Project Notes:

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

Name: Das, Aashriya

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Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
Diseases that can occur and their factors Are there stem cells in menstrual effluent?			
Ways to accessibly study Space Medicine	Article #4		9/7/25
What are the gaps in Space Medicine Research in every discipline of medicine, and what can we do to fill these gaps?	Article #1	Cells	8/20/25

How do scientists discover the underlying mechanisms that cause certain events when exposed to microgravity?

What are the implications Of simulated microgravity In health care? **Article #9** **ScienceDirect 9/7/25**

How do you culture Plant Stem Cells?

How do you create a DIY clinostat? **Patent 1** **Justia 10/26/25**

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Literature Search Parameters:

These searches were performed between (Start Date of reading) and XX/XX/2019.
List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
WPI Gordon Library Search Engine	Simulations in space medicine	I found three articles I was interested in: "Simulation and control for telerobots in space medicine", "Motigravity: A New VR System to Increase Performance and Safety in SpaceOperations Simulation and Rehabilitation Medicine", and "Behavior of stem cells under outer-space microgravity and ground-based microgravity simulation".
WPI Gordon Library Search Engine	epilepsy OR seizures in space OR outerspace	I found these articles: **Psychology in Outerspace: Personality, Individual Difference, and Demographic Predictors of Beliefs About Extraterrestrial Life, Analysis of High-Dimensional Phase Space via Poincaré Section for Patient-Specific Seizure Detection, Glia and epilepsy: excitability and inflammation,**

WPI Gordon Library Search Engine	Microgravity AND seizures OR epilepsy	**Astrocytes and epilepsy**
WPI Gordon Library Search Engine	Mesenchymal Stem Cells AND Menstrual Blood Derived Stem Cells	**Small extracellular vesicles from menstrual blood-derived mesenchymal stem cells(MenSCs) as a novel therapeutic impetus in regenerative medicine**
WPI Gordon Library Search Engine	Microgravity AND Neuron (Revisit if more resources are needed)	**Implanted multichannel microelectrode array for simultaneous electrophysiological signal detection of hippocampal CA1 and DG neurons of simulated microgravity rats,Extracting oxygen from Martian air and growing proteins, neurons in microgravity, The role of different glutamate receptors in the mediation of glutamate-evoked excitation of red nucleus neurons after simulated microgravity in rat,Morphological and physiological changes in mature in vitro neuronal networks towards exposure to short-, middle- or long-term simulated microgravity,**

Influence of Microgravity on Spontaneous Calcium Activity of Primary Hippocampal Neurons,The simulated microgravity enhances the differentiation of mesenchymal stem cells into neurons, Combined Exposure to Simulated Microgravity and Acute or Chronic Radiation Reduces Neuronal Network Integrity and Survival,Formation and Structure of Transplantable Tissue Constructs Generated in Simulated Microgravity from Sertoli Cells and Neuron Precursors,Adoption of microfluidic MEA technology for electrophysiology of 3D neuronal networks exposed to suborbital conditions,Delayed Maturation of Oligodendrocyte Progenitors by Microgravity: Implications for Multiple Sclerosis and Space Flight,Effects of microgravity on human iPSC-derived neural organoids on the International Space Station, Alterations in CNS-derived blood biomarkers during 30

days simulated microgravity,Double-Step Paradigm in Microgravity: Preservation of Sensorimotor Flexibility in Altered Gravitational Force Field, Matrix Deformation with Ectopic Cells Induced by Rotational Motion in Bioengineered Neural Tissues, Space Flight Enhances Stress Pathways in Human Neural Stem Cells, Space Microgravity Alters Neural Stem Cell Division: Implications for Brain Cancer Research on Earth and in Space,PROJECT PROPOSAL: REJUVENATION OF BRAIN NEURONS USING QUANTUM ENTANGLEMENT IN NUCLEOTIDES AND CRYPTOCHROMES

Ecosia	Expenses of simulated microgravity in medicine	A scoping review on microgravity medicine: Challenges and breakthroughs in space healthcare
WPI Gordon Library Search Engine	Biological characteristics of mesenchymal stem cells	Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins
		; Simulated Microgravity Reduces Proliferation and Reorganizes the Cytoskeleton of Human Umbilical Cord Mesenchymal Stem Cells
WPI Gordon Library Search Engine	Umbilical Cord Mesenchymal Cells AND simulated microgravity	
Ecosia	literary reviews for technology that simulates microgravity	**Simulated Microgravity: Critical Review on the Use of Random Positioning Machines for Mammalian Cell Culture**
		Lab-on-a-Chip Technologies for Microgravity Simulation and Space Applications
WPI Gordon Library	c. elegans in microgravity	

; A novel microfluidic capture and monitoring method for assessing physiological damage of C. elegans under microgravity

Notch receptor GLP-1 regulates toxicity of simulated microgravity stress by activating germline-intestine communication of insulin signaling in C. elegans

Worms in Space: Epigenetic Response of C. Elegans in Simulated Microgravity

Clinorotation to Simulate Microgravity: Defining a Model Gravitome in C. elegans

https://www.nature.com/npjmgrav/

Discoveries from human stem cell research in space that are relevant to advancing cellular therapies on Earth

WPI Gordon Search Engine

Comparative analysis on bMSCs OR bone marrow mesenchymal stem cells and umbilical cord derived mesenchymal stem cells

Comparison of in vitro -cultivation of human mesenchymal stroma/stem cells derived from bone marrow and umbilical cord: In vitro -cultivation of

Commented [DA1]: The aim of these papers is to explore a potential backup project

human mesenchymal stroma/stem cells

The Protein Content of Extracellular Vesicles Derived from Expanded Human Umbilical CordBlood-Derived CD133+ and Human Bone Marrow-Derived Mesenchymal Stem Cells Partially Explains Why both Sources are Advantageous for Regenerative Medicine

Proteomic analysis of porcine mesenchymal stem cells derived from bone marrow and umbilical cord: implication of the proteins involved in the higher migration capability of bone marrow mesenchymal stem cells

Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement

An International Society for Cell and Gene Therapy Mesenchymal Stromal Cells (MSC)

OpenAI	Are there any journal articles dictating methods and procedures for handling mesenchymal stem cells? Are there any protocols for menSCs?	Committee perspectives on International Standards Organization/Technical Committee 276 Biobanking Standards for bone marrow-MSCs and umbilical cord tissue–derived MSCs for research purposes Mesenchymal stromal cells: clinical challenges and therapeutic opportunities A feasible method for the isolation of mesenchymal stem cells from menstrual blood and their exosomes
Google Patent	Biology in Microgravity	**Microfluidic Assemblies for Studying the Effects of Space Travel on Human Brain Cells**

Tags:

Tag Name	
/spacemed	/technology

/important	/issuesinfield
/diseasedetection	/epilepsyresearch
/neuronsinmicrogravity	/regenerativemedicine
/tutorial	/mesenchymalstemcells
/backup	
/plantstemcells	

Article #1 Notes: Human Health during Space Travel: State-of-the-Art Review

Article notes should be on separate sheets

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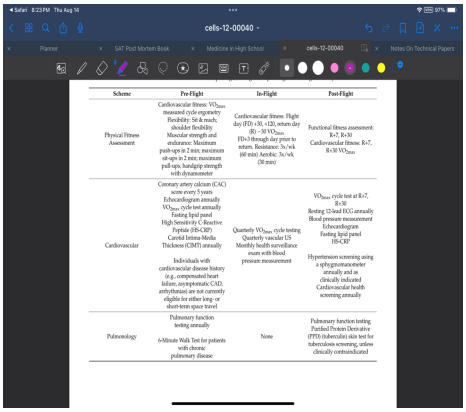
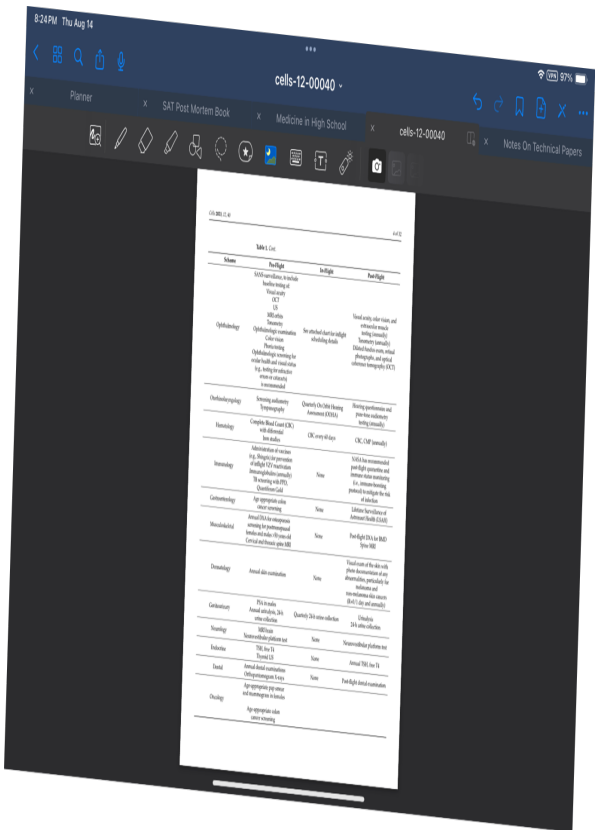
Source Title	Human Health during Space Travel: State-of-the-Art Review
Source citation (APA Format)	Krittanawong, C., Singh, N. K., Scheuring, R. A., Urquieta, E., Bershad, E. M., Macaulay, T. R., Kaplin, S., Dunn, C., Kry, S. F., Russomano, T., Shepanek, M., Stowe, R. P., Kirkpatrick, A. W., Broderick, T. J., Sibonga, J. D., Lee, A. G., & Crucian, B. E. (2022, December 22). *Human health during Space Travel: State-of-the-art review*. Cells. https://pmc.ncbi.nlm.nih.gov/articles/PMC9818606/
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC9818606/
Source type	Journal Article
Keywords	human health, space travel, space mission, space exploration, space radiation, microgravity
#Tags	/spacemed
Summary of key points + notes (include methodology)	**The article discusses the effects of different aspects of human health during prolonged durations in space, including the cardiovascular, gastrointestinal, immune, hematologic (conditions related to the blood, such as space anemia or hemolytic anemia), oncological (cancer), neurologic, ocular, and behavioral systems. The basics of this article include a discussion of exogenous factors such as space radiation (which has higher LET (Linear Energy Transfer) neutrons than radiation from atomic bombs on Earth) and microgravity -- the main concern with space radiation being the risk**

	of cancer in every body system and the alteration of the synthesis of our gut biomes, while microgravity causes viral reactivation, motion sickness, atrophy of the muscle/bone, orthostatic tolerance, and changes in anatomy. Fortunately, the article discusses potential solutions to these problems such as an AI space suit that can monitor the individual, robot-assisted surgery (for cleaner procedures in space), or anti-motion sickness AI.
Research Question/Problem/ Need	**What are the gaps in Space Medicine Research in every discipline of medicine, and what can we do to fill these gaps?**
Important Figures	

Scheme	Pre-Flight	In-Flight	Post-Flight
Physical Fitness Assessment	Cardiovascular fitness: VO_{2max} measured cycle ergometry Flexibility: Sit & reach; shoulder flexibility Muscular strength and endurance: Maximum push-ups in 2 min; maximum sit-ups in 2 min; maximum pull-ups; handgrip strength with dynamometer	Cardiovascular fitness: Flight day (FD) +30, +120, return day (R) – 30 VO_{2max} FD+3 through day prior to return. Resistance: 3x/wk (60 min) Aerobic: 3x/wk (30 min)	Functional fitness assessment: R+7, R+30 Cardiovascular fitness: R+7, R+30 VO_{2max}
Cardiovascular	Coronary artery calcium (CAC) score every 5 years Echocardiogram annually VO_{2max} cycle test annually Fasting lipid panel High Sensitivity C-Reactive Peptide (HS-CRP) Carotid Intima-Media Thickness (CIMT) annually Individuals with cardiovascular disease history (e.g., compensated heart failure, asymptomatic CAD, arrhythmias) are not currently eligible for either long- or short-term space travel	Quarterly VO_{2max} cycle testing Quarterly vascular US Monthly health surveillance exam with blood pressure measurement	VO_{2max} cycle test at R+7, R+30 Resting 12-lead ECG annually Blood pressure measurement Echocardiogram Fasting lipid panel HS-CRP Hypertension screening using a sphygmomanometer annually and as clinically indicated Cardiovascular health screening annually
Pulmonology	Pulmonary function testing annually 6-Minute Walk Test for patients with chronic pulmonary disease	None	Pulmonary function testing Purified Protein Derivative (PPD) (tuberculin) skin test for tuberculosis screening, unless clinically contraindicated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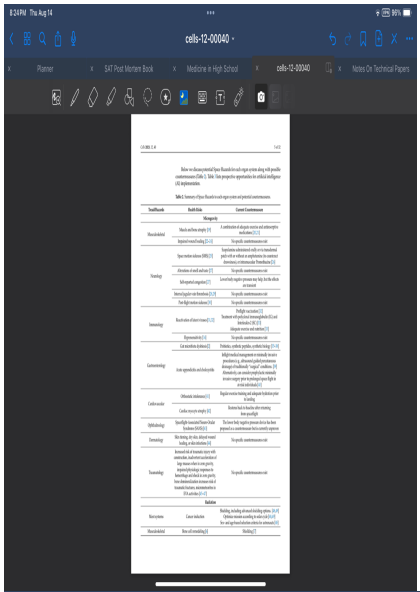
Notes On Technical Papers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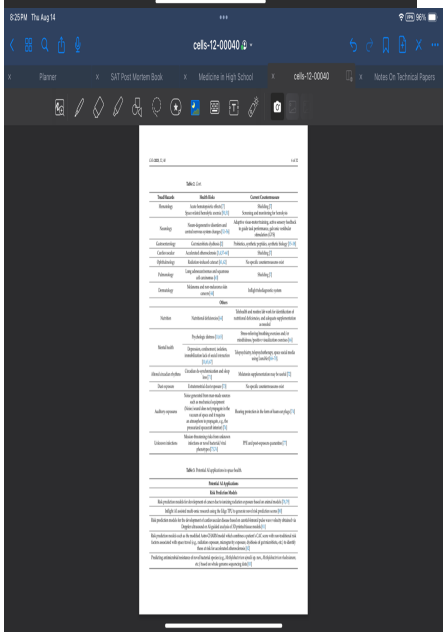
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Notes On Technical Papers

Figure 1: Common Preflight Medical Exams Today (Photos above)

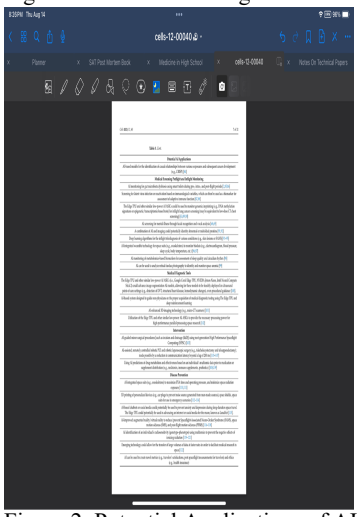


Figure 2: Potential Applications of AI in Preflight Diagnosis (Photo Above)

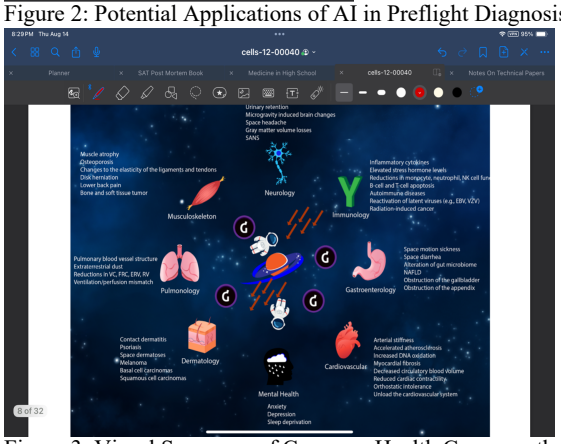


Figure 3: Visual Summary of Common Health Concerns that occur in Space

Figure 4: Visual Summary of Potential Solutions to Common Issues

VOCAB: (w/definition)

- **Aerobic Capacity:** This is the maximum rate at which an individual's body can take in and use oxygen during intense exercise. It's also known as **maximal oxygen uptake** or **VO_2 max** and is a key indicator of cardiorespiratory fitness
- **Diastolic Blood Pressure:** This is the pressure in your arteries when your heart rests between beats. In a blood pressure reading (e.g., 120/80 mmHg), the diastolic pressure is the **bottom number** (80). It reflects the minimum pressure exerted on the artery walls.
- **Physiologic Hypertrophy:** This is a healthy, adaptive enlargement of an organ or tissue, particularly the heart muscle (myocardium), in response to increased physical demand like rigorous athletic training. This "athlete's heart" is a beneficial adaptation, unlike pathological hypertrophy which is caused by disease (e.g., chronic high blood pressure).
- **Orthostatic Hypotension/Intolerance:** A condition characterized by a sudden drop in blood pressure when a person stands up from a sitting or lying position. This can cause symptoms like dizziness, lightheadedness, and fainting (syncope) because gravity pulls blood into the legs, temporarily reducing blood flow to the brain.
- **Hydrostatic Forces:** These are the forces exerted by a fluid at rest due to the force of gravity. In the body, hydrostatic pressure is the force exerted by blood against the capillary walls, pushing fluid out of the blood vessels and into the surrounding tissues.
- **Vertical Gravitational:** This term refers to the force or gradient exerted by gravity along the vertical axis of a body. On Earth, this force pulls everything towards the planet's center, influencing fluid distribution and pressure within the cardiovascular system.

	• **Internal Jugular Vein Thromboses:** This is the formation of a **blood clot (thrombus)** inside the internal jugular vein, a major blood vessel in the neck that drains blood from the brain, face, and neck. • **Anticoagulation:** The therapeutic process of using drugs (**anticoagulants**, or blood thinners) to prevent the formation of blood clots or to prevent existing clots from getting larger. • **Intracerebral Hemorrhage:** A type of stroke caused by **bleeding directly within the brain tissue**. It is often caused by a ruptured blood vessel and can lead to significant brain damage. • **Subdural Hematoma:** A collection of blood that forms between the inner layer of the dura mater and the arachnoid mater, which are two of the protective layers surrounding the brain. It is typically caused by a head injury. • **Atrial Fibrillation (AFib):** An irregular and often very rapid heart rhythm (arrhythmia) that can lead to blood clots, stroke, heart failure, and other heart-related complications. In AFib, the heart's upper chambers (atria) beat chaotically and out of sync with the lower chambers (ventricles). • **Supraventricular Arrhythmias:** A category of abnormally fast heart rhythms (tachycardias) that originate in the heart tissue **above the ventricles**, such as in the atria or the atrioventricular (AV) node. • **Echocardiography:** A non-invasive diagnostic test that uses sound waves (**ultrasound**) to create moving pictures of the heart. It allows doctors to assess the heart's size, shape, and function, including the heart chambers, valves, and blood flow. • **Endothelial Cells:** A single layer of specialized cells that line the interior surface of all blood vessels and lymphatic vessels. These cells form a crucial barrier between the blood and the rest of the body tissue and are vital for regulating blood flow, preventing clotting, and managing inflammation. • **Ionizing Radiation:** Radiation with enough energy to detach electrons from atoms or molecules, thereby **creating ions**. This type of radiation, which includes X-rays, gamma rays, and galactic cosmic rays, can damage DNA and other cellular structures. • **Accelerated Atherosclerosis:** The premature or rapid development of atherosclerosis, which is the buildup of fats, cholesterol, and other substances in and on the artery walls (plaque). This accelerated process can be triggered by factors like chronic inflammation or exposure to certain types of radiation. • **Angiogenesis:** The physiological process through which **new blood vessels form** from pre-existing vessels. It's a normal and vital process in growth and wound healing, but it also plays a key role in

	the growth of cancerous tumors. • **Linear Energy Transfer (LET):** A measure of the energy transferred by ionizing radiation to the material it travels through per unit distance. High-LET radiation (like galactic cosmic rays) deposits a large amount of energy in a small area, causing more concentrated biological damage than low-LET radiation (like X-rays). • **DNA Methylation:** An **epigenetic mechanism** where a methyl group (CH_3) is added to a DNA molecule, typically at a cytosine base. This process can change the activity of a DNA segment without changing its sequence, often acting to repress gene transcription. • **Gray (Gy) (unit):** The International System of Units (SI) unit for the absorbed dose of ionizing radiation. One gray is defined as the absorption of one joule of radiation energy per kilogram of matter (1Gy=1J/kg). • **Galactic Cosmic Ray (GCR):** High-energy particles, primarily consisting of atomic nuclei stripped of their electrons, that originate from outside our solar system, likely from supernova explosions. They travel at nearly the speed of light. • **Galactic Cosmic Radiation (GCR):** The stream of **Galactic Cosmic Rays** that permeates interstellar space. It is a **major health concern** for astronauts on long-duration missions beyond Earth's protective magnetosphere. • **Cholecystitis: Inflammation of the gallbladder**, a small organ beneath the liver. It's most often caused by gallstones blocking the tube leading out of the gallbladder. • **Appendicitis: Inflammation of the appendix**, a finger-shaped pouch that projects from the large intestine. It is a medical emergency that usually requires surgery to remove the appendix. • **Non-alcoholic Fatty Liver Disease (NAFLD):** A condition characterized by the accumulation of excess fat in the liver of individuals who consume little to no alcohol. It is the most common cause of chronic liver disease in many parts of the world. • **Epstein-Barr Virus (EBV):** A member of the herpesvirus family (HHV-4) and one of the most common human viruses. It is best known for causing **infectious mononucleosis** ("mono"). • **Varicella-Zoster Virus (VZV):** The herpesvirus (HHV-3) responsible for causing **chickenpox** (varicella) in children and **shingles** (herpes zoster) in adults, which occurs when the latent virus reactivates. • **Cytomegalovirus (CMV):** A common herpesvirus (HHV-5) that infects most people at some point in their lives, usually without causing symptoms. However, it can cause serious disease in people

with weakened immune systems and in babies infected before birth.

- **Levothyroxine:** A synthetic form of the thyroid hormone thyroxine (T4). It is a prescription medication used to treat an underactive thyroid gland (**hypothyroidism**) and to prevent certain types of goiters.
- **Karman Line:** The internationally recognized boundary between Earth's atmosphere and outer space, located at an altitude of **100 kilometers (62 miles)** above mean sea level.
- **Hypobaric + Hypoxia:** A combined condition of **low atmospheric pressure (hypobaric)** and **low oxygen levels (hypoxia)**. This environment is encountered at high altitudes and in depressurized spacecraft, posing significant physiological challenges.
- **Hypercapnia:** A condition arising from **too much carbon dioxide (CO_2) in the blood**. In spaceflight, it can occur if life support systems fail to adequately remove CO_2 from the cabin air.
- **Inflammation:** The body's complex biological response to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli and initiate the healing process.
- **Space Motion Sickness (SMS):** A form of motion sickness that affects astronauts during their first few days in a microgravity environment. It is caused by a conflict between the sensory information from the eyes and the vestibular system (inner ear), which is no longer correctly interpreting motion and orientation in the absence of gravity.
- **Intracranial Pressure (ICP):** The pressure inside the skull, exerted by brain tissue, blood, and cerebrospinal fluid (CSF). In spaceflight, a headward fluid shift is thought to potentially elevate ICP.
- **Post-flight Motion Sickness:** A form of motion sickness that can occur after an astronaut returns to Earth. The brain and vestibular system, having adapted to microgravity, must then re-adapt to the constant pull of Earth's gravity.
- **Spaceflight Associated Neuro-Ocular Syndrome (SANS):** A unique medical condition observed in astronauts on long-duration missions. It is characterized by a set of changes to the eye, including swelling of the optic disc (papilledema), flattening of the back of the eyeball, and vision changes. Its exact cause is still under investigation but is thought to be related to the headward fluid shift in microgravity.
- **Intraocular Pressure (IOP):** The fluid pressure inside the eye. It is an important aspect of eye health, and changes in IOP are monitored in astronauts as part of SANS research.

Cited references to follow up on	NASA Twins Study: A multidimensional analysis of a year-long human spaceflight
Follow up Questions	- **What is the radiation dosage needed for negative Cardiovascular effects to manifest? (The type and quality of radiation matters)** - **Preventative Strategies for AF and internal jugular vein thrombosis?** - **What about bacteria in space make them more resistant to current antibodies? Are these bacteria from space or brought to space from Earth? (Clarifying Question)** - **Plasma Volume Physiology?** - **Non-Alcoholic Fatty Liver Disease?** - **How can we prevent B-cell and T-cell apoptosis in space?** - **Can we use AI to improve the mental health of people facing isolation in the space station?** - - **Human 3D Microvessel models (This sounded cool, so I wanted to investigate it)**

Article #2 Notes: A biosensory **µvessel-gravity device for advancing vascular analysis in space medicine**

Article notes should be on separate sheets

Source Title	A biosensory **µvessel-gravity device for advancing vascular analysis in space medicine**
Source citation (APA Format)	Xu, K., Wang, X., Bai, H., Wu, G., Zhang, W., Zhou, J., Zhang, P., Zhang, X., Peng, B., Voelcker, N. H., Gao, F., & Li, J. (2025). A biosensory µvessel-gravity device for advancing vascular analysis in space medicine. *Biosensors & Bioelectronics, 268, Article 116923*. https://doi.org/10.1016/j.bios.2024.116923
Original URL	https://www-sciencedirect-com.ezpv7-web-p-u01.wpi.edu/science/article/pii/S0956566324009308
Source type	Journal Article
Keywords	Vessel-on-a-chip; Microgravity; Cell senescence; Endothelial cells; Vascular smooth muscle cells
#Tags	/spacemed /technology
Summary of key points + notes (include methodology)	Notes - Study found that simulated Microgravity causes biological aging in endothelial and vascular smooth muscle cells when grown in separate µvessels. When the cells are grown in the same µvessel, the endothelial cells stopped aging while the vascular smooth muscle cells still aged. - The continuous flow shear stress also delayed biological aging in endothelial cells and enhances tight junction integrity under Microgravity. - Study found that Microgravity causes increased stiffness, thickness, fibrosis, and aging (senscence). - Researchers have developed this µvessels in order to study why ECs (endothelial cells) and VSMCs (vasular smooth muscle cells) sense and respond to microgravity, and whether these interactions fuel aging like changes caused by microgravity. - Design: Consists of 4 independent functional units to mimic the layers of the blood vessel. Every unit has an upper and lower channel separated by a polyethylene terephthalate (PET) porous membrane. The multi-unit design allows for connections to other

	vessels/objects either in series or parallel. Before cell seeding, the µvessels were coated with Matrigel, a material that simulates the extracellular matrix. - ECs were placed on the upper channel while VSMCs were placed in the top layer of the lower channel. Certain molecules (such as alpha-smooth muscle actin or PECAM-1) were stained in order to make the cells visible. In order to simulate microgravity in the clinostat for the µvessels, a container was fabricated using 3D printing. This container could hold up to chips at a time. It has an air filter and a locking mechanism for airtight sealing/microfluidic chip operation. - Note: 12 dyn/cm^2 – What is dyn? - **Results: This experiment was a continuation of a previous study that looked at the carotid artery of rats in microgravity. After 48 hours of exposure to microgravity in the current study, there was a significant increase in protein expression of p53, p21, and y-H2AX – bio markers related to senescence.** - **Limitations: The device has several limitations, including not fully replicating complex lumen structure of blood functions essential to vascular physiology. Additionally, only being able to house 4 cells can make more comprehensive molecular biology studies more difficult. Future research should use single-cell transcriptomics and proteomics. /important/technology/spacemed**
Research Question/Problem/ Need	Due to the limitations of traditional cell cultures and animal models, studying the cardiovascular system in microgravity was difficult. This research aimed to create a device that functions as an organ-on-a-chip

