Assignments must be submitted onto Canvas as a Word doc,docx file. Be warned- exporting from GDocs to Word may change the formatting and result in a loss of points. Always double-check the formatting of your submitted work. This warning should NOT be submitted as part of your assignment.

Grant Proposal

Simulated Microgravity-Induced Stem Cell Formation from Differentiated Cells in *Physcomitrella patens*

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Author Note

Abstract (RQ) or Executive Summary (Eng)

The abstract would contain an overall summary of what you (as the author) would like to convey. It would include some of the knowledge gaps that would eventually lead to researchable questions you have identified in the field.

Keywords: emotion understanding, interest, social development, prosocial behavior, infants

Simulated Microgravity-Induced Stem Cell Formation from Differentiated Cells in *Physcomitrella patens*

II: Specific Aims

Plant stem cells have recently gained popularity in fields such as cosmetics, biotechnology, and regenerative medicine (Aggarwal et al. 2020). This proposal's objective is to find the effects simulated microgravity on cell de-differentiation in *Physcomitrella Patens*. The long-term goal is to test traditional markers of cell division under a Random Position Machine to see if the traditional mechanisms of de-differentiation have a unique response under microgravity. The central hypothesis of this proposal is that simulated microgravity will negatively alter reprogramming signals in *Physcomitrella patens* by changing CDK activity and cytoskeleton organization. The rationale is that cell reprogramming involves the cell cycle and changes in the cytoskeleton dynamics. Additionally, in an experiment down by Kume et al. 2021, *Physcomitrella patens* cultured on the ISS saw reduced photosynthetic rate and smaller chloroplasts, opposed to a trial the same study ran on hypergravity, where the photosynthetic rate increased compared to normal rates. Since microgravity reduces the photosynthetic rate, it may also impact the cell's ability to de-differentiate. Additionally, microgravity has been shown to impact the gene expression and proliferation of animal stem cells. For instance, a review of the effects of microgravity of different variations of human stem cells found that in certain types, such as mesenchymal stem cells, there was an increase of immunosuppressive ability, suggesting changes in gene expression itself, a result that could repeat itself in this experiment (Ghani et al. 2024). The work we propose here will determine how microgravity affects the key mechanisms of cellular reprogramming/de-differentiation in *Physcomitrella patens* using a low-budget Random Positioning

Machine and protoplast regeneration. This can provide new insight into how gravitational forces shape development of plants and contribute to future endeavors in space.

Specific Aim 1: Successfully build a Rotating Positioning Machine based on Open Electronics' open source GitHub Repository.

Specific Aim 2: Successfully create protoplasts through the use of driselase.

Specific Aim 3: Compare and contrast de-differentiation in microgravity versus normal gravity.

The expected outcome of this work is that the tested differentiated cells will show a lower frequency of de-differentiatiation (the ability to revert back to a stem cell). In an experiment down by Kume et al. 2021, *Physcomitrella patens* cultured on the ISS saw reduced photosynthetic rate and smaller chloroplasts, opposed to a trial the same study ran on hypergravity, where the photosynthetic rate increased compared to normal rates. Since microgravity reduces the photosynthetic rate, it may also impact the cell's ability to de-differentiate. Additionally, microgravity has been shown to impact the gene expression and proliferation of animal stem cells. For instance, a review of the effects of microgravity of different variations of human stem cells found that in certain types, such as mesenchymal stem cells, there was an increase of immunosuppressive ability, suggesting changes in gene expression itself, a result that could repeat itself in this experiment (Ghani et al. 2024).

An Overview of Stem Cells

Stem cells are cells that have not differentiated. Certain stem cells are omnipotent or multipotent, meaning that they can differentiate into multiple different types of cells, making them a critical area of study in regenerative medicine (Poliwoda et al. 2022). Plant stem cells are undifferentiated cells found in the meristem of plants. They are currently used commercially for a variety of purposes, including cosmetics and biotechnology. For instance, there has been evidence of anti-inflammatory and anti-oxidant properties in grapes (*Vitis vinifera*), lilacs (*Syringa vulgaris),* and Swiss Apples *(Uttwiler spatlauber).* (Aggarwal et al. 2020).

Because of their biological and commercial importance, understanding how plant stem cells respond to different environmental conditions is of great interest. Particularly, the effects of stem cells under simulated microgravity conditions have been studied extensively, as it is apparent that simulated microgravity alters cell proliferation, differentiation, and regeneration capabilities. As a result, there is an interest in exploring the potential for growing stem cells in space. Studying stem cells in space has allowed scientists to discover previously unknown mechanisms, disease pathways, and more. (Ghani & Zubair, 2024).

