Team UFlorida 2024

Human iPSC Bone Marrow Organoids as Clinical Model to study Sepsis Response

Undergraduate iGEM Wet Lab Captain: Alexandra Davidov
Undergraduate iGEM Dry Lab Captain: Caleb Faison
Undergraduate iGEM Human Practices Captain: Julia Jamieson
Contact: ufigemteam@gmail.com

Faculty Principal Investigator: Jing Pan, PhD
Undergraduate iGEM Advisor: Makenna Myrick
Graduate Advisors: Aditi Kumari, Yongchen Tai

Undergraduate Team Members (alphabetical order):
Shuyu Chen[1c], Alexandra Davidov[2c], Caleb Faison[3,4,5b], Jonathan French[6c], Julia Jamieson[7c], Michelle Lee[8b], Abigail Leon[6b], Anuj Master[2b], Tyler Montgomery[8c], Bella Morse[8c], Sanandan Ojha[6a], Sharad Patel[6a], Lucy Pellenbarg[9a], Kalyan Ratnakaram[7c], Jae Sarner[10b]

[1.] Behavioral and Cognitive Neuroscience, Department of Psychology, College of Liberal Arts and Sciences
[2.] Microbiology and Cell Science, Department of Microbiology and Cell Science, College of Liberal Arts and Sciences
[3.] Chemistry, Department of Chemistry, College of Liberal Arts and Sciences
[4.] Mathematics, Department of Mathematics, College of Liberal Arts and Sciences
[5.] Physics, Department of Physics, College of Liberal Arts and Sciences
[6.] Biomedical Engineering, Department of Biomedical Engineering, Herbert Wertheim College of Engineering
[7.] Biology, Department of Biology, College of Agriculture and Life Sciences
[8.] Biology, Department of Biology, College of Liberal Arts and Sciences
[9.] Public Health, Department of Public Health, College of Public Health and Health Professions
[10.] Health Science, Department of Public Health, College of Public Health and Health Professions
[a.] Freshman [b.] Sophomore [c.] Junior [d.] Senior

1. Abstract

Currently, little is known about the mechanisms of disease and inflammation in human bone marrow. Sepsis, a cascade-like infection in the bloodstream, elicits a response from the bone marrow by activating hematopoietic stem cells, altering cell differentiation, and inducing myelopoiesis. In an attempt to further the global understanding of sepsis, we are developing in vitro and in silico models of sepsis-inducing conditions to predict and quantify the immune system response to pathogenic stimuli. Our in-vitro model, created using Induced Pluripotent Stem Cell (iPSC) derived organoids, can replicate human-specific bone marrow biology and disease responses. We put forth a plan to induce an inflammatory response, similar to the one observed in septic shock, and prove that our model has a biologically similar infectious response to that seen in septic bone marrow. Additionally, using imaging, we will be able to prove that our organoid has induced vascularization and created the bone marrow niche: the environment for hematopoiesis and differentiation. Our agent-based in-silico model will complement this data by creating a virtual environment in which the mechanisms of septic immune response can be more visually understood.

2. Introduction

Sepsis is a life-threatening condition caused by the immune system's severe response to an infection[1]. This dysregulated inflammatory response can cause damage to a patient's tissues and organs, and ultimately result in death. Individuals with chronic health conditions, over the age of 65, pregnant women, and neonates are at greater risk of contracting sepsis. Approximately 48.9 million sepsis cases were reported and 11 million deaths resulted from sepsis, contributing to 19.7% of deaths worldwide in 2017[2].

Furthermore, those who survive are often left with chronic symptoms that affect their long-term well-being. Post-sepsis syndrome (PSS) is a condition that affects up to 50% of sepsis survivors and can have numerous chronic symptoms such as insomnia, hallucinations, memory loss, disabling muscle pain, and reduced organ function[3]. These consequences of sepsis make it difficult for survivors to go back to their routine and complete everyday tasks. Many studies have proven that PSS is associated with a reduced lifespan and the current 5-year mortality rate ranges between 44% to 82%[4]. This condition has a vast impact, significantly harming and impacting many people in our society.

