University of Wisconsin-Madison

Department of Biomedical Engineering

BME 400 – Design

Neural Bioreactor

Mid-Semester Report

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Abstract

Reprogramming adult cells into induced pluripotent stem cells (iPSCs), as well as their subsequent expansion and differentiation, is normally completed in adherent cell cultures. Recently, it has been proven that iPSCs can be derived and expanded in suspension cultures using a stirred suspension bioreactor. These reactors establish stable cell culture conditions by controlling the temperature as well as the level of nutrients (media), CO2 (pH), O2, and other soluble factors. The suspension components are uniformly distributed within the reactor fluid through various mixing techniques, most commonly an impeller. The process of adult cell reprogramming and iPSC expansion and differentiation can be scaled up and automated using bioreactor stirred suspension cultures.

Dr. Saha has asked our team to design a bioreactor that maximizes the production of neural progenitor cells from mouse embryonic fibroblasts in stirred suspension cultures. The project involves designing culture processes and optimizing culture conditions to reprogram adult cells to induced pluripotent stem cells (iPSCs) and differentiate those iPSCs to neural progenitors.

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Background:

Stem cells are characterized by their ability to remain pluripotent and differentiate into multiple adult cell types. In 1998, Dr. James Thomson was able to isolate cells from the inner cell mass of human embryos and developed the first human embryonic stem (hES) cell lines. These cells hold tremendous promise for advances in biomedical sciences and regenerative medicine; however, this has remained as a very controversial topic ever since the first hESCs were derived. Most recently, induced pluripotent stem cells (iPSCs) were generated by genetically modifying differentiated cells to overexpress four genes that make the adult cells revert to a pluripotent state. iPS cells also have a distinct advantage over ES cells as they exhibit key characteristics of ES cells without the ethical dilemma of embryo destruction.

Stem cell cultures are mostly done in adherent culture conditions. This system involves growing cells as monolayers on different substrates which allows for easy visual inspection under the microscope. However, in adherent culture, cell growth is limited by surface area, and thus, it requires periodic passaging utilizing enzymes to lift the cells off the surface. This system is extensively used for research applications but is not ideal for the large-scale production of therapeutic cells. Approximately 10^9 cells would be required for any relevant clinical application and it would be very expensive and unfeasible to expand this number of cells in a monolayer system [8]. Stirred-suspension bioreactors are an alternative culture system where cell growth is no longer limited by surface area but by the concentration of cells in the media. Stirred-suspension bioreactors have the capacity of producing from 10^6 to 10^7 cells per milliliter, thus, solving the issue of scalability [2]. We would initially like to design and develop a 100ml bioreactor that could potentially yield an estimate of 10^8 cells, which theoretically would be enough for a clinical-scale procedure for a single patient.

The client is interested in developing a bioreactor where adult cells can be reprogrammed to an immature, embryonic state, and then differentiated to neural progenitors. The bioreactor will culture secondary mouse embryonic fibroblasts (MEFs) that have already been encoded with the four transcription factors necessary for reprogramming - Oct 4, Sox 2, c-Myc and Klf4-, necessary for reprogramming. In order to deliver these transcription factors to the cells, the DNA that encodes their production must be introduced into the genome of the adult somatic cell using retroviral vectors such as lentiviruses. These retroviral vectors deliver the transgenes encoded for the four reprogramming factors into the cells, and if all of them are successfully integrated to the adult cell's genome, the cell will start expressing the four transgenes as functional proteins. Addition of Doxycycline to the cell culture medium results in transcriptional activation of each of the transcription factors, thereby inducing the expression of genes that are exclusively expressed in pluripotent stem cells [13]. Figure 1 shows the reprogramming process and how the overexpression of the four transcription factors causes the adult fibroblasts to revert to a pluripotent like state. Our reprogramming experiments will be mostly based on recently published articles that successfully reprogrammed MEFs in stirred suspension bioreactors [2, 11]. The differentiation experiments will be also conducted following protocols that have successfully transformed iPSCs into more specialized cells.

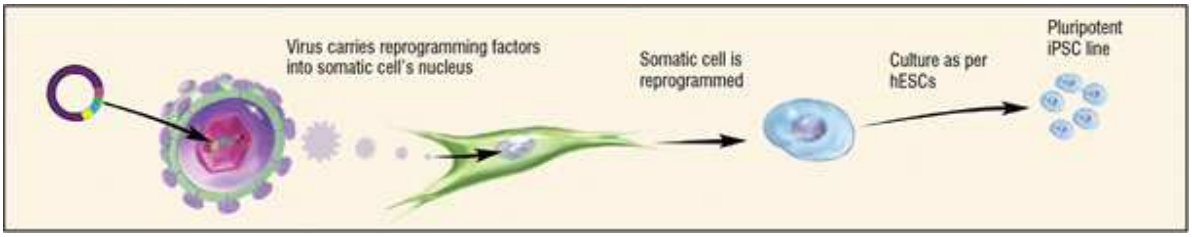

Figure 1: Generation of Induced Pluripotent Stem Cells [3]

Motivation:

Induced pluripotent stem cells have the potential to become a powerful research tool to understand and model diseases. In theory, skin fibroblasts from a patient could be obtained and reprogrammed in order to recapitulate the patient's disease in a research laboratory. These cells could be further differentiated to the cell types affected by the disease and serve as a study model [3]. Induced pluripotent stem cells have many potential clinical applications; however, it is imperative to overcome existing challenges before stem cell technologies can be translated to the clinic. One of the many current challenges is to find cost-effective ways of producing therapeutic cells. Although stirred-suspension bioreactors also present some challenges, it appears to be a suitable system to expand, reprogram, and differentiate large amounts of cells for clinical-grade stem cell therapeutics.

