OrChID-Bio: Organs-on-a-Chip with Integrated Detection of Bioluminescence

Analysis of the Discontinuation of Geron's Embryonic Stem Cell Clinical Trials

A Thesis Prospectus
In STS 4500
Presented to
The Faculty of the
School of Engineering and Applied Science
University of Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

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October 24, 2023

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

The late 1990s saw the development of Human Embryonic Stem Cells (HESC), a pluripotent cell line that can differentiate into any cell type. While HESC offers incredible potential for regenerative medicine, its use stirred ethical debates. Another major breakthrough came in 2006 when scientists demonstrated that mature cells can be ‘reprogrammed’ into versatile pluripotent cells (Euguizbal et al., 2019). These cells were termed Induced Pluripotent Stem Cells (iPSCs) and offered an alternative solution to the ethical dilemmas associated with HESC but faced their own hurdles such as incomplete reprogramming and tumorigenicity (Euguizbal et al., 2019). Despite these discoveries, viability of stem cells for human treatment still has much to be desired, a sentiment that was further exacerbated by the failed attempt of Geron Corporation's to use HESCs to treat spinal cord injuries in the late 2000s (Pollack, 2011).

In response, I propose the development of a real-time bioluminescent monitoring system that utilizes an emerging modeling technology called Organ-on-a-chip (OOAC) which could provide a solution to the issues faced by its predecessors. This integrated system will allow for real-time monitoring of enteroid cell growth without the need to put humans in harm's way while also avoiding the ethical controversies of using HESCs. As this technical project requires the construction of an intricate network composed of technical, social, and conceptual actors to support its development and implementation, understanding the mechanisms behind a successful network formation is critical to the success of this project. To examine such a network, I will draw on the STS framework of actor-network theory (ANT) to analyze the failure of Geron’s HESC Clinical Trials which tried to treat spinal cord injuries but was discontinued due to a combination of technical, social, political, and economic factors. In particular, I will investigate how interactions among technical and social factors such as the cost of specialized equipment

and training as well as public expectation and political changes contributed to the trial's failure. Failing to address both the technical and social aspects of the project will result in an isolated and incomplete conception of the project's sociotechnical context, which runs the risk of creating a system that cannot properly handle missing 'actors' that are crucial for the system's success. This means the system's ability to successfully examine and resolve the polarizing use of HESC in research will be compromised and lead to the project's failure.

Since the usage of stem-cell therapy for human treatment is sociotechnical in nature, both the technical and social aspects must be attend to. In the following sections, I describe in detail the technical project describing the design for a real-time, bioluminescence tracking system for stem cell monitoring and the STS project examining the interrelationship between the technical and non-technical actors that led to the discontinuation of the Geron Clinical Trials. The insights from this sociotechnical analysis of Geron's Clinical Trials will help me design a technical solution that optimizes the actor forces within the network to effectively reach the goal of ethical and while keeping human testing out of the equation.

Technical Project Proposal

The need for faster drug development has led to the the emergence of 'Organ-on-a-chip', a technology designed to mimic the complex physiological environment of specific organs or tissues within a microfluidic device. These devices allow researchers to study the structural and functional characteristics of human tissue (Leung et al., 2022). In particular, OOAC has potential to advance our understanding and application of stem-cell therapies, a frontier of medicine that is rapidly growing with research in liver-, heart-, lungs-, gut-, and various other organ-on-a-chips (Singh et al. 2022). Additionally, OOAC can be combined with patient-specific iPSCs to create

personalized models of human organs and internal physiology (Palasantzas et al., 2023). The University of Virginia's Moore Lab made a huge breakthrough in 2023 by successfully combining OOAC with Integrated Detection of Bioluminescence (OrChiD-Bio) which allows for detection of bioluminescence in enteroid cells: a three-dimensional structure grown from HESCs that mimics the structure and function of the enterocytes, specifically the small intestine or colon (Aminuddin, 2023). By combining naturally bioluminescent luciferase enzymes with OOAC cultivated enteroid cells, gene expression and cell behavior over a circadian time scale can be tracked using micro-photomultiplier tubes (micro-PMT). Thus, enterocyte physiology and its behavior fluctuation can be mimicked by luciferase-infused enteroid cells over the course of hours, days, and even weeks. However, extracting data from the chips is especially difficult. Currently, chips must be removed from the OOAC device and placed into a different machine, called Kronos-Dio, that lacks flow and stretch stimuli in order to detect bioluminescence. In addition, the current procedure also increases the likelihood of contaminating the enteroid cultures during transportation from OOAC to Kronos-Dio.

