Fiscal Year:	FY 2023	**Task Last Updated:**	FY 11/11/2022
PI Name:	Hammer, Bruce Ph.D.		
Project Title:	Study of Mammalian Pluripotent Stem Cells in Microgravity		
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) Developmental Biology		
Space Biology Special Category:	(1) Cell Culture		
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Zip Code:	55455-3007	**Congressional District:**	5
Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2014 Space Biology Flight NNH14ZTT001N
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No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
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Flight Program:	ISS		
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Key Personnel Changes/Previous PI:	The Principal Investigator reports that Louis Kidder, Ph.D. is no longer with the project (Ed., 11/17/22).		
COI Name (Institution):	Dutton, James Ph.D. (University of Minnesota)		
Grant/Contract No.:	NNX15AB38G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	We are investigating the effect of gravity on fundamental properties of mammalian stem cells during differentiation of 3-D cultures of induced pluripotent stem (iPS) cells. Experiments aboard the ISS, STS and ground-based simulations have demonstrated that microgravity influences gene expression, cell proliferation and differentiation in stem cells. However, the mechanism behind these observations is not clearly understood. Oct4 is a transcription factor necessary for maintaining pluripotency in mammalian stem cells. It is expressed ubiquitously in the early embryo, pluripotent ES and iPS cells and is rapidly down regulated during differentiation. In this study we will investigate how exposure to microgravity fundamentally alters the regulation of Oct4, and how these changes can affect the timing, progression and outcomes of cell differentiation. Our laboratory has created an Oct4:CreER mTmG transgenic mouse that, for the first time, allows lineage tracing of Oct4 expression in stem cells and their progeny. We will use iPS cells derived from this model to determine the influence of microgravity on the loss of pluripotency and differentiation. We are using magnetic levitation, a unique ground-based simulation of orbital free fall, to augment and compare the findings from the Spaceflight Experiment Phase (micro-15). Unfortunately astronaut handling of the samples aboard the ISS resulted in only a few EB's returned rather than the expected hundreds of EB's which resulted in a loss of science. We propose to investigate the effect of gravity on the timing and spatial arrangement of the loss of Oct4 expression in differentiating iPS cell aggregates via magnetic levitation. Using iPS cells derived from the Oct4:CreER mTmG mice and timed tamoxifen additions, it is possible over time to distinguish cells still expressing Oct4 (i.e., expressing GFP), from those that have lost Oct4 expression (expressing RFP). We will also examine the effect of gravity on gene expression in cohorts of Oct4 expressing and non-expressing cells during differentiation by comparing the results of ground-based experiments to those conducted on orbit. Finally, we will explore mechanisms behind the effect of microgravity on both Oct4 gene regulation and control of downstream gene expression by Oct4. This work will determine the effect of spaceflight on changes in Oct4 gene expression during differentiation of pluripotent stem cells and the consequences of these changes on differentiation outcomes. This will increase our understanding of fundamental stem cell behavior in microgravity.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Gravity likely influences organismal development but little is currently understood about its specific influence on stem cell differentiation. We propose to utilize a novel system to investigate how microgravity fundamentally changes the timing and spatial arrangement of the loss of Oct4 gene expression during the differentiation of stem cells. We expect this to have consequences on differentiation outcomes. Our results from this study will ultimately have a direct impact on improving the translation of human stem cell based treatments. Cell manufacturing in microgravity may speed-up the rate of iPSC (induced Pluripotency Stem Cell) differentiation, thereby reducing the time and cost to obtain a therapeutic dose of cells. If this can be done in orbit and replicated on Earth with magnetic levitation, this will have significant commercialization possibilities.
Task Progress:	A brief description of scientific goals/objectives of the research: • The gene Oct4 is a key marker of mammalian pluripotency. • The University of Minnesota UMN Oct4CreER::mTmG mouse iPSC line is the best tested, most sensitive Oct4 lineage tracing system currently available and was used for International Space Station (ISS) and ground-based microgravity simulation studies. • Magnetic levitation was used as a unique ground-based simulation of in-orbit microgravity. • Changes in the dynamics of Oct4 loss in simulated and actual microgravity was observed, indicating there are fundamental effects of the space environment on the regulation of this key gene. • This was the first project to employ ISS crew to accomplish media exchange following cell centrifugation – a standard laboratory technique that can now be used in many other ISS experiments. • This research has pioneered the self-assembly of stem cell embryoid bodies (EBs) that can be used by many other future ISS projects using cell aggregate and organoids. Progress during the current No Cost Extension period: 1) We are now preparing uniform-sized EBs, which will remove uncertainties regarding rate of differentiation, potential oxygen, and nutrient disparities as a function of EB size. The EBs are grown in EZSPHERE™ 35 mm Dishes [Diameter: 500µm, Depth: 200µm, No. of Well: 2,700/dish]. This is compatible with our sample positioning apparatus in the magnetic levitation system. 2) Optimized green fluorescent protein (GFP) and red fluorescent protein (RFP) staining protocols for EBs. This improves quantification of red and green fluorescing cells. 3) Coordinated with University of Minnesota Genomics Center for using its expertise in executing and analyzing genomic data from anticipated studies. Purchased the necessary reagents for spatial genomic analysis of EBs for 1g and simulated microgravity studies. 4) Due to a national liquid helium shortage, we are unable to secure liquid helium for the levitation magnet, which is primarily due to downtime in a helium extraction facility. The expectation is that the shortage will subside during calendar year 2023. We are planning to relocate the maglev system in early 2023 to the NASA Kennedy Space Center (KSC) microgravity simulation facility where helium supplies are anticipated to be less restrictive. This should enable us to utilize the maglev system to complete the science objectives of the grant. Future work scope and project objectives: Aim 1: Determine the effect of simulated microgravity, via magnetic levitation, on the timing and spatial arrangement of Oct4 expression in differentiating embryoid bodies /induced pluripotent stem cells (EBs/iPSC) aggregates. - Confocal microscopy will be used to determine pluripotency. Cells will be green if Oct4 is expressing and red if Oct4 is not expressing. Aim 2: Determine the effect of simulated microgravity, via magnetic levitation, on downstream gene expression in the cohorts of Oct4-expressing and non-expressing cells during cell differentiation. -Spatial genomics (Nanostring GEOMX Digital Spatial Profiling (DSP)) will be used to analyze transcriptomics.
Bibliography Type:	Description: (Last Updated:)