 | 9 |
| Cube | 45 | 9 | 64 |

Whether each trap was actually primed successfully was recorded for each participant by a moderator. When analyzed for the whole, 11-participant group, participants primed our bubble trap ("Cube") successfully 72% of the time, the Clearlink System non-DEHP extension set ("Flat") successfully 55% of the time, and Baxter high-pressure filter ("Cylinder") 18% of the time (Figure 17, left.) When divided into those with and without cell culture or bubble trap experience, the trend was similar, though those with experience were better able to prime the Clearlink System, an no participants without cell culture experience were able to correctly prime the Baxter high-pressure filter completely (Figure 17, right.)

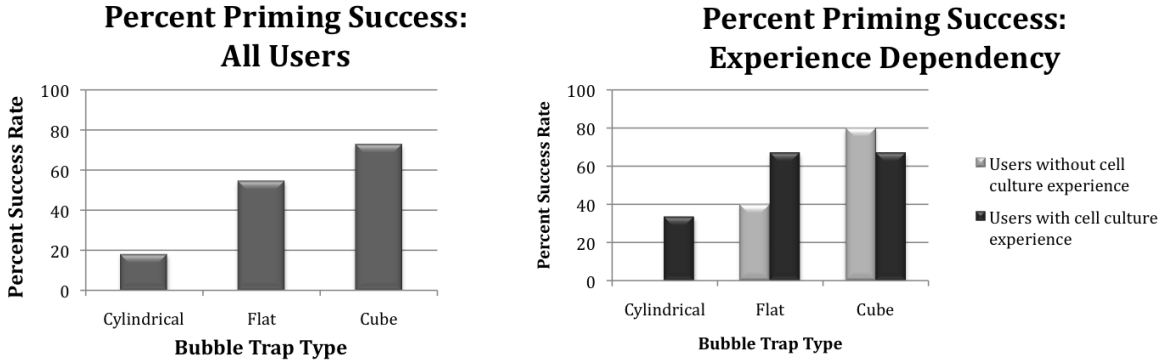



**Figure 16: When evaluated as a complete group, participants primed our bubble trap (cube) with the greatest (72%) success rate (left.) Breakdown of percent success rates between those with and without cell culture experience shows a similar trend, though those with experience were better able to prime the Clearlink trap, and those without experience were unable to prime the cylindrical trap (right.) n=11**

Because this bubble trap may be eventually integrated into a bioreactor cassette setting, participants were asked to rate the ease with which each trap could be integrated into a stacked system. Again, a difference in response is seen between those with and those without cell culture experience; those with experience think the cube would integrate best, those without experience prefer the cylindrical trap (**Error! Reference source not found.**.) Results are not statistically significant, and it is suspected that participants often misunderstood this question based on post-survey feedback.

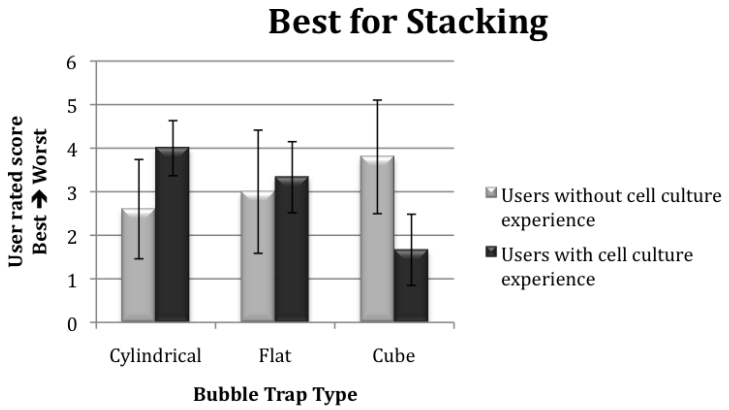



**Figure 17: Trends indicate that participants with cell culture experience think that our bubble trap (cube) would integrate best into a stacked system, those without experience think that the cylindrical trap would integrate best into a stacked system, though results are not statistically significant ($F(1,2) = 0.02$, $p > .91$), and it is suspected that this question was widely misunderstood based on participant feedback. n=11**

Direct comparison of responses about our bubble trap between those with cell culture experience and those without cell culture experience, including ratings about ease of bubble removal, shows that those with experience consistently rate our trap easier to manipulate than those without experience. This is not the case for all bubble traps evaluated in this ergonomic study (Figure 19.)

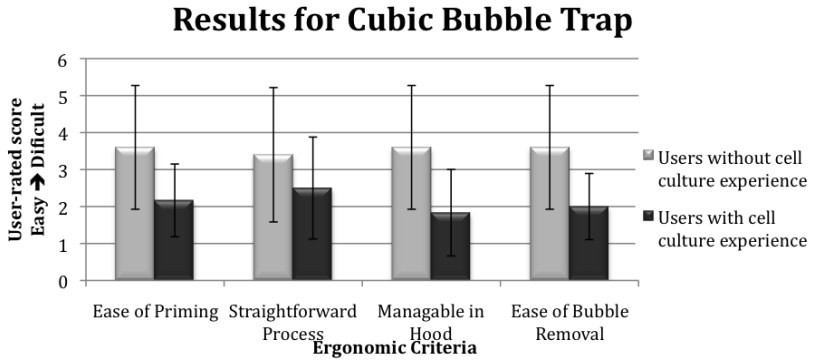



**Figure 18: Participants with cell culture experience had a better experience with our bubble trap than those without experience. This was not the case with all traps evaluated in the study; results are not statistically significant.**

Qualitative responses to survey questions suggested that the importance of minimizing the number of steps necessary to execute a procedure is more important than minimizing the complexity of the device itself. Commentary did, however, also indicate that it is important for the operator to be able to see what is happening inside of the bubble trap as they are priming. Moderator observations confirm this commentary, as participants were more likely to achieve success after misreading directions if they could see and understand what was happening inside of the bubble trap they were operating.

