New Hampshire Space Grant Consortium Summer 2024 Graduate Student Research Award Final Report

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Project Title: The Impact of Microgravity on Vascular Network Development

Background:

To effectively improve the quality of life for exploratory mission staff and make long-term space missions possible, we must understand how our molecular biology, vascular system, and new blood vessel development are impacted in space conditions, specifically microgravity. Some health issues commonly believed to be explained by gravitational field differences are the inability to maintain healthy blood pressure levels, significant bone and muscle mass loss, and bodily fluid distribution changes leading to vision issues, dehydration, and kidney stones.[1]

Wound healing consists of multiple phases generalized as inflammation, proliferation, and remodeling. Each phase is a complex process involving many cell types, cytokines, and mediators. The vascular system is vital in wound healing as it is responsible for the delivery of growth factors, cytokines, and oxygen needed for tissue repair. Biochemical signals from the wound site direct this delivery and new blood vessel growth that is vital to tissue development, known as angiogenesis.

Recently, I have collaborated to write and submit a critical review of recent advancements in biomaterials. This piece describes the relationship between the extracellular matrix's (ECM) mechanical cues and the cascade of biochemical signaling pathways responsible for wound healing. In the biomaterial community, recognition of the significant impact of mechanical cues for ECM remodeling seen in tissue repair has grown rapidly. With the knowledge of this relationship and the emphasis of importance on mechanical signals for cell behavior, it can be deduced that with mechanical unloading, cell interactions and the human vascular system will be significantly impacted.

If cell environmental sensing and conversion into biochemical signals in space can be understood, this work can be applied to tissue engineering in normo-gravity. In his proposed Cellular Tensegrity Model, Donald J. Ingber presented an explanation of the reaction mechanism of cell behavior in space as a direct result of cytoskeletal equilibrium being broken.[2] With a significantly reduced number of gravitational forces acting on the cells, tension and compression will also be significantly reduced, leading to this morphological and functional change. Given the importance of tension and compression in the human vascular system for efficient blood delivery, microgravity disrupting these forces may have significant and deleterious effects on human health.

Goals:

1. Establish a microgravity cell culturing system.
2. Evaluate the impact of microgravity on human umbilical vascular endothelial cells (HUVECs) and vascular network formation.

Methods:

The first prototype (P1) of the affordable random positioning machine for cell culture was constructed using a Bambu X1 Carbon Printer to 3D print the necessary structures from a plant-based and biodegradable compound, polylactic acid (PLA). The printed pieces include the inner frame, outer frame, stand (x2), sample stage, and 3 gears of different sizes, based on previous reports of ground-based microgravity simulation studies.[3,5]

To optimize this system for mammalian cell studies, I constructed a new sample stage that could hold multiple tissue culture dishes. With a desire to fit more samples per trial and increase testing efficiency, I also created a sample holder extension via Solidworks, and printed 2 of these pieces as well that could easily be attached to the center stage.

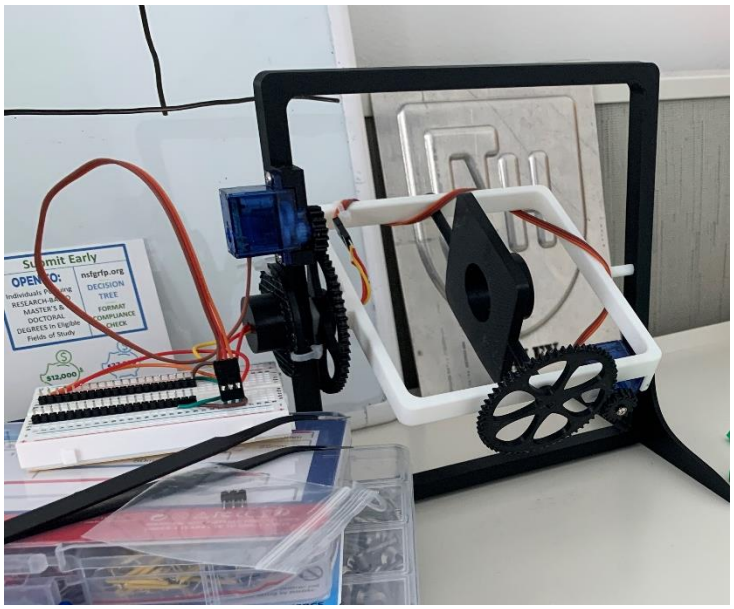


Figure 1: Random Positioning Machine (RPM) for Astrobotany Studies[5]. The sample stage has a raised cylinder on one side to allow for plant vials to be secured, one at a time.

