Bioreactor Cassette for Autologous Induced Pluripotent Stem Cells

Allison Johnson – Communicator
Kimberli Kamer – Team Leader
Elise Larson – BSAC
Laura Zeitler – BWIG

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Client: Derek Hei, Ph.D.
Adviser: Naomi Chesler, Ph.D.

ABSTRACT

Autologous induced pluripotent stem (iPS) cells show great potential for use as patient-specific medical therapeutics. 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 will be collecting data about cassette function and cell behavior within a simulated bioreactor system, which we will use to further optimize our design. First, we will culture HEK-293 cells in our current cassette design to observe general viability and growth patterns. Then, we will use IMR-90 cells, which have an iPS background, to determine the impact of the cassette design features on stem cell differentiation. We will use these testing results to improve our current cassette design. Future work for next semester includes developing a bubble trap, increasing the ergonomics of the design's sealing and seeding mechanisms, and incorporating a metabolic monitoring system.

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Background

iPS Cells

Stem cells, through which any of the body's approximate 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 grow stem cells under individually controlled conditions. Specialized culture chambers called bioreactor cassettes enable 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.

As discussed previously, it will be necessary to have an economically and ergonomically viable cell culture cassette to connect with a bioreactor system. It 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 as 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. We will design a 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, the simplest cell culture solution is to grow the cells under static conditions. In static cell culture, the cells adhere to the bottom of a polystyrene flask and are constantly bathed in culture media. Media replacement is performed in bulk. Static cell culture flasks can be 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 nearly useless for iPS cell culture. First, for the cells to

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

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

remain undifferentiated, they require constant supply of autocrine factors, 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 inefficient self-renewal (Zandstra and Nagy 2001). For culture 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. An additional problem associated with this method is that the harsh conditions of nutrient-starvation, growth in suspension, and waste accumulation would likely cause stem cell differentiation (Zhang *et al.* 2010).

One solution to some problems associated with the batch-mode stirred flask bioreactors is to use a perfusion bioreactor. In a perfusion bioreactor, fresh liquid media is constantly run through the cassette, allowing efficient waste removal and nutrient addition. Many variations of perfusion bioreactors have been employed to culture cells, and many 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 growth 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 having to grow the cells in suspension by placing microcarrier beads in the medium, which allow the cells to attach, similarly to a normal tissue culture substrate, but to still get the nutrients, oxygen, etc. 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 and the cells may not be exposed to pluripotency factors secreted by other iPS cells, also promoting differentiation.

Perhaps the most significant competing solution for us is a cell culture cassette that is designed to interface with a perfusion bioreactor. This CLINIcell cassette can be used to grow adherent cells (Figure 2). It provides a better solution than those noted previously, but it fails in several ways. First, the rectangular shape with input and output valves on top does not optimize the flow characteristics, resulting in dead space and possibly causing stem cell differentiation in some areas. Second, the plates of this cassette are thin enough that they are permeable to oxygen. This is problematic for iPS cells because they require specific oxygen content in the media. At such a low perfusion rate, the media spends a significant amount of time in the gas permeable CLINIcell cassette; thus, the oxygen content of the media might change significantly by diffusion. Third, the cassette does not provide sufficient area to grow large amounts of cells. Finally, this cassette does not yet incorporate a means to monitor the metabolism of the cells within it. This feature would be necessary if this cassette were to be incorporated into a larger-scale bioreactor cell growth system, most of which have hardware for measuring pH.

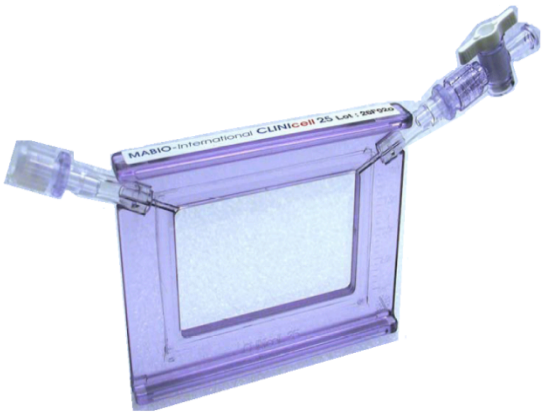

Figure 2: The CLINIcell cassette provides the most competition to our design proposal in that it allows perfusion culture with adherent cells. However, the geometry of the cassette is not optimized for flow distribution, the plates are too thin, allowing oxygen permeability, and the cassette size is too small for our purposes.

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

Ethical Considerations

The knowledge that cells grown in these bioreactor cassettes may be injected into a 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 will pay special attention to the presence of material extractables, or chemicals that could leach out of the materials forming the cassette. We will reduce the conditions under which they are more likely to form and optimize flow dynamics which could 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 will also be important that the bioreactor cassette affords security to the patient 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 built 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 top so that microscopic viewing can be done while maintaining sterility in the cassette. Similarly, valve line connections from the cassette to 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 (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 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 tracks.