Results of the ergonomic study indicate that the addition of a funneled top to our bubble trap would significantly improve its usability (See *Future Work*); participants struggled with centering the existing bubble below the top Leur port to eject it from the cassette, and disliked having to twist and turn the trap repeatedly to coalesce discreet bubbles. This redesign would minimize the number of steps necessary to execute the priming procedure, without changing visibility into the trap. Besides these design modifications, ergonomic testing revealed that it will also be important to develop a concise, informative set of directions so that the user can confidently and correctly manipulate this complex system.

## Time and Budgeting

We followed a tight schedule throughout the semester (see Appendix G for Gantt chart). Overall, our original schedule was followed; however unexpected results caused us to reevaluate our plan due as the semester progressed. One such example of these setbacks includes our first cell run, which resulted in large bubble formation. There was also a delay in prototyping the bubble trap due to the facility being moved.

There was no set testing budget for this project, however there was a prototype budget of $1,000. We did not exceed this limit with the cassette being approximately $880 and the bubble trap being $7.86. There were several unseen costs from testing consisting of common cell culture materials including but not limited to media, fetal bovine serum, PBS, various pipettes, syringes, bottles, media bags, air filters, Trypan, crystal violet and tubing.

## Future Work

Next semester we plan to replicate cell studies with HEK-293 cells and make modifications to the procedure, analysis, and ultimately the design based on the results of our testing. This will include addressing the ergonomic limitations of the cassette's design. Additionally, further testing with stem cells will be important to assess the ability to maintain an undifferentiated state. We also plan to research the possibility of using defined substrate conditions in order to avoid xenocontamination.

### IMR-90 Cell Testing

One future direction for this project will test IMR-90 cells, which are the ultimate goal of this project because they are iPS cells. The HEK-293 cell line is resilient and thus a good starting point but has less sensitivity to environmental factors than undifferentiated cells. Therefore, tests with HEK cells will not provide insight into the effects of our device on differentiation, a consideration very important to the success of our device.

Consequently, we plan to test our device with IMR-90 cells after achieving good results with the HEK-293 cells.

IMR-90 is a type of iPS cell line generated in the Thompson lab at the University of Wisconsin–Madison. Three types are available from WiscBank (1,2, or 4). These lines have been produced from feeder-free cultures, so there is no risk of xenocontamination (Deposited Cells Lines 2010). The Thompson lab used the factors OCT4, SOX2, NANOG, and LIN28 to reprogram the parental IMR-90 line back to pluripotency. They tested the pluripotency of these cells by analyzing karyotype, telomerase activity, cell surface markers analogous to those used for hESC and their ability to generate tissue from all three primary germ layers by immunohistochemical analysis of teratomas (Yu *et al.* 2007). The IMR-90 line will be more sensitive than HEK-293 cells to the environment the cassette creates, and therefore will rigorously test the culture methods associated with the cassette culture system, as well as whether the environment that the cassette creates is appropriate to maintain healthy iPS cells in an undifferentiated state. If the pluripotent cell results do not match the results of the HEK-293 cells, we will need to more rigorously optimize the flow conditions by the methods listed earlier, since we know that stem cells are more sensitive to their environment than are differentiated cells. Also if differentiation occurs, we will need to consider if some aspect of our design, such as depth, liquid volume, flow guidance, or seeding protocols places undesired stress on the sensitive iPS cells, thus leading to differentiation.

## Ergonomic Considerations

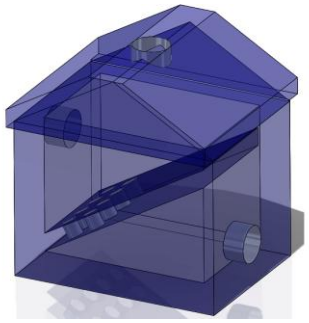


**Figure 19: Redesign of the bubble trap, based on ergonomic survey results. Sloped top guides bubbles to luer port, from which bubbles can be easily extracted with minimal trap manipulation.**

Results of the ergonomic survey suggested that redesign of the bubble trap might also be necessary. The new trap would incorporate a graded top to guide bubbles to a luer port, from which they could be easily extracted. This addition will facilitate priming the trap and removing bubbles from the trap because the bubbles will aggregate in a more localized area. In changing this feature, we will also be increasing the

overwhelm volume of the cassette, the other issue that needed to be addressed. A preliminary option that we have designed has similar dimensions to the previous design with an added "roof"-like top (Figure 19).

