Fiscal Year:	FY 2021	**Task Last Updated:**	FY 02/04/2022
PI Name:	Sekyi, Maria Ph.D.		
Project Title:	Microgravity and Partial Gravity Effects on Hepatic Organoid Steatosis and Function		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	94143-2205	**Congressional District:**	12
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2020 TRISH-RFA-2001-PD: Translational Research Institute for Space Health (TRISH) Postdoctoral Fellowships
Start Date:	08/01/2020	**End Date:**	09/30/2021
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:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: End date changed to 9/30/2021 per TRISH (Ed., 11/3/21) NOTE: End date changed to 7/31/2022 (originally 7/31/2021) per TRISH (Ed., 11/2/20)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Chang, Tammy M.D., Ph.D. (MENTOR: University of California, San Francisco)		
Grant/Contract No.:	NNX16AO69A-P0505		
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Task Description:	POSTDOCTORAL FELLOWSHIP Increasing evidence suggests that microgravity may induce detrimental effects on liver function, a serious risk for humans undertaking space exploration. To investigate this possibility, we propose the assessment of liver organoid response to microgravity and partial gravity conditions. Liver organoids are three-dimensional aggregates of hepatocyte, endothelial, and mesenchymal stem cells commonly used as a model for liver function and organogenesis. Through the use of histology to investigate cellular morphology, enzyme-linked immunosorbent assays for quantification of secreted proteins, and RNA-sequencing to evaluate changes in gene expression, we will quantify organoid response to up to 15 days of culture under altered gravitational conditions. Other readouts will include stress response to glucose or acetaminophen overdose. The proposed research is significant to the Translational Research Institute for Space Health (TRISH) and NASA's interests in establishing a sustained human presence on the Moon and Mars because it will define the hepatic health risks associated with microgravity during space exploration. As maintaining liver health is integral to maintaining human health, characterization of the effect of the gravitational perturbation on liver function and metabolism is crucial to the progression of these endeavors.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The research project aims to establish an in vitro model of microgravity and partial gravity effects on human hepatocyte-like cells, and hepatocyte-endothelial cell interactions. Because mammals exposed to spaceflight may experience deleterious effects to liver function, such a model is crucial. We have completed the first steps to establishing this model and have developed a robust organoid culture system that produces hepatic organoids, superior in hepatic function to organoids generated by traditional 2D methods. These organoids are amenable to detailed cellular and molecular characterization of hepatocytes and stromal cells, in the absence of systemic stressors intrinsic in the study of whole model organisms. Furthermore, they originate from human cells and provide an insight to human hepatic tissue behavior. As such, they can be used not only for studying the effects of gravitational perturbations on hepatic tissues, but for a wide range of pathological or basic science investigations.
Task Progress:	EDITOR's NOTE (February 2022): this Postdoctoral Fellowship ended in September 2021. REPORTING JULY 2021 Key Findings: We have established a robust culture system for translationally relevant organoids and have evaluated baseline morphological and functional attributes. To generate this culture system we tested organoids cultured on a 2D surface or those cultured in a 3D rotating wall vessel system for hepatic functional capability. We found that organoids generated from a 3D rotating wall vessel system exhibit higher levels of hepatic function compared to those generated from culture on a 2D surface. Specifically, 3D organoids express higher levels of HNF4a, Albumin, Cyp1a1, and Cyp1a2. There is a significant risk for developing nonalcoholic fatty liver disease and subsequent liver cirrhosis as a result of spaceflight and the gravitational perturbations resulting from spaceflight. C57Bl/6 mice flown on shuttle missions for 13-14 days exhibit increased levels of lipogenesis and upregulation of steatotic pathways, hallmarks of fatty liver disease. The activation of hepatic stellate cells is strongly implicated in the development of cirrhosis and should be considered in the hepatic tissue model. Thus to develop a more accurate hepatic tissue model, we also engaged in preliminary studies on organoids generated from: a. Hepatocytes alone for hepatocyte function, b. Endothelial cells and hepatocytes to simulate vascularized hepatic tissue, c. Hepatocytes, endothelial cells, and mesenchymal stem cells to simulate a more translationally relevant hepatic tissue (Mesenchymal stem cells exhibit similar phenotypic attributes to hepatic stellate cells in the liver.) We evaluated the lipid droplet formation in hepatocytes cultured alone, or co-cultured with endothelial cells and hepatic stellate cells. We found that hepatocytes cultured alone exhibit significantly higher levels of lipid droplet formation, indicative of increased levels of lipogenesis and steatosis, compared to those co-cultured with endothelial cells and stellate cells. Impact of key findings on hypothesis, technology requirements, objectives, and specific aims of the original proposal HNF4a is a nuclear factor which regulates the expression of many hepatocyte genes and is crucial for hepatic function. Albumin is a water soluble transport protein produced by the liver. In adult humans, low albumin levels are indicative of liver dysfunction. Cyp1a1 and Cyp1a2 are hepatic enzyme members of the cytochrome P450 family and are important in phase I drug and xenobiotic metabolism. These proteins are crucial indicators of a well functioning hepatocyte. Thus organoids generated from the 3D culture system are a better baseline representation of human liver tissue and will be used in further studies on the effect of gravitational perturbation on liver function. Evaluating the effect of gravitational perturbation, and the potential for the development of steatosis and fibrosis as a result of gravitational perturbation, requires the establishment of a healthy and non-steatotic baseline liver tissue. Results indicate that a co-culture system of hepatocytes, endothelial cells and mesenchymal stem cells supports the formation of a non-steatotic baseline organoid. Thus organoids generated from hepatocytes, endothelial cells, and mesenchymal stem cells will be used in further studies on the effect of gravitational perturbation on liver function. Proposed research plan for coming year: Organoid optimization and development is complete. In the coming year, we plan to run functional assessments of baseline organoid behavior concurrently with studies on microgravity effects. Organoids will be cultured for 4, 8, and 12 days in a rotating wall vessel as a baseline control, in a random positioning machine that simulates microgravity as one test group, and in a random positioning machine that simulates different levels of partial gravity as another test group. At the end of the culture period, organoids will be subjected to histological, metabolic, and gene expression assays. Histological assays will involve immunostaining for functional markers such as HNF4a, CYPs and albumin, cell specific markers such as CD31 for endothelial cells and CD271 for MSCs (mesenchymal stem cells), structural markers for ECM (extracellular matrix) proteins, and lipid dyes such as BODIPY to assay steatosis. Metabolic assays will include ELISA ((enzyme-linked immunosorbent assay) assays for secreted albumin, and CYPs. Gene expression will be quantified using qPCR, and/or RNA sequencing.

Bibliography Type:	Description: (Last Updated: 02/04/2022)
Awards	Sekyi M. "HRP Investigators' Workshop Postdoc Lightning Talk 1st Place Award, 2021 NASA Human Research Program Investigators' Workshop, Virtual, February 1-4, 2021." Feb-2021