A monitoring system, using pH readout, will indicate condition of media efflux which is indicative of the state of the cells. The monitoring system should be capable of providing automated feedback to adjust the flow rate based on the measured condition of the media. The cassette will be designed such that priming and loading of cells can be done in a sterile environment, in a straightforward, user-friendly process, 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. 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.

Project Status

During the previous semester, our work focused mainly on structural design, material selection and flow analysis. 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, with a thin silicone gasket creating a tight seal, to allow a surface on which the iPS cells grow and proliferate. Both the cell plate and top of the frame must be translucent so the researcher can visually monitor the growth of the cells.

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 cell 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. The appropriate shape will be cut out of a Corning 500 cm^2 Cell Culture Dish (Cat. # 431110) to fit into the cassette's frame and then 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 medical fields and many formulas are USP Class VI certified (Columbia Engineered Rubber Inc 2010). The silicone selected has been provided by our client and has previously been used with stem cells successfully.

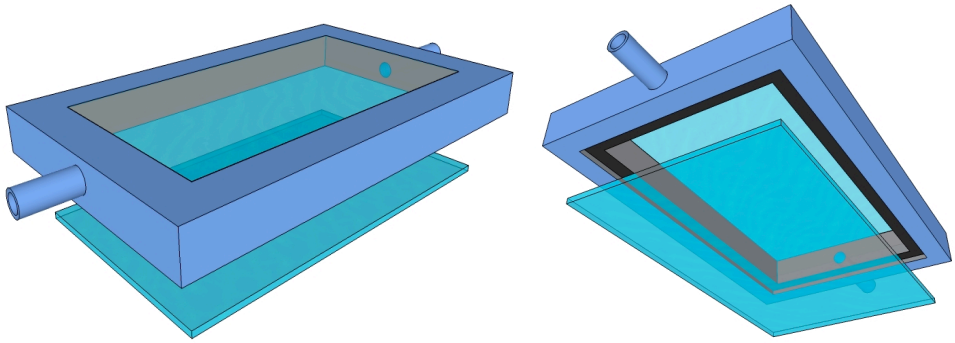

Figure 3 – Top (left) and bottom (right) view of cassette design concept. The cassette consists of a frame and cell growth plate. Both the top of the frame and cell growth plate is translucent.

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

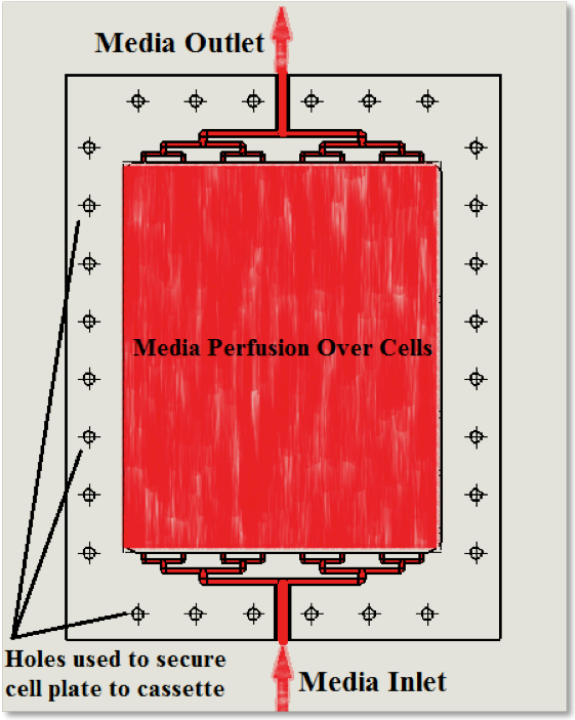

Figure 4 – Current bioreactor cassette design with mirrored balanced runners. See Appendix C for dimensions.

To resolve some of the undesirable flow patterns observed during dye testing, further modifications of the balanced runner design were completed to mirror the input to the output (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 will modify and further test this semester.