Overall Bioreactor Design:

Our system will consist of multiple components as illustrated in Figure 2. First, the main component of the device will be the vessel. Our vessel will be cylindrical and the volume of liquid will be determined by how many cells need to be produced. Initially, though, our vessel size will be designed to hold 125 mL of liquid including the impeller. Our impeller will be powered by a variable speed DC motor. The device operator will determine the rate at which the impeller will rotate. The function of the impeller is to mix gas, nutrients, and cells evenly throughout the volume, as well as to break up large clumps of cells, or aggregates, that may form.

Additionally, there will be three different

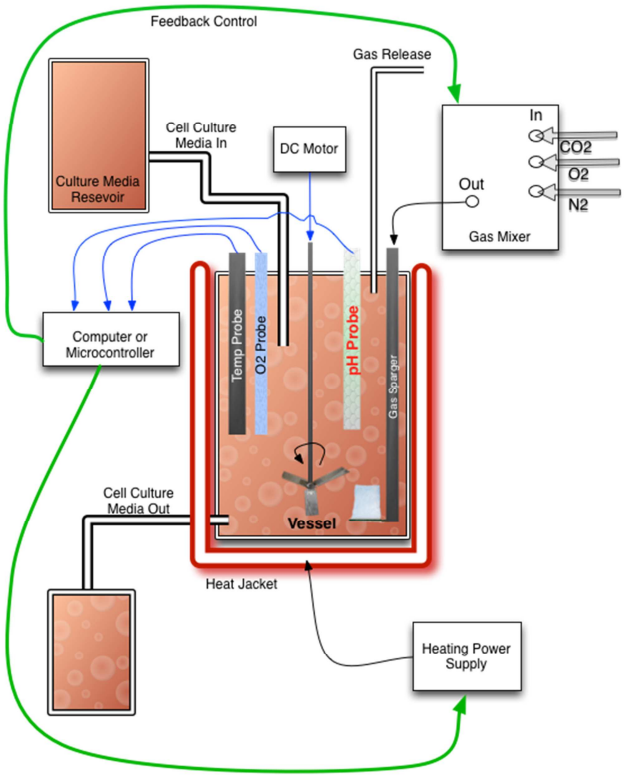

Figure 2: Stirred Suspension Bioreactor Flow Diagram

sensors, pH, dissolved oxygen, and temperature, within the vessel to monitor the conditions that the cells are exposed to. The pH sensor will measure indirectly how much dissolved CO_2 is in the cell culture media. The dissolved oxygen probe will measure how much oxygen is in the system, since the device will be closed to the outside environment. The temperature sensor will detect the temperature of the cell culture media. All three sensors will be connected to a computer or microprocessor, which will record the readings of all three sensors over the course of an experiment. Also, the computer will display the current readings of the sensor at any point in time. Lastly, the temperature sensor will provide a feedback loop to the temperature controller on whether to keep the heater on or off.

Since the device is a closed system, it will not be exposed to atmospheric air. To provide a correct level of gas levels, our system will utilize a gas mixer. This mixer will be connected to carbon dioxide, oxygen, and nitrogen sources. By varying input concentrations of nitrogen and oxygen, the user will set the oxygen concentrations to be either normal atmospheric or hypoxic conditions. The carbon dioxide levels should be maintained at a constant 5% of total gas volume.

Cell culture media will need to be changed over the course of an experiment. For initial testing, culture exchange will be performed manually, but the final product will incorporate a system that does this automatically. This will require two vessels: one that acts as a media reservoir and another for the removal of old media. Whether the system will filter out cells or remove cells within the old media has yet to be determined. If cells would be occluded from old media removal, a filter would be used to prevent cells from escaping.

Relating to whether the cells will be removed with old media is the idea of whether cells will be passaged in batch, or if they will be gradually removed with old media. For batch passage, the cells would be contained to the vessel with old media removed over time and new media replenished. The cells would eventually replicate and expand to fill the volume of the media. Once this occurs the entire contents of the vessel would need to be removed and cells would be separated from the media. The cells would then be seeded to a new vessel at the desired initial concentration and the process of growth would repeat.

Alternately, cells can be removed with the old media and separated from the removed fluid. This would require the correct rate of media removal and replenishment so the expansion rate of the cells would be the same or lower than the rate of cell removal. It is unclear how to efficiently separate cells form the removed media; therefore, additional research is required before we can decide if a batch or continuous reaction is optimal for this bioreactor design.