These flaws have major consequences when considered in conjunction. First, the lack of physiological stimuli within the Kronos-Dio means that bioluminescence data collected through its software is not entirely representative of the naturally dynamic behavior of enterocytes. Furthermore, this protocol renders real-time measurements completely impossible as bioluminescence data is not captured during the transportation timeframe. However, the most significant drawback of this method is the increased likelihood of contamination, which could kill off enteroid cells within the culture dishes, resulting in no data and a need to redo the entire culturing process over again. Considering each procedure interation takes around two weeks to complete from the initial plating and culturing of the enteroid cells to the final collection of

bioluminescence data, any error within the process can cause up to a two week setback. The technical project aims to address these drawbacks by creating an integrated system that performs real-time measurements of circadian rhythms of intestinal tissues through bioluminescent monitoring of OOAC that forgoes the need for an external tracker such as Kronos-Dio.

This project has three steps. First, a noise filtering and modeling program will be developed in Python to analyze and present bioluminescence results. Next, bioluminescent oscillations of PER2:LUC over a period of days to weeks will be collected using the existing micro-PMT system as changes in flow rate and mechanical stretch to the epithelial surface are applied to the cultured tissue model. The final task will be to demonstrate relevance for real-time bioluminescence monitoring in three different complexities of cells: Caco2 Per2:Rluc cells (an immortalized cell line), Per2:Luc mouse enteroids (samples taken directly from the animals), and human samples transfected with a Bmal1:Luc reporter by comparing resulting Real-Time collected bioluminescence data to those of Kronos-Dio collected data. Bioluminescence data used for analysis will be collected through cultures created in Moore's Lab. Two sets of cultures will be grown, one for the current procedure involving Kronos-Dio and the other for the integrated real-time bioluminescence program that will be developed. Data collected will be presented in enteroid cell count (y-axis) vs circadian time scale in hours or days (x-axis).

STS Project Proposal

In January of 2009, American biotechnology company Geron Corporation, had their revolutionary GRNOPC1 therapy for spinal cord injury (SCI) approved by the Food and Drugs Administration (FDA) for a phase I clinical trial. In October of 2010, the Geron trial became the world's first clinical trial of a HESC-based treatment (Lebkowski, 2011; Pollack, 2010).

However, despite its promising potential, Geron discontinued the HESC trials a little less than a year later along with various other stem cell programs they were funding. Geron came out with a press release stating the trials would cost the company an unsustainable $25 million per year and that the procedure was too complex and specialized to be viable in the long term (Shepherd Center, 2011). The failure of the clinical trials is often attributed to the intrinsic unpredictability of stem cell-derived treatment, complex clinical protocol, and highly specialized training required of surgeons. To elaborate, the procedure "involved several teams of surgeons, treating clinicians, study personnel, and physical therapists" across the country who were all "required to undergo specialized training" to familiarize themselves with the cells, reagents, and cell delivery devices **(**Scott, 2014). Understandably, having staff members spread so thin across the country and needing specialized equipment and training incurred an unsustainable twenty five million dollars per year financial cost that crumbled the Geron clinical trial efforts. While these factors played important roles in the discontinuation of Geron's HESC trials, they do not fully explain why the research trials ultimately failed. By only focusing on the technical aspects that led to this failure, it becomes easy to look at the issue as disconnected, individual parts rather than a holistic and intricate web of technical and non-technical actor interactions and relationships.

If we continue to view the root causes through a constricted and isolated lens, we will fall short of learning from the failure of the clinical trials, thus increasing the chances of a similar failure happening in the future. Additionally, we would fail to understand how non-technical actors can affect and interact with the technical actors to influence project outcomes. For these reasons, I argue that the procedural complexity and financial strain of Geron's HESC trial in tandem with political policy changes, social shifts, scrutiny from the research community of

using humans for testing, and external expectation from the general public contributed to the discontinuation and failure of the trials.