Murine models are the current standard for sepsis research, where sepsis is commonly induced via the administration of an external pathogen, delivery of a toxin (commonly a lipopolysaccharide), or stimulation to release internal pathogens[5]. These models have demonstrated characteristic elements of human sepsis, including inflammation,

---

[1] World Health Organization. (2023). *Sepsis*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/sepsis

[2] Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., Colombara, D. V., Ikuta, K. S., Kissoon, N., Finfer, S., Fleischmann-Struzek, C., Machado, F. R., Reinhart, K. K., Rowan, K., Seymour, C. W., Watson, R. S., West, T. E., Marinho, F., Hay, S. I., Lozano, R., … Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet (London, England), 395*(10219), 200–211. https://doi.org/10.1016/S0140-6736(19)32989-7

[3] Sepsis Alliance. (2022, June 29). Post-sepsis syndrome. https://www.sepsis.org/sepsis-basics/post-sepsis-syndrome/

[4] Van Der Slikke, E. C., Beumeler, L. F., Holmqvist, M., Linder, A., Mankowski, R. T., & Bouma, H. R.. (2023). Understanding Post-Sepsis Syndrome: How Can Clinicians Help?. *Infection and Drug Resistance, Volume 16,* 6493–6511. https://doi.org/10.2147/idr.s390947

[5] Cai, L., Rodgers, E., Schoenmann, N., & Raju, R. P. (2023). Advances in Rodent Experimental Models of Sepsis. *International journal of molecular sciences, 24*(11), 9578. https://doi.org/10.3390/ijms24119578

systemic vascular resistance, and abnormal cardiac output. One key shortcoming in current sepsis mouse models is the difference in the immune system[5]. Researchers are developing humanized models by replacing aspects of the mouse immune system with human components in attempts to enhance the accuracy of current models. However, the accuracy of rodent models is further limited due to differences in their biological pathways and disease phenotypes compared to humans [6].

Nearly 95% of drugs fail when applied to human trials after animal testing, demonstrating the limitations of animal models[7]. The FDA Modernization Act 2.0 removed the animal testing requirement of the drug approval process in 2022, promoting the use of alternatives to animal testing. The ability to verify drug efficacy without utilizing animal models enhances the utility of disease-specific in vitro models.

Organoids are 3D tissue structures most commonly derived from iPSCs[8]. The platforms recapture the key biological, morphological, and functional attributes of an organ. Organoids provide an isolated platform to study tissues outside of living organisms, eliminating many ethical issues associated with the use of animal models. Furthermore, the separation from a living being enables greater manipulation and study of the modeled organ. Researchers have created organoids representative of the brain, kidney, lung, intestine, stomach, and liver. These “mini-organs” have facilitated groundbreaking research on the microbiome, neurological processes, cancer, regenerative medicine, developmental biology, drug discovery, and disease mechanisms. A human-derived in vitro platform to model the disease would provide an in-depth understanding of the molecular processes triggered by sepsis in human bone marrow and provide a basis for future investigative drug research.

3. Project Aims

a) Aim 1: Growing and Differentiating Bone Marrow Organoids

- We will culture iPSCs on matrigel-coated plates in a basal stem cell culture medium. The iPSCs will be maintained in an undifferentiated state by regularly changing the medium and passaging cells as needed. We will perform a 7-day protocol that includes 3 phases, thawing, expanding, and passaging, to create our cell aggregates in preparation for differentiation.
- After the cells have grown to our target confluency, we will disassociate them from the plates using EDTA. The cell culture will be transferred to 6-well ultra-low-attachment plates with StemFlex supplemented with RevitaCell to promote the cells' ability to differentiate into all three germ layers. Cells will then be resuspended in media containing growth factors that will differentiate the iPSCs into Hematopoietic stem cells (HSCs). Cells will be resuspended twice in different mediums containing specific growth factors to promote the formation of the bone marrow environment.
- After allowing the cells to grow and proliferate and establish bone-marrow characteristics, we will embed them on hydrogel plates. The hydrogel contains important proteins such as collagen which will facilitate cell adhesion and establish vascularization of the organoid. The cells will be supplemented by media containing growth factors directed towards fibroblast and vascular formation.