Existing Devices:

Today there are hundreds of bioreactors on the market. These bioreactors are used for many different applications including the growth of cells or tissues in a suspension culture. Many companies offer complex bioreactors systems such as the ones offered by Eppendorf; however, these bioreactors are not applicable to our client for two main reasons. The first reason is that these bioreactors are designed for generalized or specific cell and tissue growth; unfortunately, there are no bioreactors specific to stem cells. Therefore, if our client were to purchase a bioreactor, this bioreactor would then need to be specialized for the process of reprogramming adult cells to iPSCs and then differentiating them into neural progenitors.

Figure 3: Eppendorf CelliGen BLU single-use bioreactor [9].

The second reason is that these bioreactors are simply out of our client's budget. For instance, the CelliGen BLU single-use bioreactor, Figure 3, offered by Eppendorf costs $32,000 with an additional cost of $700 per reaction vessel [9]. This is especially expensive when these bioreactors will need further customization, leading to an additional cost, for them to be useful for our client. At this point, it would be more cost effective and efficient for a bioreactor to be designed and developed for this specific process.

Client Requirements:

Our client has requested a few parameters be met, but ultimately has decided to allow us to make most of the design decisions. The biggest design consideration that must be accomplished is to produce the required amount of cells. For tissue engineering applications, the amount of cells required would be on the order of magnitude of 10^9 differentiated cells. To expand the cells seeded within the reactor, basic cell culture conditions must be met, such as oxygen and carbon dioxide concentrations, temperature as well as relative humidity.

Additionally, our client would like the bioreactor system to be an automated closed system. For the final design, you would be able to take a skin biopsy and inoculate it to the reactor to reprogram those cells into induced pluripotent stem cells. Then those reprogrammed cells would need to be expanded to a larger cell population and then reprogrammed into the desired cell type. There should be minimal interaction and maintenance needed on the bioreactor between steps to simplify the production of the desired type of cells.

Finally, the cell culture media should be serum free. This is because using serum obtained from animal sources introduces unknown factors that can affect the cells produced. Growing human cells in fetal bovine serum may contaminate the cells with infection of bovine disease or cause an immune

reaction to foreign proteins. If the cells are grown in defined conditions, these possible contaminations can be avoided.

Impeller Design Overview:

All stirred suspension bioreactors require an agitation system to control fluid flow, shear stresses, mass and heat transportation. For this bioreactor design, the agitation system will consist of a motor that will rotate a shaft that an impeller is attached to. The motor will rotate the impeller at a rate of 75 revolutions per minute, or RPM, as this was found to produce the best cellular growth [5]. This type of turbulence will cause the bioreactor's media to observe different fluid dynamics depending on the impeller design. The impeller design could cause axial or radial flow or a combination of both to occur within the bioreactor. Axial flow is the movement of the fluid along the axis of the impeller while radial flow pushes the fluid away from the impeller axis towards the vessels wall. Each flow type can be seen in Figure 4.

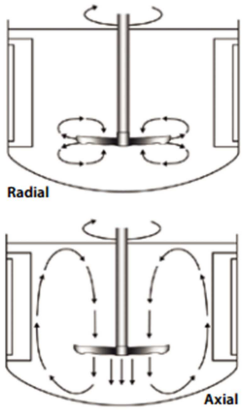

Figure 4: Graphical representations of axial and radial flow [7]

The type of flow the impeller design creates will affect the other parameters that the agitation system controls. The impeller design must be able to efficiently mix the media within the bioreactor. The mixing affects both the mass and heat transportation. Having efficient mixing will allow the heat to disperse uniformly throughout the media, causing the bioreactor to remain at a relatively constant temperature of 37° C. Also, the mixing will allow for carbon dioxide, oxygen and nutrient mass transportation to occur. This is important to ensuring that the bioreactor is at the correct conditions to allow for the cells to proliferate, reprogram and differentiate.

The impeller design must be able to produce shear stresses on the cells within an optimal range. This optimal shear stress range is specific to the cell line being used in the bioreactor, initially in this case mouse 3T3 cells. The optimal range means that the shear stress is low enough to avoid causing cell damage but high enough that the stress is able to break apart cell aggregates. A favorable shear stress value for mouse embryonic stem cells was 0.61 Pascal which gave an approximate goal for the impeller designs' shear stress [10]. To determine the optimal shear stress range and which impeller will achieve this value, testing with 3T3 cells and the impeller designs is required.

Using this information about impellers, three impeller designs were developed to meet the above parameters. Unfortunately, an impeller cannot optimize mixing and have low shear stress at the same time. To keep shear stress low, there is a loss in the mixing efficiency. Therefore, the best impeller design will create low shear stress while still keeping an appropriate mixing efficiency for mass and heat transportation.