Physcomitrella patens, their Life Cycle, and regeneration mechanisms

Physcomitrella patens is the first transgenic moss created, being capable of efficient homologous recombination, allowing for gene targeting and genome modification through knockout genes. As a result, P. patens became a landmark model organism for genome studies. Additionally, cultivation of P. patens is simpler compared to other model organisms, as it allows for the culture to be easily sterilized through means such as sodium hypochlorite.

During its life cycle, P. Patens often switches between creating sporophytes (2n, diploid phase used in asexual reproduction) and gametophytes (n, haploid phase used in sexual reproduction). Compared to other plants, gametophytes are the predominant generation, meaning that the organism reproduces sexually for more of its life than asexual reproduction. This generation begins with germination of the haploid spore. (Strotbek et al. 2013). Protonema, a type of filamentous tissue, is formed by apical growth (growth in the central stem). These protonema are further divided into caulonema cells and chloronema cells. The main differentiating factor between these cells is that chloronema contains more organelles (such as chloroplasts) than caulonema cells, making them more visible through a microscope (Strotbek et al. 2013, Vidali & Bezanilla, 2012). These cells can be stripped of their cell walls to create protoplasts with driselase, an enzymatic mixture containing pectinase, cellulase, laminarinase, xylanase, and amylase (Strotbek et al. 2013 & Protoplasts can be used to induce reprogramming, as the removal of the cell wall triggers a wound-like response allowing cells to revert to an undifferentiated state. This undifferentiated state is the stem cell. Normally, P. Patens only contain one stem cell at the tip of the main stem. An inhibitory signal prevents the creation of another stem cell unless the first stem cell is no longer detected (which can be achieved by completely isolating the differentiated cell), or there is wounding to the plant (which does not require isolating the singular differentiated cell) ()This project aims to test this ability under microgravity conditions by checking for important signals of stem cell formation, including the presence of CDK and checking the microtubules.

Factors that Can Differentiate Results from Simulated Microgravity versus Results from Outer Space

Studies under simulated microgravity can provide important insights to potential affects in space, there and factors that can create a variety of responses between the two. For instance, outer space

experiences a unique type of radiation known as space radiation. There are different types of space radiation, including galactic cosmic rays, high energy particles that posess atoms with a lack of electrons. Galactic cosmic rays have a high LET value (Linear Energy Transfer), meaning that these rays tend to concentrate large amounts of ionizing radiation to one small area. This can cause more damage than low LET radiation, which is commonly found on Earth. As a result, space radiation can have a severe impact on the behaviors of MSCs if not controlled for properly (Krittanawong et al. 2022).

Existing Microgravity Devices

The most accurate method of testing microgravity's effects on biological samples is to study the ISS. However, this is not always feasible due to the time and cost involved. As a result, devices that allow one to simulate varying levels of gravity (such as clinostats, Rotating-Wall Vessels (RWVs), and Random Positioning Machines (RPMs)) were invented. The main differentiating factor between RPMs and other devices is that RPMs have a double-axis, allowing it to replicate microgravity more efficiently (University of Toledo, 2024).

Section III: Project Goals and Methodology

Relevance/Significance:

This project has relevance in the fields of astrobiology, mechanobiology, and bioengineering. Microgravity can have significant effect on our cellular makeup. The reason why this occurs is not yet fully understood, prompting the need for more research of microgravity's effects on the cellular level. *Physcomitrella patens* is a common and simple model organism that has the ability to reprogram differentiated cells back to totipotency. This makes cell identity changes observable quickly and efficiently. Understanding this will allow for further action considering key issues such as growing food in space or biotechnology.

Innovation

The growth and photosynthetic rate of *Physcomitrella patens* has been studied extensively in the past. For example, Takemura et al. examined the effects of microgravity and hypergravity (higher g-force than on Earth) on *Physcomitrella patens* with two concurrent experiments on the ISS and on the ground.

However, the effects of microgravity on de-differentiation, a key mechanism in the response to wounding, have not been extensively studied.

Resources and Facilities:

Massachusetts Academy of Math and Science

A STEM-focused school located in Worcester in Central Massachusetts. This facility includes 3D printing technology, standard lab safety equipment, and other devices that allow for methods such as PCR analysis. This laboratory is overseen by Dr. Kevin Crowthers.

