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	PI Name:	Neelam, Srujana Ph.D.		
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Discipline:       Note         Space Biology Special Category:       (1) Cell Culture         PI Email:       oginov@xiosi.c.du       Fax:       FY         PI Organization Type:       UNVERSITY       Phone:       Congunization Special Category:       Fy         PI Address 1:       College of Letters and Science       Fy       Fy         PI Address 2:       Birge Hall, 430 Lincoln Dr       Fy       Fy         PI Meb Page:       Ty       Fy       Fy         Chyper Special Category:       Madison       State:       WI         Special Category:       Madison       State:       WI         Chyper Special Category:       Madison       State:       WI         Pi Address 2:       Binge Hall, 430 Lincoln Dr       Fy       Fy         Chyper Special Category:       Madison       State:       WI       Fy         Pi Address 2:       State Special Category:       State:       WI       Fy         Chyper Special Category:       Madison       State:       WI       Fy	Space Biology Element:	(1) Cell & Molecular Biology		
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Induction         Induction         Protocol           P1 Organization Type:         UNIVERSITY         Phone:           Organization Name:         College of Letters and Science         Image: Science           P1 Address 1:         College of Letters and Science         Image: Science           P1 Address 2:         Birge Hall, 430 Lincoln Dr         Image: Science           P1 Web Page:         State:         W1           Chy:         Madison         State:         W1           Zip Code:         53706         Congressional District:         2           Comments:         Sciencitation / Fundieng         Science Biology (ROSBio)         Science Biology (ROSBio)           Start Date:         Cound         Sulicitation / Fundieng         Science Biology (ROSBio)           Start Date:         Cound         Sulicitation / Fundieng         Science Biology (ROSBio)           Start Date:         Cound         Sulicitation / Fundieng         Science Biology (ROSBio)           Solicitation / Fundieng         Sulicitation / Fundieng         Science Biology (ROSBio)           Solicitation / Fundieng         Sulicitation / Fundieng         Science Biology (ROSBio)           Solicitation / Fundieng         Sulicitation / Fundieng         Science Biology (ROSBio)           Solof Sol Docs:	Space Biology Special Category:	(1) Cell Culture		
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Zip Code:S3706Congressional District:2Comments:Project Type:GroundSolicitation / Funding Solicitation / Source2016-17 Space Biology (ROSBio) NNH16ZTT001N-MS, PS, AB, App D,E,F: Research Using Microgravity Simulation Devices, Parabolic and Suborbital Flights, and Antarctic BalloonsStart Date:0201/2020End Date0/31/2022No. of Pst Does:No. of PhD Degrees:1No. of PhD Candidates:No. of Master' Degrees:1No. of Bachelor's Candidates:Monitoring Center:NASA KSCContact Monitor:Zhang, YeContact Phone:321-861-3253Contact Email:Ver Zhang- I Ginasa.gov321-861-3253Flight Arsignment:NOTE: End date changed to 1/31/2022 per NSSC information (Ed., 4/9/2123). This project will be contand with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be contanted with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued with Dr. Simon Gilroy from the University of Wisconsin in April J 2021. (Ed., 2/2/23). This project will be continued wit	PI Web Page:			
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Task Description:	Altered gravity is known to influence various cell functions like cell proliferation, signal transduction, and gene expression, in addition to the changes in cell morphology, focal adhesion, and cytoskeletal organization across a wide range of cell types. Such wide range of phenotypic changes in altered gravity is linked to various abnormalities observed in astronauts returning from space but the biological mechanisms resulting in these changes are not yet understood. Our preliminary results suggest that simulated microgravity significantly alters nuclear morphology. Changes in nuclear shape can alter gene expression because the nucleus houses the genome, and changes in nuclear shape can alter chromatin conformation. Therefore, we propose to understand the mechanisms by which true altered gravity impacts nuclear morphology. Our hypothesis is that the LINC (Linker of Nucleoskeleton to Cytoskeleton) complex, a nuclear envelope complex which links the cytoskeleton to the nucleus, is sensitive to altered gravity mediated effects on the nuclear structure, nuclear tension, and subsequently on gene expression. We propose to identify the effects of altered gravity on nuclear morphology and cytoskeletal organization in human breast epithelial cells flown in parabolic flight. Cells flown will be cultured in a specially designed hardware to control for temperature and humidity, and the cells will be fixed on flight at different timepoints. High resolution imaging of cell shape and nuclear shape reconstruction and quantification of shape parameters. We will characterize the mechanical forces acting on the nucleus using a Fluorescence Resonance Energy Transfer (FRET) probe biosensor to investigate the effect of altered gravity on the nuclear tension. Finally, we will identify the genes that are differentially expressed in LINC disrupted cells to understand the mechanotransduction pathway in sensing altered gravity.			
Rationale for HRP Directed Research:				
Research Impact/Earth Benefits:	Cellular structures are permanently under the force of gravity in the Earth's gravitational field. Altered gravity has been demonstrated to have profound effects at the cellular and molecular level. Changes in cell morphology, proliferation, differentiation, signal transduction, and gene expression occur after exposure to altered gravity for just a few seconds. Each of the cytoskeletal elements and focal adhesion proteins become differentially expressed in altered gravity. Despite these results, it is not yet understood how change in gravitational forces alters the expression of genes. The LINC complex (Linker of Nucleoskeleton and Cytoskeleton) connects the nucleus and the surrounding cytoskeleton to mechanically couple the nuclear lamina with the cytoskeleton. Recent advances in our understanding of how nuclear envelope and nucleoskeleton associated elements contribute to both health and disease make it clear that cells require a functional mechanical coupling mediated by the LINC complex to respond normally to external mechanical forces. This research will answer the following question: Does altered gravity induce changes in force transmission through the LINC complex and LINC-associated gene expression by analyzing fibroblasts subjected to microgravity of parabolic flight?			
Task Progress:	Performed experiments on two parabolic flights. In the first parabolic flight we performed experiments to analyze the nuclear morphology changes and differential gene expression in LINC disrupted human breast epithelial cells. In the second parabolic flight we transfected human breast epithelial cells with a Nesprin FRET construct that allows us to measure the tension in the nuclear envelope during altered gravity. We fixed the samples at 1st, 7th, and 30th parabolas along with in flight controls and ground controls. Post-flight analysis i. Microscopy Completed imaging all the samples transfected with control and FRET tension sensors with laser scanning confocal microscope. Completed imaging samples stained for nucleus and actin cytoskeleton from the first parabolic flight. The samples were collected at 1st, 7th, and 15th parabolas, inflight controls and ground controls. We are currently performing quantitative analysis on the acquired images. ii. RNA isolation and RNAseq analyses RNA was successfully isolated and RNA sequencing performed at the Interdisciplinary Center for Biotechnology Research (ICBR) University of Florida. Results are currently being analyzed to extract patterns of gene expression.			
Bibliography Type:	Description: (Last Updated: 01/08/2024)			
Articles in Peer-reviewed Journals	Neelam S, Lee A, Lane MA, Udave C, Levine HG, Zhang Y. "Module to support real-time microscopic imaging of living organisms on ground-based microgravity analogs." Appl Sci. 2021;11(7):3122. <u>https://www.mdpi.com/2076-3417/11/7/3122</u> ; , Jan-2021			