Fiscal Year:	FY 2023	**Task Last Updated:**	FY 09/12/2022
PI Name:	Schwertz, Hansjorg M.D., Ph.D.		
Project Title:	Megakaryocytes Orbiting in Outer Space and Near Earth: The MOON Study		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology		
Space Biology Cross-Element Discipline:	(1) Immunology		
Space Biology Special Category:	None		
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Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2020 Space Biology NNH20ZDA001N-SB E.12. Flight/Ground Research
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No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA ARC
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	Emilie Montenont, PhD, post-doctoral fellow left to start a new position at Miltenyi, Maryland. Marina Tristao, PhD, was on-boarded as a new post-doctoral fellow.		
COI Name (Institution):	Rondina, Matthew M.D. (University of Utah, Salt Lake City) Rowley, Jesse Ph.D. (University of Utah, Salt Lake City)		
Grant/Contract No.:	80NSSC22K0255		
Performance Goal No.:			
Performance Goal Text:			
Task Description:	Megakaryocytes (MKs) and their progeny, platelets (PLTs), are dynamic effector cells with recently discovered novel functions, which bridge the inflammatory, immune, and hemostatic continuum. Changes in bone marrow MKs, resulting in low PLT numbers, (thrombocytopenia, which occurs in astronauts during spaceflight) are associated with dysregulated host inflammatory/immune responses. MKs and PLTs sense and respond to environmental cues. MKs also differentially invest developing PLTs with RNAs and proteins that alter functions of newly-released cells, influencing cellular and host responses. Surprisingly, there is a paucity of data regarding in-flight, long-term dynamics of MK development and function, as well as PLT function and production. Given previously identified and published space-travel associated risks on dysregulated inflammation, immune responses, thrombus formation, and hemostatic systems, filling this critical knowledge gap is important for the health of spaceflight crewmembers during and after missions. Moreover, as other blood cells (e.g., red blood cells, leukocytes, etc.) may be altered by microgravity, data generated are likely to contribute to our understanding of how spaceflight affects other hematopoietic processes. This proposal is based on our robust preliminary data demonstrating that conditions mimicking microgravity (rotating wall vessel culture, RWVC) markedly alter human MK morphology and gene expression. We hypothesize that microgravity will re-program MKs and newly-released PLTs, resulting in critical changes in their transcriptome, proteome, and alterations in PLT number and function. We will determine how microgravity and space radiation conditions on board the International Space Station (ISS) alter human MK and PLT maturation/production, gene expression (DNA, RNA, and protein), and cellular function. We will study in vitro human hematopoietic progenitor cell (HPC)-derived MKs in Earth-based experiments under standard or microgravity conditions. In parallel, human MKs will be studied on the ISS. Integrated, cutting-edge OMICS toolsets (e.g., RNA-sequencing and ribosomal footprinting [Ribo-seq]), comprehensive morphologic studies, and cell production kinetic studies will be used. They will provide unprecedented insight into adaptation processes needed for MK and PLT function under conditions experienced by humans performing spaceflights. These studies will directly address crew health concerns that currently limit human space exploration and will assist in developing targeted		

countermeasures.

This proposal concurs with the major National Research Council (NRC) Decadal Survey Recommendations for cellular and molecular biology studies using state- of-the-art tools coupled with systems biology, and for studies evaluating the physiological interplay of cardiopulmonary and immune functions during application of spaceflight. Furthermore, we will address goals of the NASA Space Biology Science Plan 2016-2025, including: (1) determine the effects of the space environment on DNA function, (2) develop a systems biology-based understanding of the cellular and molecular changes to explain how gravitational changes in spaceflight effects organisms and causes phenotypic changes, and (3) identify how spaceflight affects the ability of cells to generate and maintain their complex internal cyto-architecture, processes critical for MKs and PLTs.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

Results from the proposal o have immediate mechanistic implications for the development of countermeasures for unexpected clot formation, and dysregulated systemic inflammation in ISS crew members. o may be broadly applicable to other cells, such as leukocytes, monocytes, and red blood cells, that are altered in spaceflight settings. o allowing us to translate ex vivo findings into a relevant in vivo situation via comparing longitudinal RNA expression studies in human PLTs and durable reprogramming of PLTs in astronauts (pre- vs. post-flight) mitigating cell-autonomous changes only observed in culture systems. o Identified target genes across ground and flight, cell culture and astronaut projects will be verified for functional and systems biology relevance by using cell culture CRISPR-Cas9 approaches. • Exploration Benefits o Advance knowledge will help the development of countermeasures for unexpected clot formation, and dysregulated systemic inflammation in spaceflight crew members. o Obtained data will be translatable to address known “ground-based” dysfunctions of human MKs and PLTs, may lead to the discovery of new drug targets and therapeutic interventions, and initiate new research avenues. • Earth Benefits o Since results generated may be broadly translatable to other cell types, such as leukocytes, monocytes, and red blood cells, the analysis data sets will be applicable to numerous inflammatory and pro-thrombotic conditions observed in the medical arena. Furthermore, the direct comparison of ground-simulations and ISS-flight experiments, and the integration of ex vivo findings will help in validating commonly used spaceflight simulation cell culture conditions.

