Bioreactor Cassette for Autologous Induced Pluripotent Stem Cells

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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, it is necessary to have a bioreactor cassette system capable of providing conditions to grow multiple iPS cell samples from individual patients without exchanging media between samples. To address this issue, we have broken the project into three main parts: material selection, flow analysis and monitoring systems. For material selection, polycarbonate and nylon were tested and were both sterilizable and did not harm the stem cells. Several different cassette designs were then created and analyzed with computational fluid dynamics (CFD) to determine the best geometry to achieve consistent flow. After reviewing our CFD results, we determined that our best design for flow involved a fan into a rectangle with guides. Future work includes doing further research in pH monitoring systems, making a finalized material selection, designing a method to vary flow input and physically testing our most promising designs.

Background

iPS Cells

Stem cells, through which any of the body's 200 cell types are 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 (NIH, 2010).

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. Fortunately, induced pluripotent stem (iPS) cells, or adult somatic cells reprogrammed back into a pluripotent state, have been developed. 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, is showing great progress in its potential for use in a clinical setting (NIH, 2010.) Until recently, stem cell growth has 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 are experiencing a need to grow stem cells under individually controlled conditions in specialized culture chambers called bioreactor cassettes.

Bioreactor cassettes are small, specialized culture dishes used to maintain undifferentiated colonies of stem cells. They are usually 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 (Hei, 2010). Later, they can be subjected to specific chemical or mechanical signals that direct their differentiation.

As patient-specific therapy becomes a clinical reality, it will be necessary to have a bioreactor cassette capable of providing conditions to grow 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.

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 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 an undifferentiated form. However, since cells have been used

in the biotechnology industry for a long time now, there are many methods, bioreactors, and cassettes that offer competition.

Currently, the simplest cell culture solution is to grow the cells statically. In static cell culture, the cells adhere to the bottom of a flask and are constantly bathed in culture media. Media replacement is performed in bulk. Static cell culture flasks can be purchased from Corning as shown in Figure 1. While static cell culture is a common way to grow small quantities of cells, especially for research purposes, there are many limitations associated that render it useless for large-scale iPS cell culture. First of all, for the cells to remain undifferentiated, they require constant supply of autocrine factors, which would be removed when the entire media is exchanged(Zandstra and Nagy 2001). In addition, the buildup of waste products leading up to the media exchange could cause differentiation or inefficient self-renewal (Zandstra and Nagy 2001). Finally, to be useful in large-scale therapeutic applications, it would be better to be able to grow the cells with less human intervention.

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

The biotechnology industry has found solutions to the mass production problem associated with static culture. In many mass-production, biotechnological applications for cell culture, the cells are grown 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 adaptation alters the cells in a way that makes them undesirable to be implanted back into the body as the loss of anchorage-dependent growth is associated with malignant cancers. An additional problem associated with this traditional 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-dependence, these harsh conditions are likely to cause differentiation of stem cells and the cells may not be exposed to factors secreted by other iPS cells, also promoting differentiation.

Perhaps the most significant competing solution for us, however, 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 listed previously, but it fails to provide a perfect solution for our problem. First of all, 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. The thin plates of this cassette provide another concern due to their oxygen

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/

permeability: the iPS cells we intend to design our cassette for require low oxygen content in the media and the slow perfusion rate allows oxygen to diffuse into the media if the cassette is permeable. Additionally, the cassette does not provide enough area to grow cells. Finally, if this cassette were to be used in a parallel operation, it would need to have a way to monitor the cell growth and change the perfusion rate of individual cassettes in order to compensate for different growth rates.

Ethical Considerations

The knowledge that cells grown in these bioreactor cassettes may be permanently inserted 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 material selection, process testing, and flow analysis, our team will pay special attention to the presence of material extractables, conditions under which extractables are more likely to form and interact with the cells, and motion dynamics which might encourage prolonged cellular exposure to extractables or other factors that might cause an undesirable outcome should they appear. In this way, we can ensure that cells eventually differentiated and ultimately used for research or clinical therapeutics are of the highest, most natural quality possible, and are therefore as safe as possible for the patient.

Along with safety, it will also be important that the bioreactor cassette affords security to the patient receiving or hospital 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.

This team 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, safe 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, 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. Along those lines, valve connections from the cassette to media input and output lines 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, the pH monitor must be mounted so that problems can be detected visually, and regulation of flow input must be easily accomplished soon after.