The frame of the cassette will also need to be adjusted for ergonomic considerations. Instead of screws, a clamp system will be incorporated, designed with the intent of easier maintenance of sterility during assembly and faster and more consistent assembly between trials. The framing design will also need to be compatible with a stacking and identification system within the bioreactor incubator, so that individualized patient samples can be stored and used appropriately in a clinical setting. The "Take-Out Model" (Figure 20) is a preliminary design to address these ergonomic issues. A readymade bottom tray includes flow guiding features and a cell growth area; two caps are provided with the system. The cells are seeded into the bottom tray as in static culture flasks, and a normal, flat cap can be clipped on to cover the bottom tray while cells adhere. After this process is complete, the cap can be removed, old media aspirated, and fresh media applied to the growth area. The second cap, which features a depressed center, clips into the bottom tray such that approximately 2 mm of space is left between the cell growth plate and the depressed portion of the second cap. This complete tray and cap could then be attached into a perfusion system to support continuous media exchange. The depressed portion of the cap would be transparent, allowing easy microscopic viewing. Such a design would improve speed, ease, and sterility of priming, avoid wasteful media flushing steps, and make it easier to seed evenly.

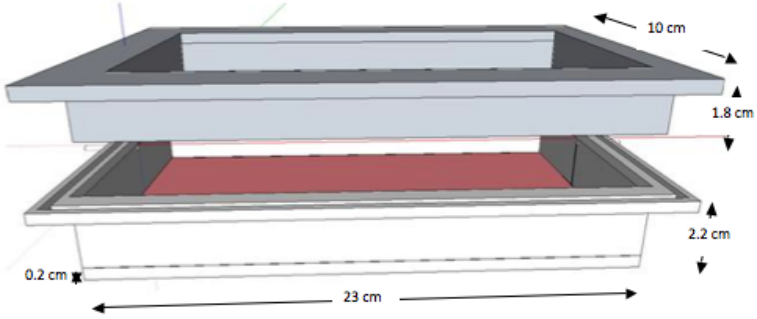



**Figure 20: "Take-Out Model." Image shows bottom tray (lower) and cell growth area (pink,) along with depressed "second cap."**

Additionally, in the future we may need to test different polystyrene plate coatings in order to further optimize growth conditions, including peptide coatings from investigators on campus and in industry. Finally, after focusing on the individual cassette, we will also need to consider the parallel growth system, including the arrangement in the

bioreactor system of multiple cassettes and how to vary the rate appropriately for each cassette independently based on cell proliferation.

### Defined Substrate Conditions

While considering how to best align with the long-term goals of this project, to culture individualized and clinical grade induced pluripotent stem cells, we understand that the future of this project must avoid xenocontamination. The gold standard for culturing both embryonic and induced pluripotent stem cells currently involves using Matrigel, a mix of mouse extracellular matrix proteins, as an adhesion substrate. Exposure to animal origins can place the cells at risk for interspecies pathogens, retroviruses, and immunogenic molecules (Lei *et al.* 2007). Several labs have been working to solve this issue using chemical synthetics or recombinant proteins. One option we may pursue is to use high densities of the synthetic peptides KGGNGEPRGDTYRAY or KGGPQVTRGDVFTMP, or low densities of GKKQRFRHRNRKG which all work through the heparin-binding domain (Klim *et al.* 2010). Another similar concept is to use recombinant vitronectin with mTeSR1 medium (Yoon *et al.* 2010). One group was successful with an E-cadherin and IgG-Fc domain fusion protein (Nagaoka *et al.* 2010). A chemical method of maintaining pluripotent stem cells was reported with the use of EHNA [*erythro*-9-(2-hydroxyl-3-nonyl)adenine] along with fibronectin coated dishes (Burton *et al.* 2010). We hope to spend some time next semester testing at least one of these xeno-free and defined culture substrates after a final prototype is designed.

## Summary

The perfusion bioreactor cassette has the potential to further the applicability of iPS cell therapy in both academic and clinical settings. During initial full system tests, we discovered bubbles formed in the bioreactor cassette, preventing optimal performance. To address this issue, we designed a bubble trap and evaluated its performance. We found our bubble trap design to be effective in cell tests, and our ergonomic study revealed that novice users primed our bubble trap design correctly more often than competing designs. Our HEK-293 cell test results revealed many areas that need to be addressed in the future.

These areas include changing the cassette housing to be more user-friendly and to promote sterile use. We will also consider the fluid flow patterns at the outlet. Other work for this project next semester includes redesigning the bubble trap with minor modifications to ease priming and bubble removal, repeating tests with HEK-293 cells, and testing with an iPS stem cell line.

## References


3D Systems Inc. 2007. DuraForm PA plastic. *Durable polyamide (nylon) material for real-world physical testing and functional use*.

Bacabac, R.G., Smit, T.H., Cowin, S.C., Van Loon, J.J.W.A., Nieuwstadt, F.T.M., Heethaar, R. and Klein-Nulend, J. 2005. Dynamic shear stress in parallel-plate flow chambers. *J Biomechanics*, 38: 159–167.

Burton, P., Adams, D., Abraham, A., Allcock,R., Jiang, Z., McCahill, A., Gilmour, J., McAbney, J., Kane, N., Baillie, G., McKenzie, F., Baker, A., Houslay, M., Mountford, J., Milligan, G. 2010. *Biochemical Society Transactions* 38 (4): 1058-1061.

Columbia Engineered Rubber, Inc. 2009. http://www.columbiaerd.com/

Deposited Cell Lines. 2010. 02 October 2010. http://www.wicell.org/index.php?option=com_oscommerce&Itemid=272

Fok, E.Y.L., Zandstra, P.W. 2005. Shear-controlled single-step mouse embryonic stem cell expansion and embryoid body-based differentiation. *Stem Cells*, 23: 1333–1342.