Important Figures	

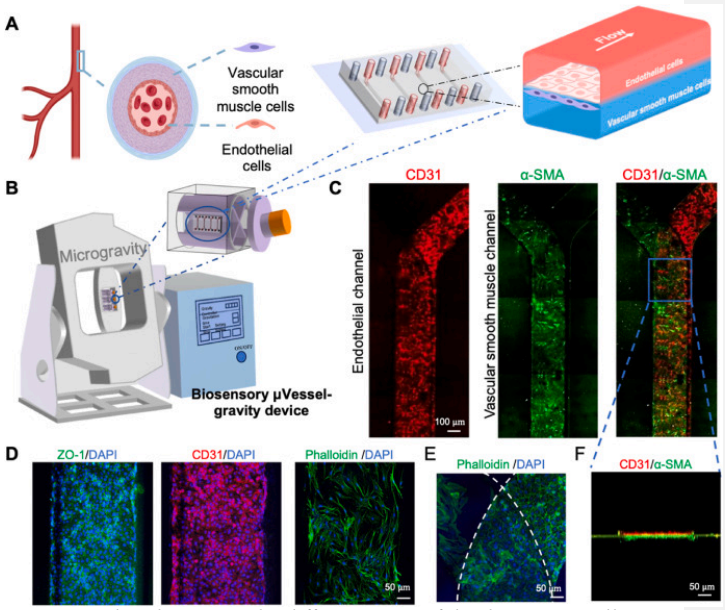
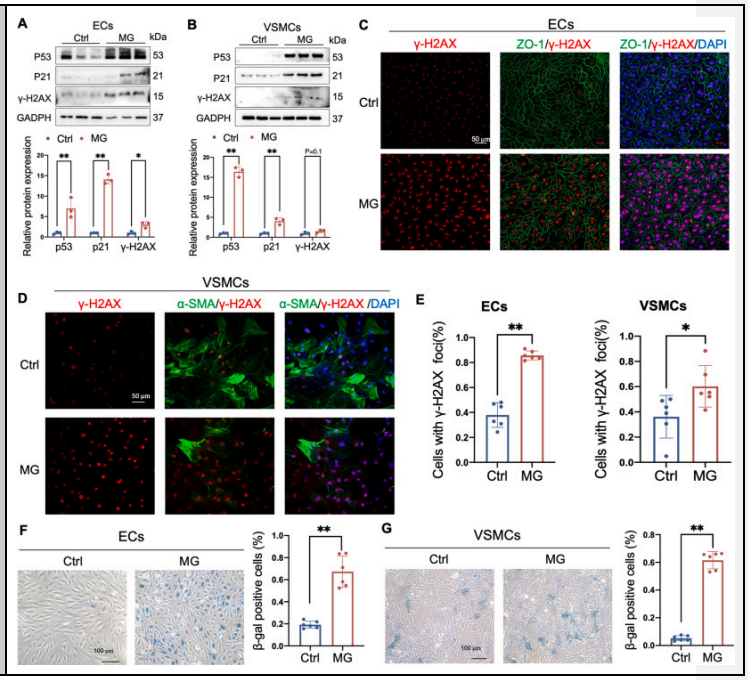


Figure 1: This showcases the different parts of the device, as well as immunofluorescence images of stained blood vessel cells.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Relative protein expression
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p21
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ZO-1/γ-H2AX
ZO-1/γ-H2AX/DAPI
D
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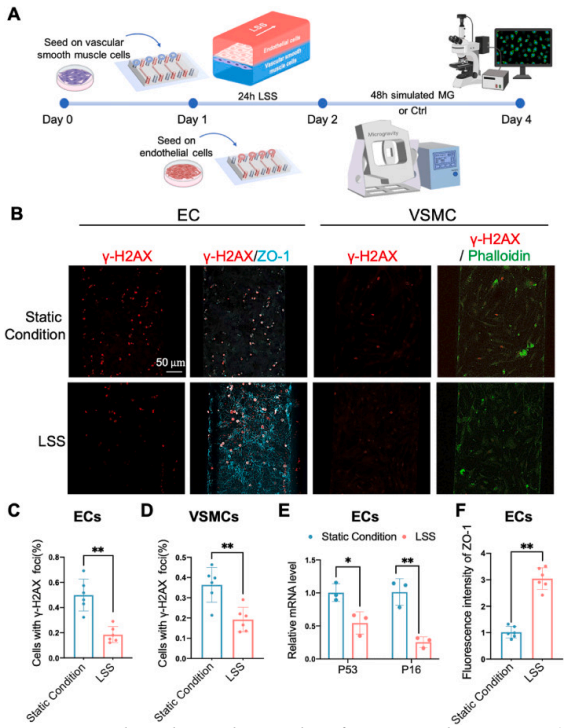
E
Cells with γ-H2AX foci(%)
F
β-gal positive cells (%)
G

	 *Figure 2 and 4: Shows the results of Laminar shear stress (LSS) on the uvessel in microgravity vs. Stable conditions. LSS has been found to play a crucial role in regulating endothelial function. The effects of LSS on vascular cell senescence in microgravity is relatively unknown, which is why the researchers decided to include it. ZO-1 expression is an indicator of intefrity and functionality of endothelial tight junctions. The data shows the LSS delays senscence in ECs and VSMCs.*
VOCAB: (w/definition)	• **Organ-on-a-chip:** A microfluidic cell culture device, typically the size of a USB stick, that contains continuously perfused chambers inhabited by living cells arranged to simulate the physiology of a human organ. These devices are used to model human physiology and disease, and to test the efficacy and toxicity of new drugs with greater accuracy than traditional cell cultures. • **Clinostat:** A device used in gravitational biology research to

simulate microgravity. It works by slowly and constantly **rotating a biological sample**, such as a plant or a cell culture, around one or two axes. This rotation averages the gravitational pull over time, effectively nullifying the net gravitational vector's influence on the sample, which mimics the conditions of free-fall or spaceflight. /important

- **3D Printing:** Also known as **additive manufacturing**, this is a process of creating a physical, three-dimensional object from a digital design. The object is built up **layer by layer** from a material like plastic, metal, or a biological gel. In science, this technology is used for everything from creating custom lab equipment to **bioprinting**, where cells and growth factors are combined to create tissue-like structures.
- **Flow shear stress modeling:** An experimental or computational technique used to study the effects of **shear stress**—the frictional force exerted by a moving fluid on biological cells. In cardiovascular research, for example, models are created to simulate how blood flow exerts shear stress on the endothelial cells lining blood vessels. This helps scientists understand how mechanical forces influence cell behavior, gene expression, and the development of diseases like atherosclerosis.

- Endothelial Cells: One of the primary components of blood vessel walls; A single layer of specialized cells that line the interior surface of all blood vessels and lymphatic vessels. These cells form a crucial barrier between the blood and the rest of the body tissue and are vital for regulating blood flow, preventing clotting, and managing inflammation.

- **Senescence:** This is the process of biological aging. At the cellular level, it refers to a state where cells have irreversibly stopped dividing but remain metabolically active. This contributes to the overall aging of an organism and is linked to age-related diseases.
- **Vascular Smooth Muscle Cells (VSMCs):** These are the primary components of blood vessel walls. Their main function is to control the diameter of blood vessels, which in turn regulates blood pressure and blood flow.
- **Mono-culture:** A mono-culture is the cultivation or growth of a single type of cell or organism in a laboratory setting. This method is used to study a specific cell type in isolation.
- **µvessel (Microvessel):** The term µvessel, or microvessel, refers to very small blood vessels like arterioles, venules, and capillaries. They are responsible for microcirculation, which is the flow of blood in the smallest parts of the circulatory system. The prefix "µ" is the

standard symbol for "micro" or one-millionth.

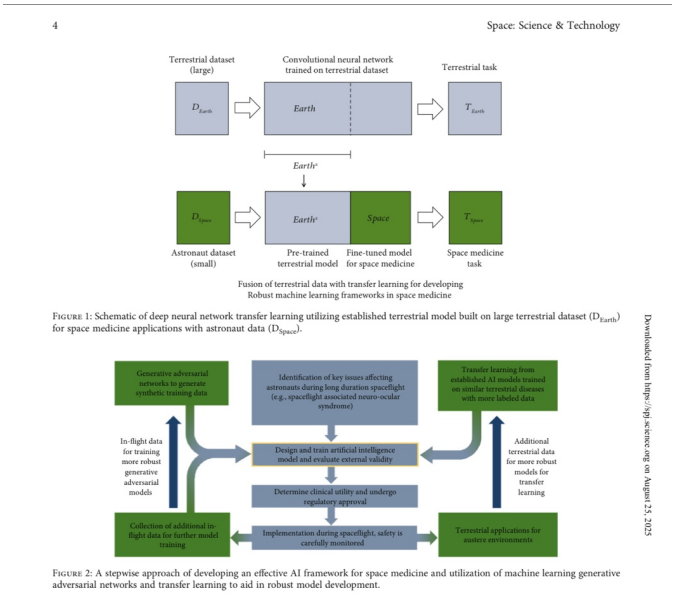
- **Junction Integrity:** This refers to the stability and strength of the connections between cells. These connections, called cell junctions, act as a seal that regulates the passage of molecules and cells through a tissue barrier.
- **Mechanosensors:** These are molecules or cellular structures that detect and respond to physical forces like pressure or tension. They convert these mechanical forces into biochemical signals that can influence cell behavior.
- **Carotid Artery:** This is a major artery on each side of the neck that supplies oxygenated blood from the heart to the head, including the brain and face.
- **Internal Elastic Lamina (IEL):** a small opening that maintains cell-cell interactions between the intima and media layers of the blood vessel. This structure separates ECs and VSMCs. The ECs poortrude through the pores of this structure to establish contact with VMSCs
- **Polyethylene terephthalate:** A common polyester material used in the fibres of clothing, thermoforming, and containers for food. According to the article, this is a porous material.
-
- **Cell Seeding:** This is the process of transferring and plating cells in a culture vessel, like a petri dish or flask. The goal is to get a uniform density of cells for an experiment. Researchers often count cells with a hemocytometer to get a precise number, which is essential for reproducibility in experiments.
- **Phalloidin Staining:** This is a technique for visualizing **actin filaments** in cells. Phalloidin, a toxin from the death cap mushroom (*Amanita phalloides*), binds specifically and tightly to F-actin (filamentous actin). By conjugating phalloidin to a fluorescent dye, researchers can stain and see the actin cytoskeleton under a microscope.
- **3D Confocal Imaging:** This is an advanced microscopy technique that uses a pinhole to block out-of-focus light from the specimen, producing a sharper image than a conventional microscope. By taking multiple images at different focal planes (a "Z-stack"), a computer can reconstruct a high-resolution 3D model of the cell or tissue. This allows researchers to visualize the spatial relationships of cellular components.
- **Western Blot Analysis:** A widely used technique to detect specific proteins in a sample. The process involves several steps:
    - **SDS-PAGE:** Proteins are separated by size using gel electrophoresis.
    - **Transfer:** The separated proteins are transferred from the gel to a membrane (e.g., nitrocellulose).
    - **Blocking:** The membrane is blocked to prevent non-

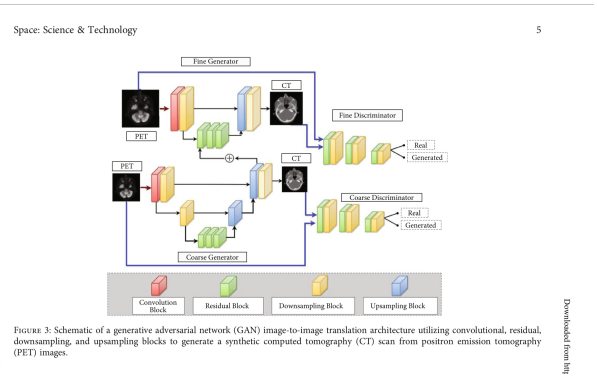
	specific antibody binding. ▪ **Antibody Probing:** The membrane is incubated with a primary antibody that binds to the protein of interest, followed by a labeled secondary antibody that binds to the primary antibody. ▪ **Detection:** The protein is visualized by a detection system (e.g., chemiluminescence). o **Senescence-Related markers:** This study included y-H2AX (a marker for double-strand DNA breaks and genomic instability),p16, and p53/p21 (both markers function in the activation of cell cycle arrest and cellular senescence). Beta-galactosidase is senescence-associated. Interleaukin (IL)-1 and IL-6 are phenotypes of cells going through senescence, causing inflammation. • **MG-induced cephalad fluid shift:** A condition where bodily fluids are redistributed to the upper body and head due to microgravity. Recent studies have shown that Piezo1 has a role in translating the altered fluid shift, causing aging-like changes in vasculature. This study found that knockdown of Piezo1 infact reduces senescence. /important • **Proteomics:** Proteomics is the large-scale study of **proteins**, which are the essential workhorses of the cell. While the **genome** (all of a person's DNA) is generally static, the **proteome** (all the proteins in a cell or organism) is dynamic, changing in response to a cell's environment or state. Proteomics aims to identify all the proteins expressed by a cell, determine their functions and structures, analyze how they interact with each other, and study any modifications they undergo. A key tool in proteomics is **mass spectrometry**, which can identify and quantify thousands of proteins in a single experiment. • **Single-cell transcriptomics:** Single-cell transcriptomics, often called single-cell RNA-sequencing (scRNA-seq), is a powerful technique for analyzing the gene expression of individual cells within a population. Unlike traditional "bulk" sequencing, which measures the average gene expression across thousands of cells, scRNA-seq reveals the unique **transcriptome** (the complete set of RNA transcripts) of each cell. This allows researchers to identify different cell types, discover rare cell populations, and trace developmental pathways, providing a much higher-resolution view of complex tissues and diseases.
Cited references to follow up on	Pospelova et al. 2019 – Pseudo-DNA damage response in senescent cells (I want to use this for my other project idea. I think I might be able to combine these two ideas of using a clinostat and organ on a chip)
Follow up Questions	How do you simulate microgravity? - Cinostat

	Why do vascular smooth muscle cells stop the aging of endothelial cells? How do ECs and VSMCs sense and respond to microgravity? Can organ on a chip technology be used for other organs?

Article #3 Notes: Challenges of Artificial Intelligence in Space Medicine

Source citation (APA Format)	Waisberg, E., Ong, J., Paladugu, P., Kamran, S. A., Zaman, N., Lee, A. G., & Tavakkoli, A. (2022). Challenges of Artificial Intelligence in Space Medicine. *Space: Science & Technology*, *2022*, Article 9852872. https://doi.org/10.34133/2022/9852872
Original URL	https://doaj.org/article/711d936a8d0a4be4bbf5a5d926724a62
Source type	Journal Article
Keywords	No Keywords in article
#Tags	/spacemed/issuesinfield
Summary of key points + notes (include methodology)	- This article claims that the future of AI in space medicine will be to detect, diagnose, and recommend treatments. One limitation of AI is that there is limited astronaut data for algorithm training. - Current AI technologies include telemedicine, wearable technology, augmented and virtual reality, and biosensors. - This article presents a scenario where humans must go to Mars in the year 2030. The distance from the space shuttle to Earth will cause a 5 to 20 minute long transmission delay. - There are different types of machine learning algoritms: unsupervised, supervised, reinforcement learning, self-supervised, multi-instance, and semi-supervised. - Many issues in space medicine have been encountered in dive and aviation medicine long before spaceflight existed. However, space does present it's unique challenges such as altered gravitational fields, radiation, confinement, and a hostile environment. /important - "In microgravity, the lack of mechanical loading on the musculoskeletal system results in a loss of bone mass + skeletal muscle atrophy"

	- The authors believe that monitoring anemia status in space can provide insights into SANS because of the neuro-ocular signs being similar to symptoms of SANS. - Example of current applications that use machine learning: OpticNet-71: Trained on Optical Coherence Tomography (OCT) images with Age-related Macular Degeneration, Diabetic Macular Edema, Drusen, and Choroidal Neovascularization. CheXNeXt can identify 14 diseases by learning from chest radiographs - The article suggests using GAN and established AI models for terrestrial diseases to implement AI. - The article also suggests that edge-computing is better than transmitting data to Earth because a. there is a time delay in data transmission and b. edge-computing reduces computational load and protects data privacy and prevents leaks. /important -
Research Question/Problem/ Need	At the moment, there are certain challenges AI faces in space medicine -- such as limited data – that make implementing AI into space medicine much more difficult. This article aims to resolve this issue.
Important Figures	 FIGURE 1: Schematic of deep neural network transfer learning utilizing established terrestrial model built on large terrestrial dataset (D_{Earth}) for space medicine applications with astronaut data (D_{Space}). FIGURE 2: A stepwise approach of developing an effective AI framework for space medicine and utilization of machine learning generative adversarial networks and transfer learning to aid in robust model development. *Figure 1 + 2: Figure 1 describes how Transfer Learning works in neural*

	networks while Figure 2 describes a potential AI framework we can work towards.  FIGURE 3: Schematic of a generative adversarial network (GAN) image-to-image translation architecture utilizing convolutional, residual, downsampling, and upsampling blocks to generate a synthetic computed tomography (CT) scan from positron emission tomography (PET) images. *Figure 3: Provides a visual for how GANs work*
VOCAB: (w/definition)	• **Deep Learning**: The usage of vast amounts of data to train multi-layered neural networks. • **Vestibulo-ocular:** refers to the connection between the vestibular system (responsible for balance and spatial orientation) and the ocular system (responsible for vision). The **vestibulo-ocular reflex (VOR)** is a key physiological mechanism that stabilizes images on the retina during head movements by producing eye movements in the opposite direction of the head movement. • **Orthostatism:** the medical term for the body's ability to maintain blood pressure and blood flow to the brain while a person changes from a lying or sitting position to a standing position. This process involves a series of physiological responses to counteract the effects of gravity, such as vasoconstriction and an increased heart rate. **Orthostatic hypotension** is a condition where this regulation fails, causing a sudden drop in blood pressure upon standing. • **Hemolysis:** is the rupture or destruction of red blood cells. This process releases hemoglobin into the surrounding fluid (plasma). Hemolysis can be caused by various factors, including certain toxins, infections, autoimmune disorders, or mechanical stress on the red blood cells. • **Papilledema:** the swelling of the optic disc, the part of the optic nerve that enters the eyeball. It is typically caused by increased intracranial pressure (pressure inside the skull) from conditions such as brain tumors, meningitis, or a cerebral hemorrhage.

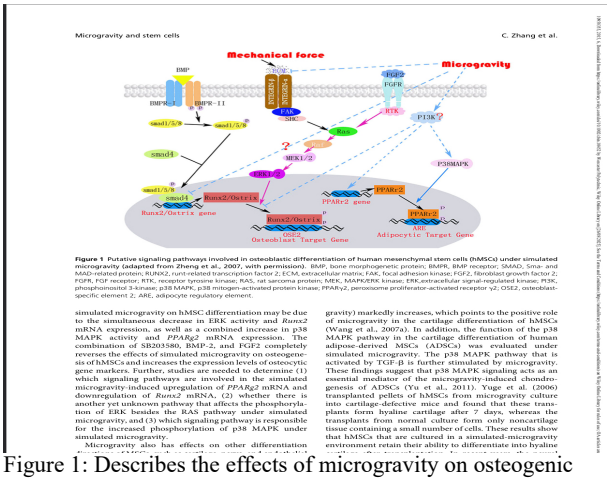
	Papilledema is a serious medical sign and often requires immediate attention. • **Optical Coherence Tomography:** a non-invasive imaging technique that uses light waves to create high-resolution, cross-sectional images of biological tissues, particularly the retina. It is analogous to an ultrasound but uses light instead of sound waves. OCT is widely used in ophthalmology to diagnose and monitor various retinal diseases like macular degeneration and diabetic macular edema. • **Age-related Macular Degeneration:** a progressive eye disease that affects the macula, the central part of the retina responsible for sharp, central vision. It is a leading cause of vision loss in older adults. AMD exists in two forms: **dry AMD**, characterized by the presence of drusen, and **wet AMD**, characterized by the growth of abnormal blood vessels (choroidal neovascularization). • **Diabetic Macular Edema:** a complication of diabetes caused by damage to blood vessels in the retina. This damage leads to fluid leaking into the macula, causing it to swell (edema). This swelling can blur or distort central vision. DME is a common cause of vision loss in people with diabetes. • **Drusen:** small, yellow deposits that accumulate under the retina. They are composed of lipids and proteins. While a few small drusen are a normal part of aging, a large number or large-sized drusen can be a sign of early-stage dry Age-Related Macular Degeneration. • **Choroidal Neovascularization:** the formation of new, abnormal blood vessels in the choroid, the layer of blood vessels between the retina and the sclera. These new vessels are often fragile and prone to leakage, which can lead to swelling and scarring of the macula. CNV is the key feature of wet Age-Related Macular Degeneration and is a significant cause of vision loss. • **Semantic Segmentation:** a computer vision task that involves classifying each pixel of an image with a corresponding class label (e.g., person, car, road). Unlike object detection, which draws a bounding box around objects, semantic segmentation provides a more granular understanding of the image by outlining the exact shape and location of objects. It is a fundamental task in fields like autonomous driving and medical image analysis. • **General adversarial networks (GANS):** A new type of deep learning technique consting of a generator and a discriminator (two architectors). The generator generates new images or data through a unique data distribution/translate one modality to a different one while the discriminator distinguishes between real and newly synthesized images. Use of GANS in medical imaging:

	synthetic data generation, image-to-image translation, and image segmentation. /important • **Fluorescein Angiography:** a diagnostic procedure used to visualize the blood vessels in the retina and choroid, the two layers at the back of the eye.This test helps ophthalmologists diagnose and monitor various retinal diseases. • **Transfer Learning:** Depp learning neural network learning technique which is used for instances where data is limited. Layers of neural network from an established convolutional neural network are transferred to a new frame. These layers are then trained on similar but larger datasets to increase their accuracy. The base network is then transfered to the new network. /important • **Six degree Head-down tilt bed rest:** Used to generate space data on Earth and can mimic the effects of microgravity on a person. However, this device can't be used to observe diseases that occur due to a long duration in space. However, this does not seem to be completely reliable, since the six-degree head-down tilt seemed to increase vascular density when the opposite happens in astronauts./important • **Edge-computing:** a distributed computing model that brings data processing and storage closer to the physical location where the data is being generated. Instead of sending all data to a centralized data center or cloud for processing, edge computing processes it at the "edge" of the network, which is often a local device or a nearby server. This approach is particularly useful for applications that require real-time analysis and low latency, as it minimizes the time it takes for data to travel back and forth. /important
Cited references to follow up on	NASA's Human Research Program has identified 30 astronaut health risks with hopes to creating countermeasures for them.
Follow up Questions	- Can we simulate disease progression in space to provide a temporary solution to the lack of actual data for AI? Will this be a reliable AI? - What effects does "increased distance" have on astronauts? Is it just the transmission delays? - What is the current impact of AI on terrestrial austere environments? - Why does the six-degree head-down tilt increase vascular density when astronauts don't seem to experience that themselves? - What is edge-computing and how can we incorporate it into AI for space medicine

Article #4 Notes: **Behavior of stem cells under outer-space microgravity and ground-based microgravity simulation**

Source Title	**Behavior of stem cells under outer-space microgravity and ground-based microgravity simulation *Revisit at a later date if more specific information is needed**
Source citation (APA Format)	Zhang, C., Li, L., Chen, J., & Wang, J. (2015). Behavior of stem cells under outer-space microgravity and ground-based microgravity simulation. *Cell Biology International*, *39*(6), 647–656. https://doi.org/10.1002/cbin.10452
Original URL	https://onlinelibrary-wiley-com.ezpv7-web-p-u01.wpi.edu/doi/full/10.1002/cbin.10452
Source type	Journal Article
Keywords	development; differentiation; microgravity; stem cells; tissue engineering
#Tags	/spacemed /issuesinfield
Summary of key points + notes (include methodology)	- Average G in microgravity is 10^-3 /important - Mouse Embryonic Stem Cells were the first embryonic stem cells (EMCs). For example, Wang et al. (2011) used a 3D Clinostat and a multidirectional G force generator to reproduce microgravity. The number of mESCs significantly decreased. However, the cell cycle was left unaffected, suggesting that the issue is adherence of cells. - Experiment 2 looked at the differentiation of ESCs by using hepatocyte-like cells in a rotating bioreactor and a biodegradable polymer scaffold(simulation of microgravity). Cells cultured in the rotating bioreactor could differentiate into hepatocyte-like cells with mature characteristics. The ones in the scaffold differentiated into liver cells. - Experiment 3 found that ESCs in microgravity can quickly form a large number of embryoid bodies. These cells could differentiate into a wide variety of cells. This suggests that microgravity

	stimulates differentiation. - Mouse embryonic fibroblasts: a study done by Kawara et al. Found that rat embryonic stem cells in microgravity were able to grow 8 times the number of it's 1 G counterpart even without leukemia inhibitory factor. - The effect of microgravity on the cytoskeletons of MSCs are easily visible /important - After 20 hours of simulated microgravity, human bone marrow cells found a large number of flat cells, a disrupted actin cytoskeleton, redistributed vinculin, and the expression of integrin alpha 2 is elevated. The number of cells that express VCAM-1 (Vascular Cell Adhesion Molecule 1) increases and the expression level of these molecules changes. - Osteogenic differentiation of MSCs are at the center of attention in respect to the musculoskeletal health of astronauts. A combination of inhibitors and activators including RUNX2, PPARy2, ERK and P38 phosphorylation through addition of BMP, FGF2 (ERK phosphorylation stimulating factor), and SB203580 (P38/MAPK inhibitory factor) can reverse the effects of osteogenic and adipogenic differentiation. /issuesinfield - Simulated microgravity increases phosphorylation of PPARg2 mRNA through an unknown signaling pathway. /important/issuesinthefield - Factors that affect the differentiation of MSCs: Telomerase activity, ***** - Further studies are needed to find which signaling pathways are involved in the simulated microgravity-induced upregulation of PPARg2 mRNA and downregulation of Runx2 mRNA, whether there is another pathway affecting the phosphorylation of ERK, and which signaling pathway is responsible for the increased phosphorylation of p38 MAPK under simulated microgravity. - Neural Differentiation of MSCs under microgravity /neuronsinmicrogravity: Neural cells under a clinostat experienced an increase in microtubule-associated protein 2 (MAP-2), tyrosine hydroxylase (TH), and choline acetyltransferase (CHAT). Neurotrophins such as nerve growth factor, brain-derived neurotrophic factor, and ciliary neurotrophic factor all increase. Simulated microgravity promotes differentiation towards a nucleus pulpous-like phenotype. TGF-Beta1 can enhance differentiation potential. - As for neural stem cells, the article concludes that microgravity promotes proliferation, or division, of neural stem cells by enhancing the mitochondria.

	- On the other hand, microgravity severely inhibits the migration of hematopoietic cells.
Research Question/Problem/ Need	What are the overall results of exposing stem cells such as Mesenchymal Stem Cells to simulated microgravity, microgravity, or hypergravity.
Important Figures	 Figure 1: Describes the effects of microgravity on osteogenic differentiation. Microgravity inhibits osteogenic differentiation and induces adipogenic differentiation.
VOCAB: (w/definition)	**Stem cells** are undifferentiated or partially differentiated cells that can differentiate into various cell types and proliferate indefinitely to produce more of the same stem cell. They serve as a repair system for the body. The two main types are embryonic stem cells, found in blastocysts, and adult stem cells, which are present in specific tissues like bone marrow and fat. **Regenerative medicine** is a field of medicine focused on developing treatments that restore, replace, or regenerate damaged or diseased cells, tissues, or organs. It includes approaches like cell therapy, tissue engineering, and the use of biomaterials and scaffolds. **Proliferation** is the rapid increase in the number of cells through cell division. In a biological context, it refers to cell growth and multiplication.