Pitched Blade Impeller:

The first design is referred to as the Pitched Blade Impeller. This design is a common impeller used for many applications including bioreactors. The impeller consists of 3 flat blades, equally spaced apart, and attached to a shaft at a 45° angle. Both axial and radial flows are supposed to be produced by this type of impeller. Because it produces both types of flow, this impeller type will have better overall mixing for mass and heat transportation than just axial or radial flow type impellers [6]. Also, the flow patterns of this impeller cause gentle mixing of the media. This means that the shear stress acting on the cells will be low and should not cause cell damage. Pitched blade impellers have been used on many mammalian cell lines and specifically should be compatible with the mouse 3T3 cell line.

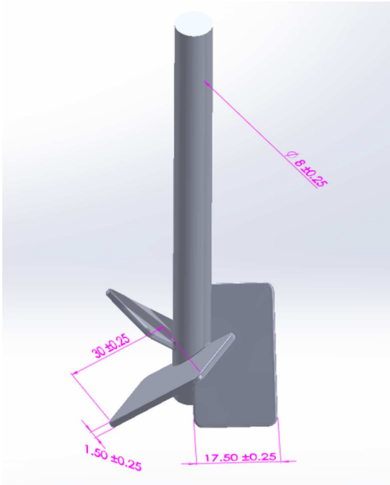

Figure 5: Solidworks model of pitched blade impeller. Dimensions in mm.

Our pitched blade impeller design follows the general form of having 3 blades attached to the shaft at a 45° angle as Figure 5 shows. The impeller blade dimensions are 17.5 mm by 30 mm by 1.5 mm. Fluid modeling was performed for the pitch blade impeller. The overall impeller geometry was rotated at 75 rpm (7.85 rad/s) within a vessel 80 mm tall and 50 mm in diameter. The vessel was filled completely with water. The fluid model was under the influence of gravity and was analyzed over a period of time. Through the flow analysis in SolidWorks, Figure 6, it was determined that this impeller design does produce both axial and radial flow simultaneously. The fluid velocities of the flow analysis are mostly within the range of 0.060 - 0.099 m/s throughout the entire vessel with some peaking around 0.119 m/s near the blades. This shows that the entire vessel is undergoing moderate mixing while keeping the shear stresses inside the vessel low.

This means that the shear stresses created by the impeller should not cause cell damage and should be able to break up the cell aggregates. Also, this impeller design should be able to spread the heat throughout the entire vessel leading to approximately a uniform temperature in the bioreactor. The mixing created by the pitched blade impeller should have the capability for carbon dioxide, oxygen and nutrient mass transportation to occur. The pitched blade impeller design meets the necessary requirements of a bioreactor to help proliferate, reprogram and differentiate cells.

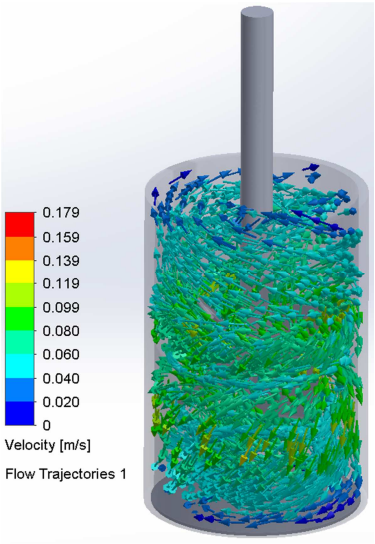

Figure 6: Solidworks flow analysis model of pitched blade impeller

Whisk Impeller:

The second impeller configuration considered for the bioreactor is the whisk impeller (Figure 7), shaped much like its kitchen namesake. This impeller features 4 flat blades evenly spaced about the central rod. Each blade has a removed interior region that extends from the central rod towards the periphery of the blade. The whisk impeller blades extend the height of the bioreactor for fluid flow generation throughout the entire vessel space. The dimensions of this design are as follows: the overall diameter is 30 mm, the blade height is 70 mm, the blade width is 1.5 mm, the removed region of each blade extends from the central rod to 5 mm from the blade perimeter in each direction, and the central rod has a diameter of 8 mm; these dimensions are shown in Figure 7.

Fluid Modeling was performed for this design to determine its efficacy for the parameters discussed in the Impeller Design Overview section. The modeling was performed under the same conditions stated for the pitch blade impeller. A snapshot of the resulting velocity profile can be seen in Figure 8. From the resulting velocity profile, it can be seen that this design creates mainly radial flow, with very little axial flow. Towards the perimeter of the vessel there are regions of static fluid, indicated by dark blue velocity vectors, which is a product of poor mixing. Additionally, there are large velocity gradients, designated by a drastic change in velocity vector color within the gaps of the blades. Large velocity gradients indicate high shear stresses within that region. This is alarming since the cells may undergo damage or death under the influence of high shear stresses.