Johnson, A.B., Kamer, K.J., Larson, E.A. and Zeitler, L.A. 2010. Bioreactor cassette for induced pluripotent stem cells. Unpublished manuscript.

Klim, J., Li, L., Wrighton, P., Piekarczyk, M., Kiessling, L. 2010. A defined glycosaminoglycan-binding substratum human pluripotent stem cells. *Nature Methods* 7: 12: 989-994.

Lane, T., 2010. Crystal Violet (CV) Staining of Cells and Clone counting. 02 October 2010. http://www.mcdb.ucla.edu/Research/Arispe/Protocols/CV_Staining_of_Cells_and.pdf

Lei,T., Jacob, S., Ajil-Zaraa, I., Dubuisson, J., Irion, O., Jaconi, M., Feki, A. 2007. Xeno-free derivation and culture of human embryonic stem cells: current status, problems, and challenges. *Cell Research* 17: 682-688.

Ling Yi, C. , and Lin, L. 2009. Current progress and prospects of induced pluripotent stem cells. *Science in China Series C Life Sciences*, *52*(7), 622-636.

Klim, J., Li, L., Wrighton, P., Piekarczyk, M., Kiessling, L. 2010. A defined glycosaminoglycan-binding substratum human pluripotent stem cells. *Nature Methods* 7: 12: 989-994.

Nagaoka, M., Si-Tayeb, K., Akaike, T., Duncan, S. 2010. *BMC Developmental Biology* 10 (60):1-12.

Schwarz, R.P. and Wolf, D.A. October 13, 1992. *Method for culturing mammalian cells in a perfused bioreactor*. US Patent #5155035.

Singh, V. 08 April 2003. *Disposable perfusion bioreactor for cell culture*. US Patent #6544788.

Stratasys Inc. Fortus 3D Production Systems. 2009. *PC-ISO.*

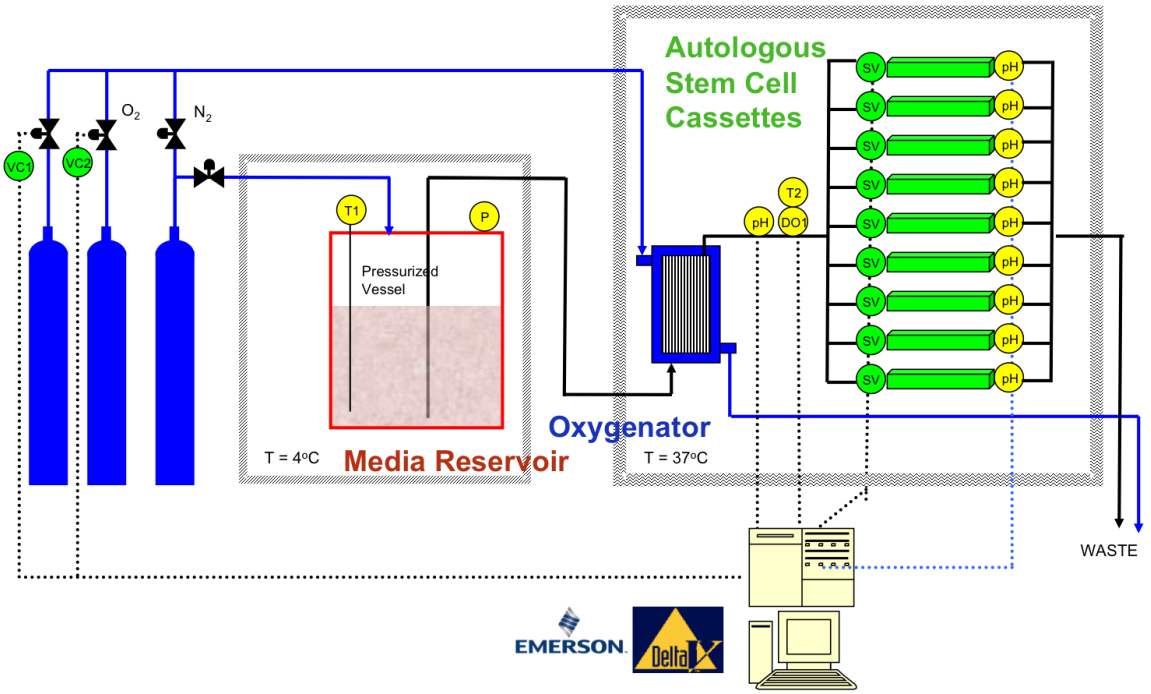
Yoon, T., Chang, B., Hyeung-Taek,K., Joo-Hyun, J., Dong-Wook,K., Dong-Youn H. 2010. Human embryonic stem cells (hESCs) cultured under distinctive feeder-free culture conditions display global gene expression patterns similar to hESCs from feeder-dependent culture conditions. *Stem Cell Rev and Rep* 6:425-437.

Yu,J., Vodyanik, M., Smuga-Otto, K., Antonsiewicz-Bourget, J., Frane, J., Tian, S., Nie, J.,Jonsdottir, G., Ruotti, V., Stewart, R., Slukvin, I., Thompson, J. 2007. Induced pluripotent stem cell lines derives from human somatic cells. Science (318): 1917-1919.

Zandstra, P.W. and Nagy, A. Stem cell bioengineering. *Annual Review of Biomedical Engineering.* 2001. 3: 275-305.