	Endocrine disorders are conditions caused by an imbalance of hormones or problems with the glands that produce them. They can affect metabolism, growth, and development. Examples include diabetes, thyroid disease, and pituitary gland disorders. **Three-dimensional-clinostat** is a device used to simulate a microgravity environment by constantly changing the orientation of a cell culture, averaging the gravity vector to near zero. It's often used in space biology research to study the effects of weightlessness on cells. **Multidirectional G force generator** is a device designed to apply forces in multiple directions, often to simulate conditions of hypergravity or altered gravity for biological experiments. **Hepatocyte** is the main parenchymal cell of the liver, making up about 70–85% of the liver's mass. These cells perform critical functions, including protein synthesis, detoxification, and metabolism. **Biodegradable polymer scaffold** is a three-dimensional structure made from a material that can be broken down naturally by the body. It provides a temporary framework for cells to grow and organize into new tissue. **Rotating bioreactor:** a device used to culture cells in a low-shear, microgravity-like environment. The rotation prevents cells from settling at the bottom and encourages the formation of three-dimensional tissue structures. **Leukemia Inhibitory Factor**: a cytokine that has diverse effects on different cells. It is particularly known for its role in maintaining the pluripotency of embryonic stem cells in culture. **Mouse embryonic fibroblasts:** cells derived from mouse embryos that are commonly used as **feeder cells** in stem cell culture. They provide a support layer that secretes factors necessary for the growth and maintenance of undifferentiated stem cells. **Differentiation multipotency:** the ability of a single stem cell to differentiate into multiple, but limited, types of specialized cells, often within a particular germ layer or tissue. **Flat cells:** are cells that have spread out and adhered to a surface, often indicating a more differentiated state or a response to gravity. Their morphology contrasts with the more rounded shape of cells in

	suspension. **Vinculin:** is a cytoskeletal protein that plays a crucial role in cell adhesion. It links integrin adhesion molecules to the actin cytoskeleton, helping to anchor the cell to the extracellular matrix. **Integrin alpha 2:** Integrin alpha-2/beta-1 is a receptor for laminin, collagen, collagen C-propeptides, fibronectin and E-cadherin. It recognizes the proline-hydroxylated sequence G-F-P-G-E-R in collagen. It is responsible for adhesion of platelets and other cells to collagens, modulation of collagen and collagenase gene expression, force generation and organization of newly synthesized extracellular matrix. **Hypergravity**: a state where the force of gravity is greater than Earth's normal gravitational pull (1g). It can be created using a centrifuge and is used to study its effects on biological systems. **Microgravity:**a condition of near-weightlessness. While often used interchangeably with zero gravity, it refers to the state where the gravitational force is very small. This is the environment found in orbiting spacecraft. **Force-sensitive cells:** cells whose behavior, such as proliferation, differentiation, or gene expression, is influenced by mechanical forces like gravity or pressure. **Force-insensitive cells:** Cells that do not show a significant response to these mechanical stimuli. **Adipogenic** : the process of forming or differentiating into adipocytes (fat cells). **Upregulation:** process in which a cell increases the number of receptors or the production of a substance in response to an external stimulus. This leads to an enhanced cellular response. **Mesenchymal stem cells (MSCs):** A critical member of the stem cell; can be found in most postnatal organs and tissues. They can be induced to differentiate into adipocytes, osteocytes, cartilage cells, neurons, hepatocytes, endothelial cells, myocardial cells, etc. Through in vitro induction. These are easy to collect and culture and are widely used in space science. /important /neuronsinmicrogravity

	Chondrogenesis: the process of cartilage formation. It involves the differentiation of mesenchymal stem cells into chondrocytes, which then produce the cartilage matrix. **Hyaline cartilage:** the most common type of cartilage in the body. It is found in the nose, larynx, and joints, providing smooth surfaces for joint movement. **Neurotrophins**: a family of proteins that support the growth, survival, and differentiation of neurons. They are crucial for nervous system development and function. **Pulposus-like phenotype in nuclei:** cells that have differentiated to resemble the notochordal cells found in the nucleus pulposus of the intervertebral disc. These cells are known for their ability to maintain disc hydration and health. **Hematopoietic Stem Cells (HSCs):** Pluripotent and self-renewing cells with a membrane-bound surface antigen CD34 in bone marrow. They have the potential to differentiate into many blood cell precursors. **Antigen:** a molecule or molecular structure that can bind to a specific antibody or T-cell receptor. The presence of an antigen in the body can trigger an immune response. **Migration ability:** refers to the capacity of stem cells to move from their niche in the bone marrow into the bloodstream or to other tissues in response to specific signals. This is a crucial process for tissue repair and hematopoiesis. **Erythroleukemia:** a rare type of acute myeloid leukemia characterized by an abnormal proliferation of red blood cell precursors in the bone marrow. **Granulocyte:** a type of white blood cell that contains granules in its cytoplasm. These cells, which include neutrophils, eosinophils, and basophils, play a key role in the immune system's response to infection. **Hematopoietic progenitor cells:** an intermediate stage between hematopoietic stem cells and mature blood cells. They have limited self-renewal capacity but are committed to differentiating into one or more specific lineages of blood cells.

	Neutrophilia: a condition characterized by an abnormally high number of neutrophils, a type of white blood cell, in the blood. It is often a sign of infection or inflammation. **Lymphopenia:** is a condition characterized by an abnormally low number of lymphocytes, a type of white blood cell, in the blood. It can be a sign of various conditions, including viral infections or immune system disorders. **Periodontal ligament stem cells:** a type of mesenchymal stem cell found in the periodontal ligament, the tissue that surrounds the roots of teeth. They can differentiate into cementoblasts, fibroblasts, and osteoblasts, making them a promising source for dental and bone tissue regeneration. **Matrix mineralization:** the process by which inorganic minerals, primarily calcium and phosphate, are deposited into an organic matrix, such as collagen. This process is essential for the formation of hard tissues like bone and dentin. **Feeder cells:** a layer of cells used in cell culture to support the growth of a different cell type. They often provide growth factors or other nutrients and can inhibit the differentiation of the target cells.
Cited references to follow up on	Chen et al. 2011 Zhang et al. 2006 (Zhang Y, Chen H, Huang H, Xu X, Tang X, Yin G, Wu J (2006) Mechanical environment of rotating bioreactor and its effect on cell growth. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi* **23**: 400–4.) Yuge et al. (2006): Yuge L, Kajiume T, Tahara H, Kawahara Y, Umeda C, Yoshimoto R, Wu SL, Yamaoka K, Asashima M, Kataoka K, Ide T (2006) Microgravity potentiates stem cell proliferation while sustaining the capability of differentiation. *Stem Cells Dev* **15**: 921–9. Dai et al. 2007 (Dai ZQ, Wang R, Ling SK, Wan YM, Li YH (2007) Simulated microgravity inhibits the proliferation and osteogenesis of rat bone marrow mesenchymal stem cells. *Cell Prolif* **40**: 671–84.) Hwang et al. 2009 (Hwang YS, Cho J, Tay F, Heng JY, Ho R, Kazarian SG, Williams DR, Boccaccini AR, Polak JM, Mantalaris A (2009) The use of murine embryonic stem cells, alginate encapsulation, and rotary microgravity bioreactor in bone tissue engineering. *Biomaterials* **30**: 499–507.)

Follow up Questions	Why does microgravity inhibit the repair of DNA? Why do mouse embryonic fibroblasts inhibit differentiation? How can one get access to Mesenchymal Stem Cells? How do hMSCs (human bone marrow derived MSCs) sense microgravity? What effects the differentiation direction of MSCs in microgravity? How can we use microgravity in biomedical engineering and automate the process? Do younger children have a greater number of stem cells? Why does microgravity change the behavior of stem cells?

Article #5 Notes: A future of personalized medicine for astronauts: Considering Genetic Variability and Biological Sex-Based Differences in Space Medicine

Article notes should be on separate sheets

Source Title	A future of personalized medicine for astronauts: Considering Genetic Variability and Biological Sex-Based Differences in Space Medicine
Source citation (APA Format)	Guo, Y., Waisberg, E., Ong, J., Kumar, R., & Lee, A. G. (2025). A future of personalized medicine for astronauts: Considering genetic variability and biologic sex-based differences in space medicine. *Acta Astronautica*, *228*, 527–531. https://doi.org/10.1016/j.actaastro.2024.12.033
Original URL	https://www-sciencedirect-com.ezpv7-web-p-u01.wpi.edu/science/article/pii/S0094576524007823
Source type	Journal Type
Keywords	Spaceflght, Space medicine, Microgravity, Genomics, Sex differences, Women's health, Female, Women in space, Personalized medicine
#Tags	/spacemed
Summary of key points + notes (include methodology)	- Genes such as SOD2, CAT, and XRCC1 play a key role in oxidative stress and DNA repair, which are crucial to mitigating the effects of cosmic radiation.

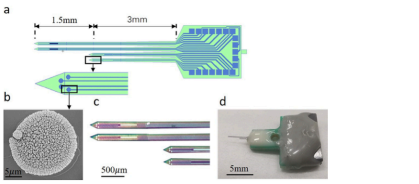
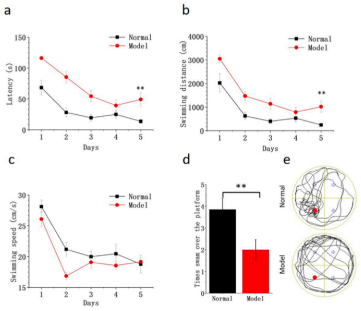
	- Asserts that astronauts can benefit from epigenetic therapies that are tailored to them. - Knowledge gaps in regard to women: The heightened risk of reproductive cancers (possibly due to estrogen modulating the p53 tumor suppressor), lack of knowledge concerning menstrual health in microgravity, the lack of research surrounding spaceflight and insulin/estrogen pathways,
Research Question/Problem/ Need	**This specific article aims to address the glaring disparities in regards to Women's Health in Space. It highlights the need to explore the effects of cosmic radiation on the female body.**
Important Figures	Y. Guo et al. **Table 1** Physiological responses to spaceflight - male and female astronauts. Body System | Observed Differences in Spaceflight | Potential Biological Mechanisms | Countermeasures/ Interventions **Cardiovascular** | Women are more susceptible to post-spaceflight orthostatic intolerance, experience greater plasma volume loss, and have reduced venous cardiac output [13,21, 22]. | Lower baseline leg vascular resistance and hormonal differences affecting vascular tone (e. g., estrogen) [3, 13]. | Compression garments, increased fluid and salt intake, lower body negative pressure (LBNP) training [21,29]. **Neuro-Ocular** | Women experience milder visual impairment symptoms and fewer clinically significant cases of Spaceflight-Associated Neuro-Ocular Syndrome (SANS) than men [35,36,39]. | Differences in intracranial pressure regulation and possibly genetic factors related to ocular structure [11,40]. | Regular visual assessments, intracranial pressure monitoring, individualized vision therapy [38,40]. **Genitourinary** | Higher incidence of urinary tract infections (UTIs) in women compared to men [22,29]. | Shorter urethra in women and altered immune responses in microgravity [3, 29]. | Prophylactic antibiotics, hydration protocols, and frequent urinary health screenings [22, 29]. **Immunologic** | Women have a higher susceptibility to radiation-induced cancers, particularly in breast and reproductive tissues [4,7,25]. | Estrogen modulation of DNA repair pathways and immune response differences [7, 24,25]. | Hormone modulation (e.g., estrogen blockers), radioprotective agents such as amifostine, targeted radiation shielding [7,25]. **Sensorimotor** | Women experience more space motion sickness (SMS) upon initial exposure to microgravity but have lower SMS upon re-entry to Earth and less rapid hearing sensitivity decline [5,29, 34]. | Sex-specific vestibular system and inner ear differences affecting balance and orientation [13,29]. | Vestibular habituation training, customized re-adaptation protocols post-flight [29,34]. **Muscular** | Women generally experience greater slow-twitch muscle loss, impacting endurance, whereas men experience faster fast-twitch muscle degradation [14, 29]. | Higher proportion of slow-twitch muscle fibers in women and hormonal influence on muscle atrophy rates [14,29]. | Resistance and endurance exercise protocols tailored to muscle fiber composition, nutritional supplementation [14, 15]. *Figure 1 shows the effects of spaceflight on both men and woman in the major body systems.*

VOCAB: (w/definition)	• **Antioxidant Therapy**: Medical treatment using antioxidants to protect cells from damage caused by harmful molecules called free radicals. • **Amifostine**: A medication given to patients receiving radiation therapy to protect healthy cells, particularly those in the salivary glands and kidneys, from damage. • **Hormone modulators**: A class of drugs that alter the effects of hormones in the body, either by mimicking them, blocking their receptors, or changing their production. • **Hypothalamic-pituitary-gonadal (HPG) axis**: A complex system of communication between the hypothalamus (a part of the brain), the pituitary gland, and the gonads (testes or ovaries). It regulates the production of sex hormones and fertility. • **Gonadotropin**: Hormones produced by the pituitary gland that stimulate the gonads to produce sex hormones. The two main types are luteinizing hormone (LH) and follicle-stimulating hormone (FSH). • **Estrogen**: The primary female sex hormone, responsible for the development of female secondary sexual characteristics and the regulation of the menstrual cycle. • **Progesterone**: A female sex hormone that plays a crucial role in preparing the uterus for pregnancy and maintaining it during gestation. It also helps regulate the menstrual cycle.
Cited references to follow up on	NASA Twins Study
Follow up Questions	N/A as of now

Article #6 Notes: Implanted multichannel microelectrode array for simultaneous electrophysiological signal detection of hippocampal CA1 and DG neurons of simulated microgravity rats

Article notes should be on separate sheets

Source Title	Implanted multichannel microelectrode array for simultaneous electrophysiological signal detection of hippocampal CA1 and DG neurons of simulated microgravity rats
Source citation (APA Format)	Wang, H., Liu, J., Lu, Z., Dai, Y., Xie, J., Xu, S., Song, Y., Xiao, G., Gao, F., Qu, L., & Cai, X. (2020). Implanted multichannel microelectrode array for simultaneous electrophysiological signal detection of hippocampal CA1 and DG neurons of simulated microgravity rats. *Biochemical and Biophysical Research Communications*, *531*(3), 357–363. https://doi.org/10.1016/j.bbrc.2020.07.079
Original URL	https://www-sciencedirect-com.ezpv7-web-p-u01.wpi.edu/science/article/pii/S0006291X20314686
Source type	Journal Article
Keywords	simulated microgravity implantable MEA neurophysiological hippocampus
#Tags	/spacemed /neuronsinmicrogravity
Summary of key points + notes (include methodology)	- Wu et al. Looked at the effects of the tail-suspension model on the memory and learning of rats. They found that after 7-12 days, spatial memory decreased; after 14-21 days, the lipid peroxidation levels in the whole brain increased. Another study looked at oxidative stress and found that after 7-21 days, MDA and H2O2 increased in concentration in the rat hippocampus. Likewise, the antioxidant SOD was reduced. - Method: Electronic signals were detected/recorded by a **128 channel neuron data recording system.** MEAs were controlled by a micro-positioner to monitor real-time positions in the brain. Isoflurane anesthesia was administered to the rats. Electrophysiological recording data were analyzed by Offline Sorter. - Design of MEA: Designed with 4 different length shanks that can detect information from different layers of the hippocampus. Modified by platinum black nanoparticles to improve signal-to-noise ratio. - Procedure: Used six-week-old adult male Sprague Dawley rats all

	weighing 220 grams. They sprayed the tail of the rats with benzoin and resin, then hung them tilted 30 degrees head down from the horizontal plane. The 28-day-tail suspension rats were tested for spatial learning and memory using the Morris water maze. The microgravity rats experienced a delay when solving the maze. - Simulated Microgravity Rats are the standard animal models for simulating microgravity exposure.
Research Question/Problem/ Need	This article aimed to fill the research gap of electrophysiological signals in simulated microgravity rats by creating a new device – a 16 channel microelectrode array (MEA). This technology aims to measure CA1 and DG at the same time in order to reduce the number of trials on rats.
Important Figures	 *Figure 1 depicts the design of the MEA.*  *Figure 2 shows the results of the experiment*

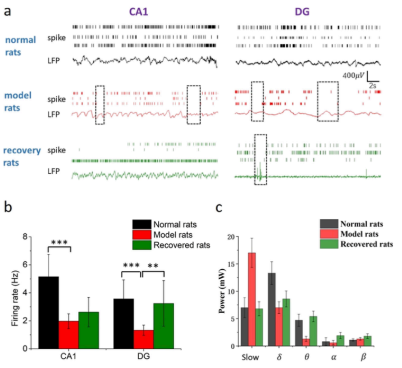


Fig. 3. Spikes, LFPs, firing rate, and power analysis of hippocampus neural signals. (a) Neural spike and LFPs recorded in CA1 and DG regions in the hippocampus from the normal, model, and recovered rats. The periods of suspension corresponded with smooth peaks in LFPs. (b) Average firing rates of neurons in the hippocampus, n indicated number of neurons. n = 17 for each group. (c) Power analysis of neurons in the hippocampus, n indicated number of channels, n = 27 for each group. (mean ± SEM; **P < 0.01; ***P < 0.001).

Figure 3 shows the electrical data collected from different areas of the hippocampus. The model rats experienced slower LFP signals than the normal rats in both CA1 and DG.

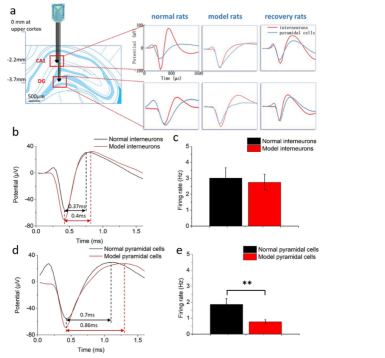


Fig. 4. The discharge pattern shows a specific difference between putative pyramidal cells and interneurons after modulation. (a) Different spike patterns of putative interneurons and pyramidal cells detected in CA1 and DG of the three groups of rats. (b) The spike of interneurons from normal and model rats, where after modulation, the spike duration had little latency. (c) The average firing rate of interneurons shows a slight drop after modulation. (d) The spike of pyramidal cells shows significant latency after modulation. (e) The firing rate shows a significant drop after modulation. (mean ± SEM; n = 21 for each group; **p < 0.01).

Figure 4 shows the differences in the results of putative pyramidal cells and interneurons after modultion.

VOCAB: (w/definition)

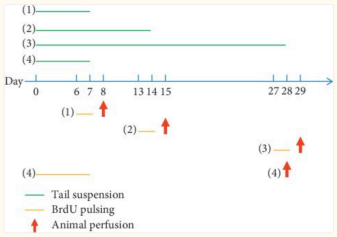
- **Tail-suspension model:** A method used in rodents to study behavioral despair, which is an indicator of depression-like states. The animal is suspended by its tail, and the duration of immobility is measured. The longer the immobility, the greater the despair.

	• **Pyramidal cells:** A common type of multipolar neuron found in the cortex and hippocampus. They are characterized by their **pyramid-shaped soma** (cell body), a single prominent apical dendrite, and multiple basal dendrites. • **Interneurons:** A diverse group of neurons that connect other neurons, forming neural circuits. They play a crucial role in regulating the activity of sensory and motor neurons, and are involved in complex functions like reflexes and neuronal oscillations. • **Hindlimb-unloading technique:** A method used in rodents to simulate the physiological effects of **microgravity** or bed rest on the musculoskeletal system. The hindlimbs are elevated to prevent weight-bearing, leading to muscle atrophy and bone loss, mimicking the effects of spaceflight. • **Bandpass filter:** An electronic filter that allows a specific range of frequencies to pass through while **blocking frequencies** both above and below this range. For example, a bandpass filter might be used to isolate a specific frequency of brain waves. • **Low-pass filter:** An electronic filter that allows frequencies **below a certain cutoff frequency** to pass through while blocking frequencies above it. They are often used to remove high-frequency noise from a signal. • **CA1:** The **Cornu Ammonis area 1**, a subfield of the hippocampus that is crucial for **memory formation**. It receives input from the CA3 region and is a major output region of the hippocampus, projecting to the subiculum and other brain areas. • **DG:** The **Dentate Gyrus**, another subfield of the hippocampus. It is one of the few brain regions where **neurogenesis**(the birth of new neurons) occurs in adult mammals. It receives input from the entorhinal cortex and projects to the CA3 region. • **Putative Pyramidal Neurons from DG:** Pyramidal neurons that are **likely** located in the Dentate Gyrus. The term "putative" is used because their exact identity is not confirmed, but their electrical properties or location strongly suggest they are this type of cell. • **Putative interneurons from CA1:** Interneurons that are likely located in the CA1 region. Similar to the above, their identity is inferred based on characteristics rather than a definitive identification. • **Symmetry Index:** A measure used to quantify the **asymmetry of a biological or physical feature**. In a biological context, it could be used to compare the activity, size, or shape of a structure on the left side of the body versus the right side. It is often calculated as a ratio or a difference divided by a sum.

	• **"Frequency Code" hypothesis:** Information is embedded in the rate of neuronal discharges.
Cited references to follow up on	
Follow up Questions	Will the recovery time be longer if the rats had spent more time in the hindlimb-unloading technique? What is the significance of putative neurons in different parts of the hippocampus?

Article #7 Notes: Treatment with Minocycline Suppresses Microglia Activation and Reverses Neural Stem Cells Loss after Simulated Microgravity

Source Title	Treatment with Minocycline Suppresses Microglia Activation and Reverses Neural Stem Cell Loss after Simulated Microgravity
Source citation (APA Format)	Cai, Y., Kong, X., Wu, Z., Liu, L., Du, J., Lin, T., Liu, Y., & Oliveira, M. S. (2020). Treatment with Minocycline Suppresses Microglia Activation and Reverses Neural Stem Cells Loss after Simulated Microgravity. *BioMed Research International*, *2020*(2020), Article 7348745. https://doi.org/10.1155/2020/7348745
Original URL	https://onlinelibrary.wiley.com/doi/10.1155/2020/7348745
Source type	Journal Article
Keywords	N/A
#Tags	/spacemed /neuronsinspace
Summary of key points + notes (include methodology)	- Learning and memory has a relationship with the neurogenesis of the denate gyrus (DG). Granule neurons are created here from Neural Stem Cells /important - The more newborn neurons an animal has, the greater its spatial learning and memory will be /important - Hippocampul neurogenesis is negatively impacted by inflammation induced by the microglia. The microglia causes this through the release

	of proinflammatory mediators such as interleukin-1beta (IL-1B), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor-alpha (TNF-alpha), and interleukin-18 (IL-18) from activated glia. These are all antineurogenic. After microgravity exposure, the number of activated microglia in the spinal cord increases. /important - Methods: o Adult Rats were suspended by the tail at a 30 degree angle (simulates microgravity) and immunohistochemistry was used to see the the changes in neural cells. - Research Question: What is the effect of microglia in reducing simulated microgravity-induced hippocampus neurogenesis - The study found that microgravity indeed reduced the proliferation of neural stem cells, but did not affect their differentiation. After using minocycline to inhibit microglia, the number of NSCs returned to normal. - Microgravity causes depression in rats, similar to the observed symptoms in humans.
Research Question/Problem/ Need	Do microglia play a role in the decrease in proliferation of the neural stem cells in microgravity.
Important Figures	Figure 1.  Open in a new tab Experimental procedure and time course of tail suspension, BrdU administration, and animal perfusion. The rats in the tail suspension (TS) groups received tail suspension for 7, 14, or 28 d (indicated by green lines in (1), (2), and (3), respectively) and were sacrificed for proliferation assays. Twenty-four hours before killing (indicated by the arrow in (1), (2), and (3), respectively), these rats received three pulses of BrdU every 8 h for 24 h (indicated by yellow lines in (1), (2), and (3), respectively). The rats used for differentiation assays first received BrdU (100 mg/kg) injection each day for 7 d (indicated by yellow line in (4)) and then were perfused at day 21 (indicated by the arrow in (4)) after treatment with control or tail suspension. *Figure 1 explains the division of the different experimental groups and the treatments they recieved*

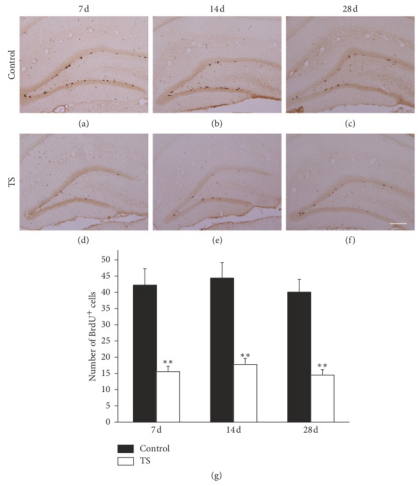


Figure 2: Microgravity suppressed the NSCs in the hippocampal dentate gyrus. Cell proliferation was assessed by BrdU labeling. Representative microphotographs show BrdU$^+$ cells in the dentate gyrus of rats in control groups (a–c) and TS groups (d–f) which received 7, 14, and 28 d tail suspension, respectively. Scale bar: 200 mm. (g) Quantification of BrdU-positive cells in the dentate gyrus showing that, relative to the control, the number of BrdU$^+$ cells was significantly decreased after 7, 14, and 28 d tail suspension. Error bars represent standard deviation (SD). $^{**}P < 0.01$, compared with the control group (Bonferroni post hoc test after one-way ANOVA).

Affects proliferation,

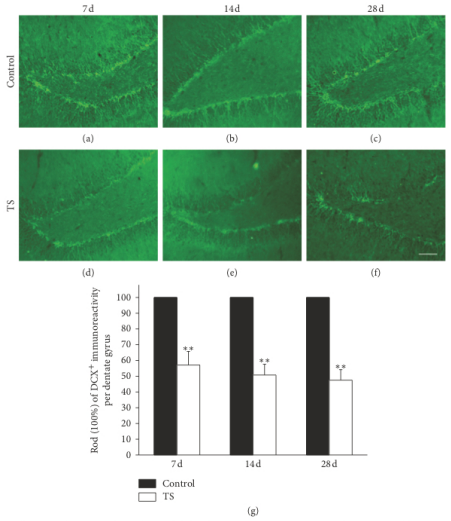


Figure 3: Microgravity decreased the number of DCX-labeled neural progenitors in the hippocampal dentate gyrus. Representative microphotographs showed DCX$^+$ cells in the dentate gyrus of rats in control groups (a–c) and TS groups (d–f) received 7, 14, and 28 d tail suspension, respectively. Scale bar: 50 mm. (g) ROD of DCX immunoreactivity in the dentate gyrus. At the time points of 7, 14, and 28 d, the ROD of TS groups was much higher than that of the control. Error bars represent SD. $^{**}P < 0.01$, compared with control group (Bonferroni post hoc test after one-way ANOVA).

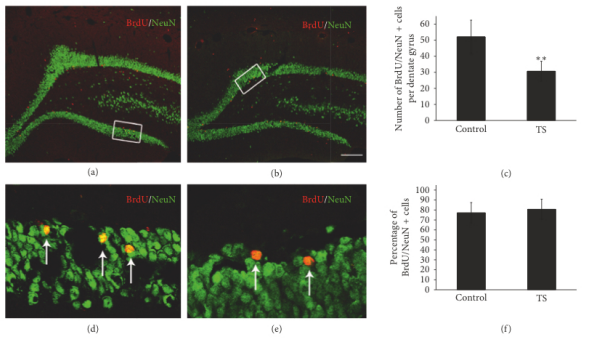


FIGURE 4: Microgravity does not affect the differentiation of NSCs in the hippocampal dentate gyrus. (a, b) Representative confocal microscope images show NeuN (green) and BrdU (red) double-labeled cells in the dentate gyrus at four weeks after BrdU injection. (d, e) The high-magnification views of the rectangular areas in their respective panels. The arrows in (d) and (e) show BrdU and NeuN double-labeled cells. Scale bars: 200 mm (a, b); 25 mm (d, e). (c) Quantification of $BrdU^+/NeuN^+$ cells in the dentate gyrus. In comparison with the control, exposure to microgravity significantly decreased the number of $BrdU^+/NeuN^+$ cells. (f) The percentage of $BrdU^+/NeuN^+$ cells to the total BrdU + cells did not differ between the control and TS groups. Error bars represent SD. $^{**}P < 0.01$, compared with the control (Bonferroni post hoc test after one-way ANOVA).

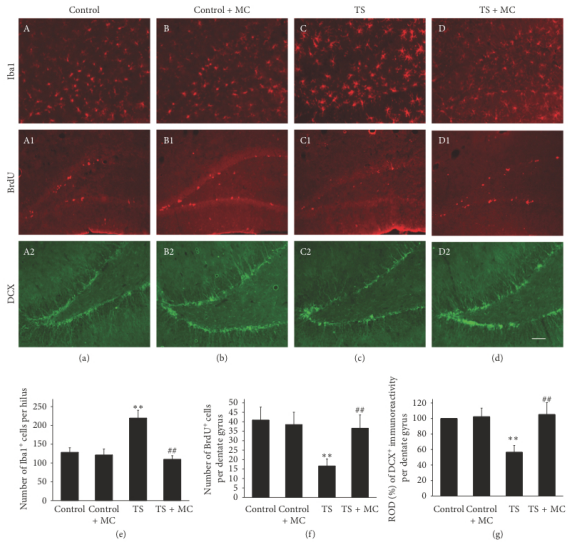


FIGURE 5: Minocycline suppressed the activation of microglia and the reduction in the number of NSCs in the dentate gyrus induced by microgravity. Representative microphotographs show that $Iba1^+$, $BrdU^+$, and DCX^+ cells in the dentate gyrus of rats in control (a), control + MC (b), TS (c), and TS + MC groups (d) received 7-day tail suspension, respectively. (e) Quantification of the ROD of Iba1 immunoreactivity in the dentate gyrus. (f) Quantification of BrdU-positive cells in the dentate gyrus. (g) Quantification of the ROD of DCX immunoreactivity in the dentate gyrus. The ROD of Iba1 immunoreactivity in TS groups was much higher than that in the control; however, the increase was restrained by minocycline treatment. The number of $BrdU^+$ cells and the ROD of DCX immunoreactivity of TS groups were much lower than those of the control; however, the decreases were reversed by minocycline treatment. Scale bar: 100 mm. Error bars represent SD. $^{**}P < 0.01$, compared with control group; $^{\#\#}P < 0.01$, compared with TS group (Bonferroni post hoc test after one-way ANOVA).

