Stirred Suspension Bioreactor for Stem Cell Culture

Ian Linsmeier, Tyler Klann, Lisa Kohli, Maria Estevez, Jeff Groskopf

Department of Biomedical Engineering

Advisor: Dr. Tracy Puccinelli **Client**: Dr. Krishanu Saha

DEPARTMENT OF Biomedical Engineering
UNIVERSITY OF WISCONSIN-MADISON

UW BMD Design

Abstract

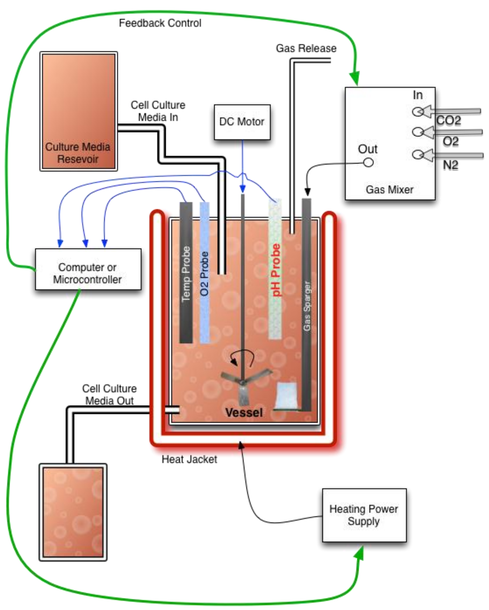
Dr. Saha has asked our team to design a bioreactor that maximizes the production of neural progenitor cells from mouse embryonic fibroblasts in stirred suspension cultures. The project involves designing a process that optimizes culture conditions to reprogram adult cells to induced pluripotent stem cells (iPSCs) and differentiate those iPSCs to neural progenitors on a large scale. It will be designed to function in an incubator with a 100 mL glass vessel, a motor-powered pitch blade impeller, and probes to monitor temperature and gas concentrations. Future work will be conducted to continue bioreactor construction, further develop design, and to test the cell response to the bioreactor.

Problem Definition

- Create a stirred suspension bioreactor which is able to:
  - Reprogram adult fibroblasts to induce pluripotent stem cells
  - Expand those iPSCs
  - Differentiate the iPSCs to neural precursor cells
- This system should require less maintenance than traditional 2D cell culture techniques when creating a large number of cells
- The final design should be able to reprogram the cells of a skin biopsy into iPSCs

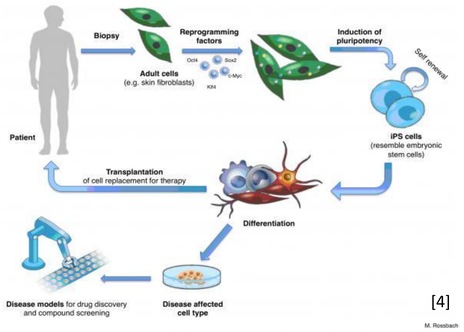
Design Criteria

- Stirred suspension culture
- Use mouse embryonic fibroblasts (MEFs)
- Reprogram MEFs into induced pluripotent stem cells (iPSCs)
- Produce 10^8 neural progenitors
- Culture Environment: 37° C , 5% CO_2



Overall Process Diagram of the Bioreactor System

Background



[4]

- Stem cell culture is mostly done in adherent culture conditions
  - 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 not ideal for the large-scale production of therapeutic cells. [5]
- Approximately 10^9 cells would be required for any relevant clinical application
  - Stirred-suspension bioreactors have the capacity of producing from 10^6 to 10^7 cells per milliliter [3]

Final Design

Probes

- Monitor cell culture conditions
  - Miniature Pt100 RTD temperature sensor
    - Temperature changes resistance
  - pH → Concentration of dissolved CO2
  - Dissolved oxygen concentration

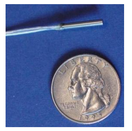
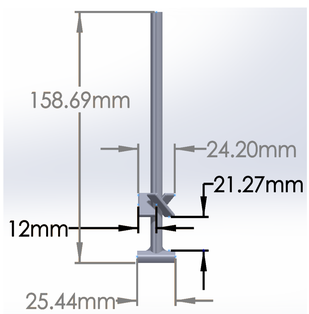


Image of a Miniature Pt100 RTD sensor [1].