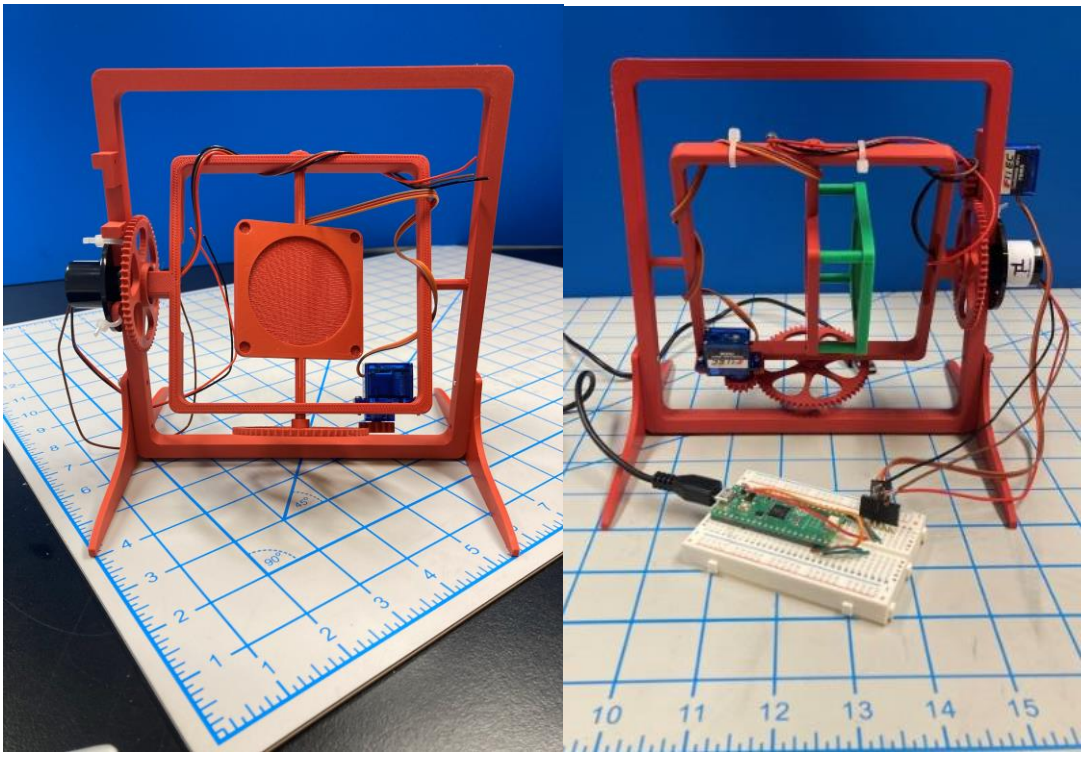


Figure 2: Random Positioning Machine (RPM) for Mammalian Cell Culture Prototype 1 with the modified stage for tissue culture plates, and one side of the stage extension attached (green).

From there, a Raspberry Pi Pico was used to store the code developed and optimized for this system. The code allows the two attached servo motors controlling the inner and outer frames to rotate continuously and independently of each other.

This continuous and independent rotation of both frames is key to the simulation of microgravity, as it utilizes the natural gravitational vector that points downwards.