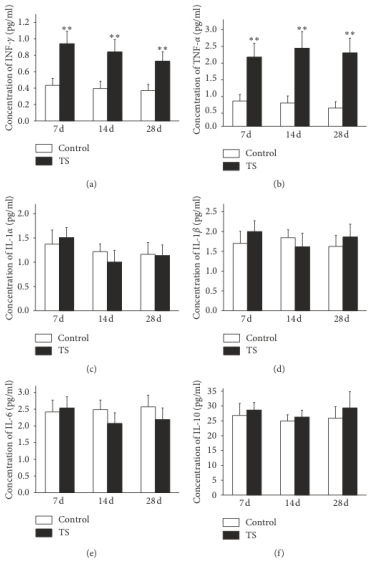


FIGURE 6: Microgravity changed the concentration of some inflammatory cytokines in rat hippocampus. The levels of INF-γ (a), TNF-α (b), IL-1α (c), IL-1β (d), IL-6 (e), and IL-10 (f) in supernatants of hippocampal tissue collected after 7-, 14-, or 28-day tail suspension were observed separately. Compared with control groups, the concentrations of INF-γ and TNF-α were much higher in TS groups at different time points. However, the concentration of other factors, such as IL-1α, IL-1β, IL-6, and IL-10, did not change much between the control and TS groups. $^{**}P < 0.01$ versus control (Bonferroni post hoc test after one-way ANOVA).

VOCAB: (w/definition)

- **Microglia:** Small cells present in the CNS that have multiple function by synergizing with other neural cells. They are involved in every step of neurogenesis as a response to damage.
- **Hippocampal Neurogenesis**: The process of generating new neurons in the hippocampus, a brain region crucial for learning and memory, which continues throughout life.
- **Immunohistochemistry**: A laboratory technique using antibodies to identify and visualize specific proteins within cells or tissue samples.
- **Minocycline**: A tetracycline antibiotic also known for its anti-inflammatory, antioxidant, and neuroprotective properties.
- **Liquid Protein Chip Analysis**: A high-throughput technology that uses microscopic beads in a liquid suspension to simultaneously analyze thousands of proteins.
- **Subgranular Zone of the Dentate Gyrus**: A region within the hippocampus where neural stem cells differentiate into new neurons.

	• **Lipopolysaccharides**: A major component of the outer membrane of Gram-negative bacteria, also known as endotoxins, which can trigger a strong inflammatory response. • **Proliferation Assay**: A laboratory method used to measure cell growth and division in response to various stimuli. • **Luminex-based Assay**: A multiplexing immunoassay that uses color-coded beads to simultaneously detect and quantify multiple analytes in a single small sample. • **Radioimmunoprecipitation Assay**: A laboratory technique that uses a radioactive label to detect and quantify specific antigens or antibodies in a sample. • **DCX**: A protein that is used as a marker for identifying and tracking immature neurons. • **Attenuation**: A gradual loss of intensity or energy over a period of time or distance. • **Retinoblastoma Protein Phosphorylation**: A process where phosphate groups are added to the retinoblastoma protein, which inactivates it and allows the cell cycle to proceed.
Cited references to follow up on	
Follow up Questions	Why does microgravity cause depressive symptoms, and what is it's correlation with microglia?

Article #8 Notes: Small extracellular vesicles from menstrual blood-derived mesenchymal stem cells (MenSCs) as a novel therapeutic impetus in regenerative medicine.

Article notes should be on separate sheets

Source Title	Small extracellular vesicles from menstrual blood-derived mesenchymal stem cells (MenSCs) as a novel therapeutic impetus in regenerative medicine.

Source citation (APA Format)	Chen, L., Qu, J., Mei, Q., Chen, X., Fang, Y., Chen, L., Li, Y., & Xiang, C. (2021). Small extracellular vesicles from menstrual blood-derived mesenchymal stem cells (MenSCs) as a novel therapeutic impetus in regenerative medicine. *Stem Cell Research & Therapy*, *12*(1), Article 433. https://doi.org/10.1186/s13287-021-02511-6
Original URL	https://go-gale-com.ezpv7-web-p-u01.wpi.edu/ps/i.do?p=AONE&u=mlin_c_worpoly&id=GALE%7CA675240806&v=2.1&it=r&aty=ip
Source type	Literary Review (Journal Article)
Keywords	Menstrual blood-derived mesenchymal stem cell (MenSC), Small extracellular vesicle, Exosomes, Cell-free therapy, Regenerative medicine
#Tags	/regenerativemedicine /important
Summary of key points + notes (include methodology)	New information on MSCs: They are heterogenous subsets of stromal/mesenchymal regenerative cells. Has lots of self-renewal potential and multi-lineage differentiation. MSC infusion is safe and effective at various doses. - List of places MSCs can be obtained: Bone marrow, umbilical cord, adipose tissue, placenta, fetal tissue, Wharton's jelly, induced pluripotent stem cells, embryonic stem cell, cervical tissue placentae, periodontal ligaments amniotic membrane/fluid, endometrium, lung, liver, dental pulp, peripheral blood, dermal tissue, synovial membranes, skeletal muscle tissue. - Menstrual blood-derived mesenchymal stem cells have not been discovered until 2007 - Compared with other sources, MenSCs have many advantages such as abundance, non-invasive isolation, high proliferaton rate, low immunological rejection, and lack of ethical issues. - MenSCs possess a doubling time of 19.4 hours, which is twice as fast as BM-MSCs. - Many studies have shown that the therapeutic benefits of MSCs are mediated through paracrine roles, specifically the secretion of growth factors, chemokines, and cytokines rather than differential abilities or cellular replacements. - MSC EV-based therapy is less likely to trigger an immune-repulsion response compared to traditional MSC-based therapy. - Since consensus has not been reached in regards to the biomarkers of EV subtypes, all MSC exosomes/microvessels are referred to as MSC-derived small EVs. - MenSC-derived small EVs promote axonal regeneration after nerve injury. They also present CD9, CD63, CD81, HSP70, HSP90, and TSG101 with the

	exclusion of Rab5 and calnexin. Additionally MenSCs exhibit octamerbinding transcription factor 4 (OCT-4), a unique marker not present in other sources. - MenSCs show higher potential in healing neurodegenerative disorders compared to other sources. - Challenges: Lack of research regarding the miRNAs of MenSCs, establishing a unified standard of collecting MenSC-derived small EVs, lack of research concerning long-term safety, since current purification methods stem from the manufactoring of viruses or viral-like particles. If these are present in the medium or the recipient cell, they have the risk of being enriched in final exosome extraction. Additionally, the abundance of small RNAs poses risk for the stability of nucleic acid chains. Lastly, the lack of engineered MenSC-derived studies.
Research Question/Problem/ Need	Identifies the current research concerning MenSC-derived EVs and evaluates its current therapeutic potential. Highlights current challenges with MenSCs.
Important Figures	**Table 1** Biological functions of extracellular vesicles (EVs) in body fluids *Figure 1: Describes each of the different subtypes of Extracellular Vessels.* *Figure 2: Describes the therapeutic benefits of each of the biomarkers present in*

EVs functions	Exosomes	Microvesicles	Apoptotic bodies
Origin	Endosomal multivesicular bodies	Cell surface	Apoptotic cell surfaces
Generation	Intracellular vesicle traffic	Plasma membrane	Plasma membrane
Size	30–150 nm	50–1000 nm	100–5000 nm
Markers	Tetraspanins (CD9/63/81), Alix, HSP70/90, flotillin, TSG101, clathrin, GM130, MHC	Annexin V, selectins, integrins, flotillin-2, CD40, metalloproteinases	Histones, Annexin V
Cargos	Proteins, lipids, mRNA, miRNA, DNA, carbohydrates	Proteins, mRNA, miRNA	Proteins, mRNA, miRNA, fragment of DNA

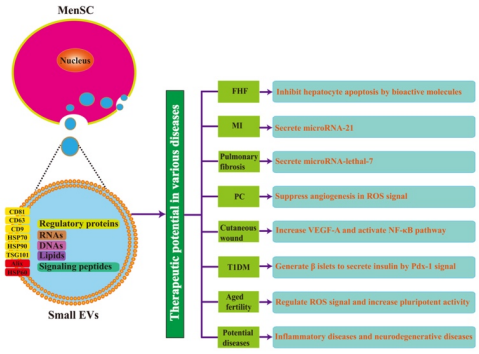
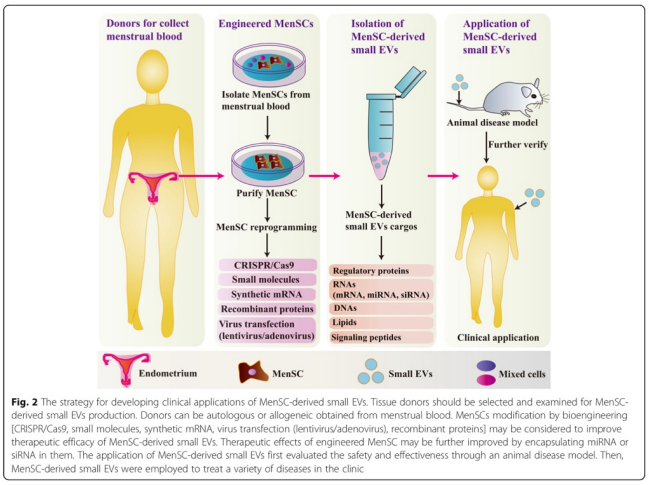


Fig. 1 Identification of MenSC-derived small EVs and their therapeutic potentials for tissue repair in various diseases. Small EVs from MenSCs consist of regulatory proteins, RNAs, and DNAs, lipids, and siginaling peptides promoting regenerative repair of wounded cells and tissues. MenSC-derived small EVs are positive for the expression of CD9, CD63, CD81, HSP70, HSP90, and TSG101, and they are negative for Rab5 and calnexin. The expression of HSP60 and Alix, which are positive for universal MSC-derived small EVs, need to be recognized for further verification. The therapeutic potential of MenSC-derived small EVs in various diseases, including fulminant hepatic failure (FHF; via inhibition of hepatocyte apoptosis by bioactive molecules), myocardial infarction (MI, via secreted microRNA-21), pulmonary fibrosis (via secreted microRNA-lethal-7), prostate cancer (PC; via suppression of angiogenesis by ROS signaling), cutaneous wound (via increase in VEGF-A and activation of NF-κB pathway), type-1 diabetes mellitus (T1DM; via generation of β islets to secrete insulin by Pdx-1 signaling), aged fertility (via regulation of ROS signaling and increase in pluripotent activity), and some potential diseases (such as inflammatory and neurodegenerative diseases)

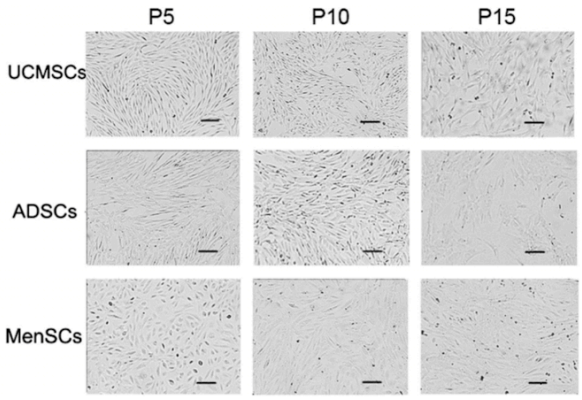
	MenSC-derived EVs.  **Fig. 2** The strategy for developing clinical applications of MenSC-derived small EVs. Tissue donors should be selected and examined for MenSC-derived small EVs production. Donors can be autologous or allogeneic obtained from menstrual blood. MenSCs modification by bioengineering [CRISPR/Cas9, small molecules, synthetic mRNA, virus transfection (lentivirus/adenovirus), recombinant proteins] may be considered to improve therapeutic efficacy of MenSC-derived small EVs. Therapeutic effects of engineered MenSC may be further improved by encapsulating miRNA or siRNA in them. The application of MenSC-derived small EVs first evaluated the safety and effectiveness through an animal disease model. Then, MenSC-derived small EVs were employed to treat a variety of diseases in the clinic *Figure 3: Describes the current strategy for studying MenSC-derived EVs in clinical trials.*
VOCAB: (w/definition)	• **BM-MSCs**: Bone Marrow Mesenechymal Stem Cells • **Immunoregulation:** The control of immune responses; the balance between recognizing foreign antigens and maintaining self-tolerance • **Extracellular Vesicles:** Released from the endosomal compartments and found in all types of cells. They are traditionally divided into exosomes, microvesicles, and apoptotic bodies based on size, origin, biogenesis, and cargo. • **Small EVs:** Small extracellular vessels have the potential to stimulate target cells, transfer membrane receptors, deliver proteins/genetic information, and eventually cause epigenetic differences in recipient cells. • **Immunosuppression**: The reduction or prevention of an immune response, either naturally due to a condition or intentionally induced with drugs. • **MHC-II**: A type of protein found on the surface of certain immune cells that presents foreign substances (antigens) to T helper cells to start an immune response. • **Secretome**: The complete set of molecules, such as proteins and lipids, that a cell or tissue secretes into its surrounding environment. • **Biogenesis**: The process by which living organisms or biological substances are produced, including the formation of cellular components. • **Microarray**: A laboratory tool used to analyze the activity of thousands of genes at once by applying a sample to a solid surface with tiny spots of known DNA sequences.

	• **Reactive Oxygen Species**: Highly reactive molecules containing oxygen that are naturally produced but can cause cellular damage at high levels. • **Transcriptomics**: The study of all the RNA transcripts produced by a cell, which provides a snapshot of gene activity. • **Neddylation**: A post-translational modification where a protein called NEDD8 is attached to a target protein, which is important for regulating protein function. • **Heterogeneous Cell Population**: A group of cells that are not all the same, differing in size, function, or other characteristics. • **Sporadic**: A term used to describe a disease or condition that occurs randomly in a population, without a clear genetic or environmental cause. • **Gingival**: An anatomical term that refers to the gums.
Cited references to follow up on	Mesenchymal stem cell-derived exosomes from different sources selectively promote neritic outgrowth.
Follow up Questions	How high is the proliferation rate of MenSCs compared to other MSCs? Why is the immunological rejection for MenSCs low compared to others? What do the different biomarkers listed in the article do? How can we modify the purification methods to exclude viral-like particles?

Article #9 Notes: Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins

Source Title	Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins
Source citation (APA Format)	Chen, J.-Y., Mou, X.-Z., Du, X.-C., & Xiang, C. (2015). Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins. *Asian Pacific Journal of Tropical Medicine*, *8*(9), 725–731. https://doi.org/10.1016/j.apjtm.2015.07.022
Original URL	https://www-sciencedirect-com.ezpv7-web-p-u01.wpi.edu/science/article/pii/S1995764515000991

Source type	Journal Article
Keywords	Adipose, Umbilical cord, Menstrual blood, Mesenchymal stem cell
#Tags	/mesenchymalstemcells /regenerativemedicine /important
Summary of key points + notes (include methodology)	- Proliferation Rate decreased in this order: UCMSCs > MenSCs > ADSCs. However, colony forming rate decreased in this order: MenSCs > ADSCs > UCMSCs - Methadology: MSCs were isolated from adipose tissue, umbilical cord tissue (UC), and Menstrual Blood, and compared with one another. - MenSCs have higher extraction efficiency, colony-forming ability, and long time passage capacity. The proliferation capacity is inferior to UCMSCs. They could more easily differentiate into neurons, but not osteogenic or chondrogenic cells. /important. MTT Assay was used to select cells of the same generation. To determine cell-colony formation, cell cloning (Used to test self-renewal) experiments were conducted while the P5 cells were in the logarithmic growth phase. The cultures were terminated when the clones became visible in the culture plates. ANOVA was conducted using SPSS 16.0 software /important - When examining MenSCs for OCT-4, Nanog, and SSEA-4, there were a range of results. Ex. One study found that they expressed only OCT-4, one found that a small portion of them expressed SSEA-4, and one found high expression of SSEA-4. This is most likely due to differences in experimental methods. - Low immune regulation makes MSCs critical for allotransplantation. (Less likely to cause harm to patient due to immune responses) - ADSCs have different cytokine secretion, making it have a stronger immunomodulatory function. - BMSCs have a different proliferation and differentiation potential based on age. That's why alternative sources are being explored. - Results: *Different amounts of cells were derived from each source - **All three MSCs**: produced long, spindle cells in vitro. Differences become more apparent during cell culture. - **Cell-Cell Contact Inhibition:** ADSCS + MenSCs were significantly inhibited while UCMSCs were not -> Possible explanation + implications: Cell-Cell contact inhibition occurs because cells want to stay as far away from each other as possible. When there is no more space, cells **stop proliferating**. This suggests that ADSCs and MenSCs stop proliferating when the maximum capacity is reached. - **Cell Volume:** MenSCs had the largest cell volume and UCMSCs were the smallest. Possible explanation + implications: MenSCs were larger in size than UCMSCs. - **Isolation Rate:** MenSCs had a higher isolation rate. -> Most likely the rate at

	which cells are isolated from a culture. - **/important:** UCMSCs experienced higher amplification potential followed by MenSCs and ADSCs, while MenSCs had higher passage ability than UCMSCs and ADSCs. - **Clonality**: MenSCs had higher clonality than the other two. - **CD44:** Adhesion molecule that supports cell migration. It was 10 fold higher in MenSCs than in others.
Research Question/Problem/ Need	Investigate the differences among mesenchymal stem cells (MSCs) derived from different tissues and their impacts on clinical applications.
Important Figures	 Figure 1. Morphological observations of MSCs from umbilical cord (UCSCs), adipose tissue (ADSCs), and menstrual blood (MenSCs) at t tenth (P10), and twentieth (P20) passages. Scale bar, 100 µm. *Figure 1: Shows morphological differences of MSCs at different passages. Shows that MenSCs had a higher cell volume than other two.*

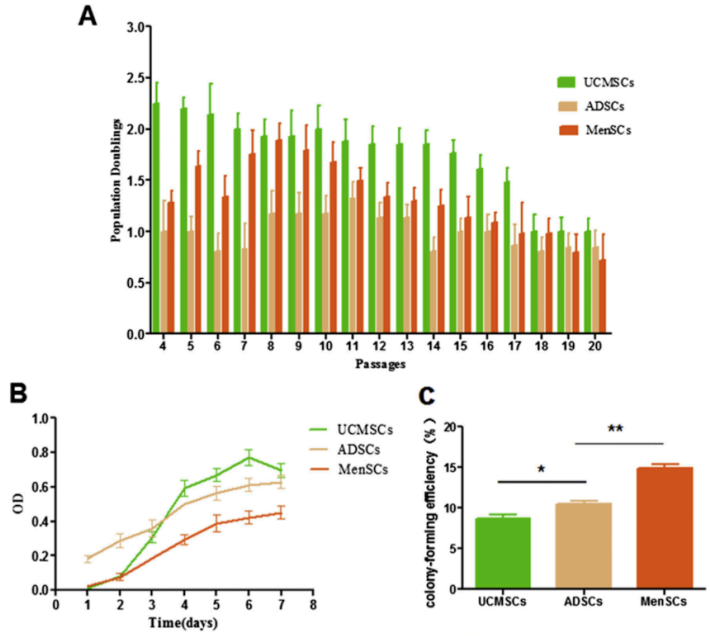


Figure 2. Comparison of proliferation characteristics of MSCs from umbilical cord (UCSCs), adipose tissue (ADSCs), and menstrual blood (Men
(A) Population doubling (n = 6); (B) growth curves; and (C) colony-forming abilities (n = 6) of UCSCs, ADSCs, and MenSCs.

Figure 2: A: Population Doubling time remained higher for UCMSCs consistently. B: UCMSCs had a faster doubling time (about 21 hours) while MenSCs and ADSCs had a doubling time of about 26 and 30 hours respectively. C: MenSCs have a higher colony forming efficiency than others.

Immunophenotypic analysis of ADSCs, UMSCs and MenSCs at passage 5.

Molecular marker	ADSCs (%) (n = 6)	MenSCs (%) (n = 6)	UCMSCs (%) (n = 6)
CD29	90.14 ± 0.20	90.20 ± 0.30	91.25 ± 0.20
CD34	0.36 ± 0.20	0.26 ± 0.30	0.56 ± 0.20
CD45	0.78 ± 0.40	11.22 ± 3.00	0.80 ± 0.50
CD73	95.90 ± 1.80	95.36 ± 1.10	96.86 ± 0.40
CD90	99.64 ± 0.30	94.98 ± 1.70	99.90 ± 0.30
CD105	90.72 ± 5.60	79.64 ± 4.30	94.56 ± 2.10
CD117	10.84 ± 1.30	10.20 ± 1.60	7.56 ± 1.30
HLA-DR	0.66 ± 0.40	0.66 ± 0.30	0.44 ± 0.20
SSEA-4	0.26 ± 0.20	0.48 ± 0.30	0.80 ± 0.40

Table 1: Showcases the molecular markers found commonly in MSCs. All three had

higher levels CD29, CD73, and CD90, and CD105 These are all stromal cell markers. However, hematopoietic cell markers such as CD34 and CD45 are fount in lower levels. SSEA-4 and HLADR were not expressed, which points to low immunogenicity (unable to provoke an immune response).

Jin-Yang Chen et al/Asian Pacific Journal of Tropical Medicine 2015; 8(9): 739–746

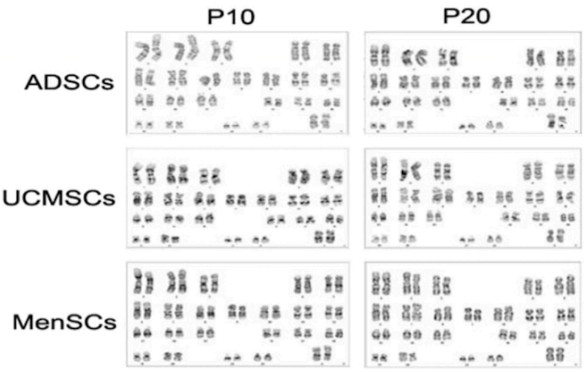


Figure 3. Karyotypes of ADSCs, UCMSCs and MenSCs at passages 10 and 20.

Figure 3: Shows the karyotypes after 10 and 20 passages (10 and 20 transfers of cells). This was done to verify the genetic stability of each cell. They found that the structures remained stable through every passage.

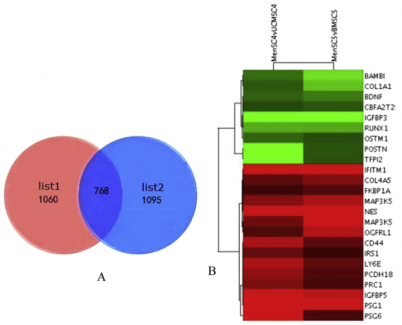


Figure 4. Dendrogram of differential gene expression.
Each color box represents the signal values of a probe on the chip (green = low signal, red = high signal). The brightness reflects the intensity of the signal. MenSC4 VS UCMSC4 indicates differential expression of genes between MenSCs and UCMSCs from the same donor. Genes that were not expressed in UCMSC4 (invalid signal) but were highly expressed in MenSC4 (valid signal) with a >10-fold change, and genes that were effectively expressed in both kinds of cells, with a >2-fold change ($P < 0.01$) comprise list 1. Genes that were not expressed in BMSC5 (invalid signal) but were highly expressed in MenSC5 (valid signal) with >10-fold change, and genes that were effectively expressed in both kinds of cells, with a >2-fold change ($P < 0.01$) comprise list 2. UCMSC4 and MenSC4 were isolated from the same donor, and BMSC5 and MenSC5 were isolated from the same donor.

Figure 4: Shows the differential gene expression analysis between MenSCs and UCMSCs. The venn diagram shows the similarities and differences between the two groups. List 1 includes genes that were not in UCMSC4 but were in MenSC4 and

genes that were found in both cells (overlap of the diagram) while list 2 describes the genes not found in BMSC but found in MenSC4. Firstly, this provides an analysis of the unique genes in MenSC4, and secondly, we can see which genes were missing from both BMSCs and UCMSCs, but were present in MenSCs. It's notable that BAMBI, IGFBP3, POSTN, TFPI2 all had a strong signal for low expression. The bars on the left of figure B are supposed to represent a dendrogram, a hierarchical tree that connects genes with increasingly similar expression patterns.

Pathways significantly enriched for differentially-expressed genes.

Pathway	Count	*P* value	Gene
Systemic lupus erythematosus	23	5.76E-22	Up: C4B;C4A;HIST1H2BC;HIST1H2BE;HIST1H2BF;HIST1H2BG;HIST1H2BI;HIST3H2A Down: H2AFX;HIST1H4A;HIST1H4B;HIST1H4C;HIST1H4D;HIST1H4E;HIST1H4F;HIST1H4H;HIST1H4I;HIST1H4J;HIST1H4K;HIST1H4L;HIST2H4B;HIST2H4A;HIST4H4
MAPK signaling pathway	16	6.21E-09	Up: ACNA2D3;RASGRP2;ARRB1;DUSP5;ARRB1;HSPA2;HSPA1A;HSPA1B;DUSP4;DUSP1;GADD45B;MAP3K5;MAPK13;MKNK2 Down: RPS6KA2;BDNF;RPS6KA2;BDNF;TGFBR1
O-Glycan biosynthesis	7	1.06E-08	Up: Down: GALNT1;GALNT10;GALNT13;GCNT1;GALNT3;GALNT5;C1GALT1
TGF-β signaling pathway	9	1.06E-07	Up: BMP2 Down: DCN;DCN;DCN;ID4;LTBP1;DCN;ID3;LTBP1;CDKN2B;ACVR2A;TGFBR1;INHBA
Complement and coagulation cascades	8	2.23E-07	Up: MASP1;PLAU;PLAU;F3;PROS1;C4B;C4A;BDKRB2 Down: CFH
p53 signaling pathway	8	2.23E-07	Up: GTSE1;GADD45B;RRM2;CCNB1;CCNB2 Down: ATM;LOC651610;IGFBP3

Figure 5: Shows the results of the Affymetrix Microarray. Shows which genes were upregulated (high expression) or downregulated (low expression).

/important Description of each pathway:
Systemic lupus erythematosus: An autoimmune disease with autoantibodies that target almost every organ. Most commonly occurs in women in their reproductive years. - https://www.youtube.com/watch?v=g9dSVkqzs7k /tutorial. Genes shown in this table have been previously associated with SLE.

MAPK signaling pathway: Associated with cancers by activating receptors/ kRAS or bRAS mutations. - https://www.youtube.com/watch?v=oDjDUUhGVsI /tutorial

O-Glycan biosynthesis: O-Glycan is a glycan linked to the oxygen atom of Serine or Threonine. They are mainly assembled in the ER and Golgi Apparatus. - https://www.youtube.com/watch?v=Vu8t7c310m0&t=110s /tutorial

TGF-Beta signaling pathway: Involved in cell growth for adult organisms and embryos. - https://www.youtube.com/watch?v=TfPHZqT7b9A /tutorial

Complement and coagulation cascades: Calls platelets to a wound. - https://www.youtube.com/watch?v=cy3a__OOa2M /tutorial

	P53 signaling pathway: A tumor suppression protein that can pause the cell cycle. Is activated due to double DNA strand breaks - https://www.youtube.com/watch?v=Y7KEQQXZxwo /tutorial
VOCAB: (w/definition)	**Adipose Tissue:** A type of connective tissue **Osteogenic Cells:** Stem cells in the bone. **Chondrogenic Cells:** Cells found in cartilage **Passage Capacity:** Transfering cells from one culture vessel to another to allow for continued growth. Passage number is the number of times cells were transferred to another vessel to avoid overconfluence. **Pluripotency:** Ability of a subject in producing multiple biological responses Markers that were expressed in amniotic fluid-derived MSCs: **Nanog:** A key regulator of embryonic development and cellular reprogramming. It has been broadly expressed in certain cancers. It promotes the proliferation of stem-cell like cancer cells. It's also a transcription factor that helps embryonic stem cells maintain their pluripotency. **SSEA-4:** An observed aspect of heterogeneity in MSCs. Often found in undifferentiated embryonic stem cells, iPSCs, and tumor cells. **OCT-4:** A protein encoded by the POU5F1 gene. It is involved in the self-renewal of undifferentiated embryonic stem cells. **Mesoderm three-line:** The middle germ layer that develops during gastrulation. **Allotransplantation:** transplantation of cells, tissues, or organs from a genetically non-identical donor that is from the same species. **Liposuction:** Process for collecting fat tissue and in this case, lipids **Confluence:** The percentage of the petri dish that is covered by adherent cells. **Centrifuge:** Uses centrifugal force to create a constant force. **Supernatant:** A solution above the solid that has been forced to the bottom of the tube: https://www.chemedx.org/JCESoft/jcesoftSubscriber/ChemPagesLab/modules/centrifu/centsuper.htm

	Stromal Cell: Another term for mesenchymal stem cells **Affymetrix Microarray:** Allows for whole genome gene expression analysis https://www.youtube.com/watch?v=B7vRpnMkQFM /tutorial
Cited references to follow up on	
Follow up Questions	Does colony formation have any implications for regenerative medicine? Should we instead compare UCMSCs and MSCs? Why were Mouse anti-human monoclonal antibodies inserted into the solution? I noticed one of those antibodies were against SSEA-4, is there a connection there?