---

[6] Perlman R. L. (2016). Mouse models of human disease: An evolutionary perspective. *Evolution, medicine, and public health, 2016*(1), 170–176. https://doi.org/10.1093/emph/eow014

[7] Adashi, E. Y., O'Mahony, D. P., & Cohen, I. G. (2023). The FDA Modernization Act 2.0: Drug Testing in Animals is Rendered Optional. *The American journal of medicine, 136*(9), 853–854. https://doi.org/10.1016/j.amjmed.2023.03.033

[8] Zhao, Z., Chen, X., Dowbaj, A. M., Sljukic, A., Bratlie, K., Lin, L., Fong, E. L. S., Balachander, G. M., Chen, Z., Soragni, A., Huch, M., Zeng, Y. A., Wang, Q., & Yu, H. (2022). Organoids. *Nature reviews. Methods primers, 2,* 94. https://doi.org/10.1038/s43586-022-00174-y

[8] iGEM, 2023. “Team Wiki.” October 2023, https://gitlab.igem.org/2023/software-tools/uflorida.

Dry Lab Aim 1:

- Using the NetLogo software, we will create a virtual environment modeling our bone marrow organoids. This environment will consist of different classes of variables, referred to as 'agents,' that can be programmed to reflect the properties of various physical and biological quantities (including cells, molecules, and viruses).
- The model will at a minimum include agent classes for each of the following: pro-inflammatory cytokines, anti-inflammatory cytokines, leukocytes, pathogens, and HSCs. Existing literature has shown these classes to be commonly used markers for simulating biological systems. They provide the most critical information about the evolution of a biological system, while still allowing the model to remain as simple as possible (saving a significant amount of computational power and lowering program run-times)
- Agent classes will be defined using existing literature and utilized to simulate the various intercellular signaling molecules involved with the human immune system. Once a virtual steady-state can be reached in our model, Aim 1 will be complete.

b) Aim 2: Validate Bone Marrow Organoids

- We will image our cells and show the vascularization that has formed, proving our organoids have developed into a representative model. Additionally, we will use immunofluorescence imaging to show the presence of specific cell types, such as CD34 cells, a primary marker for HSCs and hematopoietic stem precursor cells. We will perform flow cytometry to analyze the chemical and physical characteristics of our cells and particles.

c) Aim 3: Inducing Sepsis-like Infection

- We will induce a sepsis-like response in our organoid using inflammatory cytokines such as IL-6, TNF-α, and IL-1. These cytokines will create a "cytokine storm" which in the human body is responsible for immune activation leading to excessive inflammation. This inflammatory activation can seriously damage cells and organs, often leading to multi-organ failure and death.
- Following this, we will analyze the cell necrosis that has occurred as a result of significant inflammation (which results from infection). We will also test for immune markers to see the extent to which the immune system was activated. This model can serve as a mechanistic model to examine the processes of sepsis at different stages, and see how we are affected at a molecular level. Insight about immune responses will give us more knowledge and pathways to treat sepsis in the future, and truly understand the way this rapid infection develops.

Dry Lab Aim 2:

- After creating a healthy, virtual, steady-state cell environment, we will add onto our model by introducing a pathogen agent into the cell system. Once this agent is added to the system, we will work to ensure that the simulated immune response system evolves in a natural manner and in accordance with known experimental data in related fields. This will be quantified by the relative levels of each biomarker at various timesteps in the program.
- To further improve the accuracy and usefulness of our model, we will fit it to the data collected from our wet lab organoid experiments. Doing this will give our program the power to make informed predictions on the evolution of the human immune response in bone marrow tissue given any set of starting parameters.
- Once complete, our model will allow for users to modulate the initial levels of many key variables, including infection severity, cytokine responsiveness, and cell count. All of

these values will be given a range in which the user can vary them, and these ranges will be determined by existing literature.