Design Criteria and Considerations

Our client would like a cost-effective cassette system that allows several different samples to be connected to one perfusion bioreactor without exchange of media or cross-contamination. The cassette will be transparent and have an area of approximately 350 cm^2. It will facilitate confluent growth and adherence, encourage appropriate fluid flow coverage, and monitor the metabolism of growing iPS cells through pH. The perfusion

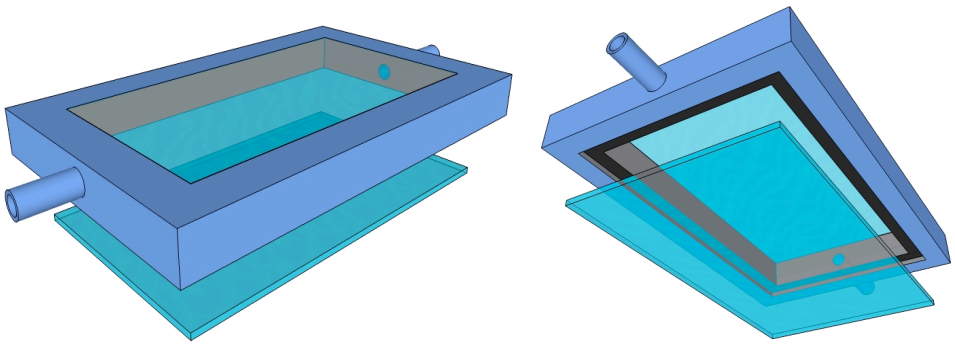

Figure 3 – Top (left) and bottom (right) view of cassette design concept. The cassette consists of a frame and cell growth plate. Both the top of the frame and cell growth plate is transparent.

interface will also allow variable control of flow rate and volume of nutrient media supplied to each cassette, while maintaining physiological conditions within them. Both components will be sterilizable with multiple autoclave cycles, composed of polymers not known to affect stem cell fate, and disposable. The cassette and interface will be designed such that sterility can be maintained if the cassettes need to be removed from the bioreactor for microscopic viewing (See Appendix B). The cassette will consist of two main parts – a frame and cell growth plate (Figure 3). The frame will have luer lock connections for input and output of media and have geometry that creates consistent flow across the entire cassette. The cell growth plate will lock into the bottom of the frame to allow a surface for the iPS cells to grow and proliferate on. Both the cell plate and top of the frame must be transparent so the researcher can visually monitor the growth of the cells.

Materials

There are a few requirements materials must meet in order for them to work for this device. First, there must be no extractables that could cause cell death, differentiation, or any other negative effects. Using USP Class VI certified or Food Grade materials will help avoid this problem. The second material prerequisite is that the cell growth plate and topside of the frame must be transparent. This allows easy visual monitoring of the cells. The final requirement is that the material must be sterilizable – specifically by autoclaving since it is the cheapest and easiest method in most labs. We also will keep in mind that gamma sterilization would be a more ideal method in a mass production situation.

We have decided to use tissue culture polystyrene for the cell growth plate. 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. This material will be ideal since it is USP Class VI certified and already used to grow iPS cells successfully.

Two different materials are being considered for the cassette's frame: Nylon and PC-ISO (polycarbonate). These materials were selected because they are USP Class VI certified (Stratasys Inc, 2009; 3D Systems Inc, 2007) and are compatible with rapid prototyping. Additionally, both materials have been proven to withstand multiple cycles in an autoclave through testing. We have also tested their compatibility with H9 embryonic stem cells: six semi-circular disks of each material were created and placed in a 6-well plate with the cells

for three days. Growth was scored and it was determined that the materials did not negatively impact the cells. A potential problem with both of these materials is transparency – nylon is opaque and PC-ISO is only translucent. Another transparent material, such as glass or Lexan, might be inserted into the center of the top frame, solving this potential setback.

To provide a tight seal between the frame and cell plate, we have decided to use a silicone gasket. 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 because of its previous use with stem cells.

Flow

Flow is a very important aspect of this design – irregular or forceful flow patterns such as eddy currents could cause enough mechanical force on the iPS cells to cause them to differentiate. Additionally, dead spots in the flow would starve and kill cells in those areas. Geometry of the cassette will primarily govern the fluid dynamics within it. The height of the cassette must promote minimal use of media without creating high shear forces on the cells, which could lead to undesired differentiation (Zhang *et al.* 2010). To find the best flow pattern, different geometric designs were evaluated using a computational fluid dynamics (CFD) computer simulation program, CFX through Ansys 14.0.

All of the following designs give a maximum of 23x15x2 mm cellular growth volume. Each input and output valve is designed to taper from a 3 mm diameter luer locking source to a 2 mm diameter circular opening in the cassette. See Appendix C for dimensions of each design.

Simple Rectangle

The simple rectangle design (Figure 4) is the easiest and most obvious solution to the problem. The rectangle is already employed in some static culture systems as it maximizes the

Figure 4: The simple rectangle is easy to prototype and maximizes surface area but does not yield the best fluid flow.

amount of cell growth due to it s larger surface area. For our prototype, the rectangle would make production the easiest as the cutting of the cell plate shape requires skilled use of a hot-wire device. Negatively, more media perfuses through the larger volume of space, so this design likely will not be as efficient at reducing media use. The biggest concern with this design is that there may be dead space in the corners.