Article #10 Notes: Simulated Microgravity Reduces Proliferation and Reorganizes the Cytoskeleton of Human Umbilical Cord Mesenchymal Stem Cells

Source Title	Simulated Microgravity Reduces Proliferation and Reorganizes the Cytoskeleton of Human Umbilical Cord Mesenchymal Stem Cells
Source citation (APA Format)	Ho, C., Hoang, S., Doan, C., Le, H., Tran, D., & Le, L. (2020). Simulated Microgravity Reduces Proliferation and Reorganizes the Cytoskeleton of Human Umbilical Cord Mesenchymal Stem Cells. *Physiol. Res.*, *69*, 897–906. https://doi.org/10.33549/physiolres.934472
Original URL	https://www.biomed.cas.cz/physiolres/pdf/2020/69_897.pdf
Source type	Journal Article
Keywords	Cell proliferation • Cytoskeleton • Human umbilical cord mesenchymal stem cells • Simulated microgravity

#Tags	/spacemed /regenerativemedicine
Summary of key points + notes (include methodology)	Background: - Many studies have found decreased proliferation in MSCs, but found changes in differentiation direction in SMG vs hypergravity. Methodology: **WST-1 Assay:** Uses a color reagent in order to assess cell viability through the measurement of cellular metabolic activity. **Flow Cytometry Analysis:** Uses a flow cytometer (a device that uses Lasers to produce both scattered and fluorescent light signals. These signals are read by photodiodes or photomultiplier tubes, then converted into an electronic signal for a computer to analyze.) for a parametric analysis single cells in a solution. Multiple different dyes are used in flow cytometry analysis, such as fluorescently conjugated antibodies, DNA binding dyes, viability dyes, ion indicator dyes and fluorescent expression proteins **Western Blot Analysis:** A widely used technique to detect specific proteins in a sample. The process involves several steps: ▪ **SDS-PAGE:** Proteins are separated by size using gel electrophoresis. ▪ **Transfer:** The separated proteins are transferred from the gel to a membrane (e.g., nitrocellulose). ▪ **Blocking:** The membrane is blocked to prevent non-specific antibody binding. ▪ **Antibody Probing:** The membrane is incubated with a primary antibody that binds to the protein of interest, followed by a labeled secondary antibody that binds to the primary antibody. ▪ **Detection:** The protein is visualized by a detection system (e.g., chemiluminescence). **RT-PCR:** Reverse transcription polymerase chain reaction: Reverse transcribes RNA to DNA to amplify DNA targets. This is used to measure the amount of a specific RNA. **Hoescht 33342 staining:** Increases nuclear intensity to view chromatin condensation. **One-way ANOVA analysis:** Used to determine whether two or more sample means' are statistically significant. **Results**: **WST-1 Assay** showed that UCMCs have lower proliferation from the control. **Flow cytometry analysis** found that the percentage of SMG-exposed hucMSCs in G0/G1 was higher than the control group -> Suggests that the cells can't get past the growth/development phase and therefore are unable to proliferate.

	Western Blot Analysis found there was a reduction in the rate of **cyclin A1 and A2 (cyclin-CDK complexes that phosphorylate proteins specifically in the S and G2 phase (responsible for duplicating DNA and Organelles. A1 specifically is important for spermatogenesis)**. This initiates the phosphorylation cascade needed to initiate the responses in the S phase). Cyclin-kinase 4 and 6 were also depleted (Activated by D-type cyclins and is often the driving force of many types of cancers. - https://pmc.ncbi.nlm.nih.gov/articles/PMC9048628/ Found in G1 – S phase - https://www.sciencedirect.com/science/article/pii/S136876462400061X) Found that the total nuclear intensity was lower than the control group. The nuclear area and nuclear-shape value did not change. Used the cell app of the Cytell microscope. -> Suggests that the chromatin condensation was lower in SMG. **Western Blot Analysis + RT-PCR Analysis:** found a downregulation (decrease rate) of B-actin and a-tubulin compared to the control. -> results in the remodeling of microfilaments, since microtubules are made of tubulin and actin. Background: - The cytoskeleton is essential for cell proliferation, shape maintenance, and internal cellular organization. Implication/Further Research: - Mechanisms underlying this decrease is not well understood. It is believed that this is not due to apoptosis. It is hypothesized that changes in the cell cycle progression lead to the inhibition of cell proliferation.
Research Question/Problem/ Need	What is the effect of simulated microgravity through a 3D clinostat on the cytoskeleton proteins of the human Mesenchymal Stem Cells from the umbilical cord?

Important Figures

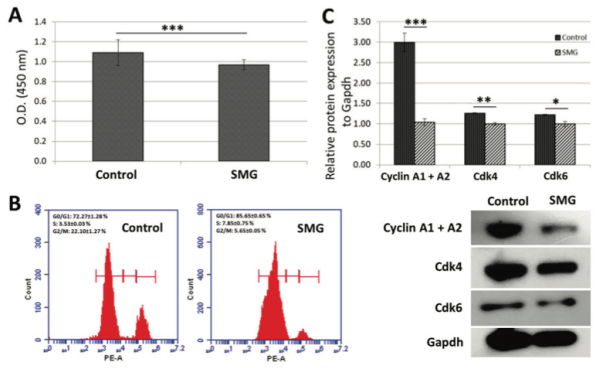


Fig. 1. The proliferation of hucMSCs from control and simulated microgravity (SMG) groups. (**A**) hucMSC proliferation assessed by a WST-1 kit. In the control group, the OD value of hucMSCs was higher than the SMG group. (**B**) Flow cytometry analysis of hucMSCs. In the SMG group, hucMSCs showed a higher G0/G1 ratio than the control group, resulting in the induction of cell cycle arrest in the hucMSCs. (**C**) The expression of major regulators was estimated by western blot. In the SMG group, the expression of cell cycle-related proteins in hucMSCs was downregulated. *** indicates a significant difference compared with the control group (P<0.001). ** indicates a significant difference compared with the control group (P<0.01). * indicates a significant difference compared with the control group (P<0.05).

Figure 1:

Important Vocab + Notes on Figures:

- Figure A: Absorbance Value/OD Value: The Optical Density Value was found to be lower in SMG group than the control group. The three stars suggests that the p-value (The probability that the results occurred by chance) is less than or equal to 0.001. The error bars for the control are larger than those of the SMG group, suggesting a higher variation for the control group than the SMG group. The error bars have little overlap, meaning that there is a slight statistically significant difference. SMG group: 0.97 +- 0.05 and control group 1.09 +- 0.13
- Figure B: Results of Flow Cytometry in a flow cytometry histogram. Proportion of cells in G0/G1 that were treated with SMG were higher than the control. The y-axis dictates the count of cells at a certain proportion. The x-axis dictates fluorescent intensity of phycoerythrin (fluorescent marker). Taller peaks indicate more cells at a fluorescence level. Every cell phase has a different fluorescence (indicated by the red bar). G0/G1 has a lower fluorescence while G2/M phase has the highest fluorescence.
- Figure C: Shows the results of Western Blot Analysis to estimate the number of regulators in each group. Interestingly, the different types of protein expressions have different statistical significance values, most likely due to the difference in the size of each data point (Mainly due to the equation to find standard deviation)
- The picture below the graph shows the downregulation of each protein in the Western Blot Analysis

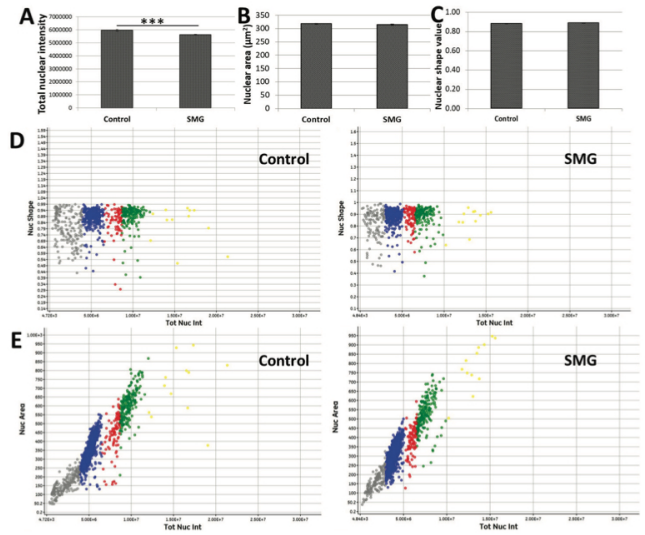


Fig. 2. The evaluation of nuclear morphology in human umbilical cord mesenchymal stem cells (hucMSCs). (**A**), total nuclear intensity, (**B**) nuclear area, and (**C**) nuclear shape of hucMSCs generated by the cell cycle app. In the control group, the nuclei of the hucMSCs showed a higher intensity than those from the simulated microgravity (SMG) group. There was no difference in the nuclear area or nuclear-shape value between the control and SMG groups. (**D**) The distribution of nuclear-shape values in relation to total nuclear intensity. (**E**) The distribution of the nuclear area in the relation to total nuclear intensity. Blue color indicates the G0/G1 phase, red indicates the S phase, green indicates the G2/M phase, grey indicates <2n, and yellow indicates >4n. *** indicates a significant difference compared with the control group (P<0.001).

Figure 2:
Figure A - C: Describes the nuclear intensity, nuclear area, and nuclear shape of the two groups using images of the cells. There is a difference in the nuclear intensity graph, but no difference in nuclear area and nuclear shape. This data was generated by the cell cycle app.
Figure D: Shows the distribution of the nuclear shape values in relation to total nuclear intensity. The y-axis shows nuclear shape values while the x-axis shows the total nuclear intensity. The data points in SMG yielded similar results to the control, suggesting little difference. Grey = <2n, yellow indicates >4n, Blue = G0/G1, red = S, green = G2/M.
Figure E: Same graph as Figure D, but shows the nuclear area rather than the nuclear shape values. The results in the two graphs are similar.

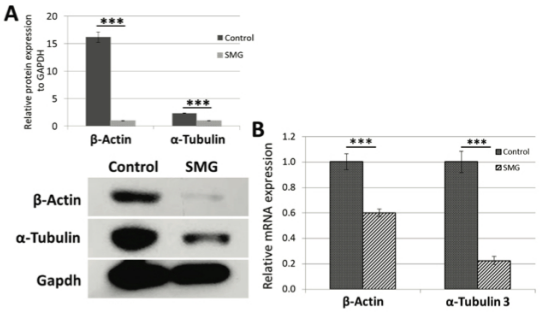


Fig. 3. The expression of β-actin and α-tubulin in human umbilical cord mesenchymal stem cells (hucMSCs). (**A**) A western blot analysis shows the expression β-actin and α-tubulin protein. In the simulated microgravity (SMG) group, hucMSCs demonstrated a lower expression of cytoskeletal proteins than the control group. (**B**) A qRT-PCR showing the transcript expression of β-actin and α-tubulin 3. The β-actin and α-tubulin 3 transcripts were attenuated in hucMSCs under SMG. *** indicates a significant difference compared with the control group (P<0.001).

Fig 3: Shows the expression of actin and tubulin, two proteins in the microtubules (essential for cell division). The levels of actin in the blot analysis are severely decreased from normal, and tubulin also decreased. Fig A shows the relative protein expression to GAPDH, showing how much of the area is covered by the proteins. Fig B shows the mRNA expression of actin and tubulin as revealed by the RT-PCR analysis.

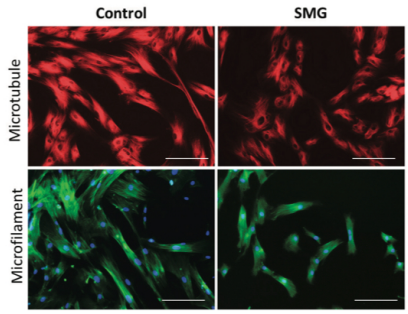


Fig. 4. Cytoskeleton staining of human umbilical cord mesenchymal stem cells. Microtubules were stained with SiR-tubulin (red). Microfilaments were stained with phalloidin (green). The nucleus was counterstained with H33342 (blue). (Magnification ×100), Scale bar = 200 μm.

Figure 4: Shows the cytoskeletons and microfilaments of both groups. The blue dots represent the stained nuclei.

VOCAB: (w/definition)

WST-1 Assay: Uses a color reagent in order to assess cell viability through the measurement of cellular metabolic activity.

Flow Cytometry Analysis: Uses a flow cytometer (a device that uses lazers to

produce both scattered and fluorescent light signals. These signals are read by photodiodes or photomultiplier tubes, then converted into an electronic signal for a computer to analyze.) for a parametric analysis of single cells in a solution. Multiple different dyes are used in flow cytometry analysis, such as fluorescently conjugated antibodies, DNA binding dyes, viability dyes, ion indicator dyes and fluorescent expression proteins. - https://pmc.ncbi.nlm.nih.gov/articles/PMC5939936/

Western Blot Analysis: A widely used technique to detect specific proteins in a sample. The process involves several steps:

- **SDS-PAGE:** Proteins are separated by size using gel electrophoresis.
- **Transfer:** The separated proteins are transferred from the gel to a membrane (e.g., nitrocellulose).
- **Blocking:** The membrane is blocked to prevent non-specific antibody binding.
- **Antibody Probing:** The membrane is incubated with a primary antibody that binds to the protein of interest, followed by a labeled secondary antibody that binds to the primary antibody.
- **Detection:** The protein is visualized by a detection system (e.g., chemiluminescence).

Nuclear Intensity: Average or integrated optical density (brightness) of a nucleus in an image, often reflecting how strongly the nucleus is stained with a dye/antibody (e.g., DAPI for DNA, or a transcription factor antibody in immunofluorescence). This tells the relative expression level or localization of a protein/nucleic acid inside the nucleus. Measured from microscopy images and calculating the mean pixel intensity.

Nuclear Area: The 2D of the nucleus in an image. Info on nuclear size, which can change in different conditions.

Nuclear Shape Value: Quantitative descriptors of the nuclear morphology, which includes things like aspect ratio, circularity (4pi x area / perimeter^2, if value is close to one, perfect circle), and solidity (how irregular the boundary is area/convex area).

Cyclin-Dependent Kinases: proteins that kinases that phosphorylate another protein to start a phosphorylation cascade.

Glyceraldehyde-3-phosphate dehydrogenase: An enzyme involved in glycolysis.

IGF-1: Insulin-like growth factor 1; a molecule similar to insulin that plays an

	important role in childhood growth and development. **Mesenchymal stem cells:** Stem cells with the ability to differentiate into any type of cell. They are able to transfer mitochondria to injured cells, deliver small therapeutics using EVs, and can be transplanted inspite of immune rejection. However, the ability to differentiate broadly creates a risk of unintentional differentiation, causing harmful effects. **Propidium Iodide:** An agent that can be used to stain cells and nucleic acids. **TRPC1:** Transient receptor potential canonical type 1; A type of ion channel located in the cell membrane.
Cited references to follow up on	https://www.youtube.com/watch?v=EJafMtUriRw (Not cited, but found during exploration of vocab. Shows how to measure nuclear intensity using ImageJ) Touchstone et al. 2019 Benavides et al. 2014 -> Can be a cool literature review on why there are mixed results in MSC proliferation Plett et al. 2004 Tan et al. 2018 Yan et al. 2015
Follow up Questions	What are the mechanisms behind this proliferation decrease? What are the differentiation directions of the humUCMSCs in SMG?

Article #11 Notes: TrpA1 is a shear stress mechanosensing channel regulating intestinal stem cell proliferation in *Drosophila*

Source Title	TrpA1 is a shear stress mechanosensing channel regulating intestinal stem cell proliferation in *Drosophilia* *Reread
Source citation (APA Format)	Jiaxin Gong *et al.* TrpA1 is a shear stress mechanosensing channel regulating intestinal stem cell proliferation in *Drosophila*.*Sci. Adv.***9**,eadc9660(2023).DOI:10.1126/sciadv.adc9660

Original URL	https://www.science.org/doi/10.1126/sciadv.adc9660
Source type	Journal Article
Keywords	N/A
#Tags	
Summary of key points + notes (include methodology)	Methadology: - Measured the mechanical forces through a genetically encoded CA^2+ indicator GCaMP6s. - This experiment was done ex vivo (after death). The team dissected the midgut to place it into the microfluidic chamber, a device used to simulate shear stress. The posterior midgut was cut open to expose epithelial cells from the lumen side, and a pump was connected to the microfluidic chamber to provide laminar flow. The flow is positively correlated with the magnitude of shear stress (in the human gastrointestinal tract, 30 dyne/cm^2). For the *Drosophila* midgut, they used a strength of 0.5 dyne/cm^2. - Provided stretching simulation by pushing a section of the gut down over a cavity, measuring the Ca^2+ responses in a neighboring region. - Provided compression stimulation by directly pushing the midgut with a fire polished glass. - Results: TrpA1 has been found to be mechanosensitive and in turn increases the rate of mitosis. However, the mitotic counts in TrpA1-FLAG flies is reduced. TrpA1-FLAG has a FLAG tag added to the C-terminus, suggesting that the N and C-termini have important roles in mechanosensing.
Research Question/Problem/ Need	What are the effects of mechanical forces in regulating adult stem cells and tissue growth.

Important Figures

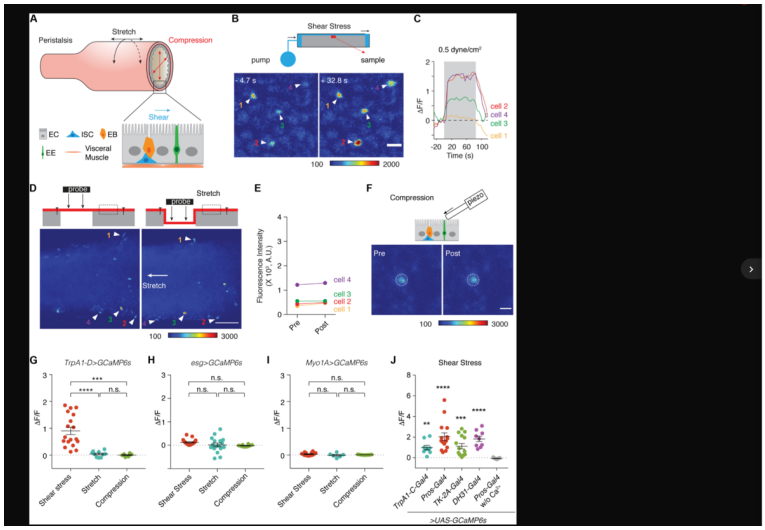


Figure 1: Describes the procedures used.

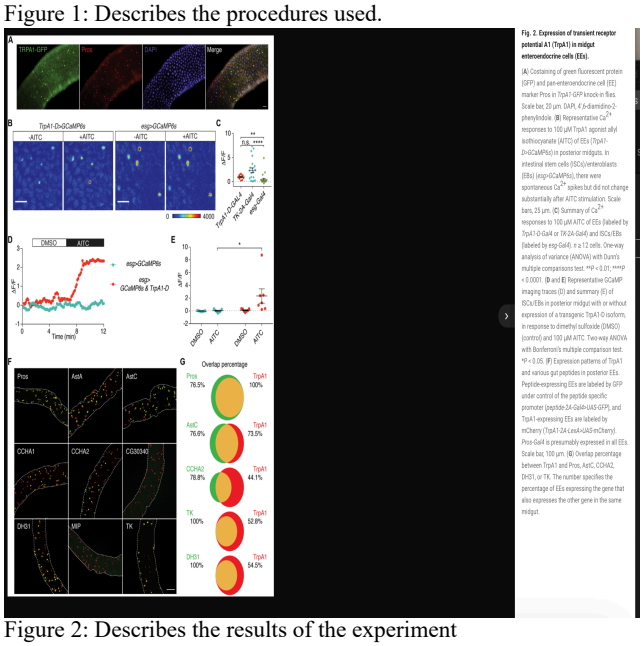


Figure 2: Describes the results of the experiment

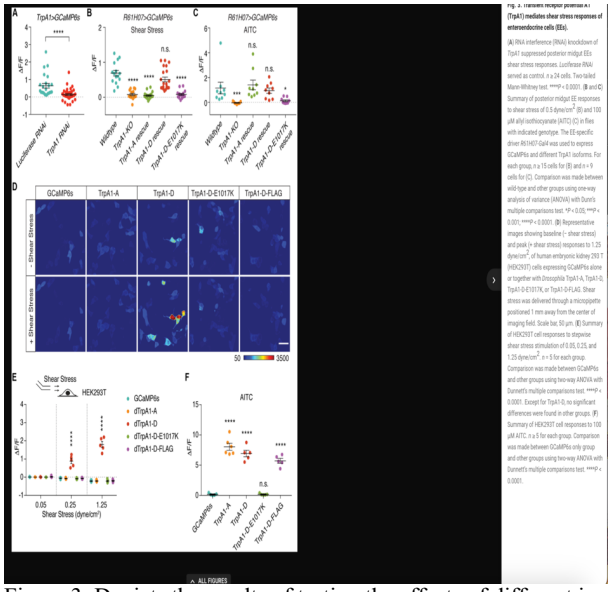


Figure 3: Depicts the results of testing the effects of different isomers of TrpA1.

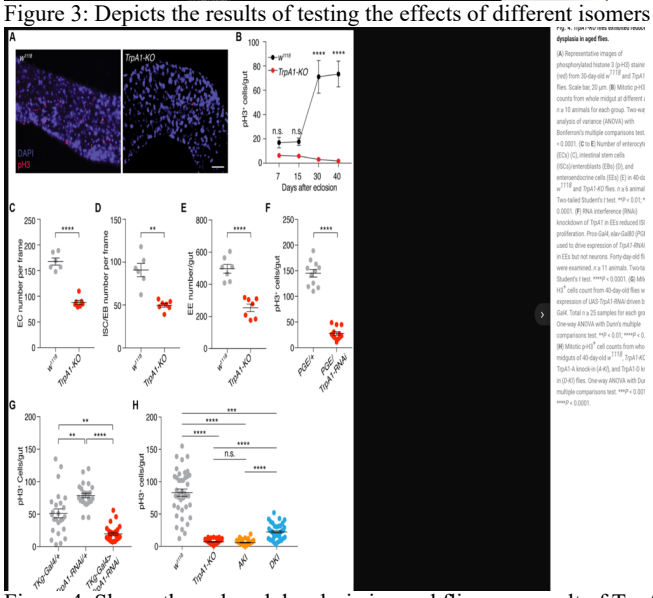


Figure 4: Shows the reduced dysplasia in aged flies as a result of TrpA1-KO.

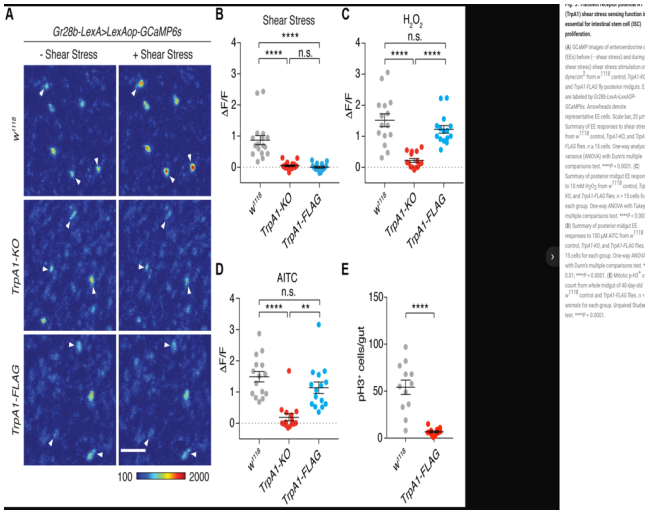


Figure 5: Shows how TrpA1 shear stress sensing function is essential for intestinal stem cell proliferation.

VOCAB: (w/definition)

Shear Stress, Stretch, and Compression: "Natural mechanical forces produced by gut peristalsis and passing of food and fluid."

Piezo Actuator: Made up of mechanical and electrical circuits that convert electrical signals to physical motion. This applies piezoelectric elements such as extremely small changes in elongation in the nanometer range, in part due to applied voltage.

Enteroendocrine Cells: "Smaller cells that are situated closer to the basal side of the midgut epithelium and serve to support various functions of the intestine." These are specialized epithelial cells.

Enterocytes: Cells that contain tight junctions in order to support absorption in the midgut epithelium.

Enteroblasts: Cells that divide and differentiate to replace other cells in the midgut.

***Drosophilia* TrpA1**: "a polymodal sensory ion channel that is known to be activated by heat and noxious chemicals and is involved in mechanical nociception."

Microfluidic Devices: Used to simulate sheer stress, a natural process that occurs

	in the gut. **Laminar Flow:** Equation: shear stress = 6(flow rate)n/(width x height^2). A type of fluid flow characterized by smooth, parallel layers, with no disruption between the layers. The equation provided calculates the shear stress exerted by this type of flow. This is the formula for a rectangular microchannel. **GCaMP Imaging:** A technique used to visualize and measure calcium signaling in living cells. GCaMP is a genetically encoded calcium indicator. When calcium ions bind to it, the protein fluoresces, allowing researchers to observe and quantify changes in intracellular calcium levels, which are often a proxy for neural activity or cellular responses. **Knock-in alleles:** Genetically engineered organisms where a specific gene or sequence has been inserted ("knocked in") into a specific location in the genome. This technique is used to study gene function, as it allows researchers to introduce a modified version of a gene (e.g., a fluorescently tagged version) to see how it affects the organism. **Transgenic Rescue Assays:** Experiments designed to "rescue" a mutant phenotype. If a specific gene is mutated, leading to a visible defect, a transgenic rescue assay involves introducing a functional copy of the same gene (a "transgene") into the organism. If the normal phenotype is restored, it confirms that the original gene mutation was the direct cause of the defect. **Heterologous cells:** Cells from a different species or tissue type than the one being studied. For example, a human gene might be expressed and studied in a mouse cell line. This is often done for simpler or more controlled experimental conditions. **Transfect:** The process of introducing foreign genetic material (like DNA or RNA) into eukaryotic cells. This is a fundamental technique in molecular biology used to study gene function and protein expression. **Age-related dysplasia:** Abnormal cell growth or development that is associated with the process of aging. In the gut, this can involve abnormal changes in the intestinal lining cells, which may be a precursor to cancer. **Gut motility:** The movement of the digestive system. This includes the involuntary muscle contractions (peristalsis) that move food and waste along the gastrointestinal tract. **Soft lithography:** A group of techniques used to fabricate or replicate structures using an elastomeric (stretchable) material, typically polydimethylsiloxane (PDMS). It is widely used in microfluidics to create the tiny channels and devices.

	Polydimethylsiloxane: A type of silicone-based polymer widely used in soft lithography and microfluidics. It is an ideal material for these applications because it is transparent, gas-permeable, non-toxic, and flexible, which allows researchers to create complex microstructures and simulate physiological environments.
Cited references to follow up on	Du et al. (23)
Follow up Questions	- Could we use this information to increase proliferation of intestinal stem cells for certain diseases? - What does the process for this experiment look like in real life? - Does shear stress cause damage to cells? Is that why TrpA1 is activated to increase proliferation? - To what degree can differences in results be chalked up to differences in methodology?