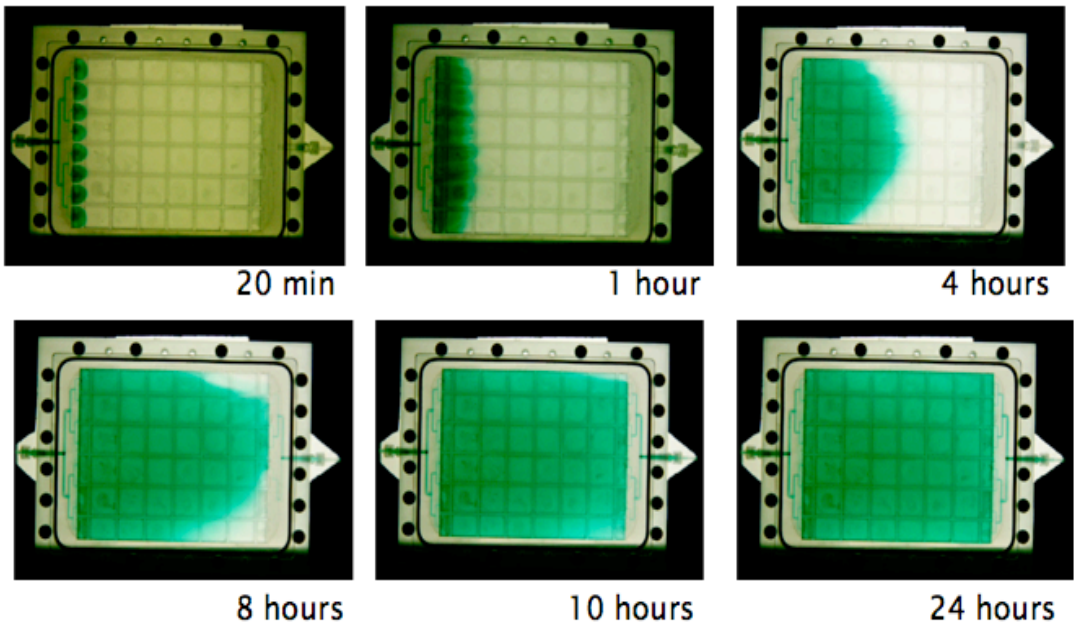

Figure 5 – Flow patterns of mirrored balanced runner cassette. This design avoids backflow and recirculation zones.

Methods: Testing and Analysis Plan

Because cassette construction and desirable flow characteristics have been achieved, at this stage in design, it is appropriate to conduct cell studies to assess our device and confirm that it meets the biological requirements needed for cell culture. This approach will allow us to elucidate and manage any unforeseen problems before final design optimization takes place. Preliminary tests will involve HEK-293 cells to evaluate growth patterns and general cell viability, as compared to a static culture control. HEK-293 cells are human embryonic kidney cells that have been transformed with adenovirus 5 to immortalize them. They are not considered a stem cell because they have been terminally differentiated (Product Description 2010). HEK-293 cells are hardy and can be left in static culture for several days without changing the media. They also grow fast and are less dependent on good cell culture technique. They will be a useful cell line with which we can practice our techniques and trouble-shoot our system before using an iPS cell line. If we do not see viability or cellular growth distribution that is comparable to or better than the static culture flask, we will need to go back to the fluid flow design phase of our project. This might include changing the balanced runner system to one that better manages bubbles or more evenly distributes the flow. We may reevaluate our straw design from last semester, change the outlet design, or change the number of balanced runners where the inlet flow meets the cells.

When cell viability and distribution are satisfactory in the cassette system using HEK-293 cells (if results are superior to or comparable to those observed in the static culture control), we will move onto a more complex cell type, IMR-90, to assess the effects of the cassette on undifferentiated 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 are putting undesired stress on the sensitive iPS cells and thus leading to differentiation.

Cell Studies

A HEK-293 cell bank has been prepared for testing purposes, comprised of 15 vials of 1 x 10^6 cells/vial. We plan to load the cassette with cells 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 1.24dyn/cm^2 (Appendix D); because 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 will generally be used for the bioreactor test, as that is how the device will be used in the future. Using one or two of our banked cell vials, we will grow the cells in static culture for at least one passage so they can recover from the thaw. Then we will seed both our cassette and static culture control T225 cm flask with 2 x 10^4 cells/cm^2. After leaving the cell suspension for at least 30 minutes at 37˚ C for the cells to adhere to the polystyrene, we will turn on the perfusion system which will supply DMEM/Glutamax plus 10% dialyzed FBS media including alamarBlue dye to the cassette. The media will then perfuse at one volume change per day. Efflux samples will be collected every 12 hours and frozen down for future absorbance analysis. We will end the perfusion after four days, as pilot static cultures have indicated that near confluency is achieved in a 225 cm^2 flask after this time period. Absorbance measurements of the efflux samples will provide quantitative measurements of cell proliferation. Upon termination, we will stain

the cells in the cassette and static culture flask with a few milliliters of Trypan blue, a cell viability indicator. After phase microscopy analysis including photos, we will fix the same cells with 10% formalin and stain with 0.05% crystal violet in order to compare the confluency and growth patterns macroscopically. We will use image analysis software such as ImageJ to count the percent live cells in the stained samples. The general testing procedure will follow the flow chart (Figure 6).