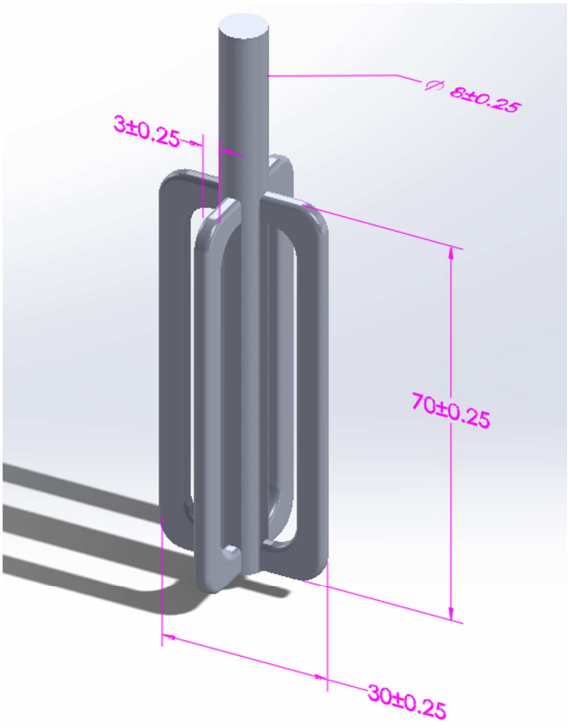

Figure 7: Whisk Impeller. The second impeller design was modeled in SolidWorks, a CAD program, and the dimensions are displayed in millimeters.

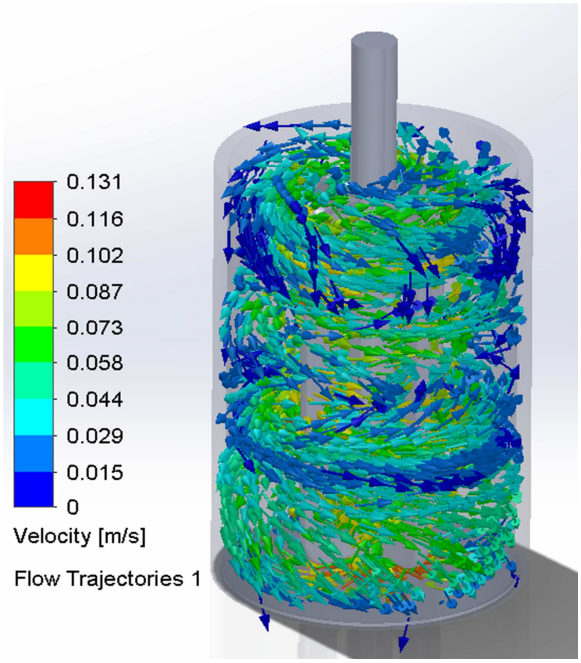

Figure 8: Whisk Impeller Fluid Model. This is a snapshot of the resulting velocity profile for the fluid simulation of the whisk impeller. The legend on the left shows the magnitudes of the relative velocities

Flat Blade:

The third design considered for the bioreactor is the flat blade impeller (Figure 9). This design was modeled after the impeller used for spinner flasks marketed by various companies. Spinner flasks have been proven as viable small-scale bioreactors for the production mouse induced pluripotent stem cells [2] [11]. This impeller has two triangular blades protruding from the central rod. These blades have a slight inward taper as they extend the entire height of the bioreactor vessel. The large base diameter of this design is intended to create uniform flow throughout the entire fluid region. The dimensions of this impeller are as follows: the overall diameter is 43 mm, the blade width is 1.5 mm, the blade height is 75 mm, and the central rod is 3 mm in diameter; these dimensions are shown in in Figure 9.

Fluid modeling was performed for the flat blade impeller under the same conditions stated for the pitch blade impeller. However, the rotating geometry was changed to match the geometry of the flat blade impeller. A snapshot of the resulting velocity vector field is shown in Figure 10. From the fluid model, it can be seen that the flat blade impeller creates mean radial flow, indicating low shear stress, except for near the base of the impeller. However, this design fails to generate axial flow or substantial mixing; this was concluded from the uniform flow in the radial direction and overall lack of turbulence.

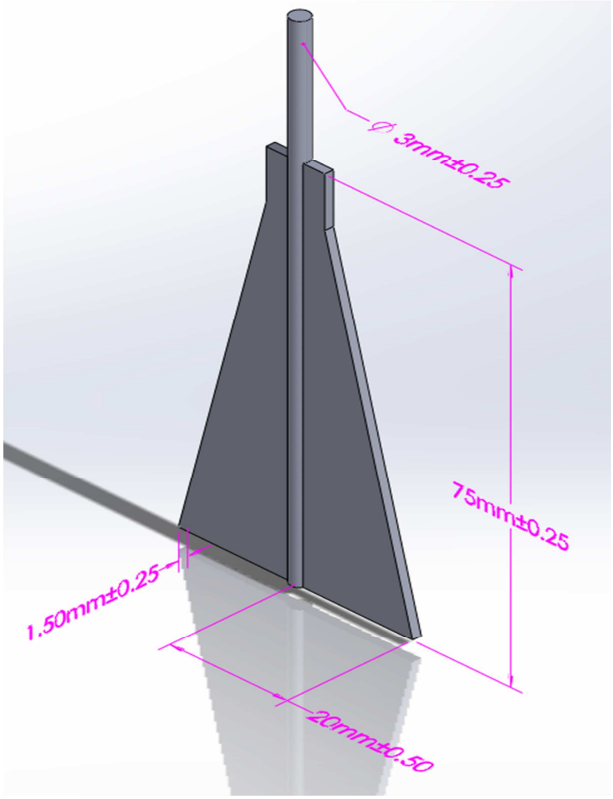

Figure 9: The third impeller design was modeled in SolidWorks with the dimensions show above in millimeters.