Diamond

The diamond design (Figure 5) was conceived in attempt to guide the flow of fluid toward the edges from the input valve to the output valve. Cutting this design from a pre-purchased polystyrene cell plate would likely be as easy as the simple rectangle. The design also has a similar cell growth surface area to the simple rectangle, though slightly smaller.

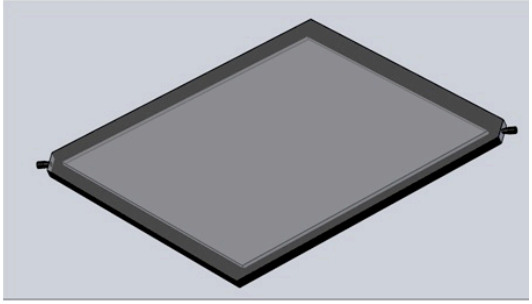

Figure 5: The diamond is also easy to prototype and but also does not yield the best fluid flow.

Ellipse

An elliptical shape (Figure 6) was designed as it was thought to achieve the same function as the diamond without the harsh corners which might collect eddy currents. The gentle slope of the curve might guide the flow to the peripheral frame was well as the interior of the plate. The smoothing of the edges also maximizes the efficiency of media changes per day. Unfortunately, this design would be very hard to cut accurately from pre-purchased polystyrene without the use of a water jet.

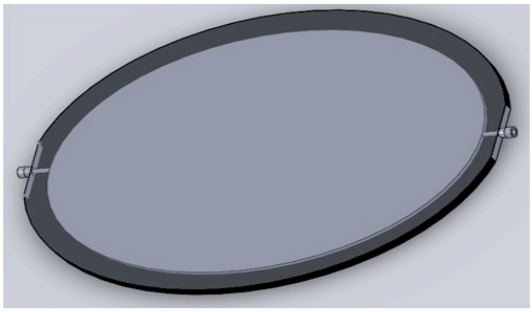

Figure 6: The ellipse geometry shown in 3D (top) and 2d with dimensions (bottom) is difficult to prototype and but may yield good fluid flow while minimizing media waste.

Fan into Rectangle with Flow Straighteners

The fan into rectangle model with flow straighteners (Figure 7) was created from both a combination of the diamond model with current static culture systems. The sloped part of the "fan" guides the flow to the periphery, until it reaches the straighteners, where flow is pointed in the correct direction to perfuse to the outlet. These flow straighteners, however, add considerable difficulty to the production of the prototype as they cannot be attached to the pre-purchased polystyrene cell growth plate, but must be fabricated as a component of the frame itself. Additionally, the surface area in which the cells can grow would be maintained at the expense of a small total cassette size, as it is intended for cell growth to occur in the region following the flow straighteners only.

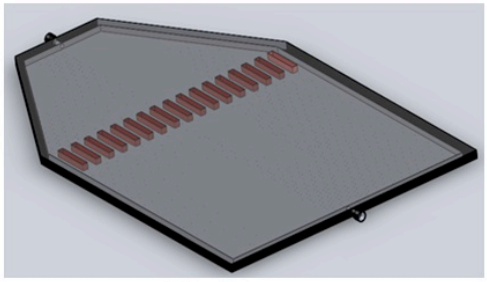

Figure 7: The fan into rectangle with flow straighteners is difficult to prototype and wastes some media

Fan into Rectangle with Guides

The fan into rectangle with guides (Figure 8) directs fluid to the periphery while simultaneously straightening flow. This approach saves space compared to the fan into rectangle with flow straighteners, but is more intricate, and would require a more precise fabrication technique. Accidental occlusion of one lane might be more likely, as a result of the very narrow entry of the fluid from the input valve into the fan guides . Cutting the cell growth plate for our prototyping purposes would remain as easy as the rectangle because cell growth would only occur after the flow guides. The use of media is not as conserved as other designs due to the increased volume of space taken up by the flow guides.

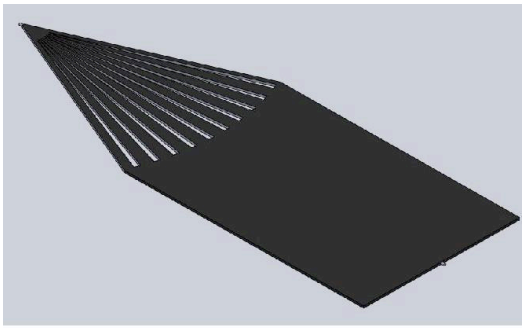

Figure 8: The fan into rectangle with flow straigheners is more difficult to prototype and wastes some media but may yield the best fluid flow.