Zhang, H., Wang, W., Quan, C. and Fan, S. Engineering Considerations for Process Development in Mammalian Cell Cultivation. 2010. *Current Pharmaceutical Biotechnology*. *11*: 103-112.

## Appendix A: Bioreactor Schematic




Schematic for the perfusion bioreactor used in this project. Media is perfused through cassettes in parallel so that a single bioreactor can be used without exchange of media. The perfusion rate could be adjusted through clamping or otherwise restricting flow to some of the lines leading to individual cassettes.

## Appendix B: Product Design Specifications

### Project Design Specifications—Bioreactor Cassette

December 08, 2010

Team: Ali Johnson, Kim Kamer, Elise Larson, Laura Zeitler
Client: Derek Hei, PhD – Technical Director, Waisman Clinical Biomanufacturing Facility
Advisor: Naomi Chesler, PhD

**Function:**

The bioreactor cassette will provide appropriate conditions to culture multiple samples (from different patients) of iPS cells without exchanging media between samples. The cassette will be translucent with a transparent growth plate and have a cell growth area of 238 $cm^2$. It will facilitate confluent healthy growth and adherence by encouraging appropriate fluid flow coverage. The perfusion interface will allow variable control of flow rate and volume of nutrient media supplied to each cassette, while maintaining physiological growth conditions within them. A trap mechanism will be included before or incorporated within the cassette to remove bubbles that may occlude flow tracks.

The cassette will be designed such that loading and priming of cells can be done in a sterile environment, in a straightforward, user-friendly process. We will particularly address the ease of bubble removal at this stage.

Components will be sterilizable with gamma irradiation or steam. Materials will be disposable and composed of polymers known to not affect stem cell fate. The cassette and interface will be designed such that sterility can be maintained if iPS cells need to be removed from the bioreactor for microscopic analysis.

**Client Requirements:**

- Steam or gamma sterilizable
- Connects to bioreactor interface and allows variable media perfusion flow
- Gas-impermeable cell growth plate and cassette material
- Optically translucent
- No extractables, or chemical leaching, in contact with media
- Induce and maintain confluent cell growth
- Ergonomic loading/priming procedure
- Mechanism to prevent bubbles from occluding media flow

**Design Requirements:**

1) Physical and Operational Characteristics
    a) *Performance requirements* – Must provide an appropriate cell growth environment with proficient perfusion of media. Cell products must be high viability and comparable or better than static culture.
    b) *Safety* – Must not contain any chemicals or substances that will negatively influence the cell, cell growth or initiate differentiation. Cassette must prevent contamination of sample for similar reasons.

c) *Accuracy and Reliability* – Must provide appropriate culture conditions that allow healthy growth and do not initiate differentiation. Must monitor and maintain these conditions.
d) *Life in Service* – Prototype: sterilizable, withstands repeated use (at least 10) and fluid submersion.
Final product: One-time use, up to 3 months
e) *Shelf Life* – Able to withstand a basic medical storage environment
f) *Operating Environment* – Must work properly at 37° C and in constant exposure to a liquid media.
g) *Ergonomics* – Should not interfere negatively with the user's ability to monitor the cells. Loading and priming should be straightforward when working under sterile conditions, and promote even seeding. Bubble removal at the priming stage should be easy.
h) *Size* – Cell growth area of 238 $cm^2$, depth less than 2mm, less than 60mL volume reservoir
i) *Weight* – Under 1 kg/cassette
j) *Materials* – sterilizable, translucent, allow cell growth, not influence differentiation
k) *Aesthetics* – Transparent cell plate

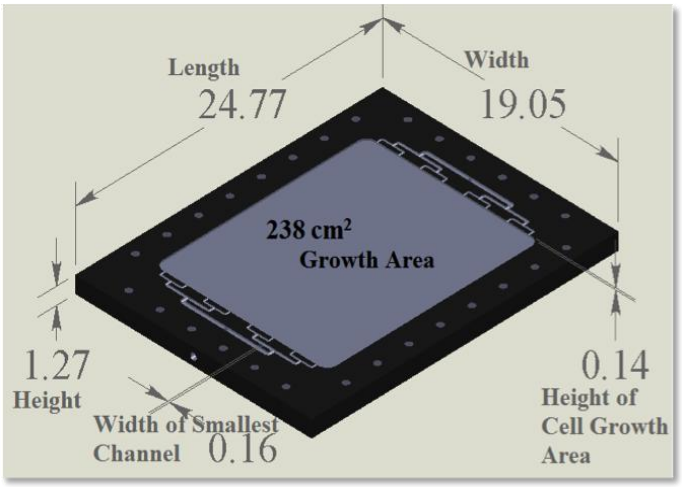
2) Production Characteristics
a) *Quantity* – One, but should be designed with the intent of mass production in the future.
b) *Target Product Cost* – $1,000 for prototype

3) Miscellaneous
a) *Standards and Specifications* – Uses USP Class VI materials, adheres to Good Manufacturing Practice Guidelines and Good Tissue Practices
b) *Customer* – Medical Research Community
c) *Patient-related concerns* – Must not negatively influence the cells. Must maintain independence of each sample. Each cassette must be easily identifiable.
d) *Competition* – There are currently different culture systems but none that allow for several different samples with no exchange in media, or have gas impermeable membranes.