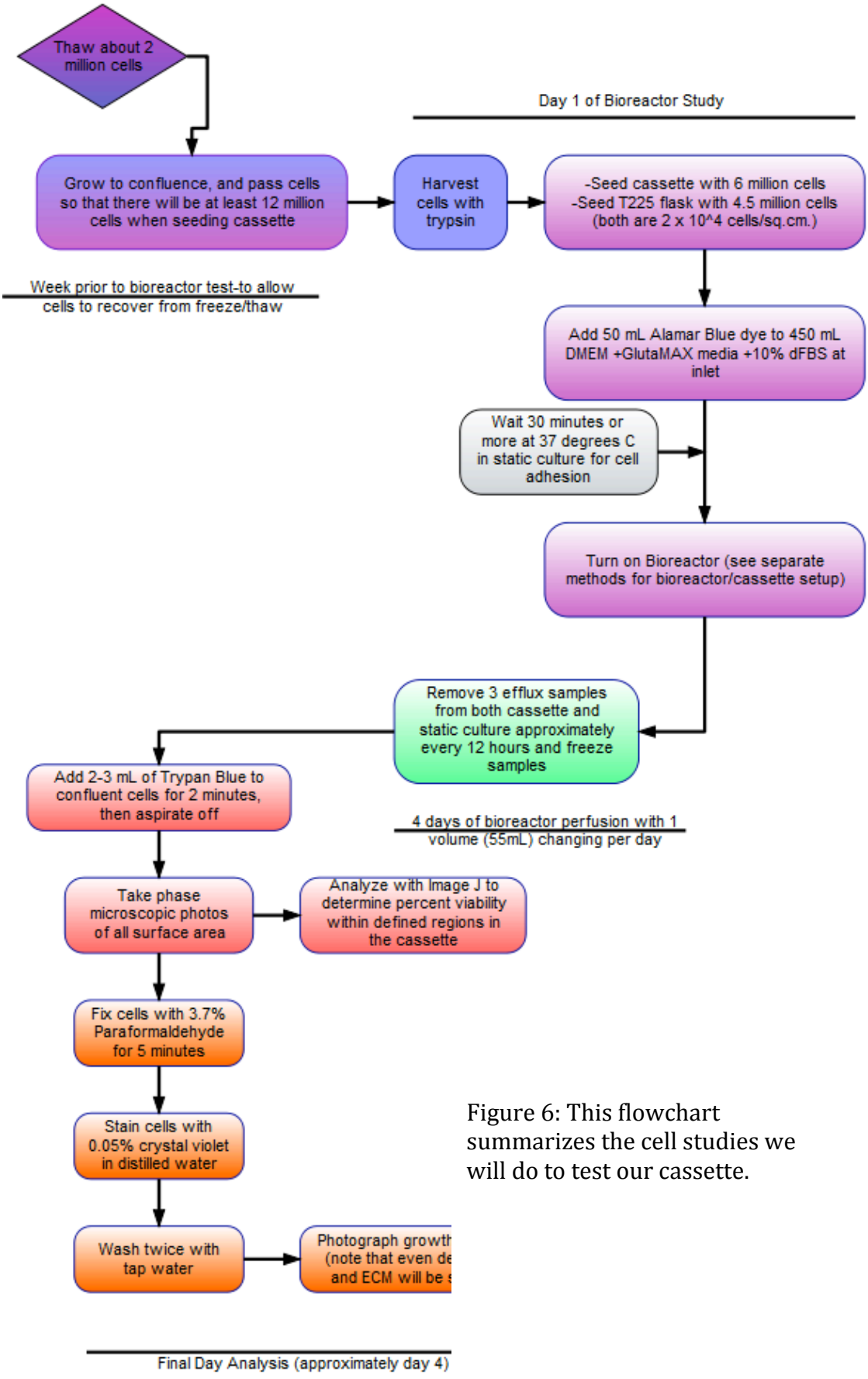

Figure 6: This flowchart summarizes the cell studies we will do to test our cassette.

Proliferation Measurements

AlamarBlue, or an equivalent dye available from our client, will be used to measure cell proliferation and cytotoxicity. AlamarBlue uses the natural reducing power of cells to convert a blue-colored substrate, resazurin, to a red product, resofurin, which is fluorescent. This assay is known to work well with HEK-293, does not have cytotoxic effects, and can be read in either absorbance or fluorescence (alamarBlue, 2010). The sample is also preserved because measurements are taken from the media efflux. We can obtain the relative number of cells proliferating as long as the dye has been incubated for the same amount of time each day prior to taking the sample. The exact number of cells per cassette can also be obtained by calibrating a standard curve. In order to create a standard curve, we will need to set up several separate flasks with a similar surface area at different cell densities. We will add alamarBlue to these samples of known cell number and measure the absorbance. By comparing our experimental values to this standard curve, we would have a quantitative measurement of the number of cells in the cassette or flask at several time points. We plan on taking three samples every 12 hours. These samples can be frozen down until they are ready to be read either by a spectrophotometer or a fluorescent plate reader.

Resulting data will allow us to compare the relative growth curves of the static culture flask to the perfusion cassette. We expect proliferation in the perfusion cassette to slightly exceed that of the static culture flask because slow perfusion of the media over the cell growth area should continually replenish nutrients available to the cells and remove harmful metabolic waste products (Figure 7). We will be confident in the design of our cassette, however, if proliferation rates in it are close to those seen in the static culture flask— since labor involved in operation and maintenance of the cassette system could be significantly reduced compared to the associated static control flask.

If we do not observe these expected results, it is possible that the perfusion rate is removing excreted factors that promote cell growth, bubbles trapped in the cassette are preventing uniform media supply, or cells were poorly distributed during seeding. These potential causes can be further explored in our endpoints analysis tests (See Endpoint Analysis), a solution can be determined based on observed results, and design and

procedure optimization – likely to ease stress on the cells during seeding, or remove bubbles that might be inhibiting media distribution of cell growth - can be performed before testing with IMR-90 cells begins.

This analysis will help justify when to perform endpoint analysis for our growth distribution and viability tests and ensure that we are not measuring the cells when they have undergone growth arrest or cell death.