Article #12 Notes: Discoveries from Human Stem Cell Research in Space that are relevant to advancing cellular therapies on Earth

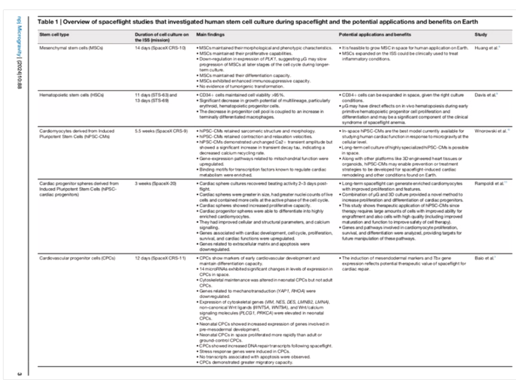
Article notes should be on separate sheets

Source Title	Discoveries from Human Stem Cell Research in Space that are relevant to advancing cellular therapies on Earth
Source citation (APA Format)	Ghani, F., & Zubair, A. (2024). Discoveries from Human Stem Cell Research in Space that are Relevant to Advancing Cellular Therapies on Earth. *Npj Microgravity*, *10*, 88. https://doi.org/10.1038/s41526-024-00425-0
Original URL	https://www.nature.com/articles/s41526-024-00425-0
Source type	Journal Article
Keywords	N/A

#Tags	/spacemedicine /mesenchymalstemcells
Summary of key points + notes (include methodology)	This study looks at the effects of real microgravity on different types of stem cells. Benefits of microgravity: - Creates a 3D cell culture by removing the effects of gravity - Increases cell proliferation Benefits and Drawbacks of MSCs - A challenge is maintaining stem cell properties and efficient expansion - Study by Huang et al. found that MSCs maintained proliferation and phenotype characteristics. - MSCs in space showed enhanced immunosuppressive abilities (prevents recipient's immune system from attacking the cells) - Conflicting studies on whether microgravity increases proliferation or decreases it Benefits and Drawbacks of Hematopoietic Stem Cells - Significant decline in myeloid progenitor cells and erythroid progenitor cells - An increase in macrophages - Suggests that spaceflight anemia could be a result of a suppression of erythropoiesis - Preserves stemness (suggested by reduced differentiation) - Inhibits migration potential, cell-cycle progression, and differentiation patterns and impair DNA damage repair. Benefits and Drawbacks of cardiomyocytes derived from iPSCs - Cardiomyocytes experience differential gene expression as a result of microgravity. There was upregulation of genes that were associated with proliferation and survival. - The cardiomyocytes had high enrichment and high viability. - Cardiomyocytes were able to be successfully cultured in microgravity Cardiovascular Progenitor Cells - Increased expression of DNA repair genes and paracrine factors - Enhanced migration - Reduced mechanotransduction and changes in cytoskeletal modifications - Neonatal CPCs exhibited increased expression of developmental markers while adult CPCs did not. Potentially causing adults to

Commented [DA2]: Effect of organoids in simulated microgravity?

	overexpress in YAP1 enable progenitors to have similar regeneration potential. Neural Stem Cells from iPSCs /neurons in microgravity - Preserved stemness and were able to proliferate after being unattended in the same culture medium. Markers for stemness that were elevated include *Sox2, Pax6,* and *Notch1*. - Tendency to differentiate into neurons in space -> Increased expression of mature neuron marker *Map2* and decrease in astrocyte marker *Gfap* and oligodendrocyte marker *Gal* and *Olig2.* - Neuroblasts placed in an NSM culture after returning on Earth proliferated for over a week before deciding to mature. - Growth rate was reduced. What makes this a great tool for neurodegenerative diseases is that the neural cells continued proliferating even after arriving on Earth and being placed in the Neural Specification Media. - Some cells from different studies showed enhanced stress responses and autophagy-like behavior and increased energy production demands - NSCs from hiPSCs exhibited an increased number of abnormal cell division
Research Question/Problem/ Need	What are the effects of real microgravity on different types of stem cells?
Important Figures	



npj Microgravity | (2024)10:88

Table 1 | Overview of spaceflight studies that investigated human stem cell culture during spaceflight and the potential applications and benefits on Earth

Stem cell type	Duration of cell culture on the ISS (mission)	Main findings	Potential applications and benefits	Study
Mesenchymal stem cells (MSCs)	14 days (SpaceX CRS-10)	• MSCs maintained their morphological and phenotypic characteristics. • MSCs maintained their proliferative capabilities. • Down-regulation in expression of *PLK1*, suggesting μG may slow progression of MSCs at later stages of the cell cycle during longer-term culture. • MSCs maintained their differentiation capacity. • MSCs exhibited enhanced immunosuppressive capacity. • No evidence of tumorigenic transformation.	• It is feasible to grow MSC in space for human application on Earth. • MSCs expanded on the ISS could be clinically used to treat inflammatory conditions.	Huang et al.[8]
Hematopoietic stem cells (HSCs)	11 days (STS-63) and 13 days (STS-69)	• CD34+ cells maintained cell viability >95%. • Significant decrease in growth potential of multilineage, particularly erythroid, hematopoietic progenitor cells. • The decrease in progenitor cell pool is coupled to an increase in terminally differentiated macrophages.	• CD34+ cells can be expanded in space, given the right culture conditions. • μG may have direct effects on in vivo hematopoiesis during early primitive hematopoietic progenitor cell proliferation and differentiation and may be a significant component of the clinical syndrome of spaceflight anemia.	Davis et al.[9]
Cardiomyocytes derived from Induced Pluripotent Stem Cells (hiPSC-CMs)	5.5 weeks (SpaceX CRS-9)	• hiPSC-CMs retained sarcomeric structure and morphology. • hiPSC-CMs retained contraction and relaxation velocities. • hiPSC-CMs demonstrated unchanged Ca2+ transient amplitude but showed a significant increase in transient decay tau, indicating a decreased calcium recycling rate. • Gene-expression pathways related to mitochondrial function were upregulated. • Binding motifs for transcription factors known to regulate cardiac metabolism were enriched.	• In-space hiPSC-CMs are the best model currently available for studying human cardiac function in response to microgravity at the cellular level. • Long-term cell culture of highly specialized hiPSC-CMs is possible in space. • Along with other platforms like 3D engineered heart tissues or organoids, hiPSC-CMs may enable prevention or treatment strategies to be developed for spaceflight-induced cardiac remodeling and other conditions found on Earth.	Wnorowski et al.[10]
Cardiac progenitor spheres derived from Induced Pluripotent Stem Cells (hiPSC-cardiac progenitors)	3 weeks (SpaceX-20)	• Cardiac sphere cultures recovered beating activity 2–3 days post-flight. • Cardiac spheres were greater in size, had greater nuclei counts of live cells and contained more cells at the active phase of the cell cycle. • Cardiac spheres showed increased proliferative capacity. • Cardiac progenitor spheres were able to differentiate into highly enriched cardiomyocytes. • They had improved cellular and structural parameters, and calcium signalling. • Genes associated with cardiac development, cell cycle, proliferation, survival, and cardiac functions were upregulated. • Genes related to extracellular matrix and apoptosis were downregulated.	• Long-term spaceflight can generate enriched cardiomyocytes with improved proliferation and features. • Combination of μG and 3D culture provided a novel method to increase proliferation and differentiation of cardiac progenitors. • This study shows therapeutic application of hiPSC-CMs since therapy requires large amounts of cells with improved ability for engraftment and also cells with high quality (including improved maturation and function to improve safety of cell therapy). • Genes and pathways involved in cardiomyocyte proliferation, survival, and differentiation were analyzed, providing targets for future manipulation of these pathways.	Rampoldi et al.[11]
Cardiovascular progenitor cells (CPCs)	12 days (SpaceX CRS-11)	• CPCs show markers of early cardiovascular development and maintain differentiation capacity. • 14 microRNAs exhibited significant changes in levels of expression in CPCs in space. • Cytoskeletal maintenance was altered in neonatal CPCs but not adult CPCs. • Genes related to mechanotransduction (*YAP1, RHOA*) were downregulated. • Expression of cytoskeletal genes (*VIM, NES, DES, LMNB2, LMNA*), non-canonical Wnt ligands (*WNT5A, WNT9A*), and Wnt/calcium signaling molecules (*PLCG1, PRKCA*) were elevated in neonatal CPCs. • Neonatal CPCs showed increased expression of genes involved in pre-mesodermal development. • Neonatal CPCs in space proliferated more rapidly than adult or ground-control CPCs. • CPCs showed increased DNA repair transcripts following spaceflight. • Stress response genes were induced in CPCs. • No transcripts associated with apoptosis were observed. • CPCs demonstrated greater migratory capacity.	• The induction of mesendodermal markers and *Tbx* gene expression reflects potential therapeutic value of spaceflight for cardiac repair.	Baio et al.[7]

3

Table 1 (continued) | Overview of spaceflight studies that investigated human stem cell culture during spaceflight and the potential applications and benefits on Earth

Stem cell type	Duration of cell culture on the ISS (mission)	Main findings	Potential applications and benefits	Study
	30 days (SpaceX CRS-11)	• Adult CPCs co-expressed several markers of early cardiovascular differentiation. • Adult CPCs expressed higher YAP1 levels in μG, especially after 12 days, but this declined at 30 days. • Adult CPCs maintained their viability and proliferative capabilities.	• Further studies defining the functional and safety implications of μG-activated cells in a model of cardiovascular repair would provide insight regarding μG-mediated conditioning in vivo. • Induction of adult CPCs to over-express YAP1 is a step towards conditioning these progenitors to have reparative potential closer to that of neonatal CPCs.	Camberos et al.[12]
Neural stem cells (NSCs)	39.3 days	• NSCs preserved their stemness in space. • NSCs maintained their proliferative capabilities. • NSCs in space exhibited a higher metabolic state (elevated oxygen consumption and glycolysis). • NSCs maintained their ability to become neurons in the appropriate conditions.	• NSCs can be expanded in space to increase neural cell numbers and address neurodegenerative diseases.	Cepeda et al.[13]

	Table 1: Summary of multiple space flight studies with different stem cells
VOCAB: (w/definition)	**Hematopoetic Stem Cells:** Cells that differentiate into all mature blood cell types **Myeloid Progenitor Cells:** Cells that give rise to monocyte/macrophage lineages as well as platelets, erythrocytes, and a variety of leukocytes **Macrophage:** A type of white blood cell that engulfs and digests pathogens. **Monocytes:** A type of white blood cell that turns onto macrophage or dendritic cells. **Erythrocytes:** A red blood cell **Leukocyte:** A type of blood cell made in the bone marrow that fight off infection and disease. **Erythroid progenitor cells:** Plays a key role in the process of creating red blood cells (**erythropoiesis**) **Sarcomere:** Smallest unit of muscle tissue **YAP1:** A protein in the Hippo signaling pathway that regulates cell proliferation/cardiac development. **LEO Environment:** Low Earth Orbit Environment
Cited references to follow up on	Huang et al. 2020 Monticone, M., Liu, Y., Pujic, N. & Cancedda, R. Activation of nervous system development genes in bone marrow derived mesenchymal stem cells following spaceflight exposure. Otsuka, T et al. Simulated Microgravity Culture Enhances the Neuroprotective Effects of Human Cranial Bone-Derived Mesenchymal Stem Cells in Traumatic Brain Injury Yuge, L et al. Simulated Microgravity Maintains the Undifferentiated State

	and Enhances the Neural Repair Potential of Bone Marrow Stromal Cells Merzlikina, N.V., Buravkova, L.B & Romanov, Y.A. The Primary Effects of Clinorotation on Cultured Human Mesenchymal Stem Cells Blaber, E. A. et al. Microgravity Reduces the Differentiation and Regenerative Potential of Embryonic Stem Cells. Cepeda, C. et al. Human Neural Stem Cells Flown into Space Proliferate and Generate Young Neurons
Follow up Questions	Does stem cell exhaustion occur under microgravity? /backup How expensive is the process of creating iPSCs versus extracting MSCs? Are the positive effects of microgravity mainly seen in bMSCs? Why did Cepeda, C. et al. not have to passage their neural stem cells? Is it due to the reduced proliferation?

Commented [DA3]: Article 12 found that microgravity reduces proliferation. I wonder if this is true in real microgravity? Additionally, many studies have found the opposite affect in Bone Marrow Stem Cells. I want to look at the differences between the two cells to determine a possible cause.

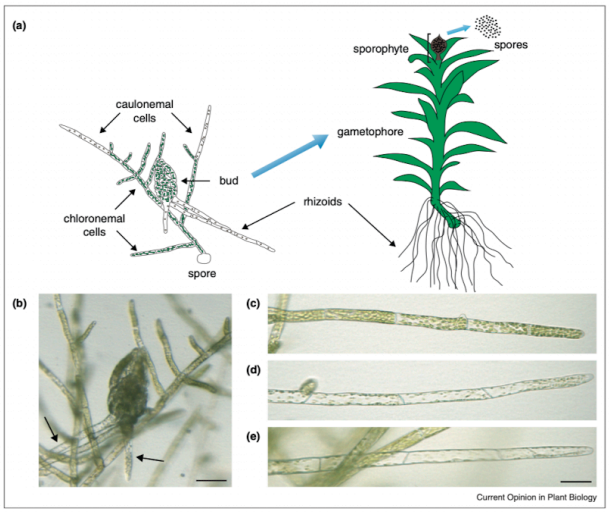
Commented [DA4]: I wonder whether the location of the cells (Embryo/Umbillical cord) had an affect on the results?

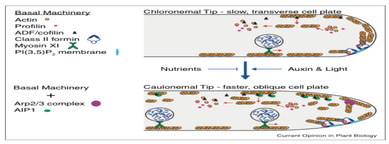
Commented [DA4R2]: *Make a mindmap of all this information so that I can create a proper hypothesis

Article #13 Notes: *Physcomitrella patens*: a model for tip cell growth and differentiation

Source Title	*Physcomitrella patens*: a model for tip cell growth and differentiation
Source citation (APA Format)	Vidali, L., & Bezanilla, M. (2012). Physcomitrella patens: A model for tip cell growth and differentiation. *Elsevier*, *15*(6), 625–631. https://doi.org/10.1016/j.pbi.2012.09.008

Original URL	https://www.sciencedirect.com/science/article/pii/S1369526612001203
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	- Moss plants grow through growth factors on the tip. The gametophyte has three filamentous tissues: chloronemata, caulonemata (which are cell types of protonema), and rhizoids - *Physcomitrella patens is a model system with great ability for reverse genetics* - /important Caulonemal cells develop within seven days of germination. They contain less chloroplasts (50 – 120) than chloronemal and have cell plates that are oblique to the long axis of the filament. These cells divide every 7 hours and at 20 mewm/hour. Mature cells that arise from caulonemal cells are often devoid of large organelles (may be harder to see the inside structure) - Chloronemata have more chloroplasts (80 – 140), have cell plates that are perpendicular to the long axis of the filament. These produce mature cells with the large organelles present - Gap in knowledge: How the actin cytoskeleton regulates the direction and rate of growth are not understood. - Actin Proteins of importance: - ADF/cofilin (RNAi-mediated silencing of Actin Depolymerizing Factor): Stunts plants and changes cells to small and depolarized. Asters form in this type of cell, most likely due to dramatic stabilization of the actin cytoskeleton. - The abundance of Class 2 Fomins suggests that fast actin polymerization is crucial to tip growth. Formin activity seems to be tightly regulated because only a fraction if they actively generate actin filaments. - A study done by Augustine et al. 2011 found that actin interactin protein 1 in *P. Patens* is crucial for rhizoid formation. Plants without these proteins are viable but only contain chloromata. //important - A transition from chloronemata to caulonemata is crucial to tip growth. - Auxin resistant mutants are unable to produce caulonemal cells. - The balance between chloronemata and caulonemata is dependent on light intensity, glucose and ammonium /important
Research	Investigating the potential of *P. Patens* as a model organism for tip growth and

Question/Problem/ Need	compares with Aribodopsis.
Important Figures	Figure 1  Tip growing cells in the moss *P. patens*. **(a)** Illustration of *P. patens* life cycle. **(b)** Young bud growing off chloronemata. Arrows indicate rhizoids emerging off the base of the developing gametophore. Scale bar is 50 μm. **(c)** Chloronemal filament contains many fully developed chloroplasts. The cell plates are perpendicular to the long axis of the filament. **(d)** Caulonemal filament has fewer less developed chloroplasts and the cell plates are oblique to the long axis of the filament. **(e)** While similar to caulonemata, rhizoids do not branch and the cell plates can be either perpendicular or oblique to the long axis of the filament. **(c–e)** Scale bar is 10 μm, shown in **e**. Figure 1: Describes the structures of the organs and cells of the moss P. patens. Figure 1A describes the general areas of cloronemal and caulonemal cells. Figure 1B showcases a bud growing off chloronemata. Figure 1C, 1D, and 1E show the differences between Caulonomel, chloronemal, and rhizoids cells, 1D being Caulonomela, 1E being rhizoids, and 1C being chloronemal.



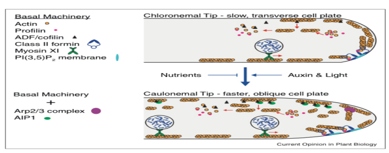
Model for tip growth in *P. patens*. The components of the basal machinery, which are essential for tip growth, are depicted in the top chloronemal cell. Class II formins are anchored at the cell cortex by binding to PI(3,5)P_2. Together with profilin, class II formins generate cortical actin filaments that are turned over by ADF-induced depolymerization. The cortical actin filaments are likely tracks for myosin XI, which transports exocytic vesicles to the tip of the cell. Additional factors necessary for differentiation of caulonemata and rhizoids are shown in the bottom caulonemal cell. Arp2/3 complex helps to polymerize more cortical actin. AIP1 works together with ADF to rapidly disassemble the actin network providing for rapid turnover of the actin cytoskeleton. More cortical actin provides additional tracks allowing for faster delivery of exocytic material to the cell apex.

Table 1

Actin cytoskeletal mutants and their loss of function phenotypes

Actin cytoskeleton protein	Loss of function method	Phenotype	Reference
Essential for tip growth			
Profilin	Transient RNAi	Essential, small plants composed of small spherical cells, altered actin organization	[10]
Actin depolymerizing factor (ADF)/cofilin	Transient RNAi	Essential, small plants composed of few unpolarized cells, altered actin organization, reduced actin dynamics	[6,7••]
Class II formins	Transient RNAi	Essential, small plants composed of small spherical cells, altered actin organization	[11••]
Myosin XI	Transient RNAi	Essential, small plants composed of small spherical cells, altered actin organization, normal actin dynamics	[14•]
Essential for tip cell differentiation			
ARPC1 (subunit of Arp2/3)	Stable RNAi	No caulonemata and rhizoids, shorter chloronemal cells	[5]
ARPC4 (subunit of Arp2/3)	Knock out	No caulonemata and rhizoids, shorter chloronemal cells	[18]
ARP3 (subunit of Arp2/3)	Knock out	No caulonemata and rhizoids, loss of polarized actin at apex	[19]
Brick (subunit of WAVE)	Knock out	Small depolarized protonemal cells with abnormal cell divisions, no rhizoids, altered actin organization	[20]
Actin Interacting Protein 1 (AIP1)	Transient RNAi & knock out	No caulonemata and rhizoids, shorter chloronemal cells, altered actin organization, reduced severing of actin filaments	[7••]
Other defects			
Class I formins	Transient RNAi	Small polarized plants, possible defects in cell division	[11••]
Myosin VIII	Transient RNAi & knock out	Smaller polarized plants with protonemal patterning defects and early gametophore formation	[47]

Figure 2: Describes the process of tip growth in a chloronemal vs. Caulonemal tip.



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Myosin VIII	Transient RNAi & knock out	Smaller polarized plants with protonemal patterning defects and early gametophore formation	[47]

Table 1: Describes the loss of function of different Actin cytoskeleton.

VOCAB: (w/definition)

Tip growth: "Polarized secretion of new extensible cell wall material to the apex of the cell, paired with turgor-driven cell expansion."

Protonema: A filamentous tissue that consist of chloronemal and caulonemal cells. These are the first tissue to arise from the spore.

Spore: A unit of sexual or asexual reproduction that can be adapted for dispersal or survival.

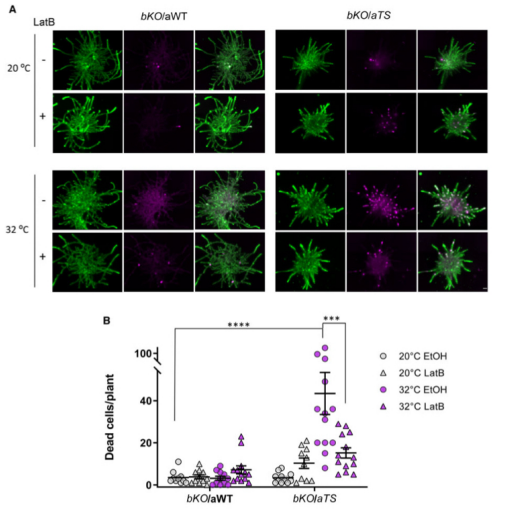
Rhizoid: Develops from the epidermal cells (The boundary between the internal and external environment) on the **gametophore** (structure where reproductive

	organs are born) **Aster:** consists of the centrosome and associated microtubules **Profilin:** A small actin monomer binding protein that is essential for many essential actin-based processes, such as tip growth, plant viability, and actin organization. **Formins:** Actin polymerization proteins. The poly-L-proline rich formin homology1 domain captures profilin-actin complexes. In P. Patens, Class II formins are essential for tip growth while class I are not. **Complementation:** The capacity of a segment of genetic material to rescue the phenotype of a mutation. Shows that a copy of a gene affected by a mutation is contained within the segment of genetic materials. **N-terminal PTEN domain:** PTEN proteins regulate rhizoid and gametophore development. **Phosphoinositides:** Plays multiple roles in eukaryotic cells. They play a prominent role in the signaling between membrane domains and the cytoskeleton. Identifies and properties to membranes. **Cytoplasmic Streaming:** Flow of the cytoplasm inside the cell. Myosin XIs are responsible for cytoplasm streaming in P. Patens **Motility:** Ability of an organism to move independently using energy. P. Patens do not possess the motility of organelles characteristic of cytoplasmic screening. **Scar/Wave (Wave Complex):** Five protein complex that activates nucleation. **Auxin:** A phytohormone that stimulates plant growth and cell division. elongation. Also play a role in cell differentiation and division. **Candidate Gene Approach:** **Dof Family of Transcription Factors:** Factors that have plant specific DNA-binding domains and are involved in a myriad of plant-specific functions. May be regulating cell differentiation in response to environmental nutrient conditions. **Nucleation:** The first step in actin polymerization
Cited references to follow up on	

Follow up Questions	- What is poly-L-proline binding and how does it aid actin organization - What is the difference between each Formin Class? - What is the PTEN domain? - There are a lot of unknowns in this article. I wonder if any of these unknowns had been resolved by now? - Are there studies on ROP genes now? (Small GTPases) - Why are caulonomal cells affected by Auxin and AIP1 mutations?

Article #14 Notes: Myosin XI drives polarized growth by vesicle focusing and local enrichment of F-actin in *Physcomitrium patens*

Source Title	Myosin XI drives polarized growth by vesicle focusing and local enrichment of F-actin in *Physcomitrium patens*
Source citation (APA Format)	Giulia, G., Wisanpitayakorn, P., Bibeau, J., Liu, Y.-C., Furt, F., Pierce, E., Parker, S., Tüzel, E., & Vidali, L. (2021). Myosin XI drives polarized growth by vesicle focusing and local enrichment of F-actin in Physcomitrium patens. *Plant Physiology*, *187*(4), 2509–2529. https://doi.org/10.1093/plphys/kiab435
Original URL	https://academic.oup.com/plphys/article/187/4/2509/6374457?guestAccessKey=03652345-fbdb-4742-8f64-ef16e294b6d4&login=true
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include	- Myosin XI anticipates F-actin accumulation at the cell tip. - By using *Physcomitrium patens* with an allele for myosin XI that is temperature sensitive. Loss of Myosin XI alters tip cell morphology,

methodology)	vacuolar homeostasis, and cell viability. However, this did not happen after F-actin depolymerization, suggesting that Myosin XI function does not depend on F-actin depolymerization - Methadology: Started with culturing a moss line with the TS allele for myosin XI. They did this by mutagenizibg residues through higher temperatures. - RNAi-based complementation assay to identify amino acids in the myosin XI protein that have temperature sensitivity. - Plants were then exposed at 20 degrees celsius and 32 degrees celsius for 3 days, then imahed.
Research Question/Problem/ Need	What drives growth in P. Patens?
Important Figures	 **Figure 2** Myosin XI *bKO/aTS* cells die after 24 h exposure at 32°C, and depolymerization of F-actin via LatB reverts the phenotype. A, Representative *bKO*/aWT and myosin *bKO/aTS* plants exposed at 32°C for 24 h, with ethanol (vehicle control) or LatB (20 μM). The plants were 11 d old when exposed to the high temperature. In (A), green represents calcofluor staining and magenta the dead cells stained with propidium iodide. (B) Quantification of the number of dead cells per plant. Number of plants analyzed: *bKO*/aWT 20°C ethanol, 10; *bKO*/aWT 20°C LatB, 11; *bKO*/aWT 32°C ethanol, 11; *bKO*/aWT 32°C LatB, 13; *bKO/aTS* 20°C ethanol, 11; *bKO/aTS* 20°C LatB, 10; *bKO/aTS* 32°C ethanol, 13; and *bKO/aTS* 32°C LatB, 13. Differences between groups were tested via a three-way ANOVA (adjusted *P*-values ****$P < 0.0001$, ***$P < 0.001$) and Tukey's post hoc tests. Error bars represent the SEM. Scale bar = 100 μm.

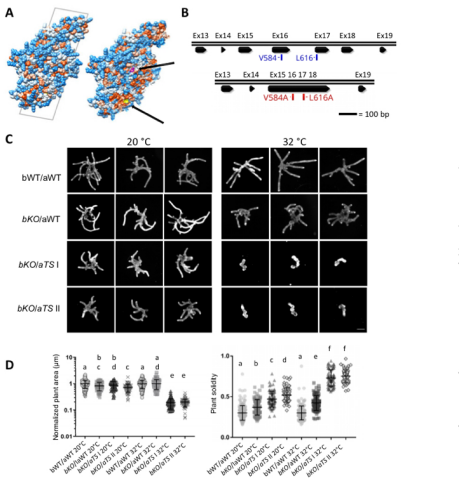


Figure 1 Two point mutations (V584A and L616A) render myosin XI protein TS. A, Myosin XIa motor domain modeled on the crystal structure of Myosin Vc from *Homo Sapiens* with SWISS-MODEL. The amino acid hydrophobicity was color-coded on the Kyte–Doolittle scale; low hydrophobicity is depicted in blue, high in orange-red. The surface of the domain was sliced to show the two point mutations buried in the hydrophobic core, Valine in yellow and Leucine in Magenta (arrows). B, Allele structure of for the myosin XIa gene in the lines *bKO/aWT* (top) and *bKO/aTS* (bottom). C, Representative images of 1-week-old control and mutant plants grown at 20˚C and 32˚C. The plants shown at the top row are the parental line with myosin XI bWT/aWT. In (C), the cellulose is stained with calcofluor-white. Myosin XI *bKO/aTS* I and II represent two independent lines harboring the TS allele. Scale bar = 50 µm. D, Quantification of normalized plant area and solidity of control and mutant plants grown at 20˚C and 32˚C. Number of plants analyzed: bWT/aWT 20˚C, 174; bWT/aWT 32˚C, 163; *bKO/aWT* 20˚C, 96; *bKO/aWT* 32˚C, 92; *bKO/aTS* I 20˚C, 76; *bKO/aTS* I 32˚C, 78; *bKO/aTS* II 20˚C, 80; and *bKO/aTS* II 32˚C, 73. Error bars represent the SD of the mean. Comparison among groups performed via two-way ANOVA and Tukey's post hoc tests, each letter corresponds to a significantly different group (adjusted $P < 0.05$).