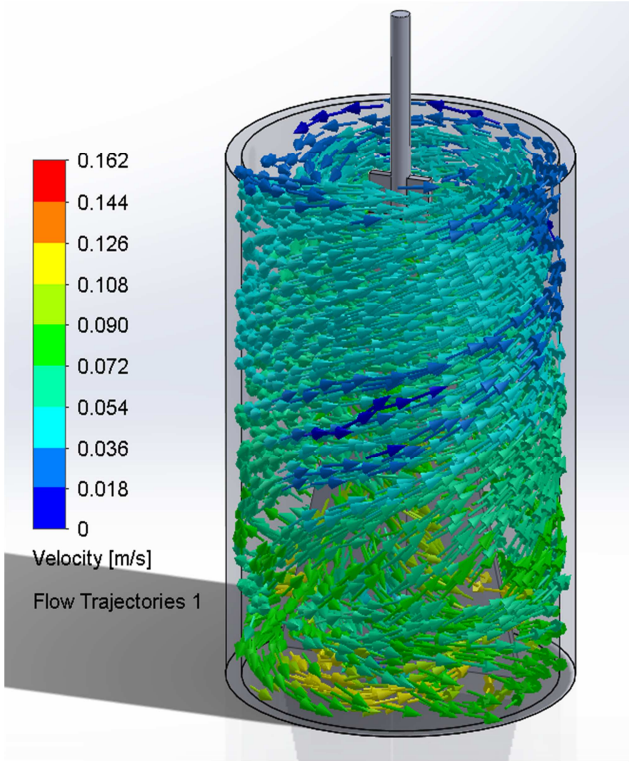

Figure 10: Flat Blade Fluid Model. This is a snapshot of the resulting velocity profile for the fluid simulation of the flat blade impeller. The legend on the left shows the magnitudes of the relative velocities (meters/second) within the vessel.

Design Matrix:

The design matrix (Table 1) used to evaluate the three impeller designs was based upon five categories: optimal mean shear stress, radial and axial flow, mixing, power consumption and cost. The three impeller designs were rated from 1-10 for each category. The individual category scores were multiplied by the corresponding weight percentage, accumulating to a maximum total score of 10 for a single design.

Table 1: Design Matrix. This matrix contains the five categories that each impeller design was rated against. The pitch blade impeller (highlighted in yellow) scored the highest and was chosen for the final design.

Designs	Optimal Mean Shear Stress	Radial & Axial Flow	Mixing (Mass & Temp)	Power Consumption	Cost	Total
Weighting	30%	25%	30%	5%	10%	10
Pitch Blade	7	8	8	8	8	7.7
Whisk Impeller	5	4	4	7	6	4.65
Flat Blade	8	6	7	5	4	6.65

Optimal mean shear stress and mixing were the two categories that were weighted the highest at 30% each. These two design parameters are most important for creating viable cell culture conditions within the bioreactor; the rationale for their importance is outlined in the Impeller Design Overview section. A single design cannot simultaneously optimize both of these features since there is a trade-off from one another. This means that improving one will adversely affect the other and vice versa. Therefore, the ideal design will achieve mean shear stress within an acceptable range, while creating enough mixing to provide nutrients and diffused gas to the cells within the uniformly heated media.

Mean shear stress is a function of the velocity gradients within the flowing fluid. The change in velocity over distance was analyzed for each design based off of the fluid modeling. The least amount of velocity variation, indicated by little color change between the relative velocity vectors, was desired for this category. The design with the optimal mean shear stress was flat blade impeller due to its uniform flow in the radial direction. The pitch blade impeller was a close second in this category.

The degree of mixing generated by each impeller was also determined through the fluid modeling. For this category the fluid velocity animation videos were analyzed. The level of mixing was

based upon the change in both the magnitude and direction of velocity throughout the entire fluid space. The flat blade impeller created flow throughout the entire fluid volume, but failed to create changes in the magnitude or direction of the flow velocity. The whisk blade impeller generated a high level of mixing near the center of the vessel; however, the presence of dead space severely hurt its evaluation for this category. The pitch blade impeller provided the highest level of mixing due to the constantly changing velocity directions within the fluid.

The generation of radial and axial flow was the category that was weighted the next highest at 25%. Fluid modeling was again used to evaluate this category. The generation of flow in the radial and axial directions in the vessel is more self-explanatory from the velocity profiles. It can be seen that the pitch blade impeller best generated flow in both directions. This flow generation is a product of the 45^{o} angled impeller blades that equally create flow along the two axes.