Computational Fluid Dynamics Analysis

After deciding on designs to analyze, we used computational fluid dynamics with CFX (using Ansys 14.0 software). These calculations are inherently dependent on the boundary conditions: we chose a no-slip wall boundary condition for all surfaces except the inlet and outlet, set the inlet velocity to be the velocity needed to go through 3 volume changes per day (which was less than 1 mm/s for all of the cassette geometries we analyzed), and we set the outlet pressure to be 0 Pa. We used these boundary conditions for both velocity contour and streamline analyses.

The velocity contours can be seen in Appendix D. Overall, we were looking for the velocity to be as consistent as possible over the cell growth area. The rectangle geometry velocity contours reveal many areas of concern, including areas of abrupt velocity change. The diamond geometry seems to fare better than the square, with fairly consistent velocity in the center, with minor problem areas on the edges. Contrary to our *a priori* expectations, the ellipse velocity contour map exposes many areas with variable velocity. Assessing our more complicated geometries—the fan with straighteners and the guided geometry—we find that the velocity contours look much more promising for the guided geometry. It is important here to point out that the cells would only be grown on the cell plate, which would be placed downstream of the straighteners. Therefore, the velocity contour prior to the cell plate is not a variable that we are particularly concerned with optimizing. Overall, the velocity contour analyses pointed to the guided geometry as having the best flow characteristics.

We also used CFD to look at the streamlines through each geometry (Appendix D). We analyzed each cassette using 100 streamlines with the same boundary conditions as previously. Perhaps the most convincing reason that we think that the guided model has the best flow characteristics is because it indeed works as intended, guiding the flow outward. The streamlines show that the flow is being directed outward toward the edges in this model, whereas, in the others, the streamlines remained centered near the middle of the cassettes.

Input Valve

The cassette will provide a stable and secure attachment to the bioreactor via luer locks. Since the rate of autocrine factors and waste products released from the cells changes throughout the proliferation process, the rate of media must be variable. This change will be done either manually with a valve system (limiting the amount of the media going to some cassettes, while maintaining higher perfusion for others) or automated with a computerized system. Additionally, maintaining sterility is a concern for disconnecting and reconnecting the cassette for visual inspection, and this must be addressed in our future work.

Monitoring Systems

In order to know when the cells are ready for use, the design requires a monitoring system. One way this can be done is through pH. As the proliferation processes continue, the output media decreases in pH due to increases in lactic acid production from the cells (Zandstra and Nagy 2001). Therefore, if the pH of the output media can be measured, the state of the cells can be monitored. Fiber Optic pH Sensors such as those found with Polestar (Polestar Technologies, Inc., 2009) and Ocean Optics (Ocean Optics, 2010) could be connected to the media output of the cassette to due such monitoring . This data would then be sent to a computer which would alert the researcher that the cell growth in a certain cassette has reached an optimal level.

Design Evaluation

All of the flow designs were evaluated on a scale of one to ten and weighted on a variety of design criteria (Table 1). The flow analysis was determined to be the most important design characteristic and given the most weight because an irregular flow could cause negative cellular effects such as cell death or differentiation. Feasibility of prototype, maximizing cell production, and efficient media use were the next highest considerations because all of these aspects affect cost-efficiency of the design; we want to be able to maximize cell growth while minimizing price. Mass production was determined to be less

important, as this characteristic does not cause many problems with modern production technology. Based on the results, the fan into rectangle with flow guides was most favorable and therefore is the design we will pursue.

Table 1 - Design matrix that displays our evaluation on a scale of one to ten and weighted on a variety of design criteria for all design concepts.

	Weight					
Fluid Analysis	0.60	4	7	6	8	9.5
Feasibility for prototype	0.15	10	9	4	9	9
Mass-Production	0.05	9.5	9	9	9	9
Maximize Cell Production	0.10	10	9	9	10	10
Efficient Media Use	0.10	8	8	8	7	7
	Score	6.18	7.70	6.35	8.30	9.20

Future Work

After choosing a cassette geometry that looks promising according to our theoretical CFD calculations, the next step is to optimize the details of this design and subsequently fabricate the cassette and test this geometry physically. Our collaborators at MSOE will use a rapid-prototyping printer to make the cassettes from our SolidWorks files. To test flow in the cassettes, we plan to employ dye and salt gradient studies. Dye studies would involve perfusing water through the cassette and adding dye to the water to visualize the flow. If we notice significant dead spots with this test, we will need to make adjustments to the geometry. This test is only qualitative, but it is an easy first test. The salt gradient test, on the other hand, allows us a quantitative assessment of our design. In this test, water is perfused through the cassette as in the dye study, but this time we will increase the concentration of salt in the water in a gradient. By measuring the concentration of salt at the output (through conductivity), we will be able to calculate time constants for the

cassette to show how quickly the entire volume of media is perfused through the cassette. If there are dead spaces, the time constant will be longer. Both of these tests will help us to evaluate and optimize our geometry. The final flow test we will perform is to grow cells in the cassette and make sure the cells can grow uniformly and normally across the plate.