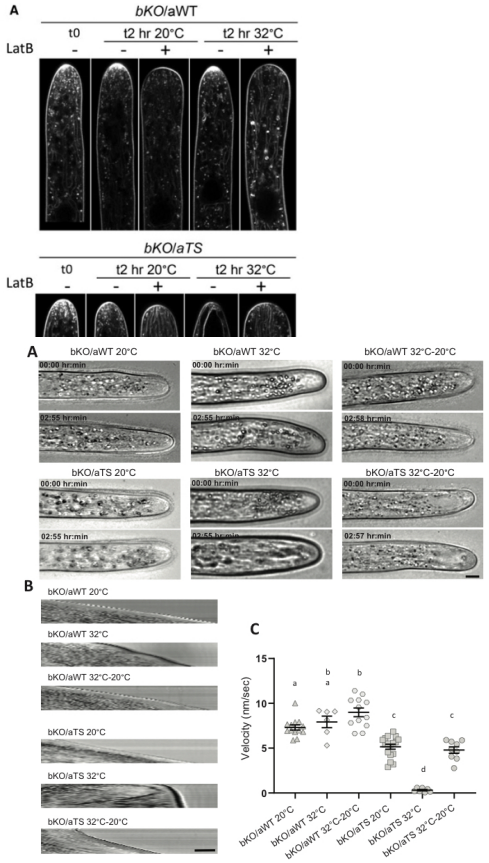


Figure 4 Time-lapse of myosin XI *bKO/aTS* cells at 32°C show reversible growth defects. A, Representative images of myosin XI *bKO*/aWT and *bKO/aTS* cells incubated at different temperatures. Cells were imaged in a microscope enclosed in a temperature-controlled chamber. Cells are shown before (top) and after (bottom) 3 h at the specified temperature. In the case of the 32–20°C, the cells were incubated first for 30 min at 32°C and imaged at 20°C. Scale bar = 10 μm. B, Representative kymographs depicting cell growth rates. The vertical total size represent ~3 h, the horizontal scale bar = 10 μm. C, Growth velocity (nm/s) of *bKO*/aWT and *bKO/aTS* cells as quantified from kymographs. Number of cells analyzed: *bKO*/aWT at 20°C, 13; *bKO/aTS* at 20°C, 17; *bKO*/aWT at 32°C, 6; *bKO/aTS* at 32°C, 7; *bKO*/aWT at 32–20°C, 12; and *bKO/aTS* at 32–20°C, 9. Differences were tested via one-way ANOVA and Tukey's post hoc tests, error bars represent SEM, each letter corresponds to a significantly different group (adjusted $P < 0.05$).

VOCAB: (w/definition)	**Myosin XI:** a plant "motor" that is involved in vesicular transport, organelle motility, and plant growth

Cited references to follow up on	N/A
Follow up Questions	What further experiments were conducted after this study, as this is a multi-stage study?

Article #15 Notes: Cells reprogramming to stem cells inhibit the reprogramming of adjacent cells in the moss *Physcomitrella patens*

Commented [DA5]: Very interesting! Definitely will revisit and contact the professors.

Source Title	Cells reprogramming to stem cells inhibit the reprogramming of adjacent cells in the moss *Physcomitrella patens*
Source citation (APA Format)	Sato, Y., Sugimoto, N., Hirai, T., Imai, A., Kubo, M., Hiwatashi, Y., Nishiyama, T., & Hasebe, M. (2017). Cells reprogramming to stem cells inhibit the reprogramming of adjacent cells in the moss Physcomitrella patens. *Scientific Reports*, *7*. https://doi.org/10.1038/s41598-017-01786-1
Original URL	https://www.nature.com/articles/s41598-017-01786-1
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	- Cells were isolated from a **gametaphore leaf** //important - Ferns have one single stem cell in the meristem //plantstemcells (Cannot use ferns in experimental design). Differentiated cells in ferns divide to form a stem cell. Additionally, if there is an absence of stem cells, then a

	single stem cell is regenerated. - Conclusion: Stem Cells produce an inhibitory signal that prevents surrounding cells from creating a stem cell. "When a single prothallus cell is isolated from adjacent cells by needle ablation, the further the isolated cell is from the original stem cell, the more quickly it reprograms into a stem cell." **This suggests a spatial gradiant formed by the inhibitory factor. Concentration near the apical stem cell is higher.** - Mosses have the ability to form stem cells from differentiated cell **after wounding** //important //plantstemcells. Mosses have a single stem cell, like ferns, but their differentiated cells can be reprogrammed to a stem cell without cell division. Additionally, **in P. Patens, multiple differentiated leaf cells experiencing a cut are reprogrammed to become chloronema apical stem cells just within 48h without any phytohormones.** - A single isolated leaf cell reprograms to a stem cell. This is in the abscence of other cells, meaning the inhibitory factor is not present. - The size of a chloronema apical stem cell is larger than that of a gametophore leaf cell //important //plantstemcells - Two adjacent differentiated cells inhibit the adjacent cell from reprogramming. Only one becomes the stem cell - The probability of one differentiated cell, the both cells reprogrammed, or neither is represented by p^2, 2p(1 – p), and (1 – p)^2; the Hardy-Weinberg equilibrium, which was used to determine the probabilities. - When two cells were aligned perpendicular to the leaf axis, no inhibition of cell-fate occured. //important - Phytohormone supplementation did not affect cell-date dependent inhibition - Growing conditions for P. Patens: A wild type was cultured for 4-5 weeks on BCDAT medium under continuous white light at 25 degrees Celsius. Reprogramming analysis involved the third to fifth gametophore leaf. Protonemata were cultured on BCDAT medium for 3-5 days.
Research Question/Problem/ Need	To prove the existence of an anisotropic inhibitory signal that regulates stem cell formation in *Physcomitrella patens*

Important Figures	

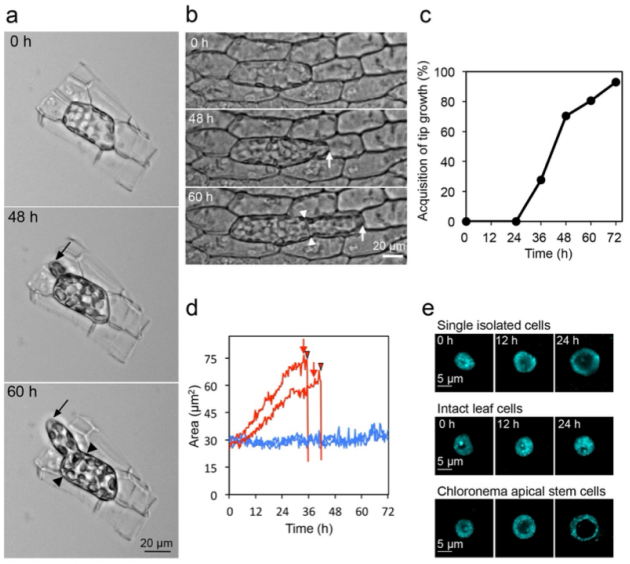


Figure 1. Conversion of an isolated gametophore leaf cell into a putative chloronema apical stem cell. (**a**) Time course images of reprogramming from a single leaf cell isolated with a carbon knife to a putative chloronema apical stem cell. Time after isolation and scales are indicated. (**b**) Time course images of stem cell formation as a single leaf cell isolated by laser ablation converts into a chloronema apical stem cell. Arrows indicate positions of a growing tip and arrowheads indicate a newly formed cell plate. Time after isolation and scales are indicated. (**c**) Percentage of single isolated cells (n = 98) exhibiting tip growth after isolation. (**d**) Changes of nuclear size during stem cell formation, analyzed by tracking HTB2-mRFP signals in single isolated cells (red) and those in intact leaf cells (blue). Arrows and arrowheads indicate time points when tip growth started and when cytokinesis was detected, respectively. (**e**) Fluorescence images of nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI) in single isolated cells, intact leaf cells, and chloronema apical stem cells. Images of chloronema apical stem cells with different size of nuclei are shown. Time after isolation and scales are indicated.

Figure 1: Figure 1A shows a single differentiated cell giving rise to a stem cell. Figure 1B shows multiple differentiated cells creating one stem cell. Figure 1C shows the percentages of isolated cells exhibiting tip growth. Figure 1D shows the nuclear size during stem cell formation. Figure 1E shows flueroscence images of nuclei in single isolated cells, intact leaf cells, and chloronema apical stem cells.

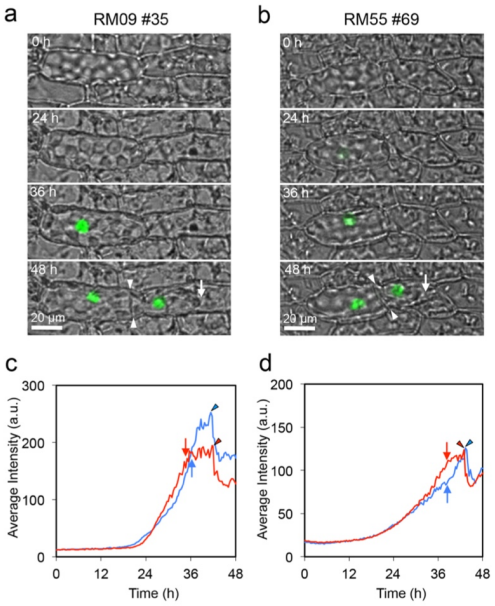


Figure 2. Promoter activities of protonema-specific genes *RM09* and *RM55* in a single isolated leaf cell. (**a**–**d**) Time course images (**a**,**b**) and change of average fluorescence intensity (**c**,**d**) of GFP signals in single isolated leaf cells. Isolated leaf cells of the protonema-specific marker lines (RM09 #35 and RM55 #69) were incubated on BCDAT medium. Twenty-four out of 27 in RM09 #35 and 33 out of 41 isolated cells in RM55 #69 showed tip growth until 72 h. *RM09* and *RM55* genes are specifically expressed in protonemata and not in leaf cells[44]. Images were taken at 20-min intervals for 48 h. Fluorescence images of GFP (green) are overlaid with bright field images (**a**,**b**). Time after isolation and scales are indicated. Arrows and arrowheads in (**a**) and (**b**) indicate positions of tip growth and newly formed cell plates, respectively. Those in (**c**) and (**d**) indicate time points when tip growth started and when cytokinesis was detected, respectively. Measurements from two independent cells are shown in red and blue (**c**,**d**).

Figure 2: All figures show data for single isolated leaf cell. All figures show changes in GFP signals in single isolated leaf cells.

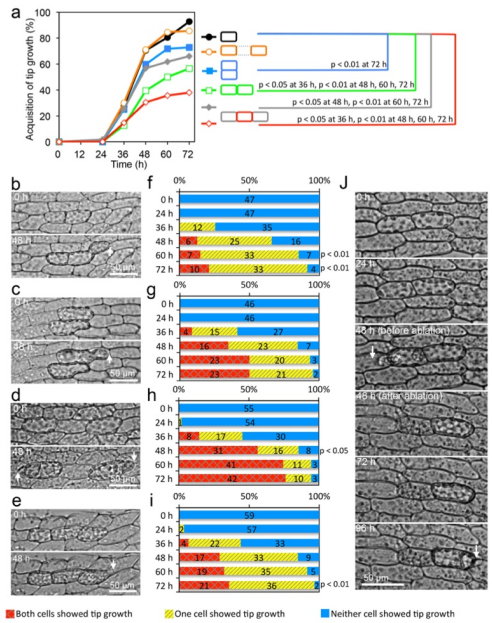


Figure 3. Intercellular communication during stem cell formation. (**a**) Percentages of the number of cells with tip growth in the total number of examined cells in each type of isolated cells. Single isolated cells (black solid circle, n = 98 single isolated cells, identical to Fig. 1c), two cells separated by a dead cell (orange open circle, n = 110 edge cells in 55 three aligned cells), two cells aligned parallel (green open square, n = 94 edge cells in 47 paired cells) or perpendicular (blue solid square, n = 92 edge cells in 46 paired cells) to a proximal-distal leaf axis, middle cells in aligned three cells (red open rhombus, n = 95 middle cells in 95 three aligned cells), and both edge cells of three aligned cells without tip growth in middle cells (gray solid rhombus, n = 118 edge cells in 59 three aligned cells) were examined. Statistical significance of difference from single isolated cells was examined by Fisher's test. (**b**–**i**) Images (**b**–**e**) and cell fates (**f**–**i**) in isolated pairs of cells aligned parallel (**b**,**f**) or perpendicular (**c**,**g**) to the leaf proximal-distal axis, isolated pairs of cells separated by a dead cell (**d**,**h**), and

Figure 3: Statistical analysis of the probabilities of both cells showing tip growth, once cell showing tip growth, or neither cell showing tip growth based on time.

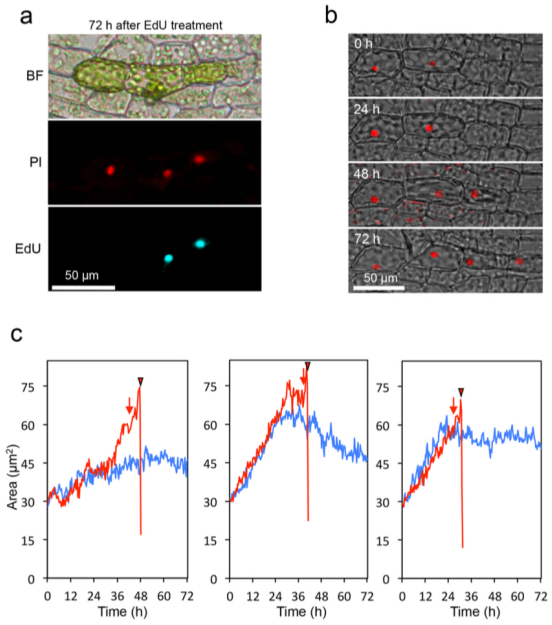


Figure 4. Changes of nuclei in isolated two adjacent cells. (**a**) Bright field (BF), propidium iodide (PI) fluorescence (red), and 5-ethynyl-2′-deoxyuridine (EdU) fluorescence (cyan) images of two adjacent cells incubated with 30 μM EdU for 72 h after isolation. (**b**) Sequential fluorescence images of the HTB2-mRFP signal were overlaid with bright-field images in isolated two adjacent cells aligned parallel to the leaf axis. Time after isolation is indicated. (**c**) Nuclear size analyzed by tracking the mRFP signal in isolated pairs of adjacent cells aligned parallel to the leaf axis. Nuclear sizes of cells with (red and magenta) or without (blue and cyan) tip growth and cell division are indicated. Arrows and arrowheads designate the time of commencement of tip growth and cell division, respectively.

Figure 4 analyzes the changes in nuclei between two adjacent cells.

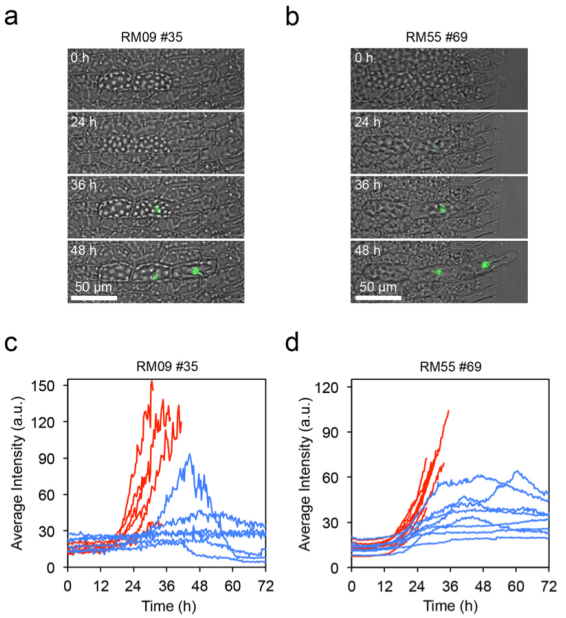


Figure 5. Promoter activities of protonema-specific genes *RM09* and *RM55* in isolated two adjacent cells. (**a**–**d**) Time course images (**a**,**b**) and changes of average fluorescence intensity (**c**,**d**) of GFP signals in isolated adjacent cells in RM09 #35 (**a**,**c**) and RM55 #69 (**b**,**d**) lines, respectively. Thirty-two out of 63 and 9 out of 26 pairs showed tip growth in only one cell. GFP (green) are overlaid with bright field images (**a**,**b**). Time after isolation and scales are indicated. Measurements of average intensity from independent cells (n = 6 in (**d**), n = 8 in (**e**)) are shown until the time of tip growth acquisition (**c**,**d**). Cells with and without tip growth were indicated in red and blue, respectively (**c**,**d**).

Figure 5: Looks at the changes in the fluorescence intensity of GFP signals in isolated adjacent cells.

VOCAB: (w/definition)

Anisotropic: A type of signal of which their strength varies with spatial orientation and direction

Proximinal-distal leaf axis: Governs polarized growth in plant cells.

Totipotency: Cells with the most differentiation ability.

Metazoans: An animal in the metazoa division

Fischer's Test: A statistical test for contingency tables. Usually used when sample sizes are small but can be used for all sample sizes.

Contingency table: A matrix that displays multivariate frequency distributions

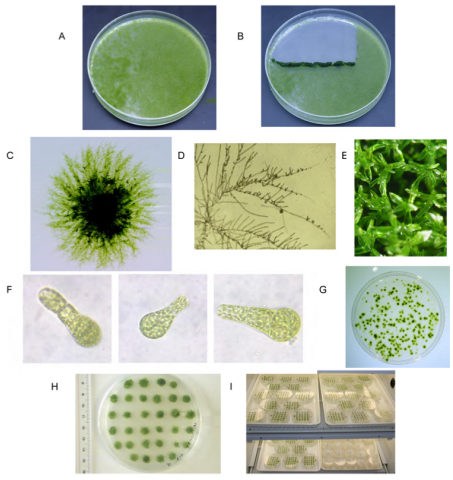
	of variables. **Hardy-Weinberg Equilibrium:** The allele distribution in a large, randomly mating population with no net immigration or emmigration.
Cited references to follow up on	Ishikawa, M. et al. Physcomitrella cyclin-dependent kinase A links cell cycle reactivation to other cellular changes during reprogramming of leaf cells.
Follow up Questions	Can I test the effects of this stem cell reprogramming under microgravity? How is it decided which differentiated cell will become the stem cell when they are adjacent? What is this inhibitory molecule?

Article #16 Notes: Isolation and Regeneration of Protoplasts of the Moss *Physcomitrella patens*

Commented [DA6]: Ask Dr. C whether these types of protocol articles are acceptable.

Commented [DA6R2]: Note that Protoplast isolation only is necessary if I am making any changes to gene expression

Source Title	Isolation and Regeneration of Protoplasts of the Moss *Physcomitrella patens*
Source citation (APA Format)	Cove, D., Perroud, P.-F., Charron, A., McDaniel, S., Khandelwal, A., & Quatrano, R. (2009). Isolation and Regeneration of Protoplasts of the Moss Physcomitrella patens. *Cold Spring Harbor Laboratory Press*, *4*(2). https://doi.org/doi:10.1101/pdb.prot5140
Original URL	https://mcdaniellab.biology.ufl.edu/wp-content/uploads/sites/37/Protoplast-isolation-and-regeneration.pdf
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	Overview of procedure: first, protonemal tissue is grown on **cellophane-overlay plates**, then harvested and treated with **0.5% Driselase** to induce protoplasts. - Materials: - BCB medium (link to how to create BCB medium)

	- D-Mannitol Solution - Driselase Solution - P.Patens, protonemal tissue - Protoplast regeneration medium for bottom layer (PRMB) - Protoplast regeneration medium for top layer (make sure to keep this medium at 45 degrees in a water bath. - Protoplast wash (PW) solution for moss - Centrifuge - Hemocytometer - Pipettes Key Notes from Methods: Estimated Time: 5 – 7 days
Research Question/Problem/ Need	A protocol on how to isolate protoplasts from the gametophyte tissue of *Physcomitrella patens*.
Important Figures	 FIGURE 1. Three-day-old filaments regenerating from *P. patens* protoplasts on PRMB medium. *Figure 1: An example of three-day old filaments from P. patens protoplasts.*

VOCAB: (w/definition)	**Driselase:** A cell wall degrading enzyme containing cellulase, hemicellulose, pectinase, etc. Effective for removing cell walls. More information: https://www.creative-enzymes.com/similar/driselase_205.html **Protoplasts:** A plant cell stripped of it's cell wall. **Hemocytometer:** A device used to estimate protoplast density
Cited references to follow up on	Culturing the Moss *Physcomitrella patens* (Cove et al. 2009b) -> Growth on cellophane overlay plates Somatic Hybridization in the Moss *Physcomitrella patens* Using PEG-Induced Protoplast Fusion (Cove et al. 2009c)
Follow up Questions	Did the procedure for using a hemocytometer change with the rise of ImageJ and Fiji? [p]

Article #17 Notes: Culturing the Moss *Physcomitrella patens*

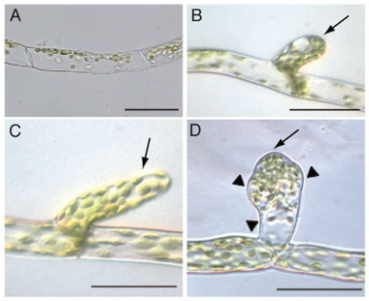
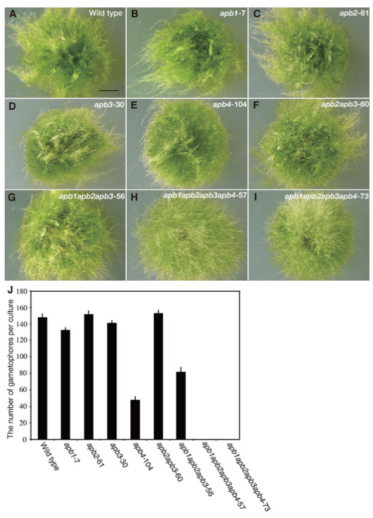
Source Title	Culturing the Moss *Physcomitrella patens*
Source citation (APA Format)	Cove, D., Perroud, P.-F., Charron, A., McDaniel, S., Khandelwal, A., & Quatrano, R. (2009). Ulturing the Moss Physcomitrellapatens. *Cold Spring Harbor Laboratory Press*, *4*(2). https://doi.org/10.1101/pdb.prot5136
Original URL	https://mcdaniellab.biology.ufl.edu/wp-content/uploads/sites/37/Culturing-the-moss-Physcomitrlla.pdf
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key	Overview: Gametophytes are axenically cultured on agar-based media and in

points + notes (include methodology)	shake liquid cultures. Requires materials such as Liquid nitrogen (or a device that can reach 10 degrees celsius) Suggests using standard microbiological procedures for spreading spores. Must be cultured for 10 days /important Then, the protonomal cells need to be blended and incubated for 7 days.
Research Question/Problem/ Need	Dictates a procedure to culture *Physcomitrella patens*.
Important Figures	
VOCAB: (w/definition)	**Axenically:** Germ-free/sterile **Microcentrifuge:** Used to hold smaller amounts of liquids (less than 5 ml). A centrifuge is a device that uses centrifugal force to separate liquids. **Micropore Tape:** Tape with tiny pores.
Cited references to follow up on	N/A
Follow up Questions	What are the standard protocals for spreading spores? How does this differ from simply germinating the seeds?

Article #18 Notes: **AP2-type transcription factors determine stem cell identity in the moss Physcomitrella patens**

Source Title	**AP2-type transcription factors determine stem cell identity in the moss Physcomitrella patens**
Source citation (APA Format)	Aoyama, T., Hiwatashi, Y., Shigyo, M., Kofuji, R., Kubo, M., Ito, M., & Hasebe, M. (2012). AP2-type transcription factors determine stem cell identity in the moss Physcomitrella patens. *Development and STEM Cells*, *139*(17), 3120–3129. https://doi.org/10.1242/dev.076091
Original URL	https://journals.biologists.com/dev/article/139/17/3120/45281/AP2-type-transcription-factors-determine-stem-cell
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	- While flowering plants only create stem cells in the sporophyte and gametophyte - Both protonema and gametophores come from apical cells - The stem cells in the gametophyte generation only have a small amount of already reported transcription factors due to lack if resources - Transcription factors that also exist in A. Thaliana include are of the Aintegumenta, plethora, and ABP family. - APB genes do not exist in protonema stem cells, only in gametophore. - Materials and methods: wild type *P. Patens* Bruch and Schimp. Susp. *Patens* collected in Gransden Woods was grown in BCDAT medium at 25 degrees celsius under continuous light, then transferred to BCDATG medium + cultivated for seven days under red light (specifically for auxin and cytokinin activity) - Conclusions: - APB genes are the main regulators of P. Patens - Disruption of all 4 AP2 transcription factors APBs cause deformations in apical cells

Commented [DA7]: Investigate how to isolate protoplasts from gametophores

Research Question/Problem/ Need	What are hidden transcription factors in gametophyte stem cell formation of *Physcomitrella patens*?
Important Figures	 **Fig. 1. Formation of secondary protonema apical cells and gametophore apical cells from caulonema cells.** (**A**) Caulonema cells. (**B**) A side branch initial cell (arrow) is formed from a caulonema cell. (**C**) Approximately 92% of side branch initial cells are fated to become a secondary protonema apical cell (arrow). (**D**) Approximately 5% of side branch initial cells are fated to become a gametophore apical cell (arrow) and divide to form gametophore cells (arrowheads). Scale bars: 50 μm.  **Fig. 2. The number of gametophores in APB single, double, triple and quadruple disruption lines.** (**A–I**) Protonema cultures of wild type (A), *apb1-7* (B), *apb2-61* (C), *apb3-30* (D), *apb4-104* (E), *apb2apb3-60* (F), *apb1apb2apb3-56* (G), *apb1apb2apb3apb4* (*apb*-quadruple)-57 (H) and *apb*-quadruple-73 (I). A pinch of protonemata was incubated on BCDAT medium for 16 days. Scale bar: 2 mm. (**J**) The number of gametophores per protonema culture. Bars represent the mean ± s.e.m. derived from data of five independent colonies.

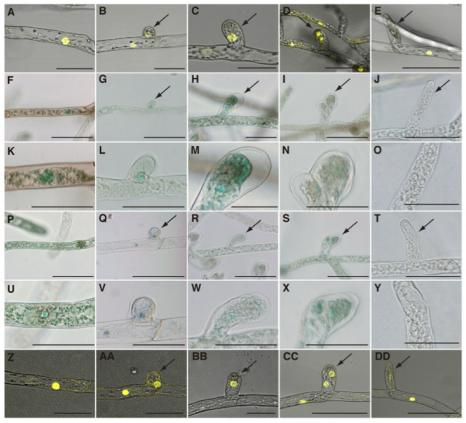


Fig. 4. APB-reporter fusion proteins were detected during gametophore apical cell formation but not during secondary protonema apical cell formation. (**A-DD**) Composites of bright-field and fluorescence images of APB1-Citrine (A-E) and APB4-Citrine (Z-DD) lines and bright-field images of APB2-GUS (F-O) and APB3-GUS (P-Y) lines in a caulonema cell before the initiation of an apical cell (A,F,K,P,U,Z), in a protruded side branch initial cell (arrows) and a parental caulonema cell just after cell division (B,G,L,Q,V,AA), in a swollen gametophore apical cell (C,H,M,R,W,BB; arrow), in a gametophore apical cell (arrows) and its daughter cell (D,I,N,S,X,CC) and in a secondary protonema apical cell (arrows in E,J,O,T,Y,DD). Magnified pictures of F, G, H, I, J, P, Q, R, S and T are shown in K, L, M, N, O, U, V, W, X and Y, respectively. Scale bars: 50 µm (A-C,K-O,U-BB), 100 µm (D-J,P-T,CC,DD).

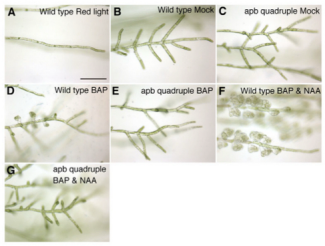


Fig. 3. Gametophore apical cells are replaced by secondary protonema apical cells in *apb*-quadruple lines. (**A–G**) The wild type (A,B,D,F) and the *apb*-quadruple-57 disruption line (C,E,G). Protonemata were grown in red light for one week (A) and then incubated in polarized white light for two days in the absence of BAP and NAA (B,C) or in the presence of 1 µM BAP (D,E) or 1 µM of both BAP and NAA (F,G). Scale bar: 200 µm.

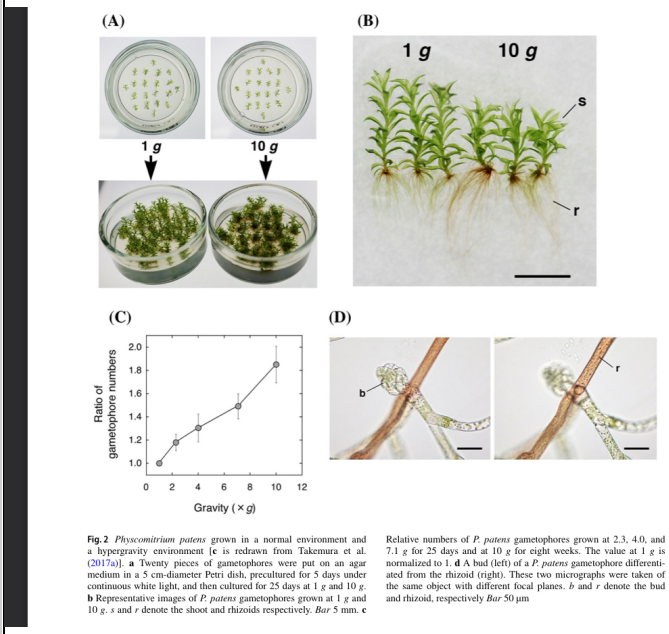
VOCAB: (w/definition)	- **Orthologous:** homologous sequences that originate from the same ancestor. - **Primary chloronema apical cell:** a stem cell that exhibits tip growth and is created during germination.