The final impeller design will be 3D printed using the Viper si2 SLA printer which uses an epoxy material called Accura 60. Thus, the cost of each design was estimated based on the volume of Accura 60 required to print each design. Since each impeller is fairly small and relatively the same volume, cost was only weighted 10% in the design matrix. It was estimated that the flat blade would cost the most, followed by the whisk impeller, and lastly the pitch blade impeller. Since the pitch blade was the cheapest, it scored the highest in the design matrix for this category.

Classical impeller design is based around the amount of power necessary to generate flow within a reaction vessel [4]. Although the impeller designs presented for the bioreactor are small enough where power consumption should not be an issue, it was deemed necessary to include it in the evaluation of each design. Since the bioreactor is being designed with the intention to be scaled up in size in the future, power use would become a more important factor and thus should be given at least some consideration. For this reason power consumption was allotted a 5% weight within the design matrix. The level of consumed power for each design was estimated based upon the surface area of the blades normal to the rotational direction of the impeller. This is the effective area that generates the fluid flow. Generally, increasing this surface area will increase the amount of power consumed by the impeller [4].]. The pitch blade impeller scored the highest due to its small three blades, which consumed the least amount of power. The whisk impeller was a close second for this category.

Based upon the aforementioned design considerations, the overall scores were summed for each design and they are as follows: the pitch blade impeller scored a 7.7/10, the whisk impeller scored a 4.65/10, and the flat blade impeller scored a 6.65/10. As the highest scoring design, the pitch blade impeller was chosen as the final design. The flat blade impeller was also a notable design; it will be used as a control against our final design in future experiments since it has been proven effective through spinner flask experiments [2] [11].

Final Design:

Based on the scoring of the design matrix of the potential impeller designs, the pitch blade impeller design was chosen for this project. The pitch blade was chosen for its predicted success of mixing, both radially and axially. Good mixing of vessel contents should maximize the efficacy of the heating and media exchange systems by ensuring the most uniform culture possible. As will be explained in the future work, the ultimate impeller final design will either be confirmed through testing or will be modified accordingly. The impeller will be constructed from 3D printed Accura 60 plastic that has been coated with polystyrene to avoid cell contact with any cytotoxic materials. During testing, the impellers will be powered by magnetic spinners, but the final bioreactor's impellers will be motor-powered.

The vessel of the bioreactor will be a 125 mL glass beaker. The relative abundance of cylindrical beakers provides a good starting point as the price is favorable and they are readily available. Also, the cylinder is a shape that is convenient for axial mixing without any corners or extreme angles to complicate the circulation of the cells and media in the vessel. As with most beakers, the opening is on the top circular face of the cylinder, from where the impellers and probes can enter the bioreactor and form a top lid to create a closed system.

To heat the bioreactor, a heating blanket was chosen as the best method in the case of this small scale bioreactor. The heating blanket will be wrapped around the exterior of the vessel as shown in Figure 11. Other options considered included an external heating cartridge, an internal heating cartridge, infrared radiation heating, or heat baths. However, those options were deemed respectively to be inefficient, a potential hindrance to the intended fluid dynamic of the vessel contents, unnecessarily complicated, or not ideal for an extended period of time due to fouling [1]. To manage heating, a temperature probe will detect the temperature and send its measurements to a microcontroller, which would then turn on or off a solid state relay connected to the heating blanket. The microcontroller will dictate whether the heating blanket is actively heating depending on whether the feedback data indicates if more heating is necessary to maintain the specified goal temperature of 37°C.

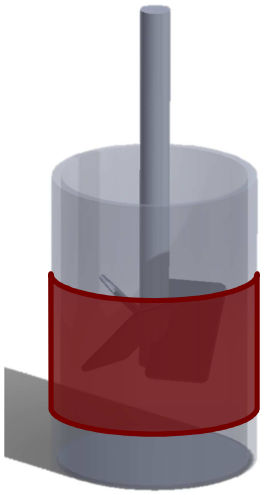

Figure 11: Bioreactor with Pitch Blade Impeller and Heating Blanket

The bioreactor will require a gas supply system to maintain proper levels of about 5% CO_2 to control pH and the assigned hypoxic O_2 concentration. The bioreactor will also require a media exchange system to refresh the cell media as necessary.

Ethical Considerations:

There is a great deal of ethical controversy that surrounds stem cell research. The majority of these ethical concerns arise from the derivation of human embryonic stem cells. Specifically, embryonic stem cells are extracted from the inner cell mass of a blastocyst – a 3 to 5 day old embryo - usually created through in-vitro fertilization. Many opponents of human embryonic stem cell research disapprove of the destruction of the embryo for the derivation of human stem cells, since they consider life to begin at the moment the zygote forms at conception and thus destruction of said embryo would be considered murder. Informed consent is received from the donors in all cases of stem cell derivation from in-vitro fertilized embryos; otherwise, the embryo itself would be discarded as it no longer serves any purpose for the donors [12].

However, the bioreactor design described above is intended to culture induced pluripotent stem cells, which are derived in a vastly different manner then embryonic stem cells. Instead of extraction from an embryo, induced pluripotent stem cells are derived from adult cells. After obtaining adult cells form a human donor, reprogramming factors are introduced to the cells, which reverts them to their pluripotent state. Since no embryos are destroyed in this process, there should be little to no ethical concerns about the bioreactor design or its purpose.