4. In Vitro Model

The primary goal of our in-vitro model is to create an organoid that can replicate human-specific bone marrow biology and disease responses. An in-vitro organoid model offers a better way to recreate native pathophysiological responses compared to other non-human or 2D cell-culture models. Through mesenchymal differentiation, our organoid will become a self-sufficient model consisting of a vascularized bone marrow niche that induces hematopoiesis. Bone marrow plays a significant role in disease and injury by producing and releasing more white blood cells in response to infections and more platelets in response to bleeding[9]. Furthermore, on a molecular level, during infection, the bone marrow experiences an increased flux of proinflammatory cytokines (IL-1, TNF, and IL-6)[10]. In our model, after inducing a response reflective of sepsis infection, we should be able to observe these changes in the immune response, proving our organoid is effective as a disease-testing model.

5. Agent-Based Model, Expanded

Supplementing the team's in-vitro model, a subsection of our team (the Dry-Lab) will focus on developing an in-silico model of sepsis progression in a bone marrow organoid. In this model, we will create a virtual environment in which the mechanisms of immune response can be more visually understood. This work will expand upon a previous sepsis model, created by the UFlorida iGEM team in 2023[8].

This year's model aims to provide a simple and easy to use model of disease progression that requires minimal background information in microbiology or computer science. It also aims to make a novel contribution to the field of biological modeling. Both of these aims will be discussed further below.

The previous UFlorida model utilized a series of differential equations to model the immune system under septic and aseptic conditions. It treated biomarkers in the immune system as a set of nodes, each having a positive or negative "force" on the other nodes (biomarkers). This particular model took in a series of initial values (setting the initial environmental conditions) and returned the relative levels of key biomarkers over a simulated timeframe of 200 hours. This model was shown to predict trends in the immune system with a sufficient level of accuracy to provide insight into the evolution of the immune system in response to a pathogen.

This year's iGEM team looks to expand upon this model by translating it into the space of agent-based modeling. Agent-based modeling focuses on building an environment from the ground up. Individual 'agents' are assigned specific attributes and allowed to move throughout their environment in any manner chosen by the programmer. These attributes provide the agent with specific instruction on how to interact with other agents in the environment[10].

In our model, bone marrow cells, pathogens, and various biomarkers will each be assigned their own agent class and will, thus, have their own unique attributes. These attributes will be defined according to previous literature, including advancements made by previous UFlorida iGEM teams. Utilizing the NetLogo software, we will create an

---

[9] Wang, J., Erlacher, M., & Fernandez-Orth, J. (2022). The role of inflammation in hematopoiesis and bone marrow failure: What can we learn from mouse models? *Frontiers in Immunology, 13*. https://doi.org/10.3389/fimmu.2022.951937

[10] Leimkühler, N. B., & Schneider, R. K.. (2019). Inflammatory bone marrow microenvironment. *Hematology, 2019*(1), 294–302. https://doi.org/10.1182/hematology.2019000045

[10] Downy, Allen, and Bruce West. "Agent-Based Modeling." Columbia University Mailman School of Public Health, https://www.publichealth.columbia.edu/research/population-health-methods/agent-based-modeling. Accessed 14 March 2024.

environment in which the agents (cells, pathogens, and biomarkers) can interact with each other over an indefinite series of timesteps. We will quantize the data by graphing the relative levels of each biomarker over time, as well as the pathogen levels and overall cell health. However, in addition to the graphical data, the entire system will be visible to the user throughout the entire runtime of the program. A window will allow the user to see the movement of each biomolecule and pathogen, as well as when they interact with each other. They will also be able to visibly see the number of each agent either increase or decrease in real time as they interact. This will provide a visualization of the mechanistic processes involved in immune response.

As with previous UFlorida models, we will also incorporate the ability of the user to change the initial conditions of the environment. This would include the infection severity, initial pathogen count, cytokine responsiveness, and cell count. This will allow users to see how changes in one (or more) variable(s) impact the evolution of the system over time.

6. Dissemination Plan/Global Impact

After demonstrating that sepsis can be induced and analyzed ex vivo through an iPSC-derived organoid, we intend to publish on the fabrication of this novel disease model. The in vitro platform will expand the current applications of bone marrow organoids and provide a new niche to study sepsis. The publication of the UFlorida team's research results will inform researchers across the globe of a viable method to model sepsis ex vivo in the bone marrow.