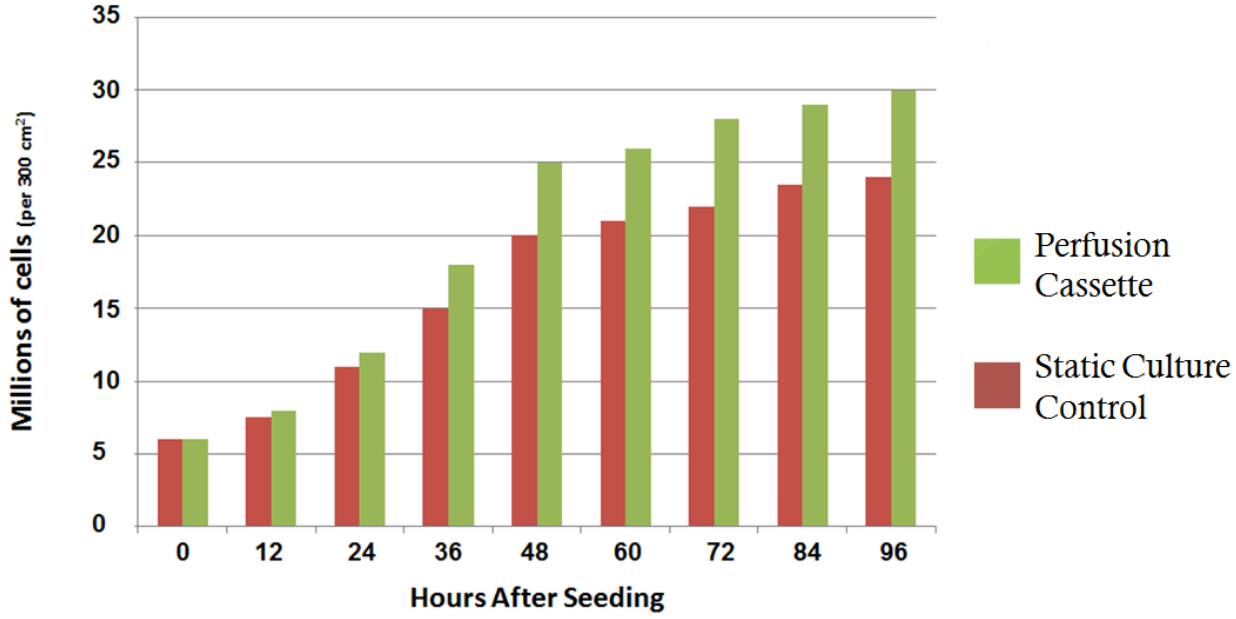

Figure 7 - Anticipated cell number (normalized over the area of the cassette) vs. time after seeding for static culture control and the perfusion cassette. We expect more growth in the perfusion cassette.

Endpoint Analysis

End point analysis of the perfusion cassette will be performed on the fourth day after seeding, when the cell density will be close to 100,000 cells/cm^2. Analysis will include a system assessment and cellular assays. We will first observe and evaluate any flaws in design that have inhibited appropriate function of the cassette when interfaced with the bioreactor system over the 4-day trial, including ergonomic issues, sterility, and cell compatibility with the setup. If viability or distribution assays reveal inadequacies in the cassette system, these flaws will be targeted in the redesign process.

A Trypan blue stain will be executed on the flat culture surface in both the cassette and control growth areas, so that viability can be related to location on the cassette. The cassette and control flasks will be divided into 12 sections and we will be able to determine viability based on spatial location of the cells within the cassette with respect to defining features of the cassette, such as cells near inlet runners or outlet corners. We expect viability to have no dependence on location in the static culture control, but that it may decrease toward the outlet in the bioreactor cassette based on our dye studies indicating that less media will be exchanged in these areas (Figure 8). We anticipate that overall live/dead ratios will be similar between the cassette and control. If viability in certain areas of the cassette is much lower than the static flask, we will need to redesign the inlet and outlet in the future to try to get more uniform growth, as it is not ethically prudent to deliver therapeutic cultures with a significant number of dead cells present. This modification could take several forms, as discussed earlier in the Methods: Testing and Analysis Plan.

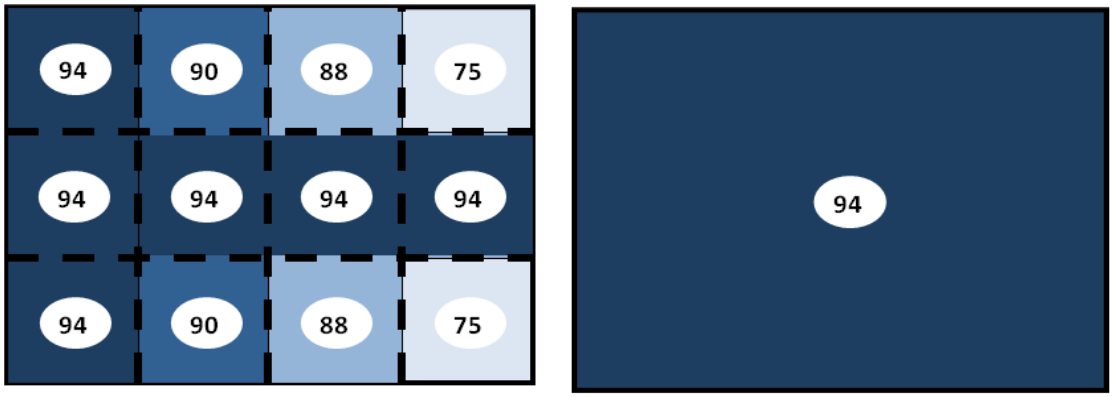

Figure 8- Expected percent of live cells with respect to area for the Trypan blue test in the perfusion cassette (left) and static culture flask (right).