Future Work:

Several components will be added to the bioreactor that each introduce several new variables to consider and will require modifications to the existing design. Therefore, it was deemed appropriate that the system be designed component by component to avoid introducing several new variables to test simultaneously. Introducing each component individually would enhance the ability to trace the origins of any unforeseen issues.

The three impeller design alternatives have been designed in SolidWorks and need to be 3D printed in Accura 60 plastic with an attachment for a magnetic stir bar and then coated with polystyrene. The team plans to test the designs by using the plastic impeller models in magnetic spinner flasks with 3T3 mouse cells. Success of the designs will be measured in cell survival and expansion. If a design does better than the selected final design, the team will likely change the design accordingly, since the design should be centered on optimizing the conditions for the cells.

The heating system has been factored into the design so the remaining steps consist of construction and testing. Due to the focus on the impeller design, the gas control and media exchange systems still require final design selection, construction, and testing. As in the case of the impeller designs, testing will be measured in cell survival and expansion. Finally, more testing of the overall bioreactor system is clearly imperative and if time permits, testing should be used to fine-tune the initial parameters set for the cells. The client has also indicated interest in a larger scale version of the

bioreactor, which will require additional testing to determine how scale-up has changed the system requirements.

References:

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Appendix A: Product Design Specifications

Neural Bioreactor

Team Members: Maria Estevez, Jeff Groskopf, Tyler Klann, Lisa Kohli, Ian Linsmeier

Date: 10/12/12

Function: Reprogramming adult cells into induced pluripotent stem cells (iPSCs), as well as their subsequent expansion and differentiation, is normally completed in adherent cell cultures. Recently, it has been proven that iPSCs can be derived and expanded in suspension cultures using a stirred suspension bioreactor. These reactors establish stable cell culture conditions by controlling the temperature as well as the level of nutrients (media), CO_2 (pH), O_2, and other soluble factors. The suspension components are uniformly distributed within the reactor fluid through various mixing techniques, most commonly an impeller. The process of adult cell reprogramming and iPSC expansion and differentiation can be scaled up and automated using bioreactor stirred suspension cultures.

Dr. Saha has asked our team to design a bioreactor that maximizes the production of neural progenitor cells from mouse embryonic fibroblasts in stirred suspension cultures. The project involves designing culture processes and optimizing culture conditions to reprogram adult cells to induced pluripotent stem cells (iPSCs) and differentiate those iPSCs to neural progenitors.

Client Requirements

- Stirred suspension culture
- Use mouse embryonic fibroblasts (MEFs)
- Reprogram MEFs into iPSCs
- Culture Environment: 37° C , 5% CO_2

Design Requirements

1) **Physical and Operational Characteristics**
    a. ***Performance Requirements:*** The bioreactor must be able maintain 37° C and 5% CO_2 for multiple weeks at a time. Most components of the bioreactor will be reusable. The bioreactor must provide an environment conducive to cell culturing and reprogramming.
    b. ***Safety:*** The bioreactor will incorporate a heating element that will heat the culture to 37° C, but a malfunction in the heating regulation system could lead to much higher temperatures that could damage the cells, microscope, or even the lab technician.
    c. ***Accuracy and Reliability:*** The bioreactor must maintain an internal temperature of 37±1°C and a CO_2 concentration of 5±.5%. The bioreactor must allow for accurate and reproducible conditions.
    d. ***Life in Service:*** The bioreactor will be autoclavable. It would be autoclaved after one use or iteration of reprogramming secondary MEFs into iPSCs, and culturing them to their desired states.

e. ***Operating Environment:*** The device will be used in a cell culture hood by a skilled lab researcher or technician. The device will only be exposed to the lab environment, which will be well controlled.
f. ***Ergonomics:*** The bioreactor must be simple to use.
g. ***Size:*** The bioreactor will be a standalone unit, meaning it will operate independently from other equipment within the lab environment. It will use a 100 mL vessel volume to contain the cell culture. All of the components of the bioreactor should be able to be transported from building to building.
h. ***Weight:*** The weight should be light enough so that one person can lift the bioreactor.
i. ***Materials:*** The materials on the inside of the bioreactor must not be cytotoxic. The bioreactor vessel and elements exposed to the cell culture must be cytophobic or coated in a material that prevents cell adhesion. The material must be impermeable to small molecules and gas to create a closed system.

2) Production Characteristics

a. ***Quantity:*** 1
b. ***Target Product Cost:*** Indeterminate

3) Miscellaneous

a. ***Customer:*** The customer would like this to be eventually used for human adult cells to be reprogrammed into iPSCs. However, we are initially going to design the bioreactor to use MEFs due to the existing protocols being readily available in literature.
b. ***Competition:*** Bioreactors exist in the market for specific applications, but the commercially available bioreactors are not tailored to the specific needs to reprogram cells into iPSCs or for later differentiation of those cells.