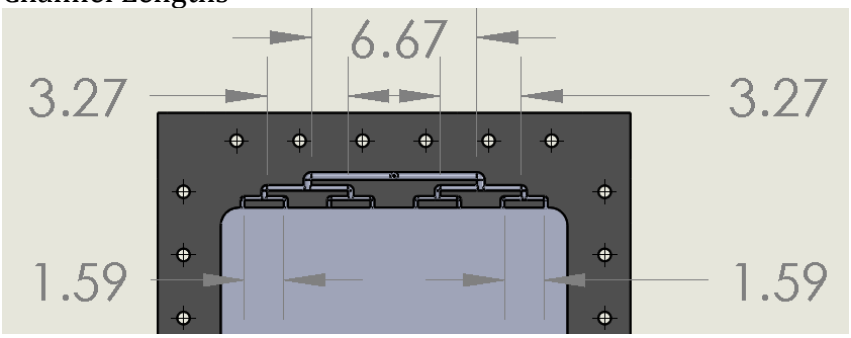
## Appendix C: Cassette Dimensions

Note: All dimensions are in cm.

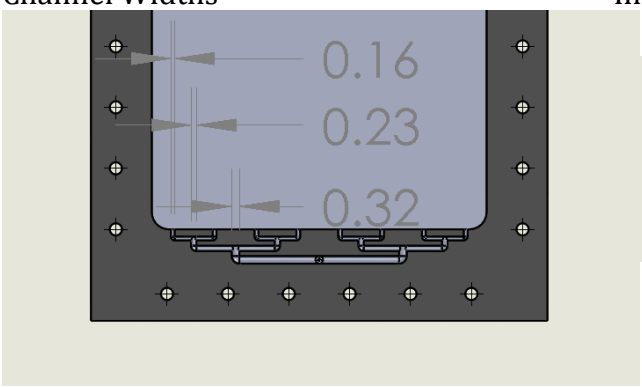
General Dimensions



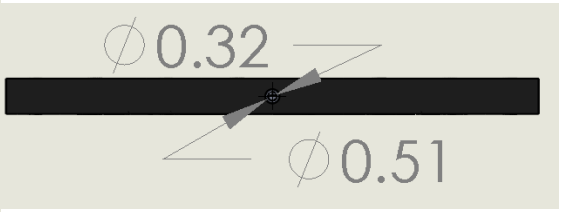

Channel Lengths




Channel Widths

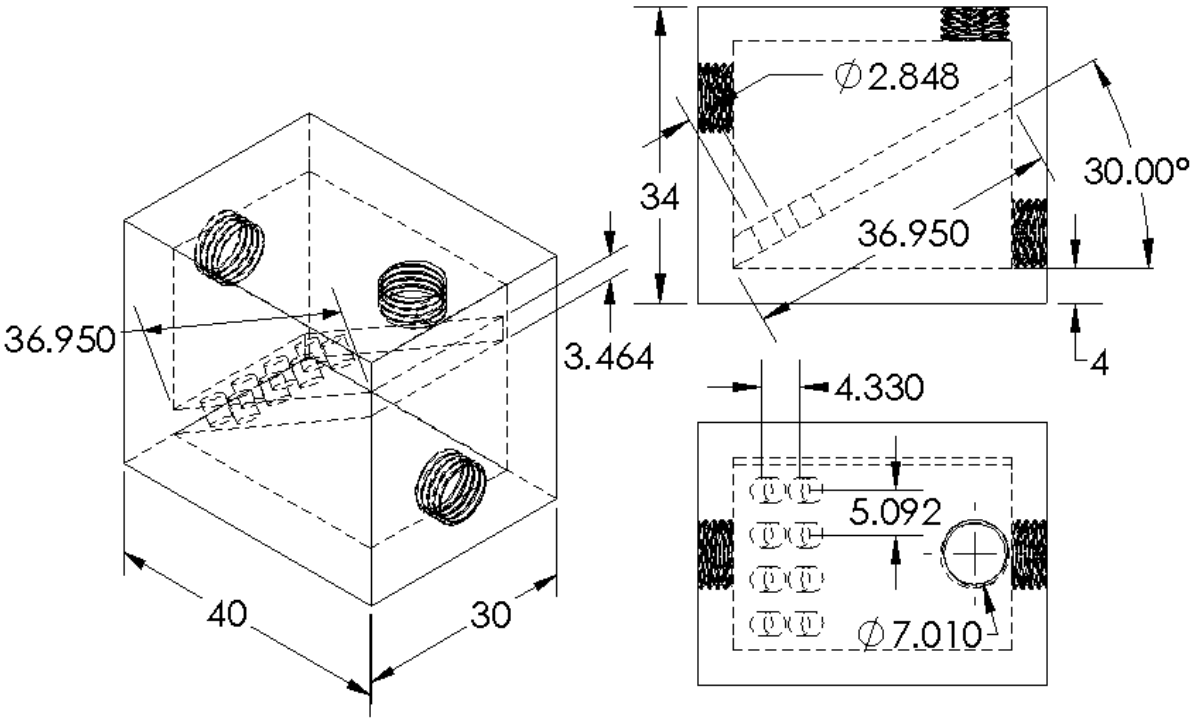



Inlet and Outlet Diameters



## Appendix D: Bubble Trap Dimensions

Note: All dimensions are in mm.