We will also obtain a semi-quantitative assessment of cell growth patterns and viability on the bioreactor cassette growth plate compared to a static control. These cell assays will determine if fluid flow dead spots evident in the dye tests inhibit the growth of cells in those areas. We will use crystal violet to stain and view the cell growth patterns on the plate and in the static culture flask. The crystal violet protocol, taken from the Iruela-Arispe lab, uses 3.7% paraformaldehyde or 10% formalin to fix the cells for 5 minutes. Then 0.05% crystal violet solution in distilled water is incubated with the cells for 30

minutes. After two wash steps with water, the plate should be stained dark enough that we can take macroscopic photos for further analysis of cell distribution and density with respect to location (Figure 9) (Lane 2010). We expect distribution in the static culture control to be similar to that in cassette because the seeding process should distribute cells everywhere which have the opportunity to secrete extracellular matrix proteins. Crystal violet will stain live and dead cells, as well as extracellular matrix proteins. If we do not see even distribution of cells, we will need to design and implement a better way to seed the cells than through the balanced runner system. This may incorporate a luer fitting from the side or corner of the cassette to inject cells with a syringe. If this method were used, we would likely inject the cells in 30 mL of media instead of 56 mL, to allow for a gas/liquid interface for the first 30 minutes while cells attach. This would allow the researcher to tilt the cassette side to side to promote the even seeding of cells, similar to how it is currently done in static culture. Then, after the cells have attached, more media will be added so that all bubbles would be removed, and perfusion can begin.

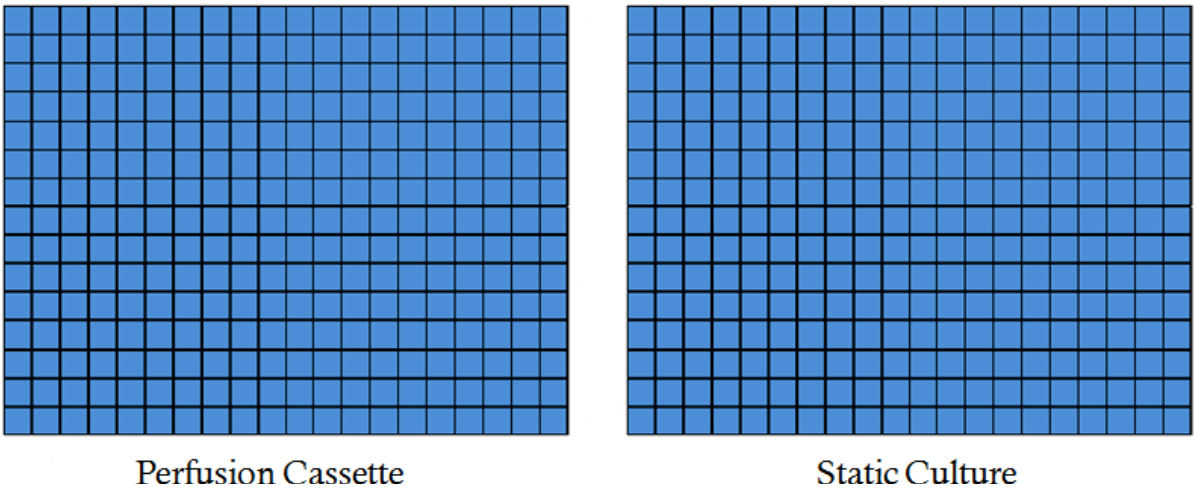

Figure 9: The expected results for the crystal violet staining show that the static culture control is expected to have the same stained quadrants as our perfusion cassette.

Future Work

This semester we plan to test with cells and make modifications to the procedure, analysis, and ultimately the design based on the results of our testing. This will include testing with IMR-90 cells and addressing problems that arise from bubbles or other

sources. We will then create a system for metabolic monitoring of the cassette, move from screws to a clamping mechanism to attach the plate to the frame, and design the system to incorporate multiple cassettes.