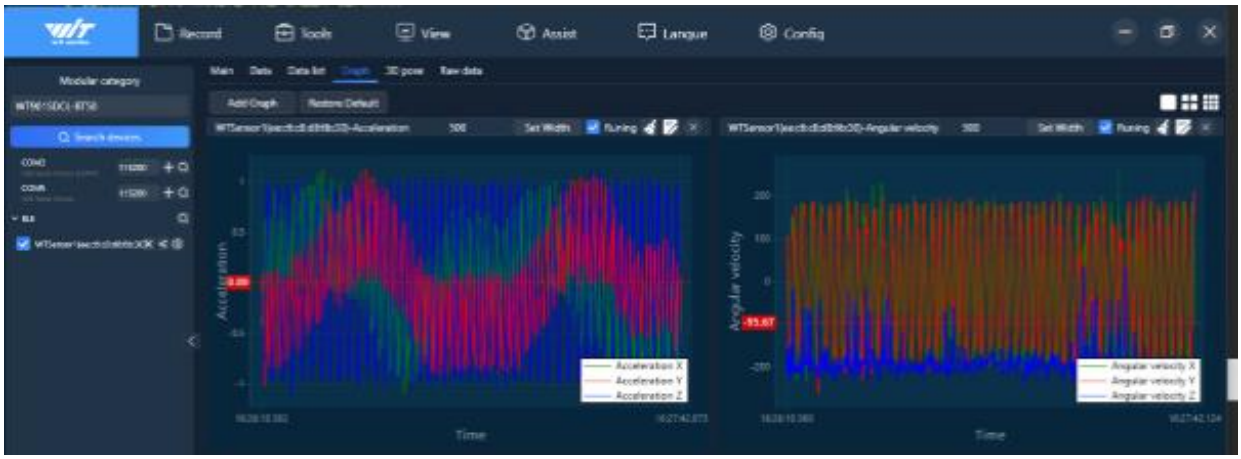
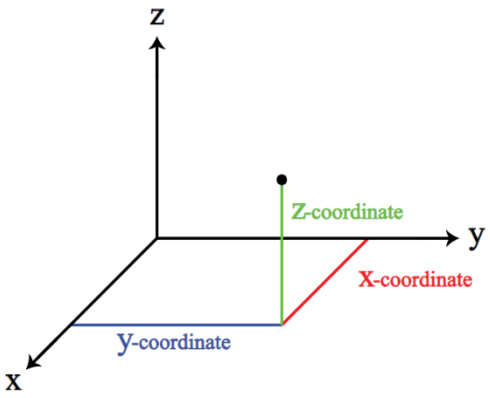


Figure 3: Microgravity Validation through WITMOTION Sensor & Software

X Direction (g):	**0.012**
Y Direction (g):	**0.009**
Z Direction (g):	**-0.011**

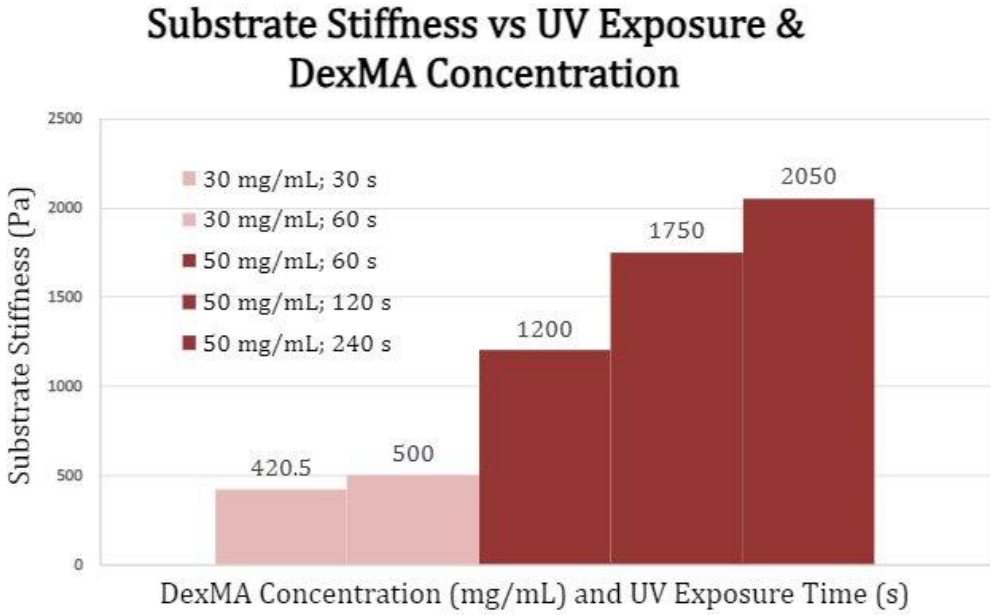
Figure 4: Average acceleration vector, with 1 g equivalent to Earth's natural gravitational force.