## Appendix E: Shear Stress Calculations

Shear stress of priming the cells through the runners:

$$\mu = 0.0078\ poise \qquad r = 0.0794\ cm$$

$$Q = \frac{60\ mL}{120\ s * 8\ runners} = 0.0625 \frac{mL}{s}/runner$$

$$\tau = \frac{4\mu Q}{\pi r^3} = \frac{4 * (0.0078\ poise) * 0.0625 \frac{mL}{s}/runner}{\pi * (0.0794\ cm)^3} = 1.24 \frac{dyn}{cm^2}$$

This wall shear stress calculation is an approximation based on a few assumptions: the Newtonian viscosity used is that of a similar DMEM media (Bacabac *et al.* 2005), the flow rate for loading the cells through the runners is based on dispensing 60 mL over 2 minutes, and the channels are assumed to be circular channels. The use of this equation for shear stress calculation is justified because this system has a small Reynolds number.

## Appendix F: Bubble Trap Ergonomics Survey & Instructional Sheets

Survey:

Bubble Trap Ergonomics Survey
**All responses will be kept anonymous. Data will be presented as bulk trend only; comments may be anonymously used to explain bulk trends**

"Priming" readies the bubble trap for use. Please follow the given instructions to prime each bubble trap. Then rate and describe your experience with each trap on the following survey.

When the traps have collected a certain amount of air, they must be purged so that it does not overflow into the outlet, negating the trap's function. Please remove the bubble from the cubic trap. Then rate and describe your experience with each trap on the following survey.

**Please use the following scale (1-5) to rate your experience with each cassette.**

**1=Very Easy, 2=Moderately Easy, 4=Moderately Difficult, 5=Very Difficult**

How easy was it to prime the cassette?

| | | | | | |
|---|---|---|---|---|---|
| Cylindrical Trap | 1 | 2 | 3 | 4 | 5 |
| Thin Trap | 1 | 2 | 3 | 4 | 5 |
| Cubic Trap | 1 | 2 | 3 | 4 | 5 |

How straightforward was the priming process?

| | | | | | |
|---|---|---|---|---|---|
| Cylindrical Trap | 1 | 2 | 3 | 4 | 5 |
| Thin Trap | 1 | 2 | 3 | 4 | 5 |
| Cubic Trap | 1 | 2 | 3 | 4 | 5 |

How easy would it be to operate this trap under a sterile hood?

| | | | | | |
|---|---|---|---|---|---|
| Cylindrical Trap | 1 | 2 | 3 | 4 | 5 |
| Thin Trap | 1 | 2 | 3 | 4 | 5 |
| Cubic Trap | 1 | 2 | 3 | 4 | 5 |

Please State:
Which trap was easiest to prime?
Why?

Which trap was most difficult to prime?
Why?

Which trap would be easiest to operate under sterile conditions?
Why?

Which would be most difficult?

Why?

**********

Please answer the following questions about your cell culture experience.

How often do you do cell culture?

Never Daily Weekly Monthly Yearly

Explain.

How often do you work with bubble traps?

Never Daily Weekly Monthly Yearly

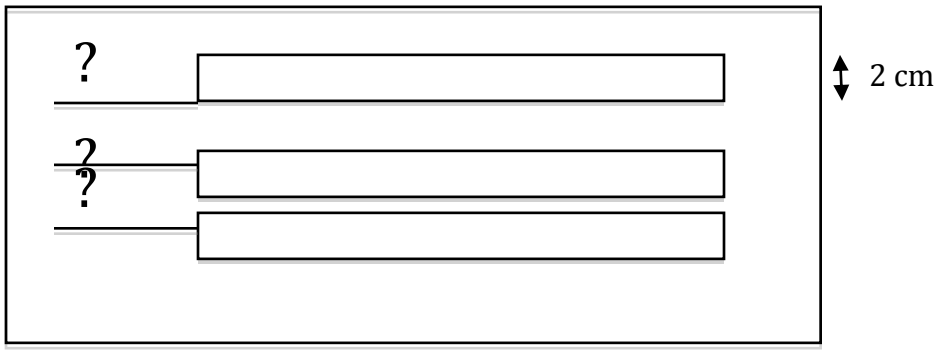
Have you ever worked with a bubble trap similar to those presented in this evaluation?

Explain.

**Using the scale from above,**
How easy was it to remove a bubble from this trap?

Cubic Trap 1 2 3 4 5

Please indicate how easy you think it would be to integrate this trap into a stacked system (See Schematic):



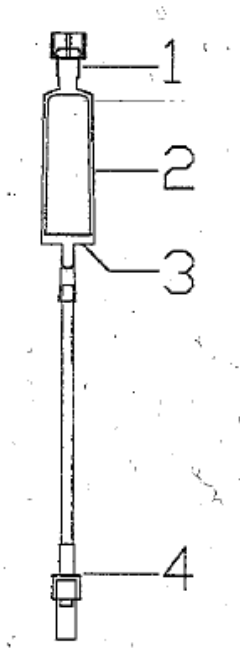

| | | | | | |
|---|---|---|---|---|---|
| Cylindrical Trap | 1 | 2 | 3 | 4 | 5 |
| Thin Trap | 1 | 2 | 3 | 4 | 5 |
| Cubic Trap | 1 | 2 | 3 | 4 | 5 |

Thank You!