In addition to flow, there are many other aspects of this design project that we intend to pursue in the future. First, we intend to incorporate a pH sensor at the output in order to monitor cell growth and to adjust perfusion rate accordingly. We will also need a method of varying the perfusion rate between individual cassettes. Finally, we will closely evaluate the behavior of cells within our cassette design after interfacing the cassettes with the bioreactor.

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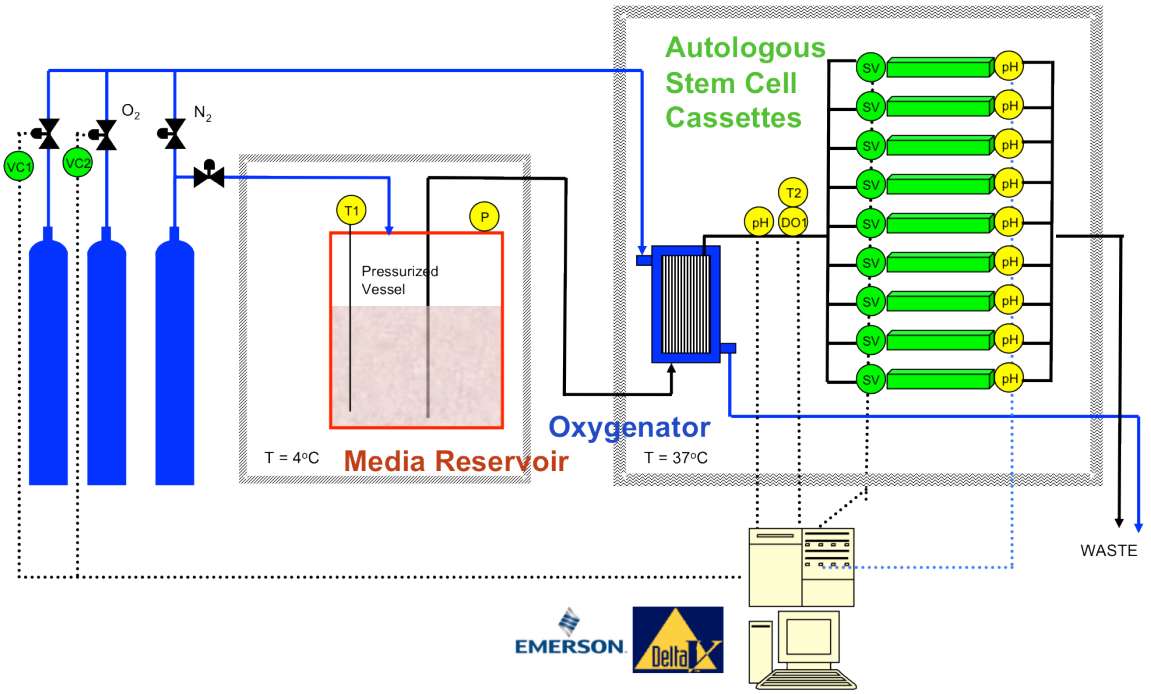
Appendix A: Bioreactor Schematic



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 in media.

Appendix B: Product Design Specifications

Project Design Specifications—Bioreactor Cassette

March 10, 2010

Team: Ali Johnson, Kim Kamer, Elise Larson, Laura Zeitler
Client: Derek Hei, PhD – Technical Director, Waisman Clinical Biomanufacturing Facility
Advisor: Willis Tompkins , 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 transparent and have an area of approximately 500 cm^2. It will facilitate confluent growth and adherence, encourage appropriate fluid flow coverage, and monitor the metabolism of growing iPS cells through pH. The perfusion interface will allow variable control of flow rate and volume of nutrient media supplied to each cassette, while maintaining physiological conditions within them. Both components will be sterilizable with gamma radiation, composed of polymers not known to affect stem cell fate, and disposable. 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 viewing.