To validate the newly constructed RPM, WITMOTION sensor and software was used to measure acceleration in the X, Y, and Z directions. With 1 g being the entirety of the gravitation vector pointing downwards in the Z direction (+ 1 g), the sensor was calibrated, and then rotated extensively with the sensor attached for a measurement over time. Attached is a screenshot of the WITMOTION software utilized for this measurement, in which the acceleration in the X, Y, and Z directions are pictured, alternating between positive and negative values. To successfully simulate microgravity, the average of these values must be as close to zero as possible in all three directions. In the table summary of acceleration in each direction averaged over

time, all three values were estimated to be around 1% of Earth's natural gravitational force, according to the sensor.

Results/Discussion:



Preliminary quantification of Dex-MA gel stiffness with a Rheolution rheometer was performed for validation of substrate physical properties. Given the results from stiffness quantification, 30 mg/mL with 60 seconds of UV exposure was utilized as a "soft" gel, and 50 mg/mL with 240 seconds of UV exposure as a "stiff" gel. In previous studies, it has been shown that endothelial cells have better spreading and proliferation capabilities on stiff rather than softer substrates.[6,7]

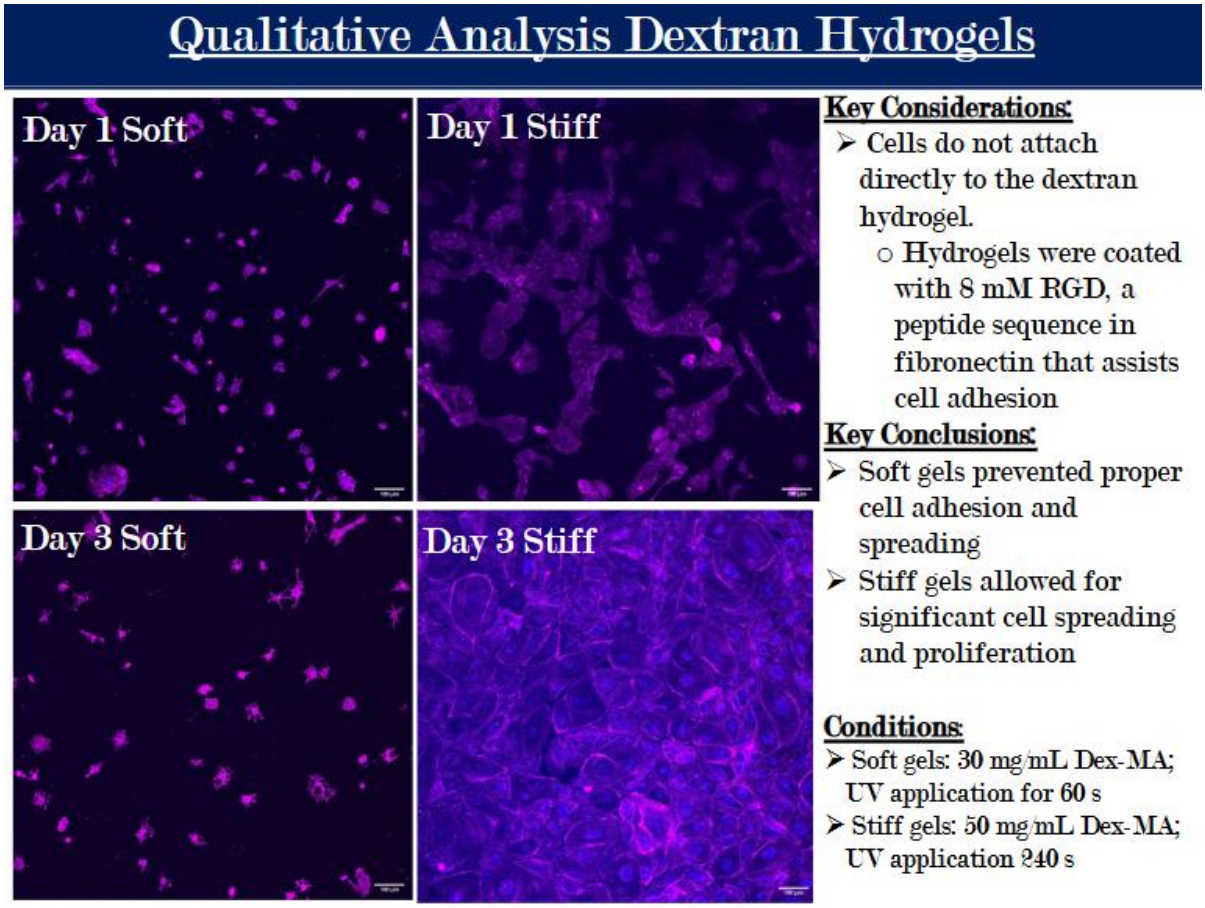


Figure 5: Soft vs. Stiff Dex-MA Hydrogels coated with 8 mM RGD[4] and seeded with HUVECs. The samples were fixed at their respective time points, day 1 and 3. The samples were stained via Phalloidin 647 and DAPI for actin fiber and nuclei visualization and qualitative analysis.

Human Umbilical Endothelial Vascular Cells (HUVECs) were grown on soft and stiff gels, respectively, for up to 7 days in a normo-gravity setting. The samples were fixed at time points of days 1, 3, and 7 to obtain comparative data throughout the timeline, detailing cell growth and proliferation rates. The cells on the soft gels were unable to properly spread and appeared to have a low live cell count. On the stiff gel, cells were able to spread and proliferate successfully. The next steps on this aspect of the project include adding a stiffer (10 kPa) gel sample to cell testing and introducing samples to the microgravity environment that has been created and validated.