Alongside the formal publication of our research data, we will spread awareness of sepsis and the research work conducted through the UFlorida iGEM Human Practices team. The Human Practices team emphasizes the social impacts and significance of an iGEM research project. The team serves as an outreach branch to emphasize education and involvement in synthetic biology in the greater community.

This team will engage in information dissemination on the impacts of sepsis and awareness of synthetic biology, specifically with relevance to in vitro models. Expanding on activities conducted last year, including tabling and passing out flyers on the University of Florida campus during Sepsis Awareness Month and maintaining an active social media presence. Objectives for the 2024 iGEM Human Practices team include promoting bioethics and hosting events at the University of Florida. The Human Practices team's specialization in education will aid in spreading awareness of sepsis and the cutting-edge synthetic biology innovations in development to inform the study of the disease and aid in the development of effective therapeutics.

7. Benefit to UF

Participation in the iGEM competition places the University of Florida on a global stage, as over 50 countries are represented at the Global Jamboree[11]. In 2023, the UFlorida iGEM team earned a Silver medal for our project "A Multifaceted Model to Study Sepsis Progression," earning the same distinction as MIT, Harvard, Princeton, Duke, Oxford, and Stanford's iGEM teams. This year we are continuing our research efforts to craft a complex in vitro model.

Furthermore, participation in iGEM and the Grand Jamboree allows undergraduate students the opportunity to network and explore research from diverse disciplines. iGEM accepts projects that fall under 15 academic "villages:" Climate Crisis, Bioremediation, Conservation, Agriculture, Food & Nutrition, Fashion & Cosmetics, Diagnostics, Therapeutics, Oncology, Infectious Diseases, Space, Foundational Advance, Software & AI, Biomanufacturing, and High School[11]. UFlorida iGEM team members immerse in a rich intellectual community through their involvement in this global synthetic biology competition. Last year, the UFlorida iGEM team

[11] iGEM Foundation. (n.d.). iGEM. https://igem.org/

collaborated with teams across the globe, including Patras Medicine (Greece), iGEM Iisertvm (India), and iGEM Bulgaria.

As the University of Florida's only fully undergraduate student-run wet laboratory research team, iGEM is a unique opportunity for students to engage in and have ownership of their research. The majority of research work is in collaboration with fellow undergraduates as the core innovators, guided by Dr. Jing Pan and his research group. iGEM is a testament to the innovation and independence of undergraduate researchers at the University of Florida.

8. Timeline

January-February:

- Decide on the 2024 project based on feedback from last year's project
- Determine members of UFlorida iGEM 2024 team
- Familiarize the team with the project through weekly meetings and literature reviews

March-April:

- Complete project proposal/description
- Begin wet-lab protocols
- Register all lab members and complete lab trainings
- Acquire funding for the project
- Begin planning Human Practices events for the upcoming year
- Create WIKI and upload the project description
- Begin creating the agent-based model

May-July:

- Start performing wet lab experiments
- Register by May 22nd
- Acquire data from experiments quantifying and qualifying our results
- Host Human Practices events
- Collaborate with other institutions and iGEM teams around the world
- Safety form due June 5th
- Village selection/project description due June 21st
- Consent forms and roster check-in are due July 17th
- All jamboree fees due July 31st

August-September:

- Complete agent-based model
- Complete Wet Lab experiments
- Write and design all sections of WIKI
- Complete project promotion video
- Complete presentation video
- Human Practices events
- Final safety forms and project promotion video due September 4th
- September 11th final roster freezes

October-November:

- Finalize WIKI
- Judging form, WIKI, project attributions form, Registry Part Pages, Project Software, all freeze on Oct 2nd
- Presentation video due October 9th
- Grand Jamboree October 23-26

9. Future Directions

Numerous other diseases besides sepsis impact the bone marrow such as aplastic anemia, Shwachman-Diamond syndrome, leukemia, and lymphoma. An organoid model can provide a new alternative to research and testing for these diseases. In future research, organoids could be created to induce one of these diseases and offer a novel way to study the disorder. Additionally, these organoids could provide an alternative route to test and screen drugs. In vitro models are a safe and efficient way to accelerate the timeline of getting a drug approved and on the market, allowing more people to have access to life-changing drugs.