IMR-90 Cell Testing

One future direction for this project will test IMR-90 cells, which are a better model of iPS cells than HEK-293 cells. The HEK-293 cell line is resilient and thus a good starting point but has less sensitivity to environmental factors than undifferentiated cells. Thus, 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. Thus, we plan to test our device with IMR-90 cells after achieving good results with the HEK-293 cells. We will also add a pH metabolic monitoring system to our testing setup after we have done viability testing. To do this, we will first adhere a pH dot, which will indicate pH via color change of the dot, at the outlet in order to monitor the efflux pH continuously. This solution will be commercially available, and operate through a chemical that undergoes a reversible, pH-sensitive reaction, allowing continuous monitoring. Results of these tests may suggest using a different flow rate or a flow rate that is a function of cell number over time or a different concentration of dissolved carbon dioxide in order to optimize the conditions based on changes in pH over time.

Bubble Trap

Results of our current testing will largely dictate the next directions of the project. If our testing with HEK-293 cells indicates that bubbles are significantly problematic, forming during perfusion and either blocking the channels of the runners or rupturing in solution, then future work will address this problem. We have already brainstormed a few plausible solutions that could be developed in the future. The basic framework of our bubble trap would consist of a structure such as that in Figure 10, with thin, tall channels that the media would flow through. In this design, the trap is an external attachment in the perfusion system prior to the cassette. It would have an air–liquid interface. If bubbles were present in the media, they are expected to rise to the top and be sequestered in the

bubble trap. Future work on this bubble trap design would include deciding on the dimensions of the channels to most effectively trap bubbles and taking pressure into consideration. If we find that this design has a problem with the buildup of pressure as it traps bubbles, we will incorporate some form of a pressure-release valve or a syringe that will allow the air volume of the trap to increase as air accumulates. Both of these potential solutions will allow us to maintain sterility while effectively removing bubbles.

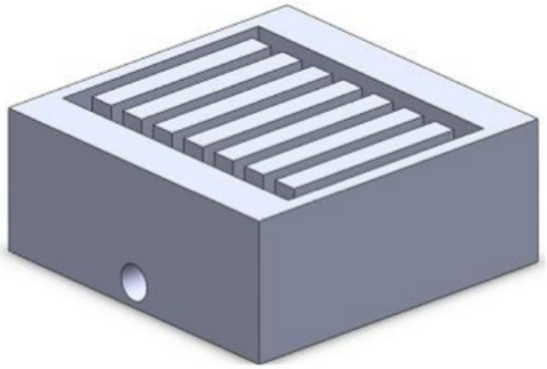

Figure 10- Possible bubble trap design. This design would be an external attachment prior to the cassette, with luer lock connections. Dimensions have not been determined yet, but the final dimensions will likely be proportional to those shown in Appendix E.

Additional Considerations

The frame of the cassette will need to be adjusted to be more ergonomic. Instead of screws, a clamp system will be incorporated, designed with the intent of easing maintenance of sterility during assembly and allowing 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. 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 (which we will likely monitor via pH).

Summary

The perfusion bioreactor cassette has the potential to further the applicability of iPS cell therapy in both academic and clinical settings. Testing with the hardy HEK-293 cell line

will expose significant problems in the operation of the cassette within the bioreactor system, and corresponding re-design, possibly to incorporate an external bubble trap, will allow us to move into testing with the more sensitive and representative IMR-90 cell line. When acceptable growth and differentiation status of these IMR-90 cells is achieved within the cassette, we will focus on simplifying the cassette frame, and developing ergonomic seeding and monitoring systems for the cassette. Because successful completion of the project would significantly reduce time committed to iPS cell culture, it has the potential to significantly increase the feasibility of therapeutic iPS cell therapies for researchers and clinicians alike.

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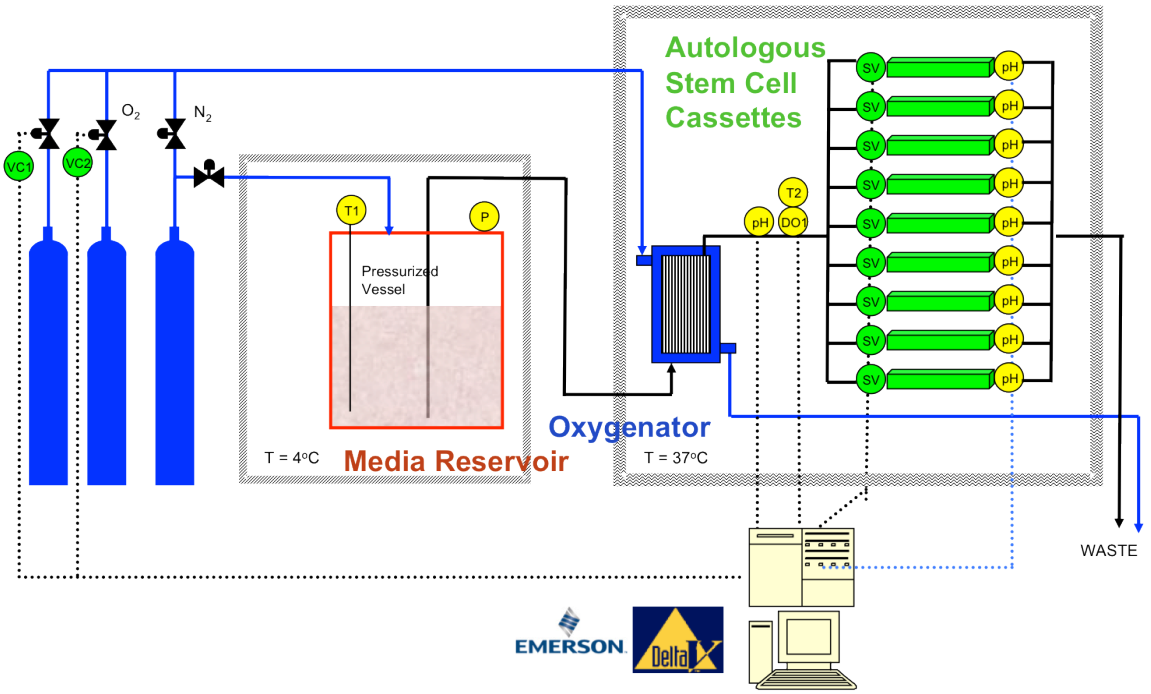
Appendix A: Bioreactor Schematic