Circuit Diagram for Interfacing Temperature Probe with an Arduino Microcontroller

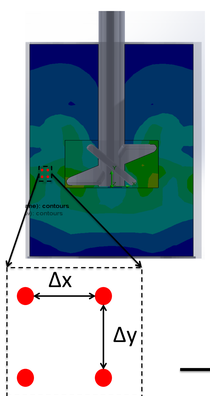
Pitch Blade Impeller

- 3D printed with Accura 60
- 3 blades at 45°
- Overall Height: 158.685 mm
- Overall Diameter: 28 mm
- Stir Bar Clasp for torque



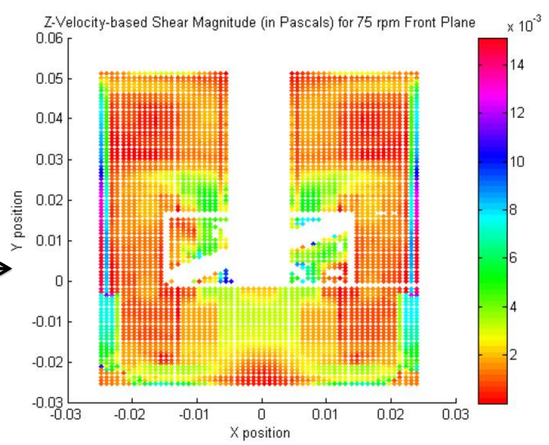
Impeller Dimensions

Fluid Modeling



2D Velocity Profile Simulation with point parameters spaced apart by fixed distances Δx & Δy

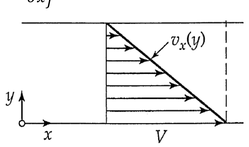
Impeller Model → Velocity Profile → Point Array → Newton's Law of Viscosity → Shear Stress Plot



Newton's Law of Viscosity

$$\sum_i \sum_j \tau_{ij} = -\mu \frac{\partial v_i}{\partial x_j} \quad i, j = 1,2,3$$

- τ_{ij} → shear stress
- μ → viscosity
- $\frac{\partial v_i}{\partial x_j}$ → velocity gradient



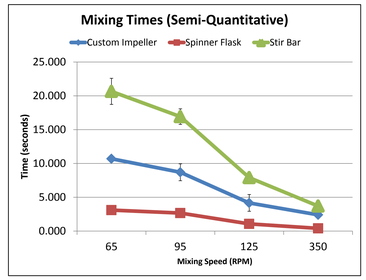
$$\tau_{xy} \cong -\mu \frac{\Delta v_x}{\Delta y}$$

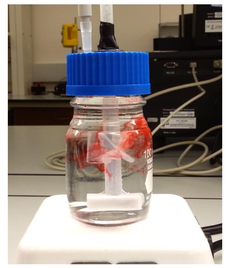
2D velocity profile and shear stress equation [6]

Testing

Time of Mixing

- Added 20 µL of dye into 115 mL H_2O in vessel with impeller
- The custom impeller provided more uniform mixing than the stir bar





Pitch Blade Impeller Mix Testing

Future Work

Cell Culture Testing

- Test survival and proliferation of 3T3 cell line in the bioreactor
- Test survival and proliferation of secondary mouse embryonic fibroblasts (2^o MEFs) in the bioreactor
- Reprogramming 2^o MEFs to iPSCs using the bioreactor
- Differentiation of the iPSCs to neural progenitors in suspension

Vessel and Impeller Design

- Impeller should be manufactured from a non-cytotoxic material that can be sterilized between uses
- Final vessel will be a modified spinner flask design
- Side openings allow for easy access to cell culture media
- Decide if the vessel will be reusable or disposable
- Incorporate baffles for increased mixing
- Manufacture a cap design that will allow for
  - Gas diffusion from incubator into vessel
  - Securing impeller and creating an axis to spin around
  - Housing probes so they will not interfere with spinning impeller

Spinner Flask Vessel [2]

References

References

[1] Auber Instruments Incorporated. *Miniature RTD Sensor.* Auber Instruments. <http://www.auberins.com/index.php?main_page=product_info&cPath=20_15&products_id=38>

[2] http://www.chemglass.com/images_product_1/DBSpinFlask.jpg

[3] Ashton, R. S., Keung, A. J., Peltier, J., & Schaffer, D. V. (2011). Progress and prospects for stem cell engineering. *Annual Review of chemical and Biomolecular engineering*, *2*, 479-502.

[4] http://www.eurostemcell.org/factsheet/reprogramming-how-turn-any-cell-body-pluripotent-stem-cell

[5] Fluri et al. Derivation, expansion and differentiation of induced pluripotent stem cells in continuous suspension cultures. Nature Methods. January 2012.

[6] Bird, R. B., Stewart, W. E., & Lightfoot, E. N. (2006). *Transport phenomena*. Wiley.

Acknowledgements

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