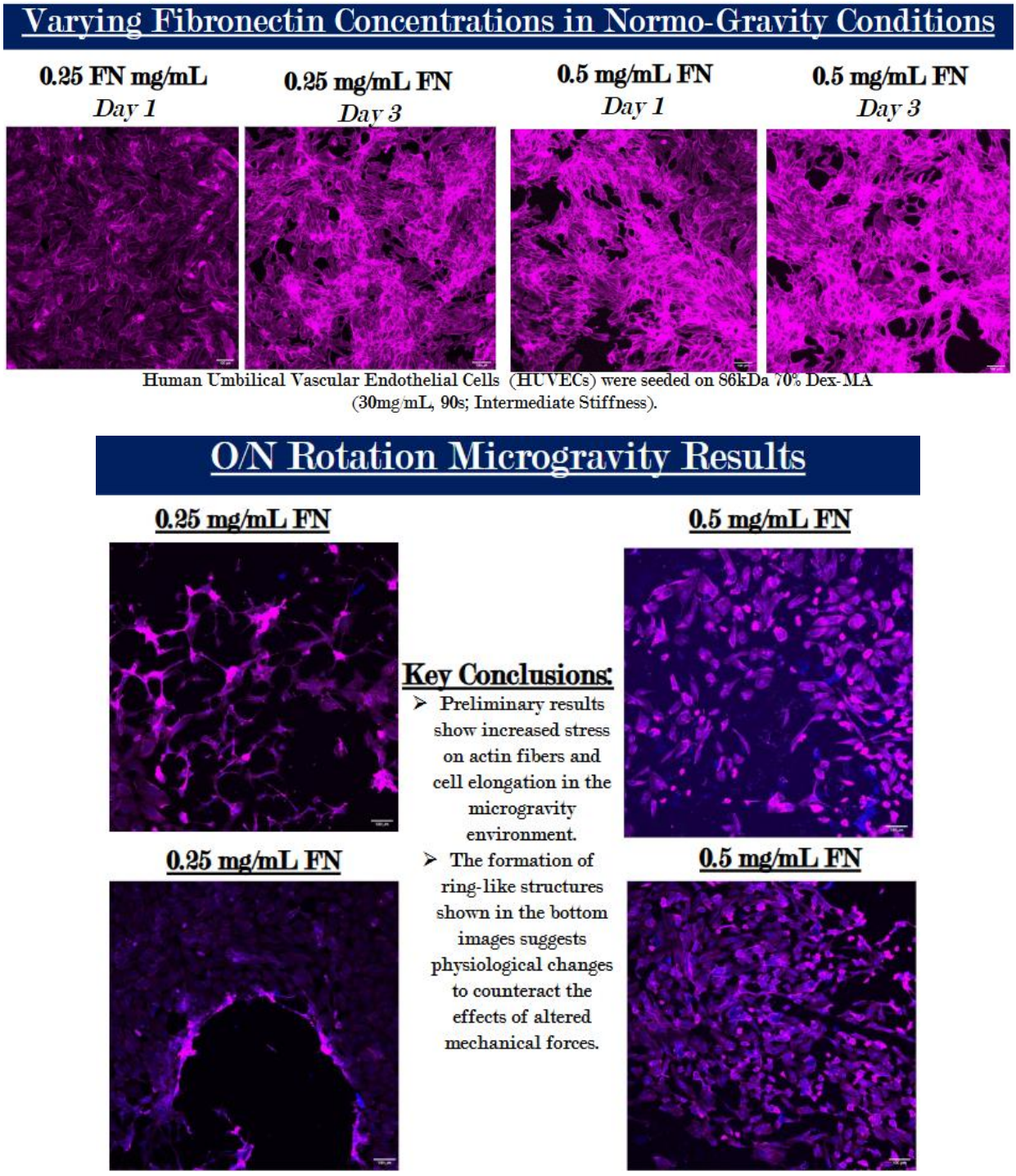


Figure 6: Varying fibronectin concentrations in normo- and microgravity settings. Samples were fixed at their respective time points, fixed, and stained with Phalloidin 647 and DAPI.

An intermediate stiffness gel (approximately 1200 Pa) was utilized for varying ECM component concentrations. Gels were coated with 0.1, 0.25, or 0.5 mg/mL fibronectin (FN), an ECM protein correlated with cell adhesion. From here, cell

samples were placed in the incubator, either in a normo-gravity setting, or in the RPM for a simulated microgravity setting.

Key differences in normo-gravity (NG) samples and microgravity (MG) samples were evaluated. The MG 0.25 mg/mL FN sample's cells appear elongated and spindle-shaped, indicative of stress and poor health. In comparison, the NG 0.25 mg/mL sample's cell growth shows good cell-cell contact, and a rounder shape, indicating a healthier cell culture. For the 0.5 mg/mL FN coated samples, the cells in MG show a rounded, cobblestone-like shape, with low cell-cell contact. In NG, cells have a much higher proportion of cell-cell contact, therefore it is likely that there is higher cell-cell communication and signaling in a NG setting.

Although more testing needs to be done to confirm the reproducibility of these findings, overall, it seems cell proliferation has a slightly reduced rate in MG environments, which could lead to increased difficulty in wound healing. Due to NG settings showing a higher degree of cell-cell contact, it is possible that heightened cell-cell signaling is partially responsible for the differences in these two settings.

Poster Presentation:

I was afforded an opportunity to present a poster on this work at the 2024 CIBBR Retreat hosted at the University of New Hampshire.

Future Work:

With the continuation of this project, I am currently creating the 2nd prototype of our affordable microgravity cell culture system with the hope of optimizing what has been constructed thus far. The second prototype (P2) will be larger to provide space for multiple samples and extra physical support with thicker frames and gears. New servo motors, larger and more powerful than those in the first prototype, will be included in the new and improved prototype (P2). There are also modifications to the sample stage and the addition of a sliding stage cover to ensure the samples are properly secured.

Other continuing work on this project includes micro- and normo-gravity evaluations of cell behavior with alternative ECM proteins, including collagen and laminin. Evaluation of cell behavior on the soft and stiff gels in a microgravity environment and test results with a stiffer gel, around 10 kPa, will also be conducted. Lastly, within the scope of this project's continuation, I would like to incorporate 3D microfluidic devices and pre-formed vasculature in a normo- vs microgravity environment for a heightened degree of accuracy in our human health model. This is an essential next step in this project to increase the complexity of this in-vitro model, where forces generated by or responsible for blood flow are interrupted under microgravity conditions.

Works Cited:

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[5] "The Factory: Simulating Microgravity with Random Positioning Machines - Video Tutorial Australia." *Core Electronics*, The Factory, Unconvengineering, Nov. 2023.

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