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

October 17, 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.

A monitoring system, using pH readout, will indicate condition of media efflux which is indicative of the state of the cells. The monitoring system should be capable of providing automated feedback to adjust the flow rate based on the measured condition of the media. 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
- Monitor pH for cell metabolic readout
- 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

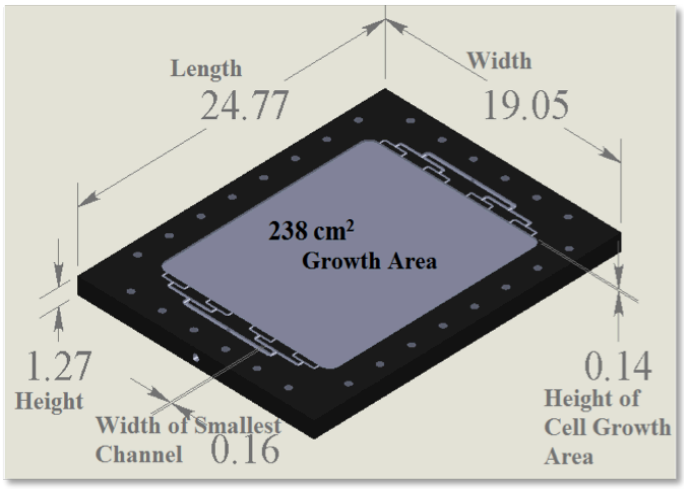
a) *Performance requirements* – Must provide an appropriate cell growth environment with proficient perfusion of media. Cell products must be high viability and comparable or better than static culture.
b) *Safety* – Must not contain any chemicals or substances that will negatively influence the cell, 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.

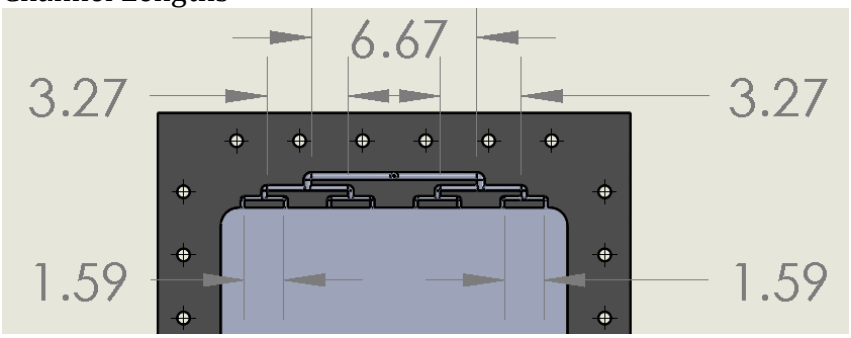
Appendix C: Cassette Dimensions

Note: All dimensions are in cm.

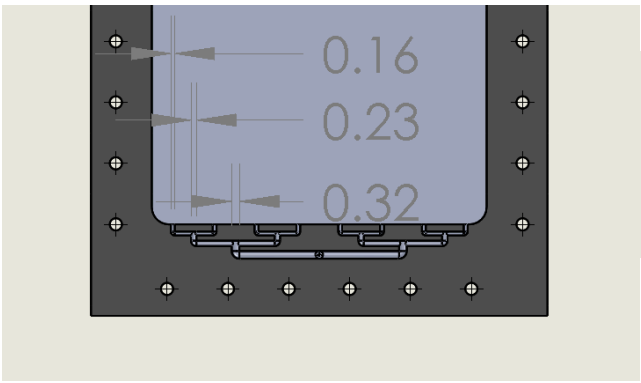
General Dimensions



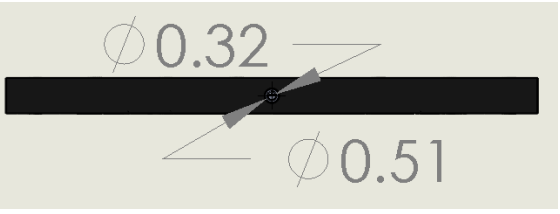
Channel Lengths



Channel Widths



Inlet and Outlet Diameters



Appendix D: 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 E: Bubble Trap Dimensions

Note: All dimensions are in mm.