Instructional Sheets (each trap printed on separate sheet, compiled here):

Cylindrical Trap

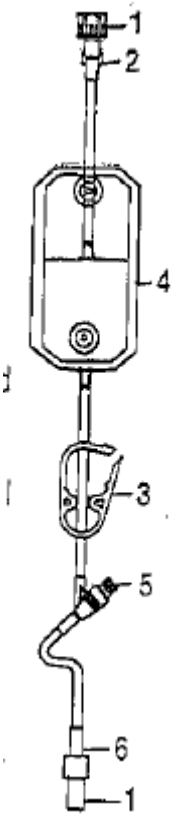
Attach female adapter (1) of filter to fluid source. Fill, invert and tap filter (2) to purge air during priming. Prime set, purge air. Hold Luer lock adapter (4) above filter (2), stop fluid flow. Attach Luer lock adapter (4) to vascular access device and rotate clockwise until secure.




Cautions:
Do not allow air to be trapped in set.
Puncturing set components may cause air embolism.
Position filter (2) upright to vent air.
Do not block air eliminating vent (3) during use.
Internal pressure not to exceed 45 psi.
********

Thin, Rectangular Trap

<u>Priming</u>



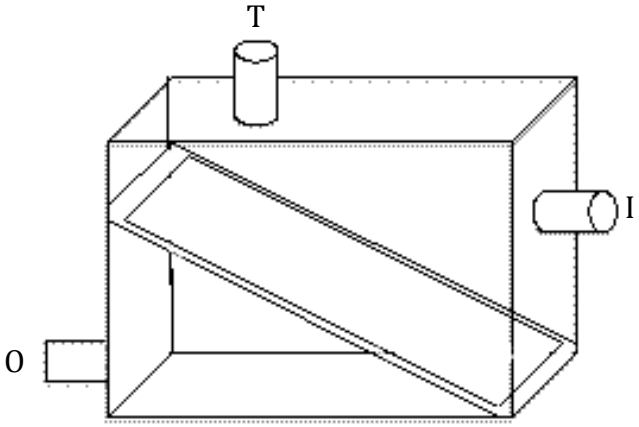

With filter (4) in vertical upright position, prime inlet side of filter (4) completely until no further drops form in drip chamber. Do not invert filter. Open On-Off clamp (3) and tap filter (4) to purge air during priming of outlet side of filter (4). Carefully inspect outlet side for trapped ar. Prime set, purge air. Attach leur lock adapter (6) to vascular access device, and rotate clockwise until secure. Ensure downstream clamp is open. Access Luer activated valve (5) by firmly pushing male Luer of connecting device directly against Luer activated surface and rotate until connection is secure.

Cautions:
Do not allow air to be trapped in set. Puncturing set components may cause air embolism. Do not regulate flow with On-Off clamp. Do not swab Luer activated surface (5) when downstream clamp is closed or valve is recessed. Ineffective swabbing may result. Replace set if valve remains recessed. Do not access Luer activated valve with needles or cannula. Attempting such access will render the product damaged, replace immediately. Use of Luer lock connection is recommended. If Luer slip connection is used, insert into valve using a firm push and twist motion. Do not leave Luer slip unattended. Trace lines before connection. Do not invert filter (4) or block air eliminating vents during priming or use. Internal pressure not to exceed 45 psi.

*******

Cubic Trap

1. Remove cap from top Luer port (T)
2. Slowly inject priming fluid into inlet port (I), until overflow is observed at outlet Leur port (O).
3. Place cap over outlet Luer port (O).
4. Continue to inject priming fluid into inlet port until overflow is observed at top Luer port (T).
5. Replace cap on top Luer port (T).




Bubble Removal:

Cubic Trap

1. Remove cap from top Leur port (T).
2. Tip bubble trap until bubble is directly under top leur port.
3. Inject priming fluid into inlet port until bubble is expelled from top Leur port.
4. Replace cap over top Leur port.

## Appendix G: Semester Gnatt Chart

**Project Schedule**

| Task | September | | | October | | | | November | | | | | December | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 13 | 20 | 27 | 4 | 11 | 18 | 25 | 1 | 8 | 15 | 22 | 29 | 6 | 13 |
| Deciding on Project Direction | | | | | | | | | | | | | | |
| Research Each Direction | | | | | | | | | | | | | | |
| Brainstorming Bubble Solutions | | | | | | | | | | | | | | |
| Create HEK Cell Bank | | | | | | | | | | | | | | |
| Preliminary Testing Plans | | | | | | | | | | | | | | |
| Testing with HEK Cells | | | | | | | | | | | | | | |
| Addressing Bubble Problems | | | | | | | | | | | | | | |
| Redesigning Cassette/Bubble Trap | | | | | | | | | | | | | | |
| Prototyping Bubble Trap | | | | | | | | | | | | | | |
| Testing Bubble Solution | | | | | | | | | | | | | | |
| Analyze testing results | | | | | | | | | | | | | | |
| Brainstorm Ideas for Next Semester | | | | | | | | | | | | | | |
| **Deliverables** | | | | | | | | | | | | | | |
| Progress Reports | | | | | | | | | | | | | | |
| PDS | | | | | | | | | | | | | | |
| Mid-semester Presentation | | | | | | | | | | | | | | |
| Mid-semester paper | | | | | | | | | | | | | | |
| Final Presentation | | | | | | | | | | | | | | |
| Final Paper | | | | | | | | | | | | | | |
| **Meetings** | | | | | | | | | | | | | | |
| MSOE team | | | | | | | | | | | | | | |
| Client/other | | | | | | | | | | | | | | |
| Advisor | | | | | | | | | | | | | | |
| **Website** | | | | | | | | | | | | | | |