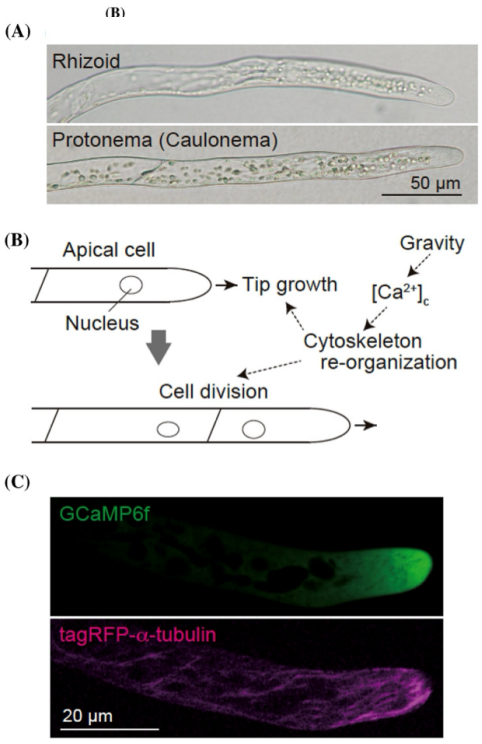
	- **Gametophores:** Stems and leaves that later transform into architonia and antheridia. - **Transformation:** process in which cells take in foreign DNA - **Southern Hybridization**: DNA labeling and primer amplification. Used to confirm correct gene targetting - **GUS**: the gene in which the promoter of a gene is inserted
Cited references to follow up on	N/A
Follow up Questions	- Why are certain transcription factors divided into families? (They have similar structures)

pay

Article #19 Notes: How plants grow under gravity conditions besides 1 g: perspectives from hypergravity and space experiments that employ bryophytes as a model organism

Source Title	How plants grow under gravity conditions besides 1 g: perspectives from hypergravity and space experiments that employ bryophytes as a model organism
Source citation (APA Format)	ne, A., Kamachi, H., Onoda, Y. *et al.* How plants grow under gravity conditions besides 1 *g*: perspectives from hypergravity and space experiments that employ bryophytes as a model organism.*Plant Mol Biol* **107**, 279–291 (2021). https://doi.org/10.1007/s11103-021-01146-
Original URL	https://link.springer.com/article/10.1007/s11103-021-01146-8
Source type	Journal Article
Keywords	N/A

#Tagste	/plantstemcells
Summary of key points + notes (include methodology)	- Aims to address the lack in access to methods used to simulate hyper gravity for prolonged plant duration culture. - For microgravity, plants were cultured on the ISS - Plants under hypergravity saw unexpected increments of chloroplast size and photosynthesis rate. Additionally, rhere was an increase in rhizoids and gametophores (involved in sexual reproduction) - Methadology: conducted a simultaneous study on microgravity on the ISS versus hypergravity using a centrifugal force machine
Research Question/Problem/ Ne	Study the effects of hypergravity (mor than 1G) on bryophytes, comparing them to normal gravity and microgravity
Important Figures	 **Fig. 2** *Physcomitrium patens* grown in a normal environment and a hypergravity environment [**c** is redrawn from Takemura et al. (2017a)]. **a** Twenty pieces of gametophores were put on an agar medium in a 5 cm-diameter Petri dish, precultured for 5 days under continuous white light, and then cultured for 25 days at 1 *g* and 10 *g*. **b** Representative images of *P. patens* gametophores grown at 1 *g* and 10 *g*. *s* and *r* denote the shoot and rhizoids respectively. *Bar* 5 mm. **c** Relative numbers of *P. patens* gametophores grown at 2.3, 4.0, and 7.1 *g* for 25 days and at 10 *g* for eight weeks. The value at 1 *g* is normalized to 1. **d** A bud (left) of a *P. patens* gametophore differentiated from the rhizoid (right). These two micrographs were taken of the same object with different focal planes. *b* and *r* denote the bud and rhizoid, respectively *Bar* 50 μm

	 **Fig. 4** Growth of *Physcomitrium patens* rhizoid and protonema, and subcellular localization of Ca^{2+} and cytoskeleton in protonemal cells. **a** Rhizoid and protonemata. Upper, rhizoid and lower image, protonema (caulonema). *Bar* 50 µm. **b** A schematic representation of tip growth and cell division in a rhizoid or a protonemal apical cell. A hypothetical model shows how gravity affects cytoskeletal re-organization through changes in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$). **c** A caulonemal cell co-expressing GCaMF6f (upper) and tagRFP-α-tubulin (lower) *Bar* 20 µm
Vocab	**Centrifugal Force:** a force that acts outward on a center
Cited references	"The life cycle of higher plants under microgravity conditions": conducted on the ISS
Follow up questions	N/A

Article #20 Notes: Transcriptome of Protoplasts Reprogrammed into Stem Cells in Physcomitrella patens

Source Title	Transcriptome of Protoplasts Reprogrammed into Stem Cells in Physcomitrella patens
Source citation (APA Format)	o, L., Zhang, L., Ge, Y., Zhu, H., & He, Y. (2012). Transcriptome of Protoplasts Reprogrammed into Stem Cells in Physcomitrella patens. *PLoS One*, *7*(4). https://doi.org/10.1371/journal.pone.0035961
Original URL	https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0035961&type=printable
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	- Reprogramming has changes associated with the cell cycle and specific cellular characteristics. Reprogramming can occur in animals, but are harder to induce. //important - Protoplasts from Physcomitrella patens easily regenerate into protonema and are useful for genetic studies. Protoplast regeneration also occurs in high frequency. Flow cytometry analysis reveals high synchronization of the cell cycle. (Meaning most cells are in the same cstage of the cell cycle at the same time). //important - Transcript levels of 4827 genes changed more than four-fold during protoplast reprogramming - Analysis of these genes found that a set of enriched Genetic Ontology terms and pathways were associated with photosynthesis, protein synthesis, and stress response. These includes decreased photosynthesis, increased fatty acid, O-glycan, flavonoid, flavone, and flavonol biosynthesis, and propionate metabolism - After 48 hours of culture, most protoplasts are successfully reprogrammed

	- Meristem identity related transcription factors: NAC2, CUC2, RD26, WOX13, BAM2, and a putative protein kinase. - It is largely hnknown how the regulators control reprogramming, but the most likely culprit are phytohormones.
Research Question/Problem/ Nee	What are the key genes and transcription factors related to differentiated cell reprogramming in Physcomitrella Patens?
Important Figures	Figure 1: Shows FCM results of each part/time to dictate the phase of the cell cycle

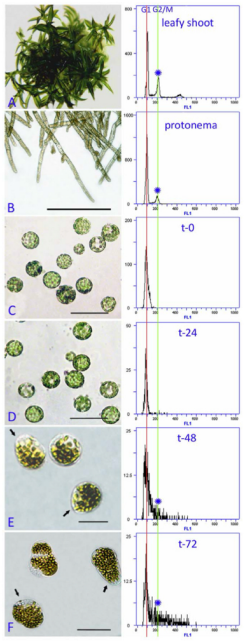


Figure 1. Morphology and evaluation of cell cycle by flow cytometry. (A) Two-month-old leafy shoots. (B) Six-day-old protonemata. (C) Freshly prepared protoplast. (D) Cells cultured for 24 hours. (E) Cells cultured for 48 hours. (F) Cells cultured for 72 hours. The stem cells are indicated by arrows in each stage. The S phase of cell cycle is represented by the area between the two red broken lines, and the G2/M phase is indicated by a blue star in each stage.
doi:10.1371/journal.pone.0035961.g001

cluded that protoplast reprogramming into stem cells occurred within 48 h.

Tag identification and quantification and depth of sequencing

To obtain global patterns of gene expression during protoplast reprogramming, RNA extracted from fresh protoplasts and cells cultured for 24, 48 and 72 h was used for DGEP analyses. More than 3.2 million raw tags (Table 1) were sequenced using the cDNA library derived from fresh protoplasts and cells cultured for 24, 48 and 72 h (Table 1). Custom Perl scripts were used for adaptor trimming and read parsing. Before mapping these tag sequences to the reference sequence, low-quality tags (tags containing 'N' and adaptor sequences) were filtered. To increase the robustness of the approach, single-copy tags in the four libraries were excluded [28]. The distribution of distinct clean tag counts over different tag abundance categories showed very similar tendencies for all four libraries (Fig. S1) [29,30]. Common and specific tags within and among samples are shown in Fig. S1.

Saturation of the library was determined by identification of unique tags. Sequencing reached saturation when no new unique tags were detected. The results shown in Fig. S2 indicated that all four sampling libraries were sequenced to saturation, and thus a full representation of the transcripts in the experimental conditions was obtained. In the four libraries fewer unique tags were identified as the number of sequencing tags increased, and reached a plateau shortly after 2 million tags were sequenced and a negligible increase in the number of genes detected in the four libraries was observed.

Mapping of short reads to the reference genome and detection of differentially expressed genes

Bowtie 0.12.7 was used to map unique consensus sequence tags (a total of two or more reads from all libraries) to the *P. patens* reference genome. Bowtie is an ultrafast, memory-efficient short-read aligner [31]. Bowtie indexes the genome with a Burrows-Wheeler index to keep its memory footprint small. This method performs effectively with DGEP data sets, which are reduced in size and complexity since reads are collapsed to unique tags before mapping. Finally, a preprocessed database of all possible CATG+17-nt tag sequences was created using reference gene sequences. All clean tags were mapped to the reference sequences and allowed no more than 1-nt mismatches. Clean tags mapped to reference sequences from multiple genes were filtered (Table 1). For genes that have multitags found in Solexa tags, the sum of all tags was considered as the gene expression value.

To compare gene expression profiles, we employed the TMM method from *edgeR* (*empirical analysis of digital gene expression in R*; to normalize the tag distribution per library and determine significance values for differentially expressed genes based on their relative abundance, which reflected the difference in number of tags between each two libraries. The *edgeR* algorithm uses an empirical Bayes approach to improve power in small sample sizes [32–34]. This approach accounts for biological and technical variation and has been implemented for tag-based data sets where small numbers of replicates are tested and standard errors disperse further from t

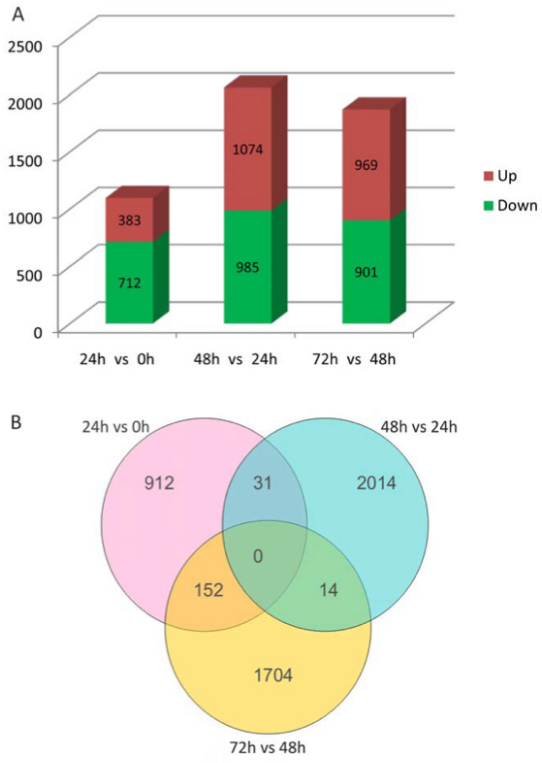


Figure 2. Genes differentially expressed at different time-points during protoplast reprogramming into stem cells. (A) Genes differentially expressed during specific phases of protoplast reprogramming into stem cells were separated into two groups on the basis of whether they were significantly up-regulated or down-regulated. (B) Venn diagrams showing the number of differentially expressed genes during specific time-points of protoplast reprogramming into stem cells.

Figure 2: upregulation and downregulation of genes.

Figure 3: DGT and PCE of five randomly selected genes.

Figure 4: shows enriched pathways over time.

Pathway_Name	P value (24h_vs_0h)	P value (48h_vs_24h)	P value (72h_vs_48h)
Nitrogen metabolism	**0.001566633**	0.341377869	0.156355902
Alanine, aspartate and glutamate metabolism	**0.003003252**	0.433168055	0.058728202
Selenoamino acid metabolism	**0.026989302**	0.078987413	0.674005365
Flavonoid biosynthesis	**0.032468515**	0.238004593	0.703204598
beta-Alanine metabolism	**0.049498538**	0.106304455	0.54854081
Porphyrin and chlorophyll metabolism	0.085563646	**0.000204566**	0.058728202
Lysine degradation	0.259496467	**0.009144941**	0.105298263
Fatty acid metabolism	0.283220555	**0.014905178**	0.127674964
Citrate cycle (TCA cycle)	0.361250811	**0.02224128**	0.061448004
C5-Branched dibasic acid metabolism		**0.033984077**	0.164550727
Glyoxylate and dicarboxylate metabolism	0.076595223	**0.034506079**	0.134889388
Oxidative phosphorylation	0.921897842	**0.035138912**	0.741859096
Pentose phosphate pathway	0.132829777	**0.040819516**	0.113558488
Valine, leucine and isoleucine biosynthesis	0.697037191	**0.040820386**	0.468305636
Spliceosome	0.986255574	0.200683453	**0.000348**
Peroxisome	0.578434766	0.052843696	**0.015050839**
Glutathione metabolism	0.388097268	0.302235402	**0.017308297**
mRNA surveillance pathway		0.301807439	**0.023749597**

Figure 5. Specific enriched pathways at specific stages during protoplast reprogramming into stem cells.
doi:10.1371/journal.pone.0035961.g005

Figure 5: shows significance levels for each pathway at all time intervals

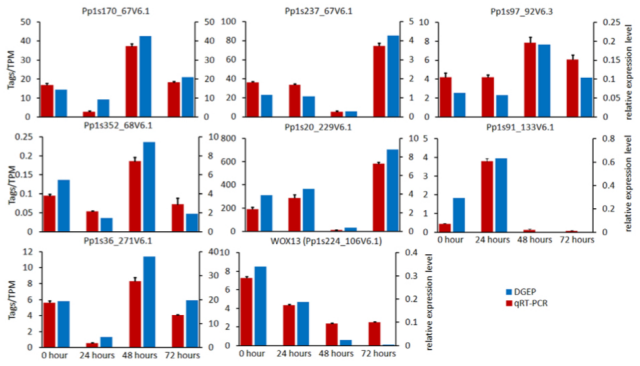


Figure 3. Digital gene expression tag profiling and quantitative real-time PCR analysis of the expression of five randomly selected genes. All real-time PCR reactions were repeated three times and the data are presented as the mean ± SD. The *x*-axis indicates the sampling time-points and cell types. The *y*-axis shows the expression levels: the left bar (red color) shows tag number per million tags by DGEP and the right (blue color) shows the relative expression level by qRT-PCR.
doi:10.1371/journal.pone.0035961.g003

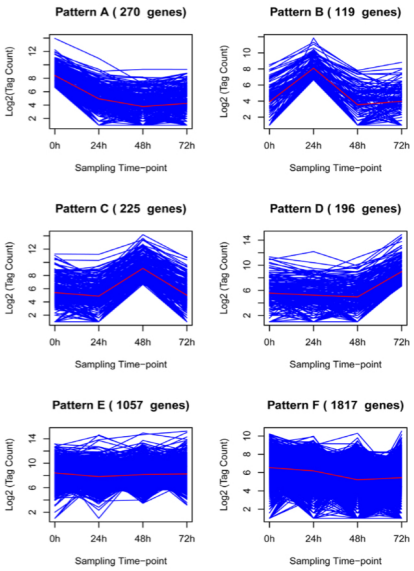


Figure 6. Patterns of gene expression by K-means cluster analysis in the developing gametophyte of *P. patens*. Differentially expressed genes across all four time-points were grouped into six patterns using the K-means clustering algorithm. The *y*-axis gives the tag count (on a $\log_2$ scale) of differentially expressed genes. Each line represents a different gene.
doi:10.1371/journal.pone.0035961.g006

Figure 6: tags present in each group at different intervals

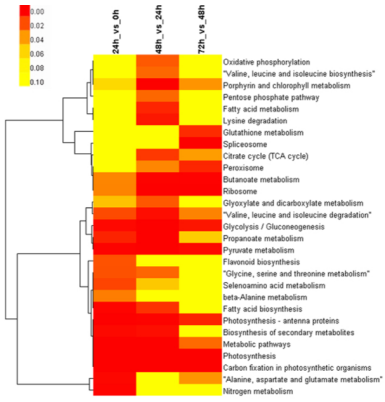


Figure 4. Heatmap of significantly enriched pathways. The yellow and red color shows the p-value of significantly enriched pathways.
doi:10.1371/journal.pone.0035961.g004

Vocab	**Asymmetric division:** a stem cell generates another stem cell and a differentiated cell. **Pyriform:** pear-shaped **Differentially expressed genes (DEGs):** genes that are differently expressed **Angiosperm:** a group in taxonomy consisting of all flowering plants **Orthologues:** genes from two different that are the same due to a speciation event. **Paralogues:** genes from the same genome that occurred from a gene duplication event. **Gymnosperms:** woody seed-producing plant **Callus Stage:** when undifferentiated, soft cells form on a wound, acting as a precursor to healing. This stage is not present during P. Patens protoplast regeneration. **NAC2:** controls age-dependent senescence and salt-promoted sensecence **RD26:** occurs in the nucleus and acts as an activator for ABA-mediated dehydration response. (Most likely due to the presence of mannitol in the culture medium) **CUC2:** expressed in the leaf sinus region for serration **Wuschel-related homeobox:** belong to homeodomain-containing transcription factors; key regulators in cell cycle and cell fate. WOX genes, as proposed by this study, may serve broader functions. **BAM2:** functions in multiple developmental processes suxh a leaf shape, size, and symmetry. Additionally, male gametophyte development and somatic cell fates and pollen mother cells are also effected by BAM2 **K-Means Clustering Analysis:** a method that assigns each point to the nearest cluster centroid and updates the centroids to minimize within-cluster variances.

Cited references to follow up on	
Follow up Questions	- Research gap: it is unknown how three WOC paralogs and NAC transcription factors function during development/protoplast regeneration. - How does reprogramming work in animals?

Article #21 Notes: **The moss Physcomitrella patens: methods and tools from cultivation to targeted analysis of gene function.**

Source Title	**The moss Physcomitrella patens: methods and tools from cultivation to targeted analysis of gene function.**
Source citation (APA Format)	tbek, C., Krinninger, S., & Frank, W. (2013). The moss Physcomitrella patens: Methods and tools from cultivation to targeted analysis of gene function. *The International Journal of Development Biology*, *57*, 553–564. https://doi.org/10.1387/ijdb.130189wf
Original URL	https://ijdb.ehu.eus/article/pdf/130189wf
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes	

(include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

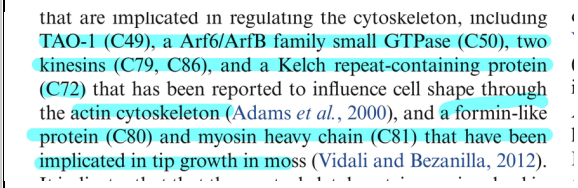
Article #22 Notes: Plant stem cells and their applications: special emphasis on their marketed products

Source Title	Plant stem cells and their applications: special emphasis on their marketed products
Source citation (APA Format)	
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC7275108/
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	
Research	

Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #23 Notes: The phosphoproteome in regenerating protoplasts from Physcomitrella patens protonemata shows changes paralleling postembryonic development in higher plants

Source Title	The phosphoproteome in regenerating protoplasts from *Physcomitrella patens* protonemata shows changes paralleling postembryonic development in higher plants
Source citation (APA Format)	
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC3991745/
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	- Through the use of a highly selective titanium dioxide based phophopeptide enrichment method and mass spectrometric technology, 300 phosphoproteins were found to be protoplast regeneration responsive. **Protoplast regeneration occurs through cell-wall synthesis, cytoskeleton construction, and cell cycle regulation**

	- Phosphorylated Proteins involved in protoplast regeneration in the primary cell wall: xyloglucan endotransglucosylase/hydrolase, SKU5 (C2), proline-rich family protein (C83), and glycone-rich protein (C84) - **Squamosa promoter binding protein: involved in cell-wall regeneration.** **Proteins in cell division during protoplast regeneration:**  that are implicated in regulating the cytoskeleton, including TAO-1 (C49), a Arf6/ArfB family small GTPase (C50), two kinesins (C79, C86), and a Kelch repeat-containing protein (C72) that has been reported to influence cell shape through the actin cytoskeleton (Adams *et al.*, 2000), and a formin-like protein (C80) and myosin heavy chain (C81) that have been implicated in tip growth in moss (Vidali and Bezanilla, 2012).
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definitionp)	**Morphogenesis:** formation of shapes and structures. **Organogenesis:** specification of organ identity. **Primary Cell Wall:** part of the cell wall containing a cellulose-xyloglucan framework, pectin, and structural protein. Y parallelm **Phragmoplast:** a structure in cytokinesis made up by parallel microtubules and actin filaments
Cited references to follow up on	
Follow up Questions	What functions do the flowering genes serve in P. patens?

Article #24 Notes: **The plant immune system**

Source Title	The plant immune system
Source citation (APA Format)	
Original URL	https://www.nature.com/articles/nature05286
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #25 Notes: **Biological and Mechanical Characterization of the Random Positioning Machine (RPM) for Microgravity Simulations**

Source Title	
Source citation (APA Format)	
Original URL	https://pmc.ncbi.nlm.nih.gov/articles/PMC8619501/
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Article #26 Notes: Gravitropism in tip-growing Cell

Source Title	Gravitropism in tip-growing Cells
Source citation (APA Format)	

Original URL	https://link-springer-com.ezpv7-web-p-u01.wpi.edu/content/pdf/10.1007/PL00008098.pdf
Source type	Journal Article
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	
Research Question/Problem/ Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

Patent #1 Notes: **Adjustable gravity simulator for tissue and organ culturing**

Source Title	**Adjustable gravity simulator for tissue and organ culturing**
Source citation (APA Format)	
Original URL	https://patents.justia.com/patent/12116560?
Source type	Patent
Keywords	N/A
#Tags	/plantstemcells
Summary of key points + notes (include methodology)	- Background: 2D Clinostats rotate with one rotation axis running perpendicular to the gravity vector and is most commonly used in plants and experiments on single cells. - RWVs have a container mounted on a **horizontal plane** and rotate on one axis with variable speed, low shear stress, and low turbulence environment. - RPMs are more efficient at simulating microgravity than RWVs and 2D Clinostats - Background urges the importance of understanding mechanical loading (traditional mechanical forces on different tissues) under microgravity, as a lack of these forces (for instance, in a rat model where the shoulder was paralyzed) causes deformation of the enthesis tissue and decreased mineralization. Description of invention: - Can house a sample and rotate independently around a first axis and a second axis. - A rotating arm which is connected to a mount and configured towards the simulation chamber. When the rotating arm rotates around the first axis, the simulation chamber rotates around the first axis as well. - **Belt tensioner** configured to move in a loop, allowing for rotation of the second axis - Added a third motor to move the sample axially and apply a mechanical

Commented [DA8]: This begs the question about existing data on plant stem cells with RPMs as opposed to the other two?

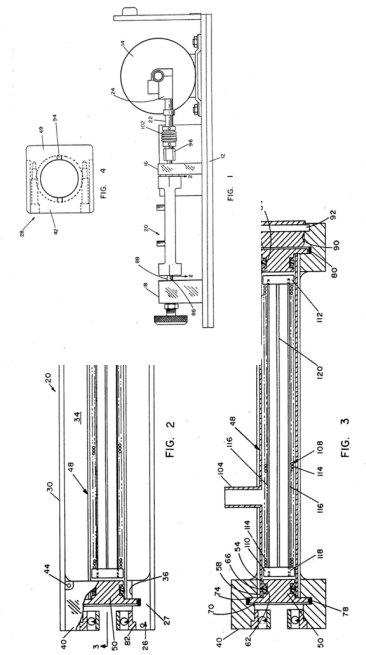
	force. - To cause the sample to experience a microgravity simulation, the first orientation and second orientation are **inverted at random intervals** to create a unique path ensuring that no orientation is revisited more than once. //important - Control monitor contains **a memory and a processor** to communicate with all motors. - Sensors that are used to test the gravitational level: accelerometers, gyroscopes, load cells, and force sensing resistors. - One can choose different ways of supplying electricity to the simulator chamber, including electromagnetic power. Materials information: - Mechanical loading device was made out of **polycarbonate (also makes up the main framework of the device due to its light and sturdy properties), aluminum, or stainless steel.** - Microgravity simulator: two 20 cm x 20 cm plates separated by 18 cm vertical support bars. -
Research Question/Problem/ Need	To create a clinostat capable of simulating mechanical forces on tissue cultures in addition to gravitational forces.
Important Figures	N/A (Figures were hidden behind a paywall) $\vec{G}\,\text{Avg} = \vec{a}\,\text{Avg} = \frac{\sum \vec{a}\, n}{N}$, Equation1, can be used to calculate the average gravitational v An equation used to solve the average gravitational vector from samples from the accelerometer. ere generated and validated with the integrated accelerometer , {right arrow over (G)}$_{Target}$=$a_x\hat{i}+a_y\hat{j}+a_z${circumflex over (k)}, ponent. This vector represents the average force due to gravity An equation to find the target vector prior to initiation of the device.
VOCAB: (w/definition)	**Tensile micromechanical strains:** microscopic elongation or deformation **Hydrostatic cyclic pressure:** repeated, varying hydrostatic pressure conditions (hoop stress) experienced by pipes over time (in this case, blood vessels)

	Enthesis tissue: connective tissue that attaches tendons or ligaments to bones. **Belt tensioner:** A device that includes a belt that runs a loop around an arm wheel, a chamber wheel, and a central wheel. In this design, the central wheel is raised relative to the chamber wheel and the arm wheel for sufficient tension. **Partial Gravity:** Any g level between theoretical zero and Earth's gravity. **Biasing:** A simulator spending more time using Earth's gravity, canceling a percentage of it. For example, when simulating microgravity, no biasing occurs. **Polymerize:** cause to form a polymer
Cited references to follow up on	N/A
Follow up Questions	How expensive is polycarbonate? Can it be added to a 3D printed design.

Patent #2 Notes: Hollow fiber clinostat for simulating microgravity in cell culture

Source Title	Hollow fiber clinostat for simulating microgravity in cell culture
Source citation (APA Format)	
Original URL	https://patentimages.storage.googleapis.com/f4/45/9b/0932ea3cf2e2a2/US5104802.pdf
Source type	Patent
Keywords	N/A
#Tags	/plantstemcells

Summary of key points + notes (include methodology)	- Traditional clinostat design with a fiber in which cells are injected. - Purpose: since cells are unable to anchor in traditional clinostats. Objectives: - Must support a cell containing fiber, the fiber being mounted for rotation. - Must include tensioning to apply a predetermined force to maintain fiver alignment. Key elements of the design: - Fiber (120 in Figure 2) is attached to 110 and 112 with wax/glue, then a coil spring is decompressed to force the end pieces apart, allowing the the fiber to stay aligned with the axis of rotation. - The diameter of the fiber determines the distance the cells are from the axis of rotation of the chamber The device was tested using thigh muscle from 12 day old chick embryos.
Research Question/Problem / Need	To create a clinostat that is able to culture cells using fiber.

Important Figures	 Figure 1: Elevational (top view) of the apparatus Figure 2: Sectional view, meaning a view of the inner machinery of the device.
VOCAB: (w/definition)	**Elastomeric Rings:** used as sealing devices to prevent air or liquids from leaking. **Flanged:** describes a portruded ridge **O-ring seals:** same function as elastomeric rings; used for sealing.
Cited references to follow up on	N/A
Follow up Questions	Why do anchorage dependent cells grow on beads?