Client Requirements:

- Steam and gamma sterilizable
- Connects to bioreactor interface and allows variable media flow
- Gas-impermeable cell growth plates
- Optically transparent
- Monitor pH
- No extractables in contact with media

Design Requirements:

1) Physical and Operational Characteristics
    a) *Performance requirements* – Must provide an appropriate cell growth environment with proficient perfusion of media.
    b) *Safety* – Must not contain any chemicals or substances that will negatively influence the cell, cell growth or initiate differentiation.
    c) *Accuracy and Reliability* – Must provide appropriate culture conditions that do not initiate differentiation
    d) *Life in Service* – One-time use, up to 1 month
    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 users ability to monitor the cells.
    h) *Size* – Cell growth area of 500 cm^2, depth of 5 mm

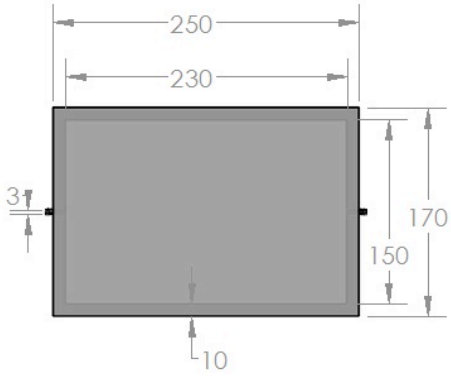
i) *Weight* – Under 1 kg/cassette
j) *Materials* – sterilizable, transparent, allow cell growth, not influence differentiation
k) *Aesthetics* – Transparent cell plate

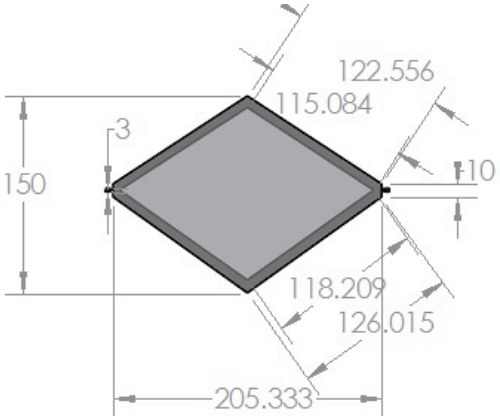
2) Production Characteristics
   a) *Quantity* – One, but should be designed with the intent of mass production in the future.
   b) *Target Product Cost* – $400
3) Miscellaneous
   a) *Standards and Specifications* – Uses USP Class VI Materials, adheres to Good Manufacturing Practice Guidelines and Good Tissue Practices
   b) *Customer* – Medical Research Community
   c) *Patient-related concerns* – Must not negatively influence the cells

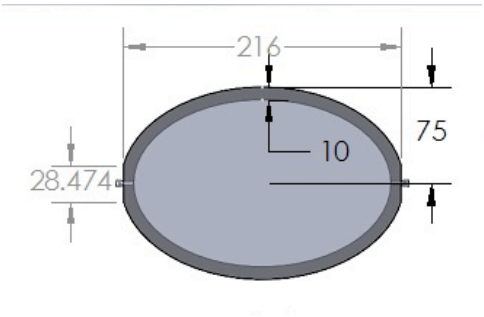
Competition – There are currently different culture systems but none that allow for several different samples with no exchange in media.