Task Progress:

The progress and accomplishments will be divided into administrative/organizational and experimental tasks.
• ADMINISTRATIVE/ORGANIZATIONAL ACCOMPLISHMENTS: o A flight hardware selection process was initiated by performing a risk-benefit-analysis and creating the required flight concept chart. This process resulted in the conception of flight hardware requirements, which were communicated to the International Space Station National Laboratory (ISSNLP). o The Principal Investigator (PI) and Project Scientist prepared the Science Requirements Document, which was submitted for further review and approval. o The request for beam time to simulate galactic cosmic rays at the NASA Space Radiation Laboratory (NSRL) at the U.S. Department of Energy Brookhaven National Laboratory, was submitted. The requested beam time for the 2023 Summer campaign was granted, the project was discussed with the staff physicists and biologists, and we are waiting for the specific timeslot to be announced. o An Institutional Review Board (IRB) application for the work with mobilized CD34+ hematopoietic stem cells was prepared and submitted to the University of Utah IRB. The project did not meet the definitions of Human Subject Research according to Federal regulations; therefore, IRB oversight is not required or necessary for projects proposed under Specific Aim 1 and 2 of this NASA proposal. o We submitted the NASA IRB application covering Human Subject Research as proposed in Specific Aim 3 of this proposal. The IRB application is currently under review, after passing the pre-review process.

• EXPERIMENTAL ACCOMPLISHMENTS: Since the flight hardware selection process is delayed for various reasons, several hardware-dependent science verification experiments cannot be performed at this time. These include, but are not limited to, biocompatibility and toxicity tests of the proposed flight hardware using CD34+ hematopoietic stem cells. In addition, cell seed density and cell survival rate verification tests can only be implemented once the flight hardware is selected and defined. Furthermore, experimental testing of microscopy capabilities, including clarity and bubble forming tests, will be performed once the flight hardware and accompanying microscopy systems are defined. Finally, the impact of media changes and the implemented media change techniques on cell culture performance can only be tested once the flight hardware is selected. The start of a new post-doctoral fellow also required intensified teaching and training experiments.

The following experiments were accomplished and generated valuable insights and data:

• Experiments related to Specific Aim 1: o In a first experiment, mobilized adult CD34+ hematopoietic stem cells performed as expected during rotating wall vessel culture. We were able to demonstrate an appropriate increase in cell numbers, and stable cell viability around 90%. Furthermore, flow cytometric data showed appropriate expression of differentiation markers (i.e., CD41, CD42b, and CD61). o In a subsequent set of experiments, we studied a multitude of different experimental conditions to define best ground control practice when performing experiments comparin to the rotating wall vessel cell culture approach. Analyzing total cell counts, viability, proplatelet formation capabilities, and flow cytometry we could confidently conclude that using cell culture dishes would be best suited serving as suspension culture control. It is important to note that keeping the cell culture media volume constant during the entire duration of the experiment, independent of the total cell count, best mimics conditions being present in the rotating wall vessel culture dish. This experiment also confirmed our previous findings, demonstrating that CD34+ hematopoietic stem cells depict a larger size when cultured under microgravity simulating conditions.

• Experiments related to Specific Aim 2: Several standard ground cell culture conditions need to be adjusted due to in-flight conditions on board the ISS. o For the proposed flight experiments, we will use pre-mixed cell culture media, already containing growth factors, instead of freshly prepared media formulations used for each media change when conducting standard ground-based experiments. We therefore tested if cell count, viability, and differentiation markers of CD34+ hematopoietic stem cells would change over the course of the experiment due to the use of freshly prepared versus pre-mixed and stored media formulations. We found that cell numbers, viability, and the flow cytometric detection of differentiation markers (i.e., CD41, and CD61) were comparable between freshly prepared and pre-mixed media formulations, indicating that the proposed flight protocol using such pre-mixed cell culture media is appropriate and will not introduce unwanted and inadvertent cell culture effects. o To further evaluate preferred cell culture conditions, we implemented experiments using differential growth factor treatment regimes. Our results demonstrated that stem cell factor is an integral part of successfully culturing CD34+ hematopoietic stem cells. However, results for cell numbers and cell culture viability were comparable and independent of having stem cell factor removed from cell culture media on day 3 or day 6 of culture. Therefore, the proposed in-flight protocol, using stem cell factor and thrombopoietin (TPO) containing media until day 6, and continuing the culture with media containing TPO only until the end of culture period, will be implemented for all cultures. o Due to ISS-introduced limitations, megakaryocytes harvested in orbit cannot be immediately frozen or even further processed towards RNA isolation and subsequent sequencing, as is custom when carrying out ground-based cell studies. Therefore, we tested several methods employing highly efficient preservation of RNA integrity, even if samples need to be stored at room temperature for prolonged periods of time (i.e., two weeks). RNA isolates from cells suspended in RNAlater, and stored at room temperature for two weeks demonstrated the highest degree of integrity, reflected in RNA integrity numbers (RIN) >8. These scores are within the “excellent range” of the Mission Success Criteria defined by the Science Requirements Document draft. o On-orbit cell culture experiments will include morphologic studies conducted using live cell imaging approaches. Since the tubulin cytoskeleton is a major contributor to regulated proplatelet formation, we selected different tubulin probes suitable for live microscopy for further testing. All tested live cell imaging dyes can be used without additional washing steps per manufacturer’s protocol, which will significantly reduce astronaut hands-on time. The experimental results demonstrated sufficient staining characteristics of the selected live imaging probes when incubating the cells for 30 minutes. In addition, using a 20x objective, we were able to readily identify detailed tubulin cytoskeletal structures within proplatelet extensions. o To address potential interference of the aforementioned cellular live stain with RNA isolation procedures, we performed experiments combining the tubulin probes for live microscopy with subsequent RNA isolation techniques. This approach enabled us to optimize established protocols. Using RNAlater as cell preservative, we found that using the tubulin live stain did not result in loss of RNA. Furthermore, RNA integrity numbers were >8, falling well within the excellent range of the Mission Success Criteria defined by the Science Requirements Document draft.

Bibliography Type: Description: (Last Updated: 09/26/2024)

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