To frame my analysis, I will draw upon the STS concept of actor-network theory. ANT claims that all technical projects are made up of a network of human and non-human actors that help achieve a particular goal, thus, providing a means to describe and analyze the complex interrelationships and interactions among actors (Cressman, 2009). More specifically, I will utilize the sub-concept of translation, which describes the process in which non-technical and technical actors enroll into a network, negotiate their roles, and work to stabilize (or destabilize) the overall network. Applying translation to my analysis will allow me to identify disruptive actors and gain a better understanding of how to optimize those actors that caused the collapse of the trials. For my analysis, I will utilize various sources of evidence including official released statements by Geron Corporation, public reports, academic papers in relation to the failure of the trials, and articles of the political and economic climate during the time period.

Conclusion

The technical project deliverable will be a fully functional monitoring program integrated into the existing OrChID-Bio system that is capable of real-time bioluminescence tracking without the need to remove cells cultured within organ-chips before bioluminescence analysis. The STS project deliverable will be a full analysis of why Geron's HESC Clinical Trials failed using ANT to determine significant disruptive actors. These findings will then be applied to my technical projects by developing measures to prevent the same mode of failure from occurring to my actor-system. The combined results of this technical report will serve to address the issue of human testing and HESCs usage in research endeavors, highlighting important considerations for

the success of human stem-cell projects and proposing the adoption of Real-Time OrChiD-Bio as an alternative to predecessor solutions.

References

Aminuddin, J. (2023, February 15). *Moore Laboratory Awarded $100,000 Grant from The Jefferson Trust - Research*. Medicine in Motion News. https://news.med.virginia.edu/research/moore-laboratory-awarded-100000-grant-from-the-jefferson-trust/

Cressman, D. (2009). *A Brief Overview of Actor-Network Theory: Punctualization, Heterogeneous Engineering & Translation*.

Eguizabal, C., Aran, B., Chuva de Sousa Lopes, S. M., Geens, M., Heindryckx, B., Panula, S., Popovic, M., Vassena, R., & Veiga, A. (2019). *Two decades of embryonic stem cells: a historical overview.* Human Reproduction Open. https://doi.org/10.1093/hropen/hoy024

Lebkowski, J. (2011). *GRNOPC1: the world's first embryonic stem cell-derived therapy.* Regenerative Medicine, 6(6s), 11–13. https://doi.org/10.2217/rme.11.77

Leung, C. M., de Haan, P., Ronaldson-Bouchard, K., Kim, G.-A., Ko, J., Rho, H. S., Chen, Z., Habibovic, P., Jeon, N. L., Takayama, S., Shuler, M. L., Vunjak-Novakovic, G., Frey, O., Verpoorte, E., & Toh, Y.-C. (2022). *A guide to the organ-on-a-chip.* Nature Reviews Methods Primers, *2*(1), 1–29. https://doi.org/10.1038/s43586-022-00118-6

Palasantzas, V., Tamargo-Rubio, I., Le, K., Slager, J., Wijmenga, C., Jonkers, I., Kumar, V., Fu, J., & Withoff, S. (2023, February 4). *iPSC-derived organ-on-a-chip models for personalized human genetics and pharmacogenomics studies*. Trends in Genetics. https://www.cell.com/trends/genetics/fulltext/S0168-9525(23)00017-3#:~:text=Organ-on-a-chip%20(OoC)%20technology%20combined,factors%20such%20as%20the%20microbiome.

Pollack, A. (2010). *Stem Cell Trial Wins Approval of F.D.A.* The New York Times. https://www.nytimes.com/2010/07/31/health/research/31stem.html

Pollack, A. (2011, November 14). *Geron Is Shutting Down Its Stem Cell Clinical Trial.* The New York Times. https://www.nytimes.com/2011/11/15/business/geron-is-shutting-down-its-stem-cell-clinical-trial.html

Scott, C. T., & Magnus, D. (2014). W*rongful Termination: Lessons From the Geron Clinical Trial.* STEM CELLS Translational Medicine, *3*(12), 1398–1401. https://doi.org/10.5966/sctm.2014-0147

Shepherd Center. (2011). *Geron to Halt Spinal Cord Injury Trial and Focus on Oncology Programs*. Shepherd Center. https://news.shepherd.org/geron-to-halt-spinal-cord-injury-trial-and-focus-on-oncology-programs/

Singh, D., Mathur, A., Arora, S., Roy, S., & Mahindroo, N. (2022). *Journey of organ on a chip technology and its role in future healthcare scenario.* Applied Surface Science Advances, 9, 100246. https://doi.org/10.1016/j.apsadv.2022.100246