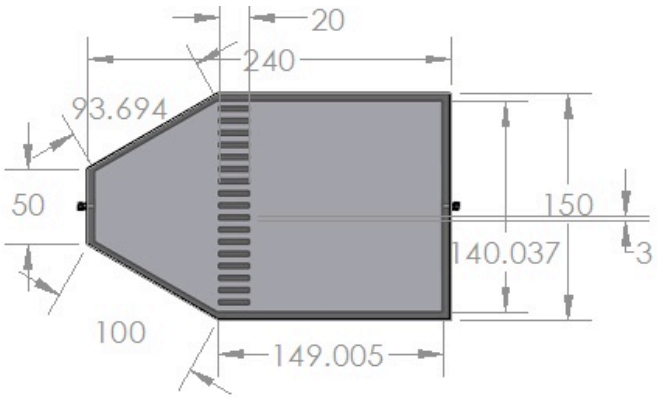
Appendix C: Dimensions for Flow Designs

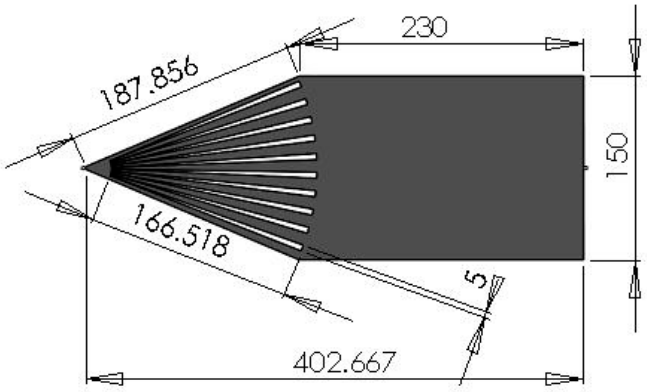
Note: All dimesnsions are in mm



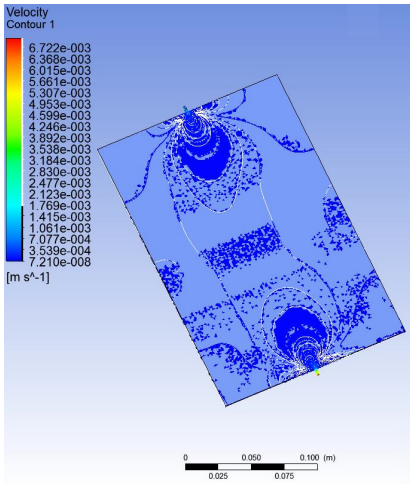


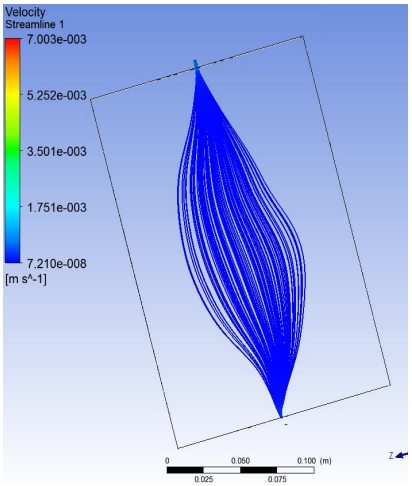




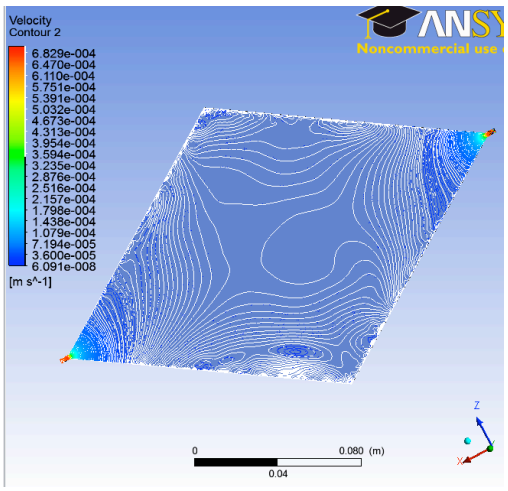


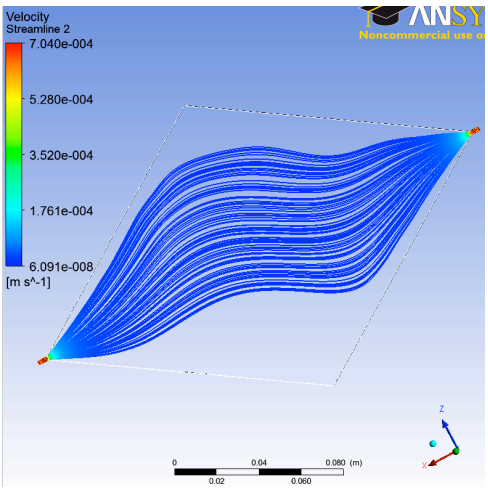
Appendix D: CFX Analysis – Contours and Streamlines



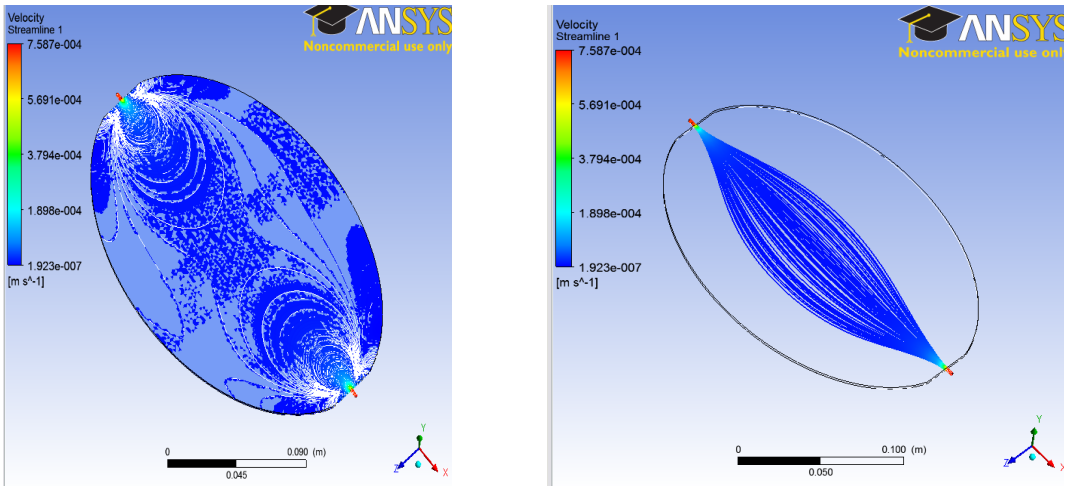


The velocity contours for the rectangle geometry are on the left and 100 streamlines through the cassette are on the right, determined using computational fluid dynamics (with Ansys CFX software). The boundary conditions used were: no-slip walls, inlet velocity of 0.76 mm/s, and outlet pressure of 0 Pa. The dimensions of the figure can be seen in Appendix C.