1. **Random Positioning Machine:**

3D-Printed Parts (using either PLA or PETG filament; designs sourced from the open-source GitHub repository CE-Random-Positioning-Machine by Core Electronics):

- 3D printer
- Experiment Platform
- Inner Frame
- Outer Frame
- End Cap
- Inner Gear
- Outer Gear
- Two Small Gear (Which will be attached to Micro Servo)
- Two Stand components

Non-3D Printed Parts:

- 6-wire 2A Slip Ring
- FS90MR Continuous Rotation Micro Servo
- Five Cable Small Cable ties
- Four Servo Screws
- Four M3x6 Bolts
- Long Header
- Breadboard
- Raspberry Pi Pico
- Micro Python
- Accelerometer (I^2C or SPI interface for easy connection to the Raspberry Pi Pico and a range of ±2g or ±4g to detect g-forces closer to zero. One may also use a smartphone application that provides an accelerometer and uses Velcro or rubber bands to attach it to the experimental platform.)

2. **Biological Materials**
   - *Physcomitrella patens* starter culture

- Four 25mm (about 0.98 in) Petri Dish
- White plant-growth LED light source
- Reagents listed in the Protocol from Cove et al. 2009 for driselase and plant culture
  - This includes:
  - Drisilase solution
  - Protonemal tissue dissected from P. Patens
  - Hemocytometer
  - BCB medium
  - D-Mannitol Solution
  - Protoplast regeneration medium for top and bottom layer
- CDK activity assay kit
- Microtubule Organization Assay (e.g. tubulin immunostaining reagents)

3. **Laboratory Equipment**
   - Laminar flow hood
   - Standard micropipettes and sterile tips
   - Light microscope/fluorescence microscope, depending on the assay
   - PCR and gel equipment
   - Centrifuge

Section III: Project Goals and Methodology

Specific Aim #1:

Construct the low-budget Random Positioning Machine design provided by Open Electronics. he objective is to successfully build the open source design and test this design using an accelerometer. Our approach will involve the CE-Random-Positioning-Machine GitHub repository. Onshape files on the GitHub will be 3D printed using PLA or PETG filament. Non-3D printed materials are listed in the *Resources and Facilities* section. Once the materials are created and gathered, they will follow the assembly instructions provided by the repository. Next, the Raspberry Pi Pico will be configured for

controlling the speed of the Micro Servos using Micro Python. The code is also provided by the GitHub Repository (OpenElectronics, 2025). For our own testing (not dictated by the repository), we will attach an accelerometer near the Experiment Platform (for maximum accuracy to the acceleration environment experienced by the sample) and configure this accelerometer to the Raspberry Pi Pico. If we use an accelerometer on a mobile phone, this step can be skipped. Our rationale for this approach is to both create and test a Random Positioning Machine to ensure that the device is configured properly and replicates microgravity as efficiently as possible.

Justification and Feasibility.

These methods are justified because a Random Positioning Machine (RPM) is one of the most accessible and validated tool for simulating microgravity without venturing to outer space. This works by continuously and randomly rotating a sample across multiple axes, averaging the gravity vector to near zero (which will be measured by the accelerometer). This can disrupt cells, tissues, or organisms by preventing them from establishing a stable gravitational reference frame. RPMs are expecially appropriate for cellular reprogramming, stem-cell behavior, and regeneration. This is due to these processes being highly sensitive to mechanical forces, often influencing their morphology.

Additionally, by using a low-budget, open-source RPM, the project is able to be completed with minimal expendages. Furthermore, the open-source documentation provides key information about the assembly of such devices, including a step by step guide on how to assemble them.

Specific Aim #2:

Generate the protoplasts using a driselase enzyme solution. This will be achieved using the protocol dictatied by Cove et al. For culturing the moss and using the driselase solution. Key parts of the protocol include using the protonemal tissue (dissected from the model organism).

Justification and Feasibility:

This is a crucial step in reprogramming the protonomal tissue. Protoplasts are a stripped version of the protonomal tissue, and by getting rid of the cell wall, the reprogramming process begins, as the plant interprets this loss as a wound. This allows us to move these cells to a petri dish for testing inside the RPM, and for assays later in the testing.

Summary of Preliminary Data. The description of the preliminary data should help to justify the specific aim and your experimental approach. Make sure to wrap the text around the figure and include a caption to provide context. This would be the preliminary data that you have acquired during your research process.

Figure 1: Pacific sand lance (*Ammodytes hexapterus*) burrowing into the sand. Mandy Lindeberg, NOAA/NMFS/AKFSC - http://www.photolib.noaa.gov/htmls/fish1917.htm

Expected Outcomes. The overall outcome of this aim is to ….. This knowledge will be used for…..

Potential Pitfalls and Alternative Strategies. We expect….

Specific Aim #2:

Please follow the section format described above for an individual-specific aim.

Section III: Resources/Equipment

Section V: Ethical Considerations

Section VI: Timeline

Section VII: Appendix

Section VIII: References

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