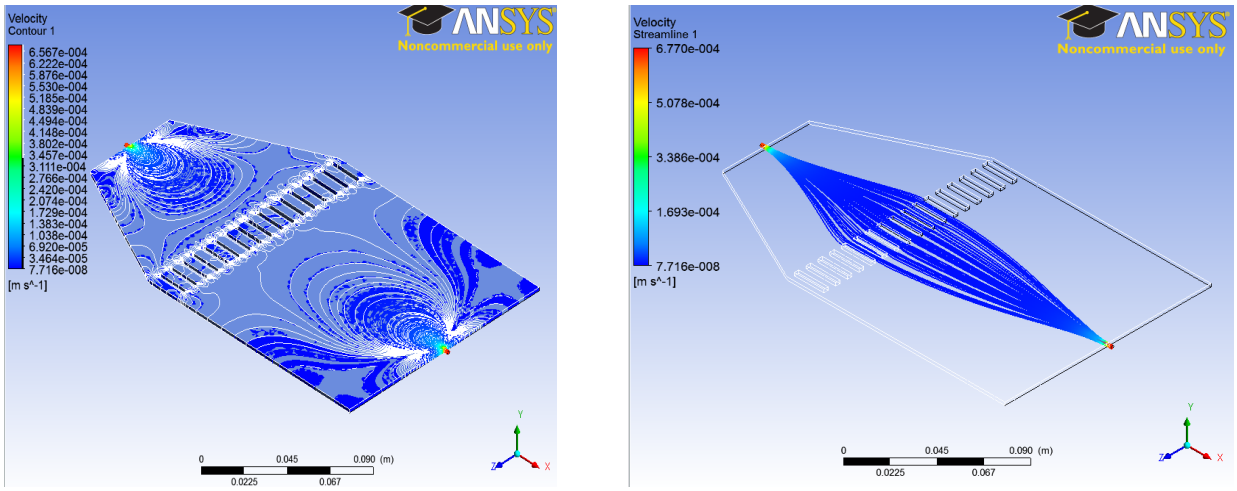




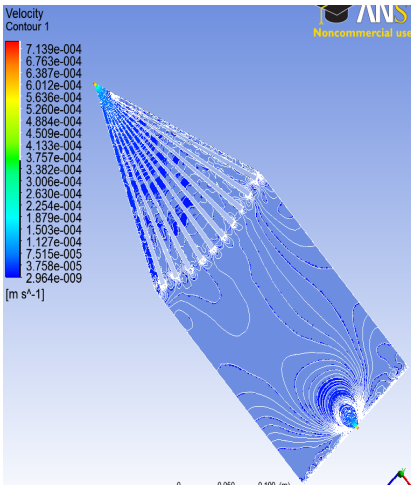
The velocity contours for the diamond geometry are on the left and 100 streamlines through the cassette are on the right, determined using computational fluid dynamics (with Ansys CFX software). The boundary conditions used were: no-slip walls, inlet velocity of 0.68 mm/s, and outlet pressure of 0 Pa. The dimensions of the figure can be seen in Appendix C.

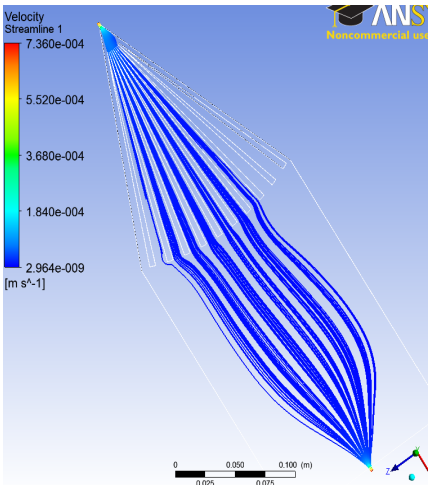


The velocity contours for the ellipse geometry determined using computational fluid dynamics (with Ansys CFX software). The boundary conditions used were: no-slip walls, inlet velocity of 0.44 mm/s, and outlet pressure of 0 Pa. The dimensions of the figure can be seen in Appendix C.



The velocity contours for the fan with straighteners geometry determined using computational fluid dynamics (with Ansys CFX software). The boundary conditions used were: no-slip walls, inlet velocity of 0.67 mm/s, and outlet pressure of 0 Pa. The dimensions of the figure can be seen in Appendix C.





The velocity contours for the guided geometry are on the left and 100 streamlines through the cassette are on the right, determined using computational fluid dynamics (with Ansys CFX software). The boundary conditions used were: no-slip walls, inlet velocity of 0.67 mm/s, and outlet pressure of 0 Pa. The dimensions of the figure